

RUMINAL AND METHANOGEN VACCINES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/422,312, filed on November 3, 2022, the entire contents of which are hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Controlling methane emissions from animals, e.g., ruminants, is a new and emerging field.

SUMMARY

[0003] The present disclosure identifies certain challenges with increases in global surface temperature due to increased levels of greenhouse gases, namely methane (CH₄). For example, the present disclosure identifies that increase in methane from agriculture represents a major source of greenhouse gas emissions. The present disclosure also identifies that reducing methane emissions from animals, e.g., ruminants, will be important to reduce greenhouse gas emissions.

[0004] Among other things, the present disclosure provides technologies for reducing methane emissions from animals, e.g., ruminants, by providing compositions for vaccinating animals, e.g., ruminants, against ruminal-associated antigens. In some embodiments, a composition comprising ruminal-associated antigens can further comprise one or more chemokines and/or cytokines. Without wishing to be bound by theory, the present disclosure proposes that a composition comprising a ruminal-associated antigen (e.g., a ruminal antigen and/or a methanogen antigen), can reduce methane emissions and/or reduce the abundance of microorganisms (e.g., methanogens) in the digestive tract of animals. In some embodiments, reducing methane emissions and/or reducing abundance of methanogens in the digestive tract of animals (e.g., ruminants) can increase the energy efficiency of animals.

[0005] This disclosure provides an isolated polynucleotide encoding one or more ruminal-associated antigens, fragments thereof, variants thereof, or variant fragments thereof.

[0006] In some embodiments, one or more ruminal-associated antigens comprises one or more ruminal antigens.

[0007] In some embodiments, one or more ruminal antigens are derived from: a polypeptide that is involved in attachment to fermenting bacteria, or a fragment or variant or variant fragment thereof.

[0008] In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 ruminal antigens.

[0009] In some embodiments, one or more ruminal-associated antigens comprises one or more methanogen antigens.

[0010] In some embodiments, one or more methanogen antigens are derived from a polypeptide found on a cell surface of a wild-type methanogen, or a fragment or variant or variant fragment thereof.

[0011] In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, or at least 20 methanogen antigens.

[0012] In some embodiments, a polynucleotide comprises about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 20, about 4 to about 20, about 5 to about 20, about 6 to about 20, about 7 to about 20, about 8 to about 20, about 9 to about 20, about 10 to about 20, or about 15 to about 20 methanogen antigens.

[0013] In some embodiments, one or more methanogen antigens are the same, e.g., having the same sequence.

[0014] In some embodiments, one or more methanogen antigens are different, e.g., having different sequences.

[0015] In some embodiments, one or more methanogen antigens comprise:

[0016] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;

[0017] (ii) one or more secreted antigens comprising a signal peptide;

- [0018] (iii) a plurality of peptides having at least 80% sequence identity to each other;
- [0019] (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
- [0020] (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity;
- [0021] (vi) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide;
- [0022] (vii) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; or
- [0023] (viii) or any combination thereof.
- [0024] In some embodiments, one or more methanogen antigens comprise:
- [0025] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
- [0026] (ii) one or more secreted antigens comprising a signal peptide;
- [0027] (iii) a plurality of peptides having at least 80% sequence identity to each other;
- [0028] (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
- [0029] (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity;
- [0030] (vi) or any combination thereof.
- [0031] In some embodiments, a polynucleotide comprises three methanogen antigens. In some embodiments, the three methanogen antigens are associated with at least three different methanogen species.
- [0032] In some embodiments, one or more methanogen antigens comprise:
- [0033] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
- [0034] (ii) one or more secreted antigens comprising a signal peptide;
- [0035] (iii) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;

- [0036] (iv) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide; or
- [0037] (v) any combination thereof.
- [0038] In some embodiments, a polynucleotide comprises five methanogen antigens. In some embodiments, five methanogen antigens are associated with at least five different methanogen species.
- [0039] In some embodiments, one or more methanogen antigens comprise:
- [0040] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
- [0041] (ii) one or more secreted antigens comprising a signal peptide;
- [0042] (iii) a plurality of peptides having at least 80% sequence identity to each other;
- [0043] (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
- [0044] (v) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; or
- [0045] (vi) any combination thereof.
- [0046] In some embodiments, a polynucleotide comprises eight methanogen antigens. In some embodiments, eight methanogen antigens are associated with at least three different methanogen species.
- [0047] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different, at least 5 different, at least 6 different, at least 7 different, at least 8 different, at least 9 different, at least 10 different, at least 11 different, at least 12 different, at least 15 different or at least 20 different methanogen species.
- [0048] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with about 2 different, about 3 different, about 4 different, about 5 different, about 6 different, about 7 different, about 8 different, about 9 different, about 10 different, about 11 different, about 12 different, about 15 different or about 20 different methanogen species.

[0049] In some embodiments, one or more methanogen antigens are derived from a polypeptide found on the cell surface of a wild-type methanogen, or a fragment or variant thereof or variant fragment thereof.

[0050] In some embodiments, one or more methanogen antigens is secreted.

[0051] In some embodiments, a methanogen antigen comprises a peptide that is involved in adhesion, attachment, or mobility, or a fragment or variant of a peptide that is involved in adhesion, attachment, mobility.

[0052] In some embodiments, a secreted methanogen antigen comprises a signal peptide. In some embodiments, a signal peptide can be predicted using a prediction algorithm and optionally wherein the prediction score is at least 0.5.

[0053] In some embodiments, one or more methanogen antigens comprise one or more peptides having at least 80% sequence identity to a methanogen protein.

[0054] In some embodiments, a polynucleotide comprises a plurality of methanogen antigens each having at least 80% sequence identity to each other.

[0055] In some embodiments, a polynucleotide comprises a plurality of methanogen antigens wherein each methanogen antigen in the plurality is associated with a different methanogen species. In some embodiments, a polynucleotide comprises a plurality of methanogen antigens, and wherein the methanogen antigens in the plurality are associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species. In some embodiments, a polynucleotide comprises a plurality of methanogen antigens and wherein each methanogen antigen in the plurality is associated with the same methanogen species. In some embodiments, a methanogen species comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanomicrobium mobile*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*, or *Methanosarcina soligelidi*. In some embodiments, a methanogen species comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, or *Methanobrevibacter oralis*. In some embodiments, a methanogen species comprises: *Methanobrevibacter ruminantium* *Methanobrevibacter smithii* *Methanobrevibacter oralis* *Methanomicrobium mobile* or *Methanosphaera stadtmanae*.

[0056] In some embodiments, a polynucleotide comprises one or more methanogen antigens comprising one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity. In some embodiments, a polypeptide having signal peptidase activity: (i) is membrane bound; (ii) has the ability to cleave one or more signal peptides; (iii) plays a role in converting a secretory protein to a mature form (e.g., from a non-secretory form to a secretory form); and/or (iv) or any combination thereof.

[0057] In some embodiments, a polynucleotide comprises one or more methanogen antigens comprising one or more peptides having: (1) at least 80% sequence identity to an Immunoglobulin (Ig)-like polypeptide, or (2) substantially similar function to an Ig-like domain-containing polypeptide. In some embodiments, an Ig-like domain-containing polypeptide comprises a polypeptide characterized as a cell-surface protein that facilitates binding to other cells and/or cell surfaces.

[0058] In some embodiments, a polynucleotide comprises one or more methanogen antigens comprising an adhesin or fragment or variant thereof, a pilli protein or a fragment or variant thereof, or a flagellin protein or a fragment or a variant thereof.

[0059] In some embodiments, a polynucleotide comprises a sequence encoding one or more methanogen antigens comprising an antigen provided in **Table 1** or a sequence with at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0060] In some embodiments, a polynucleotide comprises a sequence encoding one or more methanogen antigens comprising an antigen sequence provided in **Table 2** or a sequence with at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, a polynucleotide comprises a sequence encoding one or more methanogen antigens comprising a fragment, a variant, or a variant fragment of an antigen sequence provided in **Table 2**.

Exemplary antigen sequences provided in **Table 2** are marked with no bolding or italicizing.

[0061] In some embodiments, a polynucleotide comprises an antigen nucleic acid sequence provided in **Table 2** or a sequence with at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0062] In some embodiments, a polynucleotide comprises a sequence encoding a polypeptide having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a polypeptide sequence provided in **Table 2**.

[0063] In some embodiments, a polynucleotide comprises one or more methanogen antigens comprising one or more peptides having: (1) at least 80% sequence identity to an archaeal oligosaccharyltransferase (AglB) polypeptide, or (2) substantially similar function to an AglB polypeptide. In some embodiments, an AglB polypeptide is characterized as having N-glycosylation activity.

[0064] In some embodiments, a methanogen antigen comprises a peptide that is involved in adhesion, attachment, or mobility, or a fragment or variant of a peptide that is involved in adhesion, attachment, mobility.

[0065] In some embodiments, a methanogen antigen comprises an adhesin or fragment or variant thereof. In some embodiments, a methanogen antigen comprises an adhesin protein provided in Table 1 or a sequence with at least 85% identity thereto. In some embodiments, a methanogen antigen comprises mru1499, or a fragment or variant thereof.

[0066] In some embodiments, a methanogen antigen comprises an antigen sequence in **Table 2**, or a variant or a fragment or a variant fragment thereof. In some embodiments, a methanogen antigen comprises an antigen sequence in **Table 2** or a sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. Exemplary antigen sequences provided in **Table 2** are marked with no bolding or italicizing.

[0067] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 1, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 2, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0068] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 5, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 6, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0069] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 7, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 8, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0070] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 16, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 15, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0071] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 18, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or

100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 17, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0072] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 20, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 19, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0073] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 22, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 21, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0074] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 24, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 23, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0075] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 26, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 25, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0076] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 28, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 27, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0077] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 30, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 29, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0078] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 32, or a sequence with at least 85%, at least

86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 31, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0079] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 34, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 33, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0080] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 36, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 35, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0081] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 38, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 37, or a sequence with at least 85%, at least 86%, at least

87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0082] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 40, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 39, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0083] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 42, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 41, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0084] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 44, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 43, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0085] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 46, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 45, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0086] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 48, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 47, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0087] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 50, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 49, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0088] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 52, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or

100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 51, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0089] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 54, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 53, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0090] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 56, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 55, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0091] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 58, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 57, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0092] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 60, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 59, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0093] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 62, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 61, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0094] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 64, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 63, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0095] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 66, or a sequence with at least 85%, at least

86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 65, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0096] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 68, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 67, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0097] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 70, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 69, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0098] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 72, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 71, or a sequence with at least 85%, at least 86%, at least

87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0099] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 74, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 73, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0100] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 76, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 75, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0101] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 78, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 77, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0102] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 80, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 79, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0103] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 82, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 81, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0104] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 84, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 83, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0105] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 86, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or

100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 85, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0106] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 88, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 87, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0107] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 90, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 89, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0108] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 92, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 91, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0109] In some embodiments, a methanogen antigen comprises an antigen sequence encoded by a nucleotide sequence provided in SEQ ID NO: 94, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto. In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 93, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0110] In some embodiments, a methanogen antigen comprises a pili protein or fragment or variant thereof.

[0111] In some embodiments, a methanogen antigen comprises a flagellin protein or fragment or variant thereof.

[0112] In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 methanogen antigens.

[0113] In some embodiments, a polynucleotide comprises a signal peptide. In some embodiments, a signal peptide has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% homology to an archaeal signal peptide.

[0114] In some embodiments, a polynucleotide comprises at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% homology to a bacterial signal peptide.

[0115] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 97, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0116] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 98, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0117] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 99, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0118] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 100, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0119] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 101, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0120] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 102, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0121] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 103, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0122] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 104, or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0123] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 105, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0124] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 106, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0125] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 107, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0126] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 108, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0127] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 109, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0128] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 110, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0129] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 111, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0130] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 112, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0131] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 113, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0132] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 114, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0133] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 115, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0134] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 116, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0135] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 117, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0136] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 118, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0137] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 119, or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0138] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 120, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0139] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 121, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0140] In some embodiments, a methanogen antigen comprises an antigen sequence provided in SEQ ID NO: 122, or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0141] In some embodiments, a signal peptide is or comprises a PPA2 signal peptide, or a fragment or variant thereof.

[0142] In some embodiments, signal peptide is or comprises an SSP signal peptide, or a fragment or variant thereof.

[0143] In some embodiments, a signal peptide is or comprises a SARS-CoV-2 Spike secretion signal, or a fragment or variant thereof.

[0144] In some embodiments, a signal peptide is situated at the N terminal of the ruminal-associated antigen sequence.

[0145] In some embodiments, a polynucleotide comprises: (i) a first nucleotide sequence encoding one or more ruminal antigens, and (ii) a second nucleotide sequence encoding one or more methanogen antigens.

[0146] In some embodiments, a polynucleotide comprises: (i) a first nucleotide sequence encoding one or more ruminal antigens, (ii) a second nucleotide sequence encoding one or more methanogen antigens; and (iii) a third nucleotide sequence encoding a chemokine and/or cytokine.

[0147] In some embodiments, a polynucleotide comprises: (i) a first nucleotide sequence encoding one or more ruminal antigens, (ii) a second nucleotide sequence encoding a chemokine and/or cytokine.

[0148] In some embodiments, a polynucleotide comprises: (i) a first nucleotide sequence encoding one or more methanogen antigens; and (ii) a second nucleotide sequence encoding a chemokine and/or cytokine.

[0149] In some embodiments, a polynucleotide comprises a nucleotide encoding a chemokine and/or a cytokine. In some embodiments, a chemokine and/or cytokine are chosen from APRIL, VIP and/or CXCL10.

[0150] In some embodiments, a polynucleotide comprises a sequence encoding a chemokine or cytokine provided in SEQ ID NO: 10 or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0151] In some embodiments, a polynucleotide comprises a chemokine or cytokine nucleotide sequence provided in SEQ ID NO: 9 or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0152] In some embodiments, a polynucleotide comprises a sequence encoding a chemokine or cytokine provided in SEQ ID NO: 12 or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0153] In some embodiments, a polynucleotide comprises a chemokine or cytokine nucleotide sequence provided in SEQ ID NO: 11 or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0154] In some embodiments, a polynucleotide comprises a sequence encoding a chemokine or cytokine provided in SEQ ID NO: 14 or a sequence having at least 85%, at least 86%, at least

87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0155] In some embodiments, a polynucleotide comprises a chemokine or cytokine nucleotide sequence provided in SEQ ID NO: 13 or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least or 100% identity thereto.

[0156] In some embodiments, a nucleotide sequence encoding one or more ruminal antigens comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 ruminal antigens.

[0157] In some embodiments, a nucleotide sequence encoding one or more methanogen antigens comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 methanogen antigens.

[0158] In some embodiments, a first nucleotide sequence, a second nucleotide sequence and/or a third nucleotide sequence are situated on one polynucleotide.

[0159] In some embodiments, a first nucleotide sequence, a second nucleotide sequence and/or a third nucleotide sequence are situated on different polynucleotides.

[0160] In some embodiments, one or more ruminal antigens and/or one or more methanogen antigens are each situated on a separate nucleotide sequence.

[0161] In some embodiments, a polynucleotide comprises at least 2 methanogen antigens and the at least 2 methanogen antigens are situated on separate nucleotide sequences.

[0162] In some embodiments, a polynucleotide comprises at least 3 methanogen antigens and the at least 3 methanogen antigens are situated on separate nucleotide sequences.

[0163] In some embodiments, a polynucleotide comprises at least 4 methanogen antigens and the at least 4 methanogen antigens are situated on separate nucleotide sequences.

[0164] In some embodiments, a polynucleotide comprises at least 5 methanogen antigens and the at least 5 methanogen antigens are situated on separate nucleotide sequences.

[0165] In some embodiments, a polynucleotide comprises at least 6 methanogen antigens and the at least 6 methanogen antigens are situated on separate nucleotide sequences.

[0166] In some embodiments, a polynucleotide comprises at least 7 methanogen antigens and the at least 7 methanogen antigens are situated on separate nucleotide sequences.

[0167] In some embodiments, a polynucleotide comprises at least 8 methanogen antigens and the at least 8 methanogen antigens are situated on separate nucleotide sequences.

[0168] In some embodiments, a polynucleotide comprises at least 9 methanogen antigens and the at least 9 methanogen antigens are situated on separate nucleotide sequences.

[0169] In some embodiments, a polynucleotide comprises at least 10 methanogen antigens and the at least 10 methanogen antigens are situated on separate nucleotide sequences.

[0170] In some embodiments, one or more ruminal antigens and/or one or more methanogen antigens are situated on the same nucleotide sequence.

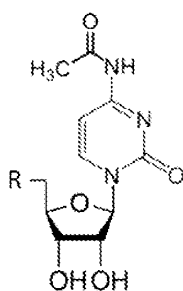
[0171] In some embodiments, a polynucleotide comprises a transmembrane domain.

[0172] In some embodiments, a polynucleotide further comprises a complement C3d-binding polypeptide from an immunoglobulin-binding protein (Sbi) of *Staphylococcus aureus*. In some embodiments, a complement C3d-binding polypeptide is or comprises one or both of domain III and domain IV of the Sbi of *Staphylococcus aureus*, or a functional fragment or a variant thereof.

[0173] In some embodiments, a polynucleotide is or comprises DNA.

[0174] In some embodiments, a polynucleotide is or comprises RNA. In some embodiments, a RNA comprises a 5' cap. In some embodiments, a RNA comprises a polyA tail.

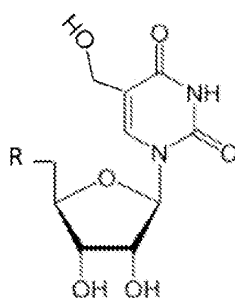
[0175] In some embodiments, a polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

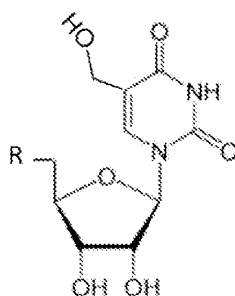
[0176] In some embodiments, a polyribonucleotide further comprises one or more modified ribonucleotides other than N4-acetylcytidine, optionally wherein the nucleoside is chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof.

[0177] In some embodiments, a nucleoside of the one or more modified ribonucleotides is 5-hydroxymethyluridine, and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

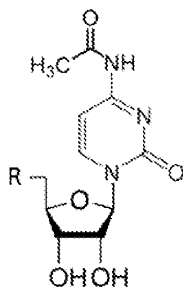
[0178] In some embodiments, a polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0179] In some embodiments, a polyribonucleotide further comprises one or more modified ribonucleotides other than 5-hydroxymethyluridine, wherein the one or more modified ribonucleotides comprises a nucleoside chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof.

[0180] In some embodiments, a nucleoside of the one or more modified ribonucleotides is N4-acetylcytidine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0181] Also provided herein is a polypeptide encoded by a polynucleotide disclosed herein.

[0182] This disclosure further provides a composition comprising one or more polyribonucleotides disclosed herein or a polypeptide disclosed herein.

[0183] In some embodiments, a composition comprises a plurality of polyribonucleotides disclosed herein. In some embodiments, each of the plurality of polyribonucleotides comprises one or more methanogen antigens. In some embodiments, each of the plurality of polyribonucleotides encodes a different methanogen antigen. In some embodiments, each of the plurality of polyribonucleotides encodes the same methanogen antigen.

[0184] In some embodiments of a composition comprising a plurality of polyribonucleotides, the plurality of polyribonucleotides comprises a mixture of polyribonucleotides, e.g., a portion that encodes the same methanogen antigen and a portion that encodes a different methanogen antigen.

[0185] In some embodiments, a composition disclosed herein comprises one or more polynucleotides comprising one or more ruminal-associated antigens, e.g., methanogen antigens. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition) comprises a plurality of polynucleotides (e.g., a plurality of RNA) each comprising an antigen. In some embodiments, a composition (e.g., a multi-component composition, a multi-component vaccine composition) comprises a plurality of polynucleotides (e.g., a plurality of RNA) each comprising one or more antigens (e.g., the same or different antigens).

[0186] In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 1% of a first polynucleotide and about 99% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 5% of a first polynucleotide and about 95% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 10% of a first polynucleotide and about 90% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 20% of a first polynucleotide and about 80% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 30% of a first polynucleotide and about 70% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 40% of a first polynucleotide and about 60% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 50% of a first polynucleotide and about 50% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 60% of a first polynucleotide and about 40% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 70% of a first polynucleotide and about 30% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 80% of a first polynucleotide and about 20% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine

composition), comprising a plurality of polynucleotides comprises about 90% of a first polynucleotide and about 10% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 99% of a first polynucleotide and about 1% of one or more additional polynucleotides.

[0187] In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 33% of a first polynucleotide, about 33% of a second polynucleotides and about 33% of a third polynucleotide.

[0188] In some embodiments, a composition is a pharmaceutical composition.

[0189] In some embodiments, a composition is an immunogenic composition.

[0190] In some embodiments, a composition is a vaccine composition.

[0191] In some embodiments, a composition is formulated for delivery with a carrier. In some embodiments, a carrier is a lipid nanoparticle, a cationic lipid, a polymeric particle.

[0192] In some embodiments, a composition is formulated for delivery without a carrier.

[0193] This disclosure provides, a method comprising administering a composition disclosed herein, to a cell, tissue or animal, e.g., a ruminant.

[0194] In some embodiments, a method is a vaccination method.

[0195] In some embodiments, an animal is a ruminant, e.g., cattle, sheep, goats, buffalo, moose, antelope, caribou, or deer.

[0196] In some embodiments, an animal is a domestic animal.

[0197] In some embodiments, a vaccine reduces methane emissions from an animal as compared to an animal not administered a vaccine or administered a different vaccine.

[0198] In some embodiments, a composition is characterized in that administration of a composition to an animal reduces methane emissions from an animal as compared to an otherwise comparable animal not administered the composition or administered a different composition. In some embodiments, a reduction in methane emissions is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% as compared to an otherwise comparable animal not administered the composition or administered a different composition.

[0199] In some embodiments, a composition is characterized in that administration of the composition to the animal reduces a population of microorganisms in the animal, as compared to an otherwise comparable animal not administered the composition or administered a different composition. In some embodiments, microorganisms are methanogens. In some embodiments, methanogen comprises a methanogen from one or more of the following clades:

Methanobrevibacter, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Methanothermobacter*, *Candidatus Methanomethylophilus*, *Thermoplasmatales*.

[0200] In some embodiments, a methanogen comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter* sp. UBA188, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeete thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.

[0201] In some embodiments, a composition is characterized in that administration of the composition to the animal increases a growth rate of the animal as compared to the growth rate of an otherwise comparable animal not administered the composition or administered a different composition. In some embodiments, an increase in growth rate comprises a daily increase in weight of the animal, optionally wherein the daily increase in weight of the animal is an increase of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of weight as compared to an otherwise comparable animal not administered the composition or administered a different composition.

[0202] In some embodiments, a method disclosed herein comprises administering one dose of the vaccine composition to the animal.

[0203] In some embodiments, a method disclosed herein comprises administering a plurality of doses of the vaccine composition to the animal.

- [0204] In some embodiments, an animal is administered a first dose of the composition followed by one or more subsequent doses of the composition.
- [0205] In some embodiments, a first dose and the one or more subsequent doses of the composition comprise the same methanogen antigens and/or ruminal antigens.
- [0206] In some embodiments, a first dose and the one or more subsequent doses of the composition comprise different methanogen antigens and/or ruminal antigens.
- [0207] In some embodiments, a composition is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or 30 times to an animal.
- [0208] In some embodiments, a composition is administered once every 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months.
- [0209] In some embodiments, a composition is administered in combination with one or more additional agents.
- [0210] In some embodiments, a one or more additional agents comprises a chemical additive, a biological feed additive.
- [0211] In some embodiments, a composition is administered in combination with one or more additional compositions.
- [0212] In some embodiments, an additional composition immunizes an animal from a disease, e.g., an infectious disease.
- [0213] Also disclosed herein is a composition comprising an isolated polynucleotide disclosed herein, or a pharmaceutical composition disclosed herein, for use in administration to (e.g., vaccination of) an animal.
- [0214] This disclosure also provides use of a composition comprising an isolated polynucleotide disclosed herein, or a pharmaceutical composition disclosed herein, in the preparation of a medicament for administration to (e.g., vaccination of) an animal.
- [0215] In some embodiments of any of the composition for use, or use disclosed herein an isolated polynucleotide or pharmaceutical composition is administered to the animal.
- [0216] In some embodiments of any of the composition for use, or use disclosed herein administration of the isolated polynucleotide or pharmaceutical composition results in:
- [0217] (i) reduced methane emissions;
- [0218] (ii) reduced abundance of one or more microorganisms (e.g., methanogens) in a digestive tract of the animal; and/or

[0219] (iii) increased growth rate of the animal,

[0220] as compared to an otherwise comparable animal not administered the composition, or administered a different composition.

[0221] In some embodiments of any of the composition for use, or use disclosed herein the animal is a ruminant or a domestic animal.

[0222] In some embodiments of any of the composition for use, or use disclosed herein a methanogen comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolini*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp.* UBA188, *Methanosarcina soligelidi*, *Methanothermobacter thermotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vannieli*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0223] **FIGS. 1A-1B** describe antigen-specific IgG titers from serum, for an exemplary multi-component RNA vaccine formulation. Experimental groups comprised of calves receiving 1 Dose (Day 20, n=5), 2 Dose (Day 20 & 34, n=5), or no dose/untreated (UTD, n=5) of the exemplary RNA vaccine formulation: 0.5 mg total of 50% OVA_HNadj (85% HNmod1, wherein HNmod1 corresponds to Ac4C and 50% mru1499_HNadj (85% HNmod1). Serum was collected at Day 20, 34, and 90. ELISAs were run for each antigen of the multi-component vaccine, respectively (OVA, mru1499). Geometric mean reciprocal titers are provided for 1 Dose and 2 Dose groups, for each timepoint, and for each timepoint to measure OVA-IgG (**FIG. 1A**) and mru1499-IgG (**FIG. 1B**) response to the exemplary RNA vaccine in cattle. **FIG. 2** describes normalized relative abundance of methanogen species in rumen fluid, for an exemplary multi-component RNA vaccine formulation. Experimental groups comprised of calves receiving 1 Dose (Day 20, n=5), 2 Dose (Day 20 & 34, n=5), or no dose/untreated (UTD, n=5) of the

exemplary RNA vaccine formulation: 0.5 mg total of 50% OVA_HNadj (85% HNmod1) and 50% mru1499_HNadj (85% HNmod1). Rumen fluid was collected at Day 0, 20, 34, and 90. Samples were sequenced, resulting in relative abundance measurements of methanogen *Methanobrevibacter (Mbb.) ruminantium* M1 for 1 Dose and 2 Dose groups, for each timepoint in response to the exemplary RNA vaccine in cattle. Each timepoint value is a delta from that group's Day 0 value. The y-axis is normalized to 1.0 with the cohort's average pre-study M1/Archaea.

[0225] **FIGS. 3A-3B** describe normalized relative abundance of methanogen and archaeal species in rumen fluid, for exemplary RNA vaccine formulations targeting methanogen proteins and with various secretion signals. Experimental groups comprised of calf groups (n=4) receiving prime/boost doses of the specified RNA vaccine formulations (0.5 mg, 100% HNmod1). Rumen fluid was collected at Day 0, 20, 34, and 90, and processed into a pellet. Samples were sequenced, resulting in relative abundance measurements of methanogen *Methanobrevibacter (Mbb.) ruminantium* M1 (**FIG. 3A**) and total Archaea (**FIG. 3B**) for each formulation group in response to the exemplary RNA vaccine in cattle. Each timepoint value is a delta from that group's Day 0 value. The y-axis is normalized to 1.0 with the cohort's average pre-study M1/Archaea.

[0226] **FIG. 4** describes normalized relative abundance of methanogen and archaeal species in rumen fluid, for exemplary RNA vaccine formulations with cytokines and with double chem mods (HNmod1/HNmod2, wherein HNMod1 corresponds to Ac4C and HNMOD2 corresponds to 5hmU). Experimental groups comprised of calf groups (n=4) receiving prime/boost doses of the specified RNA vaccine formulations (0.5 mg; respective composition described in Figure). Experimental setup is similar to FIG. 3; however, RNA vaccine formulations include cytokine and double chem mod (HNmod1/HNmod2) features. Note n=1 for F8.

[0227] **FIG. 5** describes methane emissions per feed intake, for exemplary RNA vaccine formulations targeting methanogen proteins and with various secretion signals. Experimental groups comprised of calf groups (n=4) receiving prime/boost doses of specified RNA vaccine formulations (0.5 mg, 100% HNmod1; respective composition described in Figure). Enteric methane emissions were measured by animal, as average daily CH₄ (g/d) per total daily feed intake (lbs/d), and then averaged across an 8-day measurement timeframe.

[0228] FIG. 6 describes methane emissions per feed intake, for exemplary RNA vaccine formulations with cytokines and with single chem mods (HNmod1) or double chem mods (HNmod1, HNmod2). Experimental groups comprised of calf groups (n=4) receiving prime/boost doses of specified RNA vaccine formulations (0.5 mg; respective composition described in Figure). Experimental setup is similar to FIG. 5; however, RNA vaccine formulations include cytokine features. Note n=3 for group F5 and n=2 for group F7, due to calf non-adherence to GreenFeed Systems.

[0229] FIGS. 7A-7B describe normalized relative abundance of all archaeal species in rumen fluid, for exemplary multi-component RNA vaccine formulations targeting multiple methanogen proteins. Experimental groups comprised of calf groups (n=10) receiving three doses of specified RNA vaccine formulations (0.55 mg, 100% Hnmod1; respective composition described in Table 3). Rumen fluid was collected at Day 90, 109 and 122, and processed into a pellet, in which Day 90 represents a pre-injection (prior to the third injection) sample ("PRE", 14 days prior to boost), Day 109 represents a 5-day post-injection (after the third injection) sample ("5d POST"), and Day 122 represents an 18-day post-injection (after the third injection) sample ("18d POST"). In this study, rumen collections were not taken after the first and second injections, to minimize disturbance to the rumen biome. Samples were sequenced, resulting in relative abundance measurements (e.g., % of total sequence population) for all bacterial and archaeal species in response to RNA vaccine formulations CNT(OVA) and F3. The bacterial and archaeal species tested included *M. wolinii*, *M. stadtmanae*, *M. oralis*, *M. smithii*, and *M. ruminantium*. FIG. 7A shows the relative abundance levels of all archaeal species identified at time points PRE, +5-d POST and +18-d POST. The "Other" category comprises of species with a relative abundance of < 0.01%, and include *M. mazei*, *M. soligelidi*, *M. arboriphilus*, *Thermoplasmatales sp.*, *M. formicicum*, *M. boviskoreani*, *M. mobile*, *Methanothermobacter sp.*, *Candidatus Methanomethylophilus alvus*, and *Nitrososphaera sp.* FIG. 7B shows the % change of each archaeal species relative to the PRE timepoint and normalized to the CNT(OVA).

[0230] FIGS. 8A-8B describe raw methane emissions and methane emissions per feed intake, for exemplary multi-component RNA vaccine formulations targeting multiple Methanogen proteins. Experimental groups comprised of calf groups (n=10) receiving prime/boost doses of specified RNA vaccine formulations (0.55 mg, 100% Hnmod1; respective composition described in Table 3). Enteric methane emissions were measured by animal, as

average daily CH₄ (g/d) per total daily feed intake (lbs/d), and then averaged across a 14-day timeframe post prime injection, T1 (D0 – D14), and the pre-injection baseline, T0 (D-8 - D-2). Percent Delta Methane (**FIG. 8A**) and Methane/Intake (**FIG. 8B**) are determined by dividing raw methane or methane/intake values of the later timeframe (T1) by that of the baseline (T0), and then normalized by measuring the difference in this percentage for each treatment group versus that of the control group (CNT-OVA). Error bars reflect standard error.

[0231] **FIG. 9** describes growth efficiency measurements for exemplary RNA vaccine formulations targeting Methanogen proteins and with various secretion signals, with cytokines, and with single chem mods (HNmod1) or double chem mods (HNmod1, HNmod2). Experimental groups comprised of calf groups (n=4) receiving prime/boost doses of specified RNA vaccine formulations (0.5 mg; compositions correspond to those in Fig. 5 and 6). Average Daily Gain (ADG, lb/day), a reflection of growth efficiency where a larger value reflects increased efficiency, was calculated by determining the linear regression of weight measurements between time periods. Specifically, “Pre-Injection Baseline” is the average weight gained per day from D-32 to D0 (e.g., 32-day period pre-injection), “D0-D20” is the average weight gained per day from D0 to D20 (e.g., 20-day period post prime injection), and “D20-D90” is the average weight gained per day from D20 to D90 (e.g., 70-day period post boost injection). Delta ADG represents the difference between ADG at a specified timeframe and the Pre-Injection Baseline per treatment group, and is normalized by then finding the difference in this delta and that of the control group (UTD). Not displayed is vaccine condition F8 (n=1), which had a ADG change of +1.70 lb/day at D20 and +0.91 lb/day at D90.

[0232] **FIGS. 10A-10B** describe growth efficiency measurements for exemplary multi-component RNA vaccine formulations targeting multiple Methanogen proteins. Experimental groups comprised of calf groups (n=10) receiving prime/boost doses of specified RNA vaccine formulations (0.55 mg; 100% HNmod1; respective composition described in Table 3). Average Daily Gain (ADG, lb/day), a reflection of growth efficiency where a larger value reflects increased efficiency, was calculated by determining the linear regression of weight measurements between time periods (**FIG. 10A**). Specifically, “Pre-Injection Baseline” is the average weight gained per day from D-49 to D0 (e.g., 50-day period pre-injection), “D25” is the average weight gained per day from D0 to D25 (e.g., 25-day period post prime injection), and “D90” is the average weight gained per day from D-25 to D90 (e.g., 65-day period post

boost injection). **FIG. 10B** shows Delta ADG in a pound per day unit (lb/d), representing the difference between ADG at a specified timeframe and the Pre-Injection Baseline per treatment group, then normalized by finding the difference in this delta and that of the control group (CNT-OVA). The right panel shows Delta ADG in a percentage unit (%), representing the ratio of the later time by the earlier time, then normalized by finding the difference in this percentage and that of the control group (CNT-OVA).

CERTAIN DEFINITIONS

[0233] *About or approximately:* As used herein, the terms “about” and “approximately,” when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by “about” or “approximately” in that context. For example, in some embodiments, the term “about” or “approximately” may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0234] *Administering:* As used herein, the term “administering” or “administration” typically refers to administration of a composition to an animal to achieve delivery of an agent that is, or is included in, the composition. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to an animal, e.g., a ruminant. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[0235] *Antigen:* The term “antigen”, as used herein, refers to an agent that elicits an immune response; and/or (ii) an agent that binds to a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody. In some embodiments, an antigen elicits a humoral response (e.g., including production of antigen-specific antibodies); in some embodiments, an antigen elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen). In some embodiments, an antigen comprises at least one epitope of a target protein. In some

embodiments, an epitope may be a linear epitope. In some embodiments, an epitope may be a conformational epitope. In some embodiments, an antigen binds to an antibody and may or may not induce a particular physiological response in an organism. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments other than a biologic polymer [e.g., other than a nucleic acid or amino acid polymer] etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a glycan. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (e.g., together with other materials, for example in an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source). In some embodiments, antigens utilized in accordance with the present invention are provided in a crude form. In some embodiments, an antigen is a recombinant antigen.

[0236] *Delivery/contacting:* As used interchangeably herein, the term “delivery,” “delivering,” or “contacting” refers to introduction of a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) into a target cell. A target cell can be cultured *in vitro* or *ex vivo* or be present in an animal (*in vivo*). Methods of introducing a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) into a target cell can vary with *in vitro*, *ex vivo*, or *in vivo* applications. In some embodiments, a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) can be introduced into a target cell in a cell culture by *in vitro* transfection. In some embodiments, a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) can be introduced into a target cell via delivery vehicles (e.g., nanoparticles, liposomes, and/or complexation with a cell-penetrating agent). In some embodiments, a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) can be introduced into a target cell in an animal by administering a fusion polynucleotide (e.g., as described herein) or a fusion polypeptide (e.g., as described herein) to an animal.

[0237] *Functional:* As used herein, the term “functional” is used to refer to a form or fragment of an entity that exhibits a particular property and/or activity.

[0238] *Fragment:* A “fragment” of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole.

In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a fragment comprises a polynucleotide fragment. In some embodiments, a fragment comprises a polypeptide fragment. In some embodiments, a polynucleotide fragment or a polypeptide fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (e.g., residues) as found in the whole polynucleotide or whole polypeptide. In some embodiments, a polynucleotide fragment or a polypeptide fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (e.g., residues) found in the whole polynucleotide or whole polypeptide. The whole polypeptide or whole polynucleotide may in some embodiments be referred to as the “parent” of the polynucleotide fragment or polypeptide fragment.

[0239] *Methanogen*: As used herein, a “methanogen” is a microorganism that produces methane. In some embodiments, a methanogen can inhabit a digestive tract of an animal and participate in a fermentation process. In some embodiments, a methanogen is an organism that conserves energy for ATP synthesis by producing methane gas. In some embodiments, a methanogen can inhabit a digestive tract of a ruminant. In some embodiments, a methanogen can inhabit a digestive tract of a non-ruminant. Exemplary methanogens are provided in Buan N.R. (2018) *Emerg Top life Sci* 2(4): pp. 629-646, the entire contents of which are hereby incorporated by reference. In some embodiments, a methanogen comprises: one or more methanogens from one or more of the following clades: *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Methanothermobacter*, *Candidatus Methanomethylophilus*, *Thermoplasmatales*. In some embodiments, a methanogen comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*, *Methanosarcina soligelidi*, *Methanothermobacter thermotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vannielii*.

Methanococcus maripaludis, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.

[0240] *Nucleic acid / Oligonucleotide / Polynucleotide*: As used herein, the terms “nucleic acid” and “polynucleotide” and “oligonucleotide” are used interchangeably, and refer to a polymer of 3 nucleotides or more. In some embodiments, a nucleic acid comprises DNA. In some embodiments, a nucleic acid comprises RNA. In some embodiments, a nucleic acid comprises messenger RNA (mRNA). In some embodiments, a nucleic acid is single stranded. In some embodiments, a nucleic acid is double stranded. In some embodiments, a nucleic acid comprises both single and double stranded portions. In some embodiments, a nucleic acid comprises a backbone that comprises one or more phosphodiester linkages. In some embodiments, a nucleic acid comprises a backbone that comprises both phosphodiester and non-phosphodiester linkages. For example, in some embodiments, a nucleic acid may comprise a backbone that comprises one or more phosphorothioate or 5'-N-phosphoramidite linkages and/or one or more peptide bonds, *e.g.*, as in a “peptide nucleic acid”. In some embodiments, a nucleic acid comprises one or more, or all, natural residues (*e.g.*, adenine, cytosine, deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, guanine, thymine, uracil). In some embodiments, a nucleic acid comprises one or more, or all, non-natural residues. In some embodiments, a non-natural residue comprises a nucleoside analog (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 6-O-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a non-natural residue comprises one or more modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared to those in natural residues. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or polypeptide. In some embodiments, a nucleic acid has a nucleotide sequence that comprises one or more introns. In some embodiments, a nucleic acid may be prepared by isolation from a natural source, enzymatic synthesis (*e.g.*, by

polymerization based on a complementary template, *e.g.*, *in vivo* or *in vitro*, reproduction in a recombinant cell or system, or chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, or 20,000 or more residues or nucleotides long. When a number of nucleotides is used as an indication of size, *e.g.*, of a fusion polynucleotide, a certain number of nucleotides refers to the number of nucleotides on a single strand, *e.g.*, of a fusion polynucleotide.

[0241] *Polypeptide*: The term “polypeptide”, as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids or more. Those of ordinary skill in the art will appreciate that the term “polypeptide” is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence recited herein, but also to encompass polypeptides that represent functional, biologically active, or characteristic fragments, portions or domains (*e.g.*, fragments, portions, or domains retaining at least one activity) of such complete polypeptides. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, *etc.* In some embodiments, polypeptides may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof.

[0242] *Polyribonucleotide*: As used herein, the term “polyribonucleotide” refers to a polymer of 3 ribonucleotides or more. In some embodiments, a polyribonucleotide is single stranded. In some embodiments, a polyribonucleotide is double stranded. In some embodiments, a polyribonucleotide comprises both single and double stranded portions. In some embodiments, a polyribonucleotide can comprise a backbone structure as described in the definition of “*Nucleic acid / Oligonucleotide*” above. A polyribonucleotide can be a regulatory RNA (*e.g.*, siRNA, microRNA, *etc.*), or a messenger RNA (mRNA) oligonucleotide. In some embodiments, a polyribonucleotide typically comprises at its 3' end a poly(A) region. In some embodiments, a polyribonucleotide typically comprises at its 5' end an art-recognized cap structure, *e.g.*, for

recognizing and attachment of an RNA to a ribosome to initiate translation. In some embodiments, a polyribonucleotide comprises an RNA oligonucleotide. When a number of ribonucleotides is used as an indication of size, *e.g.*, for a polyribonucleotide, a certain number of nucleotides refers to the number of ribonucleotides on a single strand.

[0243] *Ruminal-associated antigen:* As used herein, a “ruminal associated antigen” is an antigen that is expressed in a ruminant. In some embodiments, a ruminal-associated antigen is a “ruminal antigen”, *e.g.*, an antigen encoded by a nucleotide sequence naturally occurring in a ruminant’s genome. In some embodiments, a ruminal-associated antigen is encoded by a nucleotide sequence that is not naturally occurring in a ruminant’s genome, *e.g.*, has been introduced into a ruminant’s genome, or is part of a microorganism that can inhabit a digestive tract, *e.g.*, a rumen, of a ruminant. In some embodiments, a ruminal-associated antigen comprises: a methanogen antigen, an antigen from a rumen ciliate symbiotic with methanogens; an antigen from a hydrogen-producing organism; an antigen from a taxa associated with low feed efficiency, or any combination thereof. In some embodiments, a ruminal-associated antigen is or comprises a methanogen antigen.

[0244] *Variant:* As used herein, the term “variant” refers to an entity that shows significant structural identity with a reference entity but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a variant also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a “variant” of a reference entity is based on its degree of structural identity with the reference entity. For example, a variant polypeptide may differ from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (*e.g.*, carbohydrates, lipids, etc.) covalently attached to the polypeptide backbone. Alternatively or additionally, in some embodiments, a variant polypeptide does not share at least one characteristic sequence element with a reference polypeptide. In some embodiments, the reference polypeptide has one or more biological activities. In some embodiments, a variant polypeptide shares one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide lacks one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide shows a reduced level of one or more biological activities as compared with the reference polypeptide.

[0245] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, *e.g.*, RNA synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0246] The present disclosure identifies that increase in methane from agriculture represents a major source of greenhouse gas emissions. The present disclosure also identifies that reducing methane emissions from animals, *e.g.*, ruminants, will be important to reduce greenhouse gas emissions.

[0247] Methane is produced in the rumen of ruminant animals by methanogens, a subgroup of the Archaea domain. Methanogens can reduce the efficiency of nutrients and/or energy available to a ruminant as methane production by methanogens can account for 2-12% of ingested energy (*See Leahy SC et al.*, (2010) PlosOne; volume 5, issue 1; e8926, the entire contents of which are incorporated by reference). Accordingly, the present disclosure identifies that reducing methane emissions from a ruminant can be beneficial in increasing ruminant energy efficiency and reducing methane emissions into the atmosphere.

[0248] Among other things, the present disclosure provides technologies for reducing methane emissions from animals, *e.g.*, ruminants, by providing compositions for administering to animals, *e.g.*, ruminants, ruminal-associated antigens (*e.g.*, vaccinating animals (*e.g.*, ruminants) against ruminal-associated antigens). The present disclosure also provides technologies for increasing energy efficiency in animals, *e.g.*, ruminants, for administering to animals, *e.g.*, ruminants, ruminal-associated antigens (*e.g.*, vaccinating animals (*e.g.*, ruminants) against ruminal-associated antigens), thus reducing the abundance of one or more methanogens in the rumen of an animal, *e.g.*, ruminant.

[0249] Without wishing to be bound by theory, the present disclosure proposes that a composition comprising a ruminal-associated antigen (e.g., a ruminal antigen and/or a methanogen antigen), can reduce methane emissions and/or reduce the abundance of microorganisms (e.g., methanogens) in the digestive tract of animals. In some embodiments, reducing methane emissions and/or reducing abundance of methanogens in the digestive tract of animals (e.g., ruminants) can increase the energy efficiency of animals. In turn, animals (e.g., ruminants) can require less food, experience an increase in muscle mass, and/or have improved overall health. The benefits can lead to lower costs for raising animals and/or increased revenue from the sale of animals or meat obtained from such animals.

Ruminal-associated antigens

[0250] A ruminal-associated antigen as described herein comprises a ruminal antigen and/or a methanogen antigen. In some embodiments, a ruminal-associated antigen is endogenous to a ruminant, e.g., encoded by a nucleotide sequence in a ruminant genome. In some embodiments, a ruminal-associated antigen is not endogenous to a ruminant, e.g., encoded by a nucleotide sequence that is introduced to a ruminant, or encoded by a nucleotide sequence from a microorganism present in or introduced into a ruminant.

[0251] In some embodiments, a ruminant is chosen from a cattle, sheep, goat, buffalo, moose, antelope, caribou, or deer, or combinations thereof.

[0252] Exemplary ruminal-associated antigens include peptides that are involved in attachment to bacteria, e.g., fermenting bacteria, and fragments, or variants thereof.

[0253] In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 ruminal antigens.

[0254] In some embodiments, a polynucleotide comprises about 2-100, about 3-100, about 4-100, about 5-100, about 6-100, about 7-100, about 8-100, about 9-100, about 10-100, about 20-100, about 30-100, about 40-100, about 50-100, about 60-100, about 70-100, about 80-100, about 90-100, about 2-90, about 2-80, about 2-70, about 2-60, about 2-50, about 2-40, about 2-30,

about 2-20, about 2-10, about 2-15, about 2-14, about 2-13, about 2-12, about 2-11, about 2-10, about 2-9, about 2-8, about 2-7, about 2-6, about 2-5, about 2-4, about 2-3 ruminal antigens.

Methanogens and methanogen antigens

[0255] A methanogen is an anaerobic archaea characterized by the ability to conserve energy for ATP synthesis by producing methane gas, as described in Buan N.R. (2018). Methanogens can also inhabit the digestive tract of animals, e.g., ruminants and humans. Methanogens can reduce the efficiency of nutrients and/or energy available to a ruminant as methane production by methanogens can account for 2-12% of ingested energy (See Leahy SC et al., (2010) PlosOne; volume 5, issue 1; e8926, the entire contents of which are incorporated by reference). Accordingly, reducing methane emissions from a ruminant can be beneficial in increasing ruminant energy efficiency and/or reducing methane emissions into the atmosphere.

[0256] In some embodiments, a methanogen comprises a methanogen from one or more of the following clades: *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Methanothermobacter*, *Candidatus Methanomethylophilus*, *Thermoplasmatales*.

[0257] In some embodiments, a methanogen comprises a methanogen from a *Methanobrevibacter* clade. In some embodiments, a methanogen from a *Methanobrevibacter* clade comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanobrevibacter thaueri*, *Methanobrevibacter gottschalkii*, *Methanobrevibacter millerae*, *Methanobrevibacter woesei*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter wolinii*, *Methanobrevibacter olleyae*, *Methanobrevibacter boviskoreani*, or combinations thereof.

[0258] In some embodiments, a methanogen comprises a methanogen from a *Methanosphaera* clade. In some embodiments, a methanogen from a *Methanosphaera* clade comprises: *Methanosphaera stadtmanae*.

[0259] In some embodiments, a methanogen comprises a methanogen from a *Methanobacterium* clade. In some embodiments, a methanogen from a *Methanobacterium* clade comprises: *Methanobacterium bryantii* or *Methanobacterium formicicum*.

[0260] In some embodiments, a methanogen comprises a methanogen from a *Methanosarcinales* clade. In some embodiments, a methanogen from a *Methanosarcinales* clade comprises *Methanosarcina bakeri*, *Methanosarcina mazei*, or *Methanosarcina soligelidi*.

[0261] In some embodiments, a methanogen comprises a methanogen from a *Methanomicrobiales* clade. In some embodiments, a methanogen from a *Methanomicrobiales* clade comprises *Methanofollis liminatans*, *Methanospirillum hungatei*, *Methanolacinia payteri*, *Methanoculleus marisnigri*, or *Methanoculleus sp.*

[0262] In some embodiments, a methanogen comprises a methanogen from a *Methanomicrobium* clade. In some embodiments, a methanogen from a *Methanomicrobium* clade comprises *Methanomicrobium mobile*.

[0263] In some embodiments, a methanogen comprises a methanogen from a *Thermoplasmatales* clade. In some embodiments, a methanogen from a *Thermoplasmatales* clade comprises *Thermoplasma volcanium* or *Thermoplasma acidophilum*.

[0264] In some embodiments, a methanogen comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, , or any combination thereof.

[0265] A methanogen antigen comprises any protein expressed or produced in any methanogen clade, e.g., as described herein. Exemplary methanogen antigens are provided in Table 1. In some embodiments, a methanogen antigen is an antigen provided in Table 1, or a fragment thereof, or a variant thereof. In some embodiments, a methanogen antigen has a sequence with at least 85% identity to a methanogen antigen provided in Table 1. In some embodiments, a methanogen antigen is an adhesin, e.g., mru 1499.

[0266] In some embodiments, a methanogen antigen is a peptide involved in adhesion, attachment, mobility, or any combination thereof. In some embodiments, a methanogen antigen is an adhesin, a pili protein, a flagellin protein, or any combination thereof.

[0267] In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 methanogen antigens.

[0268] In some embodiments, a polynucleotide comprises about 2-100, about 3-100, about 4-100, about 5-100, about 6-100, about 7-100, about 8-100, about 9-100, about 10-100, about 20-100, about 30-100, about 40-100, about 50-100, about 60-100, about 70-100, about 80-100, about 90-100, about 2-90, about 2-80, about 2-70, about 2-60, about 2-50, about 2-40, about 2-30, about 2-20, about 2-10, about 2-15, about 2-14, about 2-13, about 2-12, about 2-11, about 2-10, about 2-9, about 2-8, about 2-7, about 2-6, about 2-5, about 2-4, about 2-3 methanogen antigens.

[0269] In some embodiments, a polynucleotide comprises one or more methanogen antigens from one or more methanogens.

[0270] In some embodiments, a methanogen antigen comprises an antigen sequence provided in **Table 2** or a fragment, or a variant or a variant fragment thereof. In some embodiments, a methanogen antigen comprises an antigen sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, identity to a sequence provided in **Table 2**. Exemplary antigen sequences provided in **Table 2** are marked with no bolding or italicizing.

[0271] In some embodiments, a methanogen antigen comprises an antigen sequence provided in any one of SEQ ID NOs: 2, 6, 8, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122 or a sequence with at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. Antigen sequences are unmarked (no bolding or italicizing) in SEQ ID

NOs: 2, 6, 8, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 97, 98, 99, 100, 101, 102, 103, 14, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122.

[0272] In some embodiments, a methanogen antigen comprises an antigen encoded by an antigen nucleotide sequence provided in any one of SEQ ID NOs: 1, 5, 7, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94 or a sequence with at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0273] Table 1: Exemplary methanogen antigens.

Locus	Protein annotation	Size (bp)
mru0019	adhesin-like protein	1220
mru0327	adhesin-like protein	2090
mru0687	adhesin-like protein	2963
mru1210	adhesin-like protein	7250
mru1222	adhesin-like protein	4055
mru1506	adhesin-like protein	857
mru2053	adhesin-like protein	3494
mru2134	adhesin-like protein	17957
mru2147	adhesin-like protein	16955
mru2178	adhesin-like protein	9239
mru0031	adhesin-like protein	4415
mru0704	adhesin-like protein	2858
mru0963	adhesin-like protein	8159
mru0976/0977	adhesin-like protein	4775
mru0020	adhesin-like protein with cysteine protease domain	6014
mru0064	adhesin-like protein	3536
mru0072	adhesin-like protein	2918
mru0076	adhesin-like protein	6605
mru0077	adhesin-like protein	9161
mru0079	adhesin-like protein	3560
mru0083	adhesin-like protein	839
mru0084	adhesin-like protein	14477
mru0085	adhesin-like protein	8030
mru0086	adhesin-like protein	10175
mru0143	adhesin-like protein with cysteine protease domain	3284
mru0160	adhesin-like protein	3176

Locus	Protein annotation	Size (bp)
mru0222	adhesin-like protein with cysteine protease domain	3302
mru0327	adhesin-like protein	2060
mru0338	adhesin-like protein	6929
mru0417/0418	adhesin-like protein	1391
mru0419	adhesin-like protein	4175
mru0727	adhesin-like protein with cysteine protease domain	3788
mru0772	adhesin-like protein with cysteine protease domain	3281
mru0839	adhesin-like protein with cysteine protease domain	8639
mru0842	adhesin-like protein with cysteine protease domain	3977
mru0978	adhesin-like protein	6606
mru0979	adhesin-like protein	8753
mru1076	adhesin-like protein	2681
mru1077	adhesin-like protein	2273
mru1246	adhesin-like protein	4619
mru1247	adhesin-like protein	5060
mru1465	adhesin-like protein	2882
mru1513	adhesin-like protein	1853
mru1650	adhesin-like protein	9161
mru1726	adhesin-like protein	6767
mru1971	adhesin-like protein	1937
mru1996	adhesin-like protein	4496
mru2043	adhesin-like protein	9530
mru2048	adhesin-like protein	5417
mru2049	adhesin-like protein	10355
mru2052	adhesin-like protein	4112
mru2054	adhesin-like protein	5054
mru2055	adhesin-like protein	8906
mru2059	adhesin-like protein	4415
mru2090	adhesin-like protein	15200
mru0004	adhesin-like protein	2237
mru0331	adhesin-like protein	1622
mru0843	adhesin-like protein with cysteine protease domain	6197
mru0015	adhesin-like protein with cysteine protease domain	3845
mru0090	adhesin-like protein	2063
mru0255	adhesin-like protein	4187
mru0450	adhesin-like protein	803
mru0723	adhesin-like protein	7727

Locus	Protein annotation	Size (bp)
mru0962	adhesin-like protein	14789
mru0970	adhesin-like protein	2483
mru1263	adhesin-like protein	2585
mru1358	adhesin-like protein	2243
mru1386	adhesin-like protein	1841
mru1387	adhesin-like protein with cysteine protease domain	2957
mru1424	adhesin-like protein	1445
mru1500	adhesin-like protein	3896
mru0493	adhesin-like protein	2447
mru0824	adhesin-like protein with transglutaminase domain	2027
mru1499	adhesin-like protein with transglutaminase domain	3032
mru1604	adhesin-like protein with transglutaminase domain	2996

Antigens from hydrogen-producing symbiotes

[0274] In some embodiments, a ruminal-associated antigen comprises an antigen from a hydrogen-producing symbiote. In some embodiments, hydrogen-producing symbiotes are microbes that produce rumen hydrogen in a way that reduces cow efficiency similar to methanogens.

[0275] In some embodiments, a ruminal-associated antigen comprises an antigen from a rumen ciliate. In some embodiments, rumen ciliates are symbiotic with methanogens. In some embodiments, rumen ciliates comprises: *Diplodinium dentatum* (syn. *Diplodinium denticulatum*), *Diploplastron affine* (syn. *Eudiplodinium affine*), *Enoploplastron triloricatum* (syn. *Ostracodinium triloricatum*), *Entodinium simplex*, *Entodinium caudatum*, *Entodinium longinucleatum*, *Epidinium ecaudatum*, *Eremoplastron bovis* (syn. *Eudiplodinium neglectum*), *Eudiplodinium maggii*, *Ostracodinium obtusum*, *Polyplastron multivesiculatum*, or combinations thereof.

[0276] In some embodiments, hydrogen-producing symbiotes supply nutrients and/or energy (e.g., hydrogen) to methanogens. In some embodiments, hydrogen-producing symbiotes which supply nutrients and/or energy (e.g., hydrogen) to methanogens comprise *Butyrivibrio proteoclasticus*, *Bacteroides thetaiotaomicron*, *Ruminococcus flavefaciens*, *Ruminococcus albus*, or combinations thereof.

[0277] In some embodiments, hydrogen-producing symbiotes comprise taxa associated with low feed efficiency. In some embodiments, hydrogen-producing symbiotes comprising taxa associated with low feed efficiency, comprise: *Veillonellaceae*, *Prevotellaceae*, *Lachnospiraceae*, *Succinivibrionaceae*, *Fibrobacteraceae*, *Anaerovibrio*, *Clostridiales*, *Prevotella*, *Ruminococcaceae*.

Exemplary multi-component compositions comprising ruminal-associated antigens

[0278] Disclosed herein are polynucleotides (e.g., RNA) comprising one or more ruminal-associated methanogens, e.g., ruminal antigens and/or methanogen antigens. Also disclosed herein are compositions comprising the same, as well as methods of making and using the same for administration to (e.g., vaccination of) an animal, e.g., a ruminant. In some embodiments, a polynucleotide disclosed herein comprises a plurality of ruminal-associated methanogens, e.g., a plurality of ruminal antigens and/or a plurality of methanogen antigens. In some embodiments, a composition comprising such a polynucleotide is also referred to as a multi-component vaccine composition or a multiplexed vaccine composition.

[0279] In some embodiments, a polynucleotide is or comprises RNA. In some embodiments, an RNA comprises a 5' cap, e.g., a 5'-5' triphosphate linked guanosine. In some embodiments, an RNA comprises a polyA tail. In some embodiments, an RNA comprises one or more untranslated regions, e.g., a 5' UTR and/or a 3' UTR.

[0280] In some embodiments, a polynucleotide is an RNA comprising: (i) a 5' cap; (ii) a 5' UTR region; (iii) a sequence encoding one or more payloads (e.g., one or more ruminal-associated antigens); (iv) a polyA tail, and (v) a 3' UTR region. In some embodiments, a polynucleotide further comprises one or more elements, such as one or more sequences encoding an additional payload, or one or more sequences that can form a secondary structure (e.g., hairpin, or an IRES), etc. In some embodiments, a polynucleotide is a messenger RNA.

[0281] In some embodiments of an isolated polynucleotide disclosed herein, a composition comprising the same, or methods of making or using a polynucleotide disclosed herein, a polynucleotide comprises one or more methanogen antigens. In some embodiments, a polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, or at least 20 methanogen antigens. In some embodiments, one or more methanogen antigens are the same, e.g., antigens having the same sequence, structure

and/or function. In some embodiments, one or more methanogen antigens are different, e.g., antigens having different sequence, structure and/or function. In some embodiments, one or more methanogen antigens include a mixture of antigens that are the same and antigens that are different. In some embodiments, one or more methanogen antigens are associated with one or more methanogen species. In some embodiments, one or more methanogen antigens are associated with a single methanogen species.

[0282] In some embodiments, one or more methanogen antigens comprise one or more, or all, or any combination of: (i) one or more peptides having at least 80% sequence identity to a methanogen protein; (ii) one or more secreted antigens, e.g., each comprising a signal peptide; (iii) a plurality of peptides having at least 80% sequence identity to each other; (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species; (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity; (vi) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide; (vii) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; or (viii) one or more methanogen antigens provided in Table 1 or sequences with at least 85% identity thereto. In some embodiments, a polynucleotide comprises one or more methanogen antigens from each of (i)-(viii).

[0283] In some embodiments, one or more methanogen antigens comprise one or more peptides having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to a methanogen protein. In some embodiments, a methanogen protein comprises a polypeptide encoded from a genome of a methanogen, or a variant or fragment thereof.

[0284] In some embodiments, one or more methanogen antigens comprise one or more secreted antigens, e.g., one or more methanogen antigens comprise a signal peptide. In some embodiments, a secreted antigen is or comprises an antigen that is a surface associated protein, or a protein that is secreted out of a cell. In some embodiments, a secreted antigen comprises a signal peptide. In some embodiments, a signal peptide can be predicted using an algorithm. In some embodiments, a prediction score of a signal peptide is at least 0.5.

[0285] In some embodiments, one or more methanogen antigens comprise a plurality of peptides having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence

identity to each other. In some embodiments, one or more methanogen antigens comprising at least 80% identity to each other are also referred to as a cluster homology.

[0286] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different, at least 5 different, at least 6 different, at least 7 different, at least 8 different, at least 9 different, at least 10 different, at least 11 different, at least 12 different, at least 15 different or at least 20 different methanogen species.

[0287] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with about 2 different, about 3 different, about 4 different, about 5 different, about 6 different, about 7 different, about 8 different, about 9 different, about 10 different, about 11 different, about 12 different, about 15 different or about 20 different methanogen species.

[0288] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with no more than 2 different, no more than 3 different, no more than 4 different, no more than 5 different, no more than 6 different, no more than 7 different, no more than 8 different, no more than 9 different, no more than 10 different, no more than 11 different, no more than 12 different, no more than 15 different or no more than 20 different methanogen species.

[0289] In some embodiments, one or more methanogen antigens comprise a plurality of peptides associated with about 2 to about 20, about 2 to about 15, about 2 to about 12, about 2 to about 11, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 20, about 4 to about 20, about 5 to about 20, about 6 to about 20, about 7 to about 20, about 8 to about 20, about 9 to about 20, about 10 to about 20, about 11 to about 20, about 12 to about 20, or about 15 to about 20 different methanogen species.

[0290] In some embodiments, one or more methanogen antigens that are associated with a methanogen species refer to antigens that can be found in a methanogen species. For example, a methanogen antigen associated with a particular methanogen species comprises an antigen encoded by a nucleotide sequence naturally occurring in said methanogen species.

[0291] In some embodiments, one or more methanogen antigens comprise a methanogen from one, or more, or all of the following clades: Methanobrevibacter, Methanosphaera,

Methanobacterium, Methanosarcinales, Methanomicrobiales, Methanothermobacter, Candidatus Methanomethylophilus, Thermoplasmatales.

[0292] In some embodiments, a methanogen species is chosen from: Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanobrevibacter oralis, Methanomicrobium mobile, Methanobrevibacter wolinii, Methanobrevibacter arboriphilus, Methanobrevibacter boviskoreani, Methanosphaera stadtmanae, Methanosarcina mazei, Methanobrevibacter thaueri, Methanobrevibacter sp. UBA188, Methanosarcina soligelidi, Methanothermobacter thermautotrophicus, Methanococcus aeolicus, Methanocaldococcus jannaschii, Methanococcus voltae, Methanococcus vanniellii, Methanococcus maripaludis, Methanopyrus kandleri, Methanocorpusculum labreanum, Methanococcoides burtonii, Methanosaeta thermophila, Methanoregula boonei, Methanosphaerula palustris, Methanoculleus marisnigri, Methanospirillum hungatei, or Methanosarcina acetivorans..

[0293] In some embodiments, a methanogen species is chosen from Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanobrevibacter oralis, Methanomicrobium mobile, Methanobrevibacter wolinii, Methanobrevibacter arboriphilus, Methanobrevibacter boviskoreani, Methanosphaera stadtmanae, Methanosarcina mazei, Methanobrevibacter thaueri, Methanobrevibacter sp. UBA188 or Methanosarcina soligelidi.

[0294] In some embodiments, a methanogen species is chosen from Methanobrevibacter ruminantium, Methanobrevibacter smithii, or Methanobrevibacter oralis.

[0295] In some embodiments, a methanogen species is chosen from Methanobrevibacter ruminantium Methanobrevibacter smithii Methanobrevibacter oralis Methanomicrobium mobile or Methanosphaera stadtmanae.

[0296] In some embodiments, one or more methanogen antigens comprise a plurality of methanogen antigens each of which is associated with the same methanogen species.

[0297] In some embodiments, one or more methanogen antigens comprise one or more peptides having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to a polypeptide having signal peptidase activity. In some embodiments, a polypeptide having signal peptidase activity has one or more (or all) of the following characteristics: (i) is membrane bound; (ii) has the ability to cleave one or more signal peptides; and/or (iii) plays a role in converting a secretory protein to a mature form (e.g., from a non-secretory form to a secretory form).

[0298] In some embodiments, one or more methanogen antigens comprise one or more peptides having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide. In some embodiments, an AglB polypeptide is characterized as having N-glycosylation activity.

[0299] In some embodiments, one or more methanogen antigens comprise one or more peptides having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide. In some embodiments, an Ig-like domain-containing polypeptide comprises a polypeptide characterized as a cell-surface protein that facilitates binding to other cells and/or cell surfaces.

[0300] An Ig-like domain containing polypeptide described herein can include: (i) transglutaminase domain-containing protein or a fragment, variant or variant fragment thereof, (ii) a pseudomurein-binding repeat-containing protein or a fragment, variant or variant fragment thereof, (iii) a right-handed parallel beta-helix repeat-containing protein or a fragment, variant or variant fragment thereof, (iv) a succinylglutamate desuccinylase/aspartoacylase family protein or a fragment, variant or variant fragment thereof, or (v) any combination of (i)-(iv).

[0301] In some embodiments, one or more methanogen antigens comprise one or more antigens provided in Table 1 or sequences with at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto. In some embodiments, one or more methanogen antigens comprise an adhesin or a fragment or a variant thereof. In some embodiments, one or more methanogen antigens comprise a pilli protein or a fragment or a variant thereof. In some embodiments, one or more methanogen antigens comprise a flagellin protein or a fragment or a variant thereof.

[0302] In some embodiments of an isolated polynucleotide disclosed herein, a composition comprising the same, or methods of making or using a polynucleotide disclosed herein, a polynucleotide comprises one or more methanogen antigens. In some embodiments, a polynucleotide comprises one or more methanogen antigens chosen from, one or more, or all, or any combination of: (i) one or more peptides having at least 80% sequence identity to a methanogen protein; (ii) one or more secreted antigens comprising a signal peptide; (iii) a plurality of peptides having at least 80% sequence identity to each other; (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5

different methanogen species; or (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity. In some embodiments, a polynucleotide comprises one or more methanogen antigens from each of (i)-(v). In some embodiments, a polynucleotide comprises three methanogen antigens. In some embodiments, three methanogen antigens are associated with at least three different methanogen species, e.g.,

Methanobrevibacter ruminantium, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*.

Exemplary multi-component compositions are described in Table 3.

[0303] In some embodiments of an isolated polynucleotide disclosed herein, a composition comprising the same, or methods of making or using a polynucleotide disclosed herein, a polynucleotide comprises one or more methanogen antigens. In some embodiments, a polynucleotide comprises one or more methanogen antigens chosen from, one or more, or all, or any combination of: (i) one or more peptides having at least 80% sequence identity to a methanogen protein; (ii) one or more secreted antigens comprising a signal peptide; (iii) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species; or (iv) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide. In some embodiments, a polynucleotide comprises one or more methanogen antigens from each of (i)-(iv). In some embodiments, a polynucleotide comprises five methanogen antigens. In some embodiments, five methanogen antigens are associated with at least five different methanogen species, e.g., *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanosphaera stadtmanae*. Exemplary multi-component compositions are described in Table 3.

[0304] In some embodiments of an isolated polynucleotide disclosed herein, a composition comprising the same, or methods of making or using a polynucleotide disclosed herein, a polynucleotide comprises one or more methanogen antigens. In some embodiments, a polynucleotide comprises one or more methanogen antigens chosen from, one or more, or all, or any combination of: (i) one or more peptides having at least 80% sequence identity to a methanogen protein; (ii) one or more secreted antigens comprising a signal peptide; (iii) a plurality of peptides having at least 80% sequence identity to each other; (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5

different methanogen species; or (v) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide. In some embodiments, a polynucleotide comprises one or more methanogen antigens from each of (i)-(v). In some embodiments, a polynucleotide comprises eight methanogen antigens. In some embodiments, eight methanogen antigens are associated with at least three different methanogen species, e.g., *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*. Exemplary multi-component compositions are described in Table 3.

[0305] In some embodiments of an isolated polynucleotide disclosed herein, a composition comprising the same, or methods of making or using a polynucleotide disclosed herein, a polynucleotide comprises one or more methanogen antigens. In some embodiments, a polynucleotide comprises one or more methanogen antigens chosen from, one or more, or all, or any combination of: (i) one or more peptides having at least 80% sequence identity to a methanogen protein; (ii) one or more secreted antigens comprising a signal peptide; (iii) a plurality of peptides having at least 80% sequence identity to each other; (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species; or (v) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide. In some embodiments, a polynucleotide comprises one or more methanogen antigens from each of (i)-(v). In some embodiments, a polynucleotide comprises 34 methanogen antigens. In some embodiments, one or more methanogen antigens are associated with at least five different methanogen species, e.g., *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*. An exemplary multi-component composition is described in Table 5.

[0306] In some embodiments, a composition disclosed herein comprises one or more polynucleotides comprising one or more ruminal-associated antigens, e.g., methanogen antigens. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition) comprises a plurality of polynucleotides (e.g., a plurality of RNA) each comprising an antigen. In some embodiments, a composition (e.g., a multi-component composition, a multi-component vaccine composition) comprises a plurality of

polynucleotides (e.g., a plurality of RNA) each comprising one or more antigens (e.g., the same or different antigens).

[0307] In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 1% of a first polynucleotide and about 99% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 5% of a first polynucleotide and about 95% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 10% of a first polynucleotide and about 90% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 20% of a first polynucleotide and about 80% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 30% of a first polynucleotide and about 70% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 40% of a first polynucleotide and about 60% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 50% of a first polynucleotide and about 50% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 60% of a first polynucleotide and about 40% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 70% of a first polynucleotide and about 30% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 80% of a first

polynucleotide and about 20% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 90% of a first polynucleotide and about 10% of one or more additional polynucleotides. In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 99% of a first polynucleotide and about 1% of one or more additional polynucleotides.

[0308] In some embodiments, a composition (e.g., a multi-component composition, e.g., a multi-component vaccine composition), comprising a plurality of polynucleotides comprises about 33% of a first polynucleotide, about 33% of a second polynucleotides and about 33% of a third polynucleotide.

[0309] Exemplary methanogen antigens disclosed herein can be obtained, e.g., from sequencing samples from a rumen of an animal, e.g., a ruminant. Sequencing samples from a rumen of an animal, e.g., a ruminant, can provide information about specific protein targets and/or specific species, e.g., methanogen species. Additionally, methanogen antigens can also be obtained by using a list, e.g., a defined list, of target species, e.g., methanogens, as would readily be ascertainable by one with knowledge in the pertinent field. Additionally or alternatively, methanogen antigens can also be obtained by using algorithms or language models such as those known in the field, to generate de novo proteins or variants of related sequence or structure.

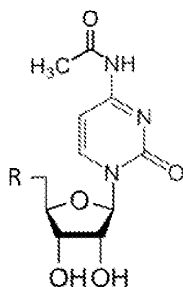
[0310] Accordingly, also provided herein is a method of identifying one or more methanogen antigens for use in a polynucleotide disclosed herein or a composition comprising the same, e.g., for vaccinating an animal, e.g., a ruminant.

Acetylated nucleotides

[0311] Among other things, provided herein are polyribonucleotides comprising one or more modified ribonucleotides including a nucleoside comprising an acetyl group. In some embodiments, a nucleoside of a modified ribonucleotide is N4-acetylcytidine and the modified ribonucleotide has: a 5' monophosphate, a 5' diphosphate or a 5' triphosphate. Additional details about polyribonucleotides comprising one or more modified ribonucleotides including a nucleoside comprising an acetyl group is provided in International Patent Application

PCT/US22/27721 filed on July 18, 2022, the entire contents of which are hereby incorporated by reference.

[0312] In some embodiments, a nucleoside of a modified ribonucleotide is N4-acetylcytidine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0313] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues. In some embodiments, at least 5% of cytidine residues in a polyribonucleotide comprise N4-acetylcytidine. In some embodiments, less than 100% of cytidine residues in a polyribonucleotide comprise N4-acetylcytidine.

[0314] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 5% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0315] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 10% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0316] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 15% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0317] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 20% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0318] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 25% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0319] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 30% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0320] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 35% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0321] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 40% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0322] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 45% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0323] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 50% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0324] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 55% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0325] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 60% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0326] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 65% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0327] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 70% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0328] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 75% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0329] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 80% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0330] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 85% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0331] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 90% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0332] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and at least 95% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0333] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues. In some embodiments, about 5% to 99%, about 5% to 95%, about 5% to 90%, about 5% to 85%, about 5% to 80%, about 5% to 75%, about 5% to 70%, about 5% to 65 %, about 5% to 60%, about 5% to 55%, about 5% to 50 %, about 5% to 45 %, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25 %, about 5% to 20%, about 5% to 15 %, about 5% to 10%, about 10% to 99%, about 15% to 99%, about 20% to 99%, about 25% to 99%, about 30% to 99%, about 35% to 99%, about 40% to 99%, about 45% to 99%, about 50% to 99%, about 55% to 99%, about 60% to 99%, about 65% to 99%, about 70% to 99%, about 80% to 99%, about 85% to 99%, about 90% to 99%, or about 95% to 99% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0334] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 60% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0335] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 65% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0336] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 70% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0337] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 75% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0338] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 80% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0339] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 85% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0340] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 90% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0341] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 95% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0342] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and more than about 99% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0343] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 5% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0344] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 10% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0345] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 15% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0346] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 20% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0347] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 25% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0348] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 30% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0349] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 35% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0350] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 40% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0351] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 45% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0352] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 50% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0353] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 55% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0354] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 60% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0355] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 65% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0356] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 75% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0357] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 80% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0358] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 85% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0359] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 90% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0360] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 95% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0361] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and about 99% of cytidine residues in a polyribonucleotide comprises N4-acetylcytidine.

[0362] In some embodiments, a polyribonucleotide disclosed herein comprises cytidine residues and 100% of cytidine residues in a polyribonucleotide comprise N4-acetylcytidine.

[0363] In some embodiments, a polyribonucleotide disclosed herein (e.g., a polyribonucleotide comprising cytidine residues with about 5%-100% cytidine residues comprising N4-acetylcytidine) comprises one or more additional modified ribonucleotides. In some embodiments, one or more additional modified ribonucleotides comprises a nucleoside chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof. In some embodiments, one or more additional modified ribonucleotides comprises a 5-hydroxymethyl group. In some embodiments, one or more additional modified ribonucleotides comprises 5-hydroxymethyluridine. In some embodiments 5%-100% of uridine residues in a polyribonucleotide comprising uridine are 5-hydroxymethyluridine.

[0364] In some embodiments, a polyribonucleotide can have a length of at least 5 nucleotides or longer. In some embodiments, a polyribonucleotide can have a length of at least 5 nucleotides, at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least 40 nucleotides, at least 45 nucleotides, at least 50 nucleotides, at least 55 nucleotides, at least 60 nucleotides, at least 65 nucleotides, at

least 70 nucleotides, at least 75 nucleotides, at least 80 nucleotides, at least 85 nucleotides, at least 90 nucleotides, at least 95 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 1000 nucleotides, at least 2000 nucleotides, at least 5000 nucleotides or longer.

[0365] In some embodiments, a polyribonucleotide can have a length of about 5 nucleotides to about 200,000 nucleotides, about 5 nucleotides to about 150,000 nucleotides, about 5 nucleotides to about 100,000 nucleotides, about 5 nucleotides to about 50,000 nucleotides, about 5 nucleotides to about 10,000 nucleotides, about 5 nucleotides to about 5000 nucleotides, about 5 nucleotides to about 1000 nucleotides, about 5 nucleotides to about 500 nucleotides, about 5 nucleotides to about 400 nucleotides, about 5 nucleotides to about 300 nucleotides, about 5 nucleotides to about 200 nucleotides, about 5 nucleotides to about 100 nucleotides, about 5 nucleotides to about 90 nucleotides, about 5 nucleotides to about 85 nucleotides, about 5 nucleotides to about 80 nucleotides, about 5 nucleotides to about 75 nucleotides, about 5 nucleotides to about 70 nucleotides, about 5 nucleotides to about 65 nucleotides, about 5 nucleotides to about 60 nucleotides, about 5 nucleotides to about 55 nucleotides, about 5 nucleotides to about 50 nucleotides, about 5 nucleotides to about 45 nucleotides, about 5 nucleotides to about 40 nucleotides, about 5 nucleotides to about 35 nucleotides, about 5 nucleotides to about 30 nucleotides, about 5 nucleotides to about 25 nucleotides, about 5 nucleotides to about 20 nucleotides, about 5 nucleotides to about 15 nucleotides, about 5 nucleotides to about 10 nucleotides.

[0366] In some embodiments, a polyribonucleotide can have a length of about 5 nucleotides to about 200,000 nucleotides, about 10 nucleotides to about 200,000 nucleotides, 15 nucleotides to about 200,000 nucleotides, about 20 nucleotides to about 200,000 nucleotides, about 30 nucleotides to about 200,000 nucleotides, about 40 nucleotides to about 200,000 nucleotides, about 50 nucleotides to about 200,000 nucleotides, about 100 nucleotides to about 200,000 nucleotides, about 200 nucleotides to about 200,000 nucleotides, about 300 nucleotides to about 200,000 nucleotides, about 400 nucleotides to about 200,000 nucleotides, about 500 nucleotides to about 200,000 nucleotides, about 1000 nucleotides to about 200,000 nucleotides, about 2000 nucleotides to about 200,000 nucleotides, about 3000 nucleotides to about 200,000 nucleotides, about 4000 nucleotides to about 200,000 nucleotides, about 5000 nucleotides to about 200,000 nucleotides, about 10,000 nucleotides to about 200,000 nucleotides, about 20,000 nucleotides to

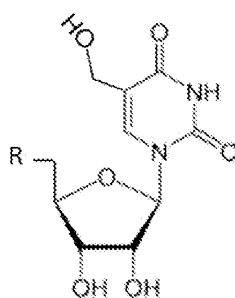
about 200,000 nucleotides, about 30,000 nucleotides to about 200,000 nucleotides, about 40,000 nucleotides to about 200,000 nucleotides, about 50,000 nucleotides to about 200,000 nucleotides, about 100,000 nucleotides to about 200,000 nucleotides, about 150,000 nucleotides to about 200,000 nucleotides.

[0367] In some embodiments, a polyribonucleotide can have a length of no more than 200,000 nucleotides, no more than 150,000 nucleotides, no more than 100,000 nucleotides, or no more than 50,000 nucleotides.

5-hydroxymethyl modified nucleotides

[0368] Among other things, provided herein are polyribonucleotides comprising one or more modified ribonucleotides including a nucleoside comprising a 5-hydroxymethyl group. In some embodiments, a nucleoside of a modified ribonucleotide is 5-hydroxymethyluridine and the modified ribonucleotide has: a 5' monophosphate, a 5' diphosphate or a 5' triphosphate. Additional details about ribonucleotides including a nucleoside comprising a 5-hydroxymethyl group is provided in International Patent Application PCT/US22/27721 filed on July 18, 2022, the entire contents of which are hereby incorporated by reference.

[0369] In some embodiments, a nucleoside of a modified ribonucleotide is 5-hydroxymethyluridine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0370] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues. In some embodiments, at least 5% of uridine residues in a polyribonucleotide comprise

5-hydroxymethyluridine. In some embodiments, less than 100% of uridine residues in a polyribonucleotide comprise 5-hydroxymethyluridine.

[0371] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 5% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0372] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 10% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0373] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 15% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0374] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 20% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0375] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 25% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0376] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 30% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0377] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 35% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0378] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 40% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0379] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 45% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0380] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 50% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0381] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 55% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0382] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 60% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0383] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 65% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0384] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 70% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0385] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 75% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0386] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 80% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0387] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 85% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0388] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 90% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0389] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 95% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0390] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and at least 99% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0391] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues. In some embodiments, about 5% to 99%, about 5% to 95%, about 5% to 90%, about 5% to 85%, about 5% to 80%, about 5% to 75%, about 5% to 70%, about 5% to 65 %, about 5% to 60%, about 5% to 55%, about 5% to 50 %, about 5% to 45 %, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25 %, about 5% to 20%, about 5% to 15 %, about 5% to 10%, about 10% to 99%, about 15% to 99%, about 20% to 99%, about 25% to 99%, about 30% to 99%, about 35% to 99%, about 40% to 99%, about 45% to 99%, about 50% to 99%, about 55% to 99%, about 60% to 99%, about 65% to 99%, about 70% to 99%, about 80% to 99%, about 85% to 99%, about 90% to 99%, or about 95% to 99% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0392] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 60% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0393] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 65% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0394] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 70% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0395] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 75% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0396] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 80% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0397] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 85% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0398] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 90% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0399] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 95% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0400] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and more than 99% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0401] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 5% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0402] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 10% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0403] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 15% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0404] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 20% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0405] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 25% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0406] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 30% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0407] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 35% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0408] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 40% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0409] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 45% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0410] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 50% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0411] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 55% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0412] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 60% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0413] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 65% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0414] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 75% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0415] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 80% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0416] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 85% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0417] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 90% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0418] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 95% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0419] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and about 99% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0420] In some embodiments, a polyribonucleotide disclosed herein comprises uridine residues and 100% of uridine residues in a polyribonucleotide comprises 5-hydroxymethyluridine.

[0421] In some embodiments, a polyribonucleotide disclosed herein (e.g., a polyribonucleotide comprising uridine residues with about 5%-100% uridine residues comprising 5-hydroxymethyluridine) comprises one or more additional modified ribonucleotides other than 5-hydroxymethyluridine. In some embodiments, one or more additional modified ribonucleotides comprises a nucleoside chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof. In some embodiments, one or more additional modified ribonucleotides comprises an acetyl group. In some embodiments, one or more additional modified ribonucleotides comprises N4-acetylcytidine. In some embodiments 5%-100% of cytidine residues in a polyribonucleotide comprising cytidine are N4-acetylcytidine.

[0422] In some embodiments, a polyribonucleotide can have a length of at least 5 nucleotides or longer. In some embodiments, a polyribonucleotide can have a length of at least 5 nucleotides, at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least 40 nucleotides, at least 45 nucleotides, at least 50 nucleotides, at least 55 nucleotides, at least 60 nucleotides, at least 65 nucleotides, at least 70 nucleotides, at least 75 nucleotides, at least 80 nucleotides, at least 85 nucleotides, at least 90 nucleotides, at least 95 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 1000 nucleotides, at least 2000 nucleotides, at least 5000 nucleotides or longer.

[0423] In some embodiments, a polyribonucleotide can have a length of about 5 nucleotides to about 200,000 nucleotides, about 5 nucleotides to about 150,000 nucleotides, about 5 nucleotides to about 100,000 nucleotides, about 5 nucleotides to about 50,000 nucleotides, about 5 nucleotides to about 10,000 nucleotides, about 5 nucleotides to about 5000 nucleotides, about 5

nucleotides to about 1000 nucleotides, about 5 nucleotides to about 500 nucleotides, about 5 nucleotides to about 400 nucleotides, about 5 nucleotides to about 300 nucleotides, about 5 nucleotides to about 200 nucleotides, about 5 nucleotides to about 100 nucleotides, about 5 nucleotides to about 90 nucleotides, about 5 nucleotides to about 85 nucleotides, about 5 nucleotides to about 80 nucleotides, about 5 nucleotides to about 75 nucleotides, about 5 nucleotides to about 70 nucleotides, about 5 nucleotides to about 65 nucleotides, about 5 nucleotides to about 60 nucleotides, about 5 nucleotides to about 55 nucleotides, about 5 nucleotides to about 50 nucleotides, about 5 nucleotides to about 45 nucleotides, about 5 nucleotides to about 40 nucleotides, about 5 nucleotides to about 35 nucleotides, about 5 nucleotides to about 30 nucleotides, about 5 nucleotides to about 25 nucleotides, about 5 nucleotides to about 20 nucleotides, about 5 nucleotides to about 15 nucleotides, about 5 nucleotides to about 10 nucleotides.

[0424] In some embodiments, a polyribonucleotide can have a length of about 5 nucleotides to about 200,000 nucleotides, about 10 nucleotides to about 200,000 nucleotides, 15 nucleotides to about 200,000 nucleotides, about 20 nucleotides to about 200,000 nucleotides, about 30 nucleotides to about 200,000 nucleotides, about 40 nucleotides to about 200,000 nucleotides, about 50 nucleotides to about 200,000 nucleotides, about 100 nucleotides to about 200,000 nucleotides, about 200 nucleotides to about 200,000 nucleotides, about 300 nucleotides to about 200,000 nucleotides, about 400 nucleotides to about 200,000 nucleotides, about 500 nucleotides to about 200,000 nucleotides, about 1000 nucleotides to about 200,000 nucleotides, about 2000 nucleotides to about 200,000 nucleotides, about 3000 nucleotides to about 200,000 nucleotides, about 4000 nucleotides to about 200,000 nucleotides, about 5000 nucleotides to about 200,000 nucleotides, about 10,000 nucleotides to about 200,000 nucleotides, about 20,000 nucleotides to about 200,000 nucleotides, about 30,000 nucleotides to about 200,000 nucleotides, about 40,000 nucleotides to about 200,000 nucleotides, about 50,000 nucleotides to about 200,000 nucleotides, about 100,000 nucleotides to about 200,000 nucleotides, about 150,000 nucleotides to about 200,000 nucleotides.

[0425] In some embodiments, a polyribonucleotide can have a length of no more than 200,000 nucleotides, no more than 150,000 nucleotides, no more than 100,000 nucleotides, or no more than 50,000 nucleotides.

SBI adjuvant

[0426] Polynucleotides disclosed herein encoding one or more ruminal-associated antigens can further comprise a sequence encoding an adjuvant.

[0427] In some embodiments, an adjuvant disclosed herein can be used to elicit and/or modulate an immune response elicited by an antigen (e.g., fragment antigen or antigen variant) described herein. In some embodiments, an adjuvant disclosed herein comprises a complement binding polypeptide. In some embodiments, a complement binding polypeptide comprises a complement C3d binding polypeptide. An exemplary C3d binding polypeptide is an immunoglobulin-binding protein (Sbi) of *Staphylococcus aureus*.

[0428] As disclosed herein, *S. aureus* binder of immunoglobulin (Sbi) is an exemplary polypeptide which can bind complement C3d (as described in Clark et al. (2011) *Mol Immunol.* 48(4): 452–462, the entire contents of which is incorporated herein by reference). Sbi comprises two immunoglobulin binding domains (Domains I and II) and two complement C3d binding domains (Domains III and IV). Sbi domains III and IV can bind C3d (in native C3, iC3b and C3dg) and can result in fluid phase consumption of C3 via activation of the alternative pathway (see Clark et al 2011). It has also been shown that Sbi can be secreted and is involved in *S. aureus* immune evasion (Burnan et al., 2008 *J. Biol. Chem.*; 283:17579–17593).

[0429] Without wishing to be bound by theory, it is believed that in some embodiments, a complement C3d-binding polypeptide from Sbi of *S. aureus* can be used as an adjuvant to enhance and/or modulate an immune response from an antigen described herein. In some embodiments, the immune response is elicited by a fragment antigen or antigen variant disclosed herein. In some embodiments, the immune response is elicited by a component of Sbi of *S. aureus*.

[0430] Exemplary Sbi adjuvants and compositions comprising the same are disclosed in International Patent Application PCT/US2022/018610 filed on March 3, 2022, the entire contents of which are hereby incorporated by reference.

Compositions

[0431] Among other things, the present disclosure provides compositions comprising one or more ruminal-associated antigens, e.g., one or more ruminal antigens and/or one or more

methanogen antigens. Compositions disclosed herein may also include polynucleotides encoding the same.

[0432] In some embodiments, a composition disclosed herein comprises a polynucleotide comprising a first nucleotide sequence encoding one or more ruminal antigens, and a second nucleotide sequence encoding one or more methanogen antigens.

[0433] In some embodiments, a first nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 ruminal antigens.

[0434] In some embodiments, a first nucleotide sequence comprises about 2-100, about 3-100, about 4-100, about 5-100, about 6-100, about 7-100, about 8-100, about 9-100, about 10-100, about 20-100, about 30-100, about 40-100, about 50-100, about 60-100, about 70-100, about 80-100, about 90-100, about 2-90, about 2-80, about 2-70, about 2-60, about 2-50, about 2-40, about 2-30, about 2-20, about 2-10, about 2-15, about 2-14, about 2-13, about 2-12, about 2-11, about 2-10, about 2-9, about 2-8, about 2-7, about 2-6, about 2-5, about 2-4, about 2-3 ruminal antigens.

[0435] In some embodiments, a second nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 methanogen antigens.

[0436] In some embodiments, a second nucleotide sequence comprises about 2-100, about 3-100, about 4-100, about 5-100, about 6-100, about 7-100, about 8-100, about 9-100, about 10-100, about 20-100, about 30-100, about 40-100, about 50-100, about 60-100, about 70-100, about 80-100, about 90-100, about 2-90, about 2-80, about 2-70, about 2-60, about 2-50, about 2-40, about 2-30, about 2-20, about 2-10, about 2-15, about 2-14, about 2-13, about 2-12, about 2-11, about 2-10, about 2-9, about 2-8, about 2-7, about 2-6, about 2-5, about 2-4, about 2-3 methanogen antigens.

[0437] In some embodiments, a composition disclosed herein comprises a polynucleotide comprising one or more ruminal-associated antigens and a polynucleotide comprising one or more cytokines and/or chemokines. In some embodiments, a cytokine and/or chemokine is one expressed in a ruminant. In some embodiments, a cytokine and/or chemokine is or comprises VIP, CXCL10, APRIL, or any combination thereof.

[0438] In some embodiments, a cytokine and/or chemokine comprises a sequence provided in any one of SEQ ID NOs: 9, 11, or 13, or a sequence with at least 85% identity thereto. Cytokine/chemokine sequences are underlined in SEQ ID NOs: 9, 11 or 13.

[0439] In some embodiments, a cytokine and/or chemokine comprises a polypeptide encoded by a cytokine and/or chemokine nucleotide sequence provided in any one of SEQ ID NOs: 8, 10 or 12, or a sequence with at least 85% identity thereto. Cytokine and/or chemokine sequences are underlined in SEQ ID NOs: 8, 10 and 12

[0440] In some embodiments, a composition disclosed herein is a pharmaceutical composition.

[0441] In some embodiments, a composition disclosed herein is an immunogenic composition.

[0442] In some embodiments, a composition disclosed herein is a vaccine composition.

[0443] In some embodiments, a composition disclosed herein is administered at a dose of about 5ng to about 1000ng, about 5ng to about 900 ng, about 5ng to about 800 ng, about 5ng to about 700 ng, about 5ng to about 600 ng, about 5ng to about 500 ng, about 5ng to about 400 ng, about 5ng to about 300 ng, about 5ng to about 200 ng, about 5ng to about 100 ng, about 5ng to about 90 ng, about 5ng to about 80 ng, about 5ng to about 70 ng, about 5ng to about 60 ng, about 5ng to about 50 ng, about 5ng to about 40 ng, about 5ng to about 30 ng, about 5ng to about 20 ng, or about 5ng to about 10ng. In some embodiments, a composition disclosed herein is administered at a dose of about 10ng to about 1000ng, about 20 ng to about 1000ng, about 30 ng to about 1000ng, about 40 ng to about 1000ng, about 50 ng to about 1000ng, about 60 ng to about 1000ng, about 70 ng to about 1000ng, about 80 ng to about 1000ng, about 90 ng to about 1000ng, about 100 ng to about 1000ng, about 200 ng to about 1000ng, about 300 ng to about 1000ng, about 40 ng to about 1000ng, about 50 ng to about 1000ng, about 60 ng to about 1000ng, about 700 ng to about 1000ng, about 800 ng to about 1000ng, or about 900 ng to about 1000ng.

[0444] In some embodiments, a composition disclosed herein is administered at a dose of about 5ng, about 10 ng, about 20 ng, about 30 ng, about 40 ng, about 50 ng, about 60 ng, about 70 ng, about 80 ng, about 90 ng, about 100 ng, 150 ng, about 200 ng, about 250 ng, about 300 ng, about 350 ng, about 400 ng, about 450 ng, about 500 ng, about 550 ng, about 600 ng, about 650 ng, about 700 ng, about 750 ng, about 800 ng, about 850 ng, about 900 ng, about 950 ng, or about 1000ng.

[0445] In some embodiments, a composition disclosed herein is administered at a dose of at least 5ng, at least 10 ng, at least 20 ng, at least 30 ng, at least 40 ng, at least 50 ng, at least 60 ng, at least 70 ng, at least 80 ng, at least 90 ng, at least 100 ng, at least 150 ng, at least 200 ng, at least 250 ng, at least 300 ng, at least 350 ng, at least 400 ng, at least 450 ng, at least 500 ng, at least 550 ng, at least 600 ng, at least 650 ng, at least 700 ng, at least 750 ng, at least 800 ng, at least 850 ng, at least 900 ng, at least 950 ng, or at least 1000ng.

Pharmaceutical compositions

[0446] In some embodiments, a composition comprising one or more ruminal-associated antigens is a pharmaceutical composition. In some embodiments, a pharmaceutical composition further comprises a pharmaceutically acceptable excipient. Pharmaceutical compositions of the present disclosure may comprise a polypeptide disclosed herein, a polynucleotide disclosed herein, or an expression vector comprising a polynucleotide disclosed herein.

[0447] In some embodiments, a pharmaceutical composition can include a pharmaceutically acceptable carrier or excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, glycerol, sugars such as mannitol, sucrose, or others, dextrose, fatty acid esters, etc., as well as combinations thereof.

[0448] A pharmaceutical composition can, if desired, be mixed with auxiliary agents (e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic

pressure, buffers, coloring, flavoring and/or aromatic substances and the like), which do not deleteriously react with the active compounds or interfere with their activity. In certain embodiments, a water-soluble carrier suitable for intravenous administration is used. In some embodiments, a pharmaceutical composition can be sterile.

[0449] A suitable pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. A pharmaceutical composition can be a liquid solution, suspension, or emulsion.

[0450] A pharmaceutical composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. The formulation of a pharmaceutical composition should suit the mode of administration. For example, in some embodiments, a composition for intravenous administration is typically a solution in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachet indicating the quantity of active agent. Where a pharmaceutical composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where a pharmaceutical composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0451] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions that are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts or cells in vitro or ex vivo. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals or cells in vitro or ex vivo is well understood, and the ordinarily skilled practitioner, e.g., a veterinary pharmacologist, can design and/or perform such modification with merely ordinary, if any, experimentation.

[0452] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a

diluent or another excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0453] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of a pharmaceutical composition described herein.

RNA Formulations

[0454] Among other things, provided herein are compositions comprising polyribonucleotides comprising ruminal-associated antigens, and formulations thereof. In some embodiments, a composition comprising a polyribonucleotide disclosed herein is formulated in a lipid nanoparticle (LNP) formulation.

[0455] In some embodiments, a polyribonucleotide disclosed herein encodes for a polypeptide. In some embodiments, a polyribonucleotide disclosed herein is or comprises a messenger RNA. In some embodiments, a composition comprising a polyribonucleotide comprising a messenger RNA is formulated in a lipid nanoparticle (LNP) formulation.

[0456] In some embodiments, the disclosure provides an LNP formulation comprising a polyribonucleotide disclosed herein for use in a pharmaceutical composition, e.g., an immunogenic composition.

Methods of using compositions disclosed herein

[0457] The disclosure provides, among other things, methods for using a polyribonucleotide disclosed herein, or a composition comprising the same.

[0458] In some embodiments, provided herein is a method of administering a polyribonucleotide disclosed herein or a composition comprising a polyribonucleotide disclosed herein to a cell, tissue or an animal, e.g., a ruminant.

[0459] In some embodiments, provided herein is an administration method, e.g., a vaccination method, comprising administering a polyribonucleotide disclosed herein or a composition comprising a polyribonucleotide disclosed herein to a cell, tissue or animal, e.g., a

ruminant. In some embodiments, a ruminant comprises a cattle, sheep, goat, buffalo, moose, antelope, caribou, or deer, or combinations thereof.

[0460] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, a composition reduces methane emissions from an animal as compared to an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, a reduction in methane emissions is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to methane emissions in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, a reduction in methane emissions is at least 5%.

[0461] In some embodiments, a reduction in methane emissions is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to methane emissions in an otherwise comparable animal not administered the composition or administered a different composition.

[0462] In some embodiments, a reduction in methane emissions about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 5% to about 10%, about 10% to about 95%, about 15% to about 95%, about 20% to about 95%, about 25% to about 95%, about 30% to about 95%, about 35% to about 95%, about 40% to about 95%, about 45% to about 95%, about 50% to about 95%, about 55% to about 95%, about 60% to about 95%, about 65% to about 95%, about 70% to about 95%, about 75% to about 95%, about 80% to about 95%, about 85% to about 95%, about 90% to about 95% as compared to methane emissions in an otherwise comparable animal not administered the composition or administered a different composition.

[0463] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, a composition reduces a population of microorganisms in an animal, as compared to an animal not administered a composition or administered a different

composition. In some embodiments, microorganisms comprise methanogens. In some embodiments, microorganisms are methanogens from: *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Thermoplasmatales*, or a combination thereof. In some embodiments, a methanogen comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof. In some embodiments, a methanogen abundance is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to abundance of a methanogen in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, a methanogen abundance is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to abundance of a methanogen in an otherwise comparable animal not administered the composition or administered a different composition.

[0464] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, a composition reduces abundance of all or substantially all methanogens (e.g., total methanogen abundance) in an animal as compared to total methanogen abundance in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, methanogen abundance, e.g., total methanogen abundance, is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to

methanogen abundance in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, methanogen abundance, e.g., total methanogen abundance, is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to methanogen abundance in an otherwise comparable animal not administered a composition or administered a different composition.

[0465] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, a composition reduces abundance of at least one methanogen species in an animal as compared to abundance of the same methanogen species in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, at least one methanogen species is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to abundance of the same methanogen species in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, at least one methanogen species is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to abundance of the same methanogen species in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, abundance of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 18, at least 19, at least 20 methanogen species is reduced.

[0466] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, a composition increases a growth rate of the animal as compared to a growth rate of an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, growth rate is assessed by measuring a weight of an animal at one or more timepoints. In some embodiments, growth rate is assessed by measuring a weight of an animal at one or more timepoints. In some embodiments, the weight of an animal is measured daily. In some embodiments, a growth rate of an animal increases over a

period of time. In some embodiments, a period of time comprises at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months or at least 12 months. In some embodiments, an increase in growth rate comprises a daily increase in weight of an animal. In some embodiments, a daily increase in weight of an animal is an increase of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to weight in an otherwise comparable animal not administered the composition or administered a different composition. In some embodiments, a daily increase in weight of an animal is an increase of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to weight in an otherwise comparable animal not administered a composition or administered a different composition.

[0467] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, the composition increases energy efficiency in an animal as compared to energy efficiency in an otherwise comparable animal not administered a composition or administered a different composition. In some embodiments, increasing energy efficiency comprises increasing digestion efficiency. In some embodiments, digestion efficiency comprises one or more, or all of the following parameters: (i) rate of weight gain, (ii) weight to intake relationship, or (iii) milk quality. In some embodiments, milk quality can be assessed by: weight gain metrics (e.g., Average Daily Gain (ADG) of cattle), intake to weight metrics (e.g., Food Conversion Ratio, or Residual Feed Intake), and/or milk composition metrics. In some embodiments, increasing digestion efficiency comprises decreasing digestion-related disorders in animals, e.g., ruminants.

[0468] In some embodiments, a composition disclosed herein is characterized in that when administered to an animal, the composition modifies output of cellulose fermentation.

[0469] In some embodiments, a composition disclosed herein is administered in one dose. In some embodiments, a composition is administered in a plurality of doses. In some embodiments, a composition is administered as a first dose of a composition followed by one or more subsequent doses of a composition. In some embodiments, a first dose and one or more

subsequent doses of a composition comprise the same methanogen antigens and/or ruminal antigens. In some embodiments, a first dose and one or more subsequent doses of a composition comprise different methanogen antigens and/or ruminal antigens.

[0470] In some embodiments, one or more methanogen antigens and/or one or more ruminal antigens are specific to an animal. In some embodiments, one or more methanogen antigens and/or one or more ruminal antigens that are specific to an animal are obtained by a method comprising (i) identifying one or more methanogen antigens and/or one or more ruminal antigens that are expressed in an animal and (ii) responsive to the identification, selecting one or more methanogen antigens and/or one or more ruminal antigens to be included in a composition. In some embodiments, one or more methanogen antigens and/or one or more ruminal antigens that are specific to an animal is different from one or more methanogen antigens and/or one or more ruminal antigens that are specific to a different animal. In some embodiments, a different animal comprises any one or all of (i) a different species of animal, (ii) an animal of a different sex, (iii) an animal of a different age, or (iv) an animal located in a different geographical location, or (v) the same animal but at a different timepoint.

[0471] In some embodiments, a composition is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or 30 times to an animal. In some embodiments, a composition is administered once every 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months.

[0472] In some embodiments, a composition is administered in combination with one or more additional agents. In some embodiments, one or more additional agents comprises a chemical additive, a biological feed additive. In some embodiments, a composition is administered in combination with one or more additional compositions. In some embodiments, the additional composition immunizes the animal from a disease, e.g., an infectious disease.

Kits

[0473] Another aspect of the present disclosure further provides a pharmaceutical pack or kit. In some embodiments, a kit can comprise a polyribonucleotide or a composition described herein, e.g., comprising a polynucleotide comprising a nucleotide sequence encoding a ruminal-associated antigen and/or a nucleotide sequence encoding a chemokine and/or cytokine. In some embodiment, kits may be used in any applicable method, e.g., methods as described herein.

SEQUENCES

[0474] Table 2: Exemplary antigen sequences

Cluster	Description	Sequence	SEQ ID NO.
M1.1	mm1499_sb i, nucleotide	ctgtctttttgcagaagctcagaataaaagctcaactttggccaccATGCTCCTGGCGGCTTGCTGATGGG TTTTGTGCTCATTAGTAGCGTCTCTGCTATCGATATATAGATGAGGCATCAAGTAGC AGCGATTGTCCGATTCTAGTATCTCTAATGACTATCTTGTGCTAACAGCGGCG ATGACTCTGTCGCGAGCTCATCAGCCCTCTTCTATTGCCGCGGATGACTCAGA CCTCTTAATAATGCTTCTTCACTCAATGTCAATTCGAGAACGGAAGTGTGTCT ACTAACAAACACGAAGACACAGAAAGCGAAATCGTTAAGGATTCTAAATACTA ACTTCCAGCAGCTCATTGCAGGCTTCTACCAAGACAAGACGACCCTGAAGGG TAGCGGCTCATCTGTCTATAGGGGCAACCTTACTATGTTACACTACGGACAGC AACGGGAAGGTTCTGGCTTCACAGAAAGTTACGTTCAATATCCTCGGAAAAAAT TATACACGAACAACCTGATTCTAAGGGGTCGCCCTCATCAACATAAATTTGGCG AAGGGGAAGTATAATATCGCTGTCTGTATGCTGGTACAGAGAAATATGCCAGT TCCAAACTTCTGTGGCGCTTACAGTCAACTTGAIGTCTACAAAATTAACACTG GAGGCTCAACGGTCAAAAAGGGAATGCATACAGTGTGACTTTACGGATGGA AACGGTAAGCCTTGAGCAGTCAAAAAGTGACACTGAACATACTGGGTAAAAA TTATACAGGACGACGGATAGCAAGGGGCTTGCAGCATAGCAATAAATCTCGC TGCAGGAAAAAATTCACACTTACAGCCAGTTACGCAGGTCCGCAAAATTACCT CAGCAGCAAAAGTGCAGTACCGTGACGGTTACAGAAAGGGGACACATCAATCA AGCTAGCGGGACCTCAATTGTAAAGGTAATTCCTATTCTTTACACTCGTTGA CGGCTCTGAAAGGGACTTGCAAAACCAAAAGGTGGCCATCAAGATCTCTGGAA AGTCACTACTCCCGCACAAACAGTAATGGCGTCGCATCCATAGCTATCAATT TGGCAGCCGGGAAGAAATATAGTATTGTGTGTTCTTATGCAAGTTCTCTAATTA TAAAGCAGCTCTCAACGGTGTCCTCTCCGTGACAAATCCGTCAACTAATCT AAAAATCTCCATTGCGAAGATTGAGGCCCGCAGCGACAAATCTCAAGCTTAT GTCAACAAGAATAAGGCTGTCCCTACACGGTTTCTGTGGCGGCACTAATCTG AAGATTTCGAGTTCCTCTATCTGATGTCTAAGGCCATAGTGAATCTGAACCTCA ACAACACCAACGCGATCACTCTGCCGAGTGGCATTTATAATGGCGCTCCGCAT	SEQ ID NO.: 1

Cluster	Description	Sequence	SEQ ID NO.
		CCAACTCCCTTAATGCCACTGTTACAAAGGCCCAATATGTTGATTTGTCCAAACG GGTGATAACTATATAGACAAGATAAAGGTTCTTCCCGCTTAIGGTACGGTGTA CAATGCCAATGGAGCGTCACTGGCAACGCAGGGTTAAACCTCTACACATTCCG GTTCCGTAAGAATTCCTTGACTTCCACAAACAATAAATATCTTCCAAATTAATGT TCCTTCGATAGCTCTGTTTCAAGGCATCCCAATGGCAGTAGCAGTAGCAATATC GTAGCTCCACTAACTCATCATCTACTACTAATTCAGCAGTGGAAAGTAGCAATTC AAGCGTTCTGGCTCTAGTACCCCTGGGGTCAACAGTTAAAGTACCAGTTTGAA GGCAGCCAGCACAAAGTGTCAATAAGGGGGGACGACTATAGCGTGACGCTCACAG ATTCCAGCGTAATGCCCTCGCCAACCCAGAAAAAATACGTTTCGGCTTTCCCTCTAG TAGTTATACCCGGACAACGAACAGCAAGGAGGGGTGCTAGCTTGACCCCTGAACCT CGCAGGAGGCAATACTCCATCAACCACTTCTACGCCGGAACGTCTGCCTACAA AGCAAGCAAACTTACTAACACCGTTACGATACTAATCATCATCATCCAGGTTTTTT TTGAAACGATATAGAAACCGCTGCGGAAACAGTTAAACGTATGTGACTAAAAAC AAAGCACTGCTAATACTGTCACTGTGCGCGGAACCCAACTGACCCCTTCTCAGT TTAGTTACGTCAATGGCAAAAGCGGATCCACAATATTAACGCTTCCAAATTCAAACTA TATTTCTCTGAAGTCTGCTTCCAGTAACTCTACTGGTGACTATTTGGATACC ACCGTGACCCGGCACAGTACATGAACCTTGACAAATCGAGTGATAAGCTTCGTT GAACTCTGATAAGATCACACCTACTTTCGCAACGGTGATATAATCTAATGGTAAA AGCGTCGGTAAGCCGAATTCAACTGTATACATTCGCAATTTGCTAAAATACIC GCTTTTACAAGACTAATAATTATTTGCCGACCTATTGCACCTTCCAGTCAAGTG CCATCGGGGTTGTCACAGCGTCGCCACCAACGTGACGATAAATAGCAAGATAA ATGCCAATATGAATCAATTCAAAAGTCGGTTGAACGAAAGAAATACTGTTTCTA ATTGTCTGCATATCTGGTTGGTACAGGACAGTCCAGTACGACTAATATAAA AAACGTGCGGGCGCAACTGACGAAAGGTCTCAATTCACCCGCCACAAAGCGCT CGCGATCTATAACTTTGTGAGAGACGACATTTCTCTATTCTATTATAGTGATTCA CGGAAGGGGCAGATGGGACGCTCTCTCCGGTAGCGGGAATTGCGTGGATCAA GCTAGCCTTGCTGGCATTTGTGACGGGTGCCCGGTATTCCTGCAAGGTACAGCC ATGCTCAGGGGTGCACATTTTCTCTGGCTGGTACAGGCCACGTGTGGGTCA AATTTGGTTGATGGAGTTTGGTACTCTGCGGACGCAACGTCAAGTTCGGAACTCC CTTGGTAACATAGTTAATTGGAAACACCAATAGCTATCACAGCATGAACAGTAT GCCGCTGTCCATTTCGGCGCGCGGCTTCTGGAGGGCGGGTTCGGGGGGGGTG	

Cluster	Description	Sequence	SEQ ID NO.
		GCTCCATCGGAAACGCTGACAAAGGCCATTAAAGGATTTCCAGGATAATAAAGCGCCACA TGATAAGTCTGCGGCCCTATGAAGCTAATAGTAAAC TGCCGAAAGATCTGCGGGGACAAG AATAACCGCTTTGTGGAAAGGCTGTCATCGAAAGGCAATAGTCCGCCACGATGAAA GAGTGAAATCAGCTAACGATGCCATAAGTAAATTGAAACGAGAAGGATTCTATCGAGAT CGACGGTTGGCACAGAGAGAGGGTGAA CAAAGCTCCAA TGGATGTCAAGGACACCTT CAGAAACAACTCGACtaatgatagaccagcccaagaacacccgaatggagctctaagctacataataccaactacact ttacaaaatgtgcgcccaaaaatgtagccattcgtatcgtcctataaaaaaaggaaagtcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
M1.1	mmu1499_sbi, amino acid	MLLAVLLMGFVLSSVSAIDIDEASSSDLSDDSSISNDYLVANSGGDDSVASSASSSIA ADDSDLNNASSNVNFENEVLSTNNNEDTESEIVKDSKNQLSSSLQASTKIKTTL KSGSSVYRGNPYYVTLTDSNGKVLASQKVTFNILGKNYTRTDSKGVASININLAK GKYNIACLYAGTENYASSKLSVALTVNLMSTKINTGGSTVKKGNAYSVTLTDGNGK ALSSQKVTNLILGKNYTRTDSKGVASIAINLAAGKKFTLTASYAGSANYLSSKVSA TVTVQKGDTSIKPSGTSIVKGNYSFTLVDGSGKGLANQKVAIKISGKSYSRITNSNG VASIAINLAAAGKKYSIVCSYAGSSNYKASSSTVLSVTNPSTNSKTFISIAKIEAAATNL KAYVNKNKAVPTTVSVGGTNLKISEFSYLMMSKAIIVNLNSNNTNAIILPSGIYNGASA SNSLNATVYKAQYVDLSKRVYNYIDKNKVPAAYGTVYNANGASLGNAGFNLYTFA FAKILDFHIKTNKYLPNYCSFDSSVFKASNGSSSSSSSTNSSTNSSSGSSSSGSGS STPAVTVKATSLKAASTSVIRGDDYSVTLTDSGNALANQKITFALSSSYTRITNSK GVASLTILNLAGGKYSITTSYAGTSAKASKLTNTVTISNSSRFLNDIETAAENVKT YVTKNKALPNTVTVAGTQLTLSQFSYVMMAKAIHNINASNSNYISLKSVAASSNSTGDY LDTTVYRAQYMNLTNRVISFVESDKITPFAIVYNSNGKSVGKAFFKLYTFAFAKIL AFYKTNNYLPTYCTFQSSAIGVVPDVATNVTINSKINANMNQKVGLEKNTVSNLS AYLVGTGOSTITTNIKNVAQLTKGLNSTATKALAIYNFVRDDISYSYSDSRKGAD GTLSSGSGNCVDQASLVVALCRAAGIPARYSHAQGCFTSSGLVTGHVWAQILVDGV WYSADATSVRNSLGNIVNWNNTNSYHSMIKQYAAVPEFGGSGSGGSGGSGGSIENAD KAIKDFQDNKAPHDKS44YEANSKLPKDLRDKNNRFEVSEK4IIVRHDERVKSANDAI SKLNEKDSIENRRLAQREVNKAPMDI/KEHLQKQLD	SEQ ID NO.: 2
M1.2	OVA_sbi, nucleotide	ctgttcttttgcagaagctcaataaagctcaacttggccaccATGGGGTCCATAGGGGCCGCGTCAAT GGAGTTTTCGTCGACGCTTTCAAAGAGATTGAAGGTCCACCACGCGAATGAAAA TATCTTCTATTGTCCTATTGCAATTATGAGTGCCCTTGCAATGGTTTACCTTGGGG CAAAAGACAGTACAAGGACACAAATCAACAAGTGTCCGATTGATAAATTGC CGGATTGGGATAGTATCGAAGCTCAGTGTGGGACGAGTGCACAGTGCATT CTTCTCTCGCGATATCCTCAATCAGATCACCAGCCTAACGACGTGACTCCTT TTCCTCTCGCTTCTCGACTTTATCGGGAAGAGCGGTATCCTATATTGCCTGAGTAT CTTCAGTGCGTAAAGAGCTGTATCGCGGGGCTCGAACCAATCAATTTTCAAA CGCGGGCCGACCAAGCTCGAGAGCTGATTAAATCTTGGGTGGAACTCAGAGCCA ACGGGATTATCCGCAACGGTCTTCAGCCGCTCTCTGCTGACTCACAGACCGCTAT GGTTTGGTGAAACGCAATCGTCTTTAAAGGGTTGTGGGAGAAAGCGTTTAAGGA	SEQ ID NO.: 3

Cluster	Description	Sequence	SEQ ID NO.
		CGAAGATACTCAGGCAATGCCATTTAGAGTTACAGAACAAGAATCCAAACCTGT CCAGATGATGACCAAAATTGGCTTGTTCAGGGTGGCTCAATGGCTTCAGAGAA AATGAAGATCTTGGAGCTTCTTTTGTCTCCGGTACTATGTCATGCTGGTCTCTG CTCCCGGATGAAGTCTCAGGTCTGGAACAACCTGGAGAGCATATATAAATTTTGAG AAACTACGGAATGACCTCTCTCAAATGTTATGGAAGAAAGAAAGATCAAGGTC TATCTCCCGGATGAAGATGGAGGAAAAATACAACTTACTTCTGTGTTGATG GCTATGGGCAATACTGATGTTTCAGCAGTTCCTGCTAATCTTACGGGATATCAA GCGCAGAACTTTTGAAGATCTCTCAAGCTGTTACCGTCTACGCTGAAATAA ACGAGGCTGGCGAGAAAGTGGTCGGTTCGGCGGAGCCGGGTGGATGCGGCG TCCGTTTCAGAAAGATTCCGAGCGGACCAATCCTTCTCTGTTTGTATCAAGCATA TTGCGACTAATGCTGTGCTCTTTTTCGGGCGCTGCGTGTCTCTGCGCGGCGG TTCTGGAGCGGGGTTCCGGGGGGGTGGCTTCATCGAAACGCTGACAAAGGCCA TTAAGGATTTCCAGGATAATAAAGCGCCACATGATAAGTCTGCGGCTATGAAGCTAAT AGTAAACTGCCGAAAGATCTGCGGGGACAAAGATAACCGCTTTGTTGAAAAGGTGTTCA TCGAAAAGGCAATAGTCCGCCACGATGAAAGAGTGAATATCAGCTAACGATGCCATAAG TAAATTGAACGAGAAGGATCTATTCGAGAATCGACGGTTGGCACAGAGAGAGGTGAAC AAAGCTCCCAATGGATGCAAGGAGCACCTTCAGAAAACAACCTCGACtaagatagaccagcctcaa gaacaccgaatggagtctctaaactaataataacacacttacaataatgtgtcccccaaatgtagccattgctatctgctcc taataaaaagaagtcttctcacattct	
M1.2	OVA_sbi, amino acid	MCSIGAASMEFCFDVFKELKVHHANENIFYCPIAIMSALAMVYLGAKDSTRTOINKV VRFDKLPFGDSIEAQCGTSVNVHSSLRDILNQITKPNDVYSFSLASRLYAEERYPIPLP EYLQCVKELYRGGLEPINFQTAAADQARELINSWVESQTNIGHIRNVLPSSVDSQTAM VLVNAIVEFKGLWEKAFKDEDTQAMPFRVTEQESKPVQMYYQIGLFRVASMASEKM KIELPFASGTMMSMLVLLPDEVSGLEQLESHINFEKLTEWTSSNVMEERKIKVYLP KMEEKYNLTSVLMAMGITDVFSANLSGSSAESLKISQAVHAHAINEAGREV GSAEAGVDAASVSEEFRAHPLFCIKHIAITNAVLFGRVSPGGGGGGGGGGGG SIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFEKTSIEKAIIRHDERIK SANDAISKLEKDSIENRRRLAQRVYNKAPMDVKEHLQKQLD*	SEQ ID NO.: 4

Cluster	Description	Sequence	SEQ ID NO.
M2.1	ssp_mru149_9_sbi_nucleotide	ctgtctttttgcagaagctcagaataaacgctcaactttggccacc ATGTTTCGTGTTCTTCGGTGCTCCTGCGCTCTGGTGTCATCAGCCGCC ATCGACATAGATGAAGCTTCCTCTCCAGCGAICTGTCCGATTCTAGCATCAGCAATGACTACCTGGTCGCTAACAGGGGACGACTCAGTCGCTTCCAGTAGTGCAAGCTCCCTCCATAGCAGCTGACGACAGGATCTCAGCAATAATGCCAGCTCCAGCAATGTGAATTCGAGAACGAGGTCTCTCAGCACCAATAAACGAGGACACAGAGAGTGAAATCGTGAAAGATAGCAAGAACCAATTGAGAGCTCCAGICTGCAAGCCAGCACAAAGACTAAGACCACCTTTGAAGGGGTCGGAAAGCAGCGTCTACCGGGGAACCCATACTACGTCAACCTGACCGGATTCAAATGGAAGGGTTCTGGCTCCAGAGAAAGTGACTTTCAACATCTGGGCAAAAATTAACCCGGACCACTGATAGTAAAGGGGTGGCTCCATCAAACTCAATCTGGCCAAAGGCAATATAATATCGCTTGCTGTACGGCGGTACAGAGAAATTACGCATCATCTAAGTTGAGTGGCTCTGACCGGTCAACCTTATGTCCACCAAAATTAATACCGGTGGATCTACCGTCAAGAGGGTAATGCAATATTCGGTTACCTGACCCTGATGGAAACGGAAAGGCCCTCTCCAGCCAGAGGTCACTTGAACATCTCTGGTAAAACTATACCCGCACTACCGACTCTAAAGGGGTGGCCAGCATCGCCATCAATCTTGCTGCAAGCAAGAAATTCACACTGCTAGCTATGCAGGCTCTGCCAACTATCTTAGTAGTAAGGTGCTGCCACAGTGACCGTCCAGAGGGCGATACTAGCATCAAAACCAAGCGAACCTCCATCGTGAAAGGAACTCATATAGCTTCACTTGGTGACGGCAGTGGGAAGGGCTCGCAATCAAAAGGTGCGCCATCAAAATCAGCGGTAAAGAGCTACTCCAGGACCACTCTAACGGTGTGGCCAGTATCGCCATCAACCTGGCCGCTGGCAAGAGTACTCTATCGTGTGTTCTATGCCGGCAGCAGCAACTACAAAGGCTTCTAGCACCGTTAGTCTGAGTGTCAACCAACCATCCACTAATAGCAAGACCTTCTCCATAGCCAAATTGAAGCTGCTGCCACTAACCTCAAGCCCTATGTGAACAAGAACAAAGCAGTGCCAAACACCCGTGAGCGTGGAGGCACTAACCTTGAAGATCAGTGAAGTTTTCATCTTGATGTCTAAGGCCATTGTGAATCTGAATAGCAATAATACCAACGCAATCACCTGCCTTCAGGTATCTACAATGGGGCAAGCGCATCTAATCACTGAACGCAACTGTCTATAAGCACAGTACGTGACCTGTCAAGAGAGTTTACAAATTATAGATAAAACAAAGGTGCCAGCCGTTACGGAAACCGTTTATAACGCTAACGGTGCTCCCTGGGGAACGGTGGCTTTAATCTGTATACCTTTGCGCTTTGCCAAGATCCTGGACTTCCACAACAACAAGTACCTGCCGAACTACTGTTCCTCGACAGTCCGCTTTAAGGCCCTTAATGGCTCAAGTAGTCCAAATAGTCA	SEQ ID NO.: 5

Cluster	Description	Sequence	SEQ ID NO.
		AGTTCACCTAACTCTAGTAGTTCTAACAAATTCAGCTCCGGCTCCAGCAACTCTA GTGGCTCTGGGTCCTCTACACCGCGCTCACCGTGAAAGGCTACCTCACCTGAAAAG CCGGCTCAACCAAGCGTTATCGGGGGCGAGCTACTCTGTTACTCTGACTGACTC CAGCGGGAATGCCCTTGCTAATCAGAAAATTACCTTCGCACCTGTCCAGCAGCTC ATATACACGCACCCACAACACAGCAAGGGCGTGGCTTCACTCACACTCAATCTCGC TGGAGGGAAGTATTCATATCACAAACCACTACCGCTGGAAACATCCGCTTACAAAGGC TTCCAAAGCTTACCACACCGTGACTATCAGCAATTTCTTCCAGATTTCTTTTGA ACGACATTGAGACAGCAGCTGAGAAATGTTAAGACTTATGTACACAAAACAAAG GCACCTTCCAAAATACTGTACCGTGGCTGGAAACCCAGCTGACACTGTCAAGTTCT CTTACGTCAATGGCAAAAAGCCATTCAACAACATAAAGGCCAGTAATAGCAACTATA TATCCCTGAAAAGCGTGGCTAGCTTAATCTCAAAACCGAGTGATTTCTTTTGGGAA CGTGACCGGGCCAGTACATGAAATCTCAAAACCGAGTGATTTCTTTTGGGAA AGCGATAAGATTACACTACCTTTTGGCAACCGTTTATAACAGCAATGGCAAGAGC GTGGCAAGGCTGAGTTCAAGCTGTACACCTTCGCTTTGGCCAAAATTTCTGGCCT TTTACAAGACCAATAACTACCTGCTACCTATTTGCACATTCACGCTAGCGCCAT TGGGTGGTGGCTGACGTGGCCACCAAGCTGACTATCAACTCCAAAGATTAAAGC TAACATGAATCAGTTCAAGTGGGGCTGAATGAGAAGAACACCGTGTCAAACTT GTCCGCTACCTGGTGGGCACAGGCCAATCCACCAATAACAACCAATATCAAGAA CGTTGCTGCACAGCTACCAAGGCGCTGAATTCACCGCAACCAAGGCCCTCGC TATATACAACCTTTGTGAGAGACGACATCTCCTACTCATACTACAGTGACTCTAGG AAGGTGCGACGCGTACCCTGTCCAGTGGAAAGTGGTAACTGTGTTGACCCAGGCC AGTCTCGTCGTGGCTCTGTGCCGCGCCGCGGGATCCCAGCTCGATATAGTCACG CACAGGGAATGTACATTTTCAAGCGGGCTGGTTACAGGGCATGTGTGGGCTCAAA TTCTGGTCGATGGCGCTGGTACTCAGCCGACGCAACATCCGTGCGGAATTCACCT GGGGAACATTGTGAATTGGAACACCAACTCCTACCATAGCATGAACACAGTATGC TGCTGTCCCTTTTGGTGGCGCGGTCTTGGGGGAGGGCGGACGGGAGGTGGCGGC TCTATCGAAAACCGCTGATAAGGCCATCAAGGATTTCCAGATAATAAGGCCCGGCATG ACAAGTCCGCTGCTACGAGGCCAATCTCCAACTGCGCGAAGGACCTCCGGGACAAAG ATAACCGCTTTGTGGAGAAAGTGTCCATTGAAAAGGCCATCTCTCCGCCACGACGAGA GGGTCAAGAGCGCCAACGATGCTATTTTCAAGCTTAACGAAAAGGACAGCATTTGAGAA CCGCCGGCTGGCACAGCGAGAAAGTGAATAAAGCACCTATGGATGTGAAGGACACCT	

Cluster	Description	Sequence	SEQ ID NO.
		<i>GCAGAAACAAC TGGAC</i> <i>taatgataagaccagcctcaagaacacaccggaatggggtctctaaagctacataataccactaca</i> <i>ctttacaaatgtgtcccccataatgtatctgctcttaataaaaaagaaagtttcttcacattct</i>	

Cluster	Description	Sequence	SEQ ID NO.
M2.1	ssp_mru149 9, amino acid	MFVFLVLLPLVSSAAIDIDEASSSDLSDDSSISNDYLVANSGLDSDSVASSASSIAAD DSDLNNASSNVNFENEVLSTNNNEDIIESEIVKDSKNQLSSSLQASIKTKTTLKGS GSSVYRGNPYVTLTDSNGKVLASQKVTFENILGKNYTRTIDSKGVASININLAKGK YNIACLYAGTIENYASSKLSVALJVNLMSTKINTGGSTVKKGNAYSVTLTDGNGKAL SSQKVILNIIIGKNYTRTIDSKGVASIAINLAAGKKFTLTASYAGSANYLSSKVSATV TVQKGDTSIKPSGTSIVKGNYSFTLVDSGKGLANQKVAIKISGKYSRTTNSNGV ASIAINLAAGKKYSIVCSYAGSSNYKASSSTVSLSVTNPSTNSKTFIAKIEAAATNLK AYVNKNKAVPTTVSVGGTNLKISEFSYLMKAIIVNLNSNTNAITLPSGIYNGASAS NSLNAVYKAQYVDLSKR VYNYIDKNKVPAA YGTVYNANGASLGNAGFNLYTFAF AKILDFHIKTNKYLPNYCSFDSSVFKASNGSSSSSSSTNSSSSTNSSSGSSSSGSS TPAVTVKATSLKAASTSVIRGDDYSVTLTDSGNALANQKITFALSSSSYTRTTNSKG VASLTLNLAGGKYSHITSYAGTSAYKASKLTNTVTISNSSRFFELNDIETAAENVKTY VTKNKALPNTVTVAGTQLTLSQFSYVMAKAHNINASNSNYISLKSVAASNSTGDYL DITVYRAQYMNLTNRVISFVESDKITPTFATVYNSNGKSVGKAFFKLYTFAFKILA FYKTNNYLPITYCTFQSSAIGVVPDVATNVTINSKINANMNQFVGLNEKNTVSNLSA YLVGTGQSTHTTNIKNVA AQLTKGLNSTATKALAIYNFVRDDISYYSYSDSRKGGADG TLSSGSGNCVDQASLVVALCRAAGIPARYSHAQGCFTSSGLVTGHVWVAQILVDGV WYSADATSVRNSLGNIVNWNTNSYHSMIKQYAAVPEFGGGGGGGGGGGGSIENAD KAIKDFQDNKAPHDKS44YEANSKLPKDLRDKNNRFTVEKVSIEK4IVRHDERVKSANDAI SKLNEKDSIENRRLAQREVNKAPMDVKEHLQKQLD*	SEQ ID NO.: 6

Cluster	Description	Sequence	SEQ ID NO.
M2.2	ppa2_mnu14 99_sbi, nucleotide	ctgtctcttttgcagaagctcagaataaacgtcaacttggccaccATGCGGGTTCCTTCTATTTTCACCG CAGTCTTGTTCCGTGCTAGTAGCGCTTTGGCTATGCCACTGAGCGGAGGGCG GATCTGGGGGTGACGGCTCTATGCTCTGAACTACACACAGAGGACGAGA CTGCACAGATACTGCAGAGGCACTCGGGTACCTCGACCTCGAGGGTG ACTTGATGTGGCGTTTGGCTTTCTCTAATTCCTAATAACAACGGCTGCT GTTCAACAACACCACTAGCCAGTATCGCCGCAAAAGAGAGGGAGTCA CCGAGGTTGTGGGAGGTTGTGAGAAAGTTGTGGCCGCAATCGACAT AGATGAAGCTTCTCTCCAGCGATCTGTCCGATTCTAGCATCAGCAATGACTAC CTGGTCGTAACAGCGGGGAGGACTCAGTCGCTTCCAGTAGTGCAGCTCCTCC ATAGCAGCTGACGACAGCGATCTCAGCAATAATGCCAGTCCAGCAATGTGAAT TTCGAGAACGAGGTCTCAGCAACCAATAGAGCAGTCCAGTCTGAGGCCAGCAAA CGTGAAGATAGCAAGAACCATTGAGCAGTCCAGTCTGAGGCCAGCAAA AGACTAAGACCACTTTGAAGGGTCCGGAAGGTTCTGGCTCCCAAGAAAGTGA ACTAGTCACTGACCGATTCAAAATGGAAGGTTCTGGCTCCCAAGAAAGTGA CTTTCAACATCTGGGCAAAATTAACCCGGACCACTGATAGTAAAGGCGTGG CCTCCATCAACATCTGGCCAAAGGCAATAATAATCGCTTGTCTGTACGC CGGTACAGAGAAATACGCATCATCTAAGTTGAGTGTGGCTCTGACCGTCAACCTT ATGTCCACCAAAATTAATACCGGTGGATCTACCGTCAAGAAAGGTAATGCATAT TCCGTTACCTGACCGATGGAAACGGAAAGGCCCTCTCCAGCCAGAGGTCAACC TTGAAACATCTCGGTAAAACTATACCCGCACTACCGACTCTAAAGGCGTGGCC AGCATCGCCATCAATCTTGTGCGAGGCAAGAAATTCACACTGACTGCTAGCTAT GCAGGCTCTGCAAACTATCTTAGTAGTAAGGTGTCTGCCACAGTGACCGTCCAG AAGGGCGATCTAGCATCAACCAAGCGGAACCTCCATCGTGAAGGAAACTC ATATAGCTTCACTTGGTGGACGGCAGTGGGAAGGGCTCGCAAAATCAAAGGT CGCCATCAAAATCAGCGGTAAAGAGCTACTCCAGGACCACTCTAACGGTGT GGCCAGTATCGCCATCAACCTGGCCGCTGGCAAGAGTACTCTATCGTGTGTCC TATGCCGGCAGCAGCAACTACAAGGCTTCCCTAGCACCGTTAGTCTGAGTGT ACCAACCCATCCACTAATAGCAAGACCTTCTCCATAGCCAAATTTGAAGTGT GCCACTAACCTCAAAGCCTATGTGAACAAGAAACAAAGCAGTGCCAAACACCGTG AGCGTCGGAGGCACTAACTTGAAGATCAGTGAGTTTCTACTTGTATGTCTAAG GCCATTGTGAATCTGAATAGCAATAATACCAACGCAATCACCTGCTCAGGT	SEQ ID NO.: 7

Cluster	Description	Sequence	SEQ ID NO.
		ATCTACAAATGGGGCAAGCGCATCTAACTCACTGAACGCAACTGTCTATAAAGCA CAGTACGTCGACCTGICAAAGAGAGGTTTACAAATATATAGATAAACAACAGGTG CCAGCCGTTACGGAAACCGTTTATAACGCTAACGGTGCCCTCCCTGGGGAACGCT GGCTTAAATCTGTATACCTTTGGCCTTTGCCAAGATCTCGACTTCCACAAAACAA ACAAGTACCTGCCGAACTACTGTTCCTTCGACAGTTCGGTCTTAAAGGCCCTCTAA TGGCTCAAGTAGCTCCAAATAGTTCAAGTTCCACTAACTCTAGTAGTTCTACCAAT TCAAGCTCCGGCTCCAGCAACTCTAGTGGCTCTGGTCCCTACACCGGCCGCTCA CCGTGAAGGCTACCTCACTGAAGGCCCTCAACACAGCGTTATCCGGGGCGACG ACTACTCTGTTACTGTGACTGACTCCAGCGGGAATGCCCTTGCTAATCAGAAAAAT TACCTTCGCACTGTCAGCAGCTCATATACACGCACCAACAACAGCAAGGGCGT GGCTTCACTCAACTCAATCTCGCTGGAGGGAAGTAATCAATCAACAACAGCTA CGCTGGAACATCCGCTTACAAGGCTTCCAAAGCTTACCAACACCGGTGACTATCAG CAATTCCTTCCAGATTCTTTTGAACGACATTGAGACAGCAGCTGAGAAATGTT AAGACTTATGTCACAAAACAAGGCACCTTCCAAATACTGTACCGTGGCTGGA ACCCAGCTGACACTGTCAACAGTCTCTTACGTCATGGCAAAAAGCCATTCAACAAC ATAACGCCAGTAATAGCAACTATATATCCCTGAAAAGCGTGGCTAGCTCTAAC TCTACTGGAGACTACCTTGACACCAACCGGTACCGGGGCCAGTACATGAATCTC ACAAACCGAGTGATTTCTTTTGTGGAAAGCGATAAGATTACACCTACCTTTTGCAA CCGTTTATAACAGCAATGGCAAGAGCGTGGGCAAGGCTGAGTTCAAGCTGTACA CCTTCGCCCTTTGCCAAAATCTTGGCCTTTTACAAGACCAATAACTACCTGCCTAC CTATTGCACATTCCAGCTAGCGCCATTGGGGTGGTGGCTGACGTGGCCACCAAC GTGACTATCAACTCCAAGATTAAACGCTAACATGAATCAGTTCAAGGTGGGGCTG AATGAGAAGAACAACCGTGTCAAACTTGTCCGCTACCTGGTGGGCAACAGGCCAA TCCACCATAACAACCAATATCAAGAACGTTGTGTGCACAGCTCACCAAAGGCCGTG AATTCCACCGCAACCAAGGCCCTCGCTATATACAACCTTTGTGAGAGACGACATC TCCTACTCATACTACAGTACTCTAGGAAGGGTGCAGACGGTACCCCTGTCCAGT GGAAGTGGTAACTGTGTGACCAGGCCAGTCTCGTGGCTGTGTGCGCGGCC GCCGGGATCCAGCTCGATATAGTCACGCACAGGGAATGTACATTTTCAAGCGGG CTGGTTACAGGGCATGTGTGGGCTCAAAATCTGTGTCGATGGGCTGTGTAATCAG CCGACGCAACATCCGTGGGGAATTCACCTGGGGAACATTTGTAATTGGAACACCA ACTCTACCATAGCATGAACAGTATGCTGTCTCCCTTTTGGTGGCGCGGCTC	

Cluster	Description	Sequence	SEQ ID NO.
		TGGGGGAGGCGGCAGCGGAGGTGGCGGCTCTATCGAATAACGGCTGATAAGGCCATCA AGGATTTCCTCAGATAATAAGGCCCGCATGACAAGTCCGCTGCTACGAGGCCAACCTC CAAAC TGCCGAAGGACCTCCGGGACAAGATAACCGCTTTGTGGAGAAAGTGTCCATT GAAAGGCCATCTCCGCCACGACGAGAGGGTCAAGAGCGCCAACGGATGCTATTTTC AAGCTTAACGAAAAGGACAGCAATTGAGAACCGCCGGCTGGCACAGCGGAGAAATGAAT AAAGCACCTATGGGATGTGAAGGAGCACCTGACAGAAACAACCTGGACtaatgataagaccctca agaacacccgaatggagictaagctacataataccaactacattacaataatgtgtcccccaaatgtagccattgtatcgtc ctaataaaaagaagtttttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
		AGATTATCGCTACCATGAAAAAATGGAGAGAAGCGATGTCTTAACCCCTGAAT CCAAAGACTATCAAGAACTCTGCTGAAGGCTATCAATAAGCAGCGGACCAAGCGCT CACCAACGGACACGGAAGAGGCTAatgatagaccgctcaagaacacccgaatgagctctaatgctacat aataccactttacattacaatgtgtcccccataatgtgagccattctgctcctaataaaaagaaagtcttcacattct	
M2.3	ppa2_cCXC L10, amino acid	MRFPSIFTAVLFAASSALAMPLSGGSGSMPLNITTEDETAQIPAEAVIGYL DLEGDFDVAVLPEFSNSTNNGLLFINFTIASIAAKEGVSRRLRLRLRLAAVP LSRNTRCSCIEISNGSVNPRSLKLEVIPASQSCPRVEHATMKKNGEKRCCLNPESKTIK NLLKAINKQRTKRSPRTRKEA	SEQ ID NO.: 10
M2.3	ppa2_cVIP, nucleotide	ctgtctttttgcaagaagctcagaataaaagctcaactttggccacc ATGCGGGTTCCTTCTATTTTACCCG CAGTCTTGTTCCGCTGCTAGTAGCGCTTTGGCTATGCCACTGAGCGGAGGCG GATCTGGGGGTCAGGCTCTATGCCCTCTGAACAACACTACACAGAGGACGAGA CTGCACAGATACTGCAGAGGCGAGTCATCGGGTACCTCGACCTCGAGGGTG ACTTTGATGTGGCCGTTTGGCTTTCTCTAATTCCTCACTAAACAACGGCTGCT GTTTCATCAACACCAACCATAGCCAGTATCGCCGCAAAAGAAGAGGGAGTCA CGGAGGTTGTTCGGGAGTTGCTGAGAAAGTTGTGCGCGCACATAGTGA TGCCGTCTTACCGACAACACTACACTAGGTGGGAAAGCAGATGGCCCGTGAAAGAA GTACCTGAACAGTATTTTGAACTaatgatagaccgctcaagaacacccgaatgagctctaatgctacataat accactttacattacaatgtgtcccccataatgtgagccattctgctcctaataaaaagaaagtcttcacattct	SEQ ID NO.: 11
M2.3	ppa2_cVIP, amino acid	MRFPSIFTAVLFAASSALAMPLSGGSGSMPLNITTEDETAQIPAEAVIGYLDL EGDFDVAVLPEFSNSTNNGLLFINFTIASIAAKEGVSRRLRLRLRLAAHSDAVFT DNYTRLRKQMAVKKYLNSILN*	SEQ ID NO.: 12
M2.5	ppa2_cAPR IL, nucleotide	ctgtctttttgcaagaagctcagaataaaagctcaactttggccacc ATGCGGGTTCCTTCTATTTTACCCG CAGTCTTGTTCCGCTGCTAGTAGCGCTTTGGCTATGCCACTGAGCGGAGGCG GATCTGGGGGTCAGGCTCTATGCCCTCTGAACAACACTACACAGAGGACGAGA CTGCACAGATACTGCAGAGGCGAGTCATCGGGTACCTCGACCTCGAGGGTG ACTTTGATGTGGCCGTTTGGCTTTCTCTAATTCCTCACTAAACAACGGCTGCT GTTTCATCAACACCAACCATAGCCAGTATCGCCGCAAAAGAAGAGGGAGTCA CGGAGGTTGTTCGGGAGTTGCTGAGAAAGTTGTGCGCGCACATAGTGA GACTCGGAAGCACAAAGAAACGAAGCGTGCTTCACTCGTCCCGATCAACAT TACAAAGCAAGGAAGATAGTGACGTCACCGAAGTGTATGTGGCAGCCAGCACTCC AGCGGGCCGGGACTTGAGGCTCAGGGCTACGTGGTGAGGGTTTGGGACGCA	SEQ ID NO.: 13

Cluster	Description	Sequence	SEQ ID NO.
		GGGGTCTATCTGCTGTATAGCCAGGTGCTGTTTCACGATGAAACTTTCACCATGG GCCAGATGGTGTCCCGGAGGCCAGGGAGGAGGAGAAACACTGTTCGGTGC ATTGAGAGCATGCCATCTAACCCTGACTGGGCTACATACTAGTGTCTACTCCGCCG GGGTGTTTCACCTTCACCCAGGGGGATATACTGAGCGTTGTTATCCACGGGCCA GAGCAAGAGCTGCTCTGAGCCCGCACGGCACCTTTTGGGGCTCGTGAAGCTCtaa tgatagaccagcctcaagacacccgaatggagctctaaagctacataataccaactttacaaatgftcccccaaatgta gccatgctatcgtcctctaataaagaaagtcttcacattct	
M2.5	ppa2_cAPR IL, amino acid	MRFPSIFTAVLFAASSALAMPLSGGSGSGSMPLNTTTEDETAQIPAEAVIGYL DLEGDFDVAVLFPNSTNNGLLFINFTIASIAAKEGVSRRLRLRLRLAAAV LTRKHKKRSVLHLVPINITSKEDSDVTEVMWQPALQGRGLEAQGYVVRVWDAG VYLLYSQVLFHDETFIMGQMVSRGQGRQETLFRCIQSMPSNPDW AYNSCYSAGV FHLHQGDILSVVIPRARAKLSLSPHGTFGLVKL*	SEQ ID NO.: 14
C1.1	mrn_1726, amino acid	MFVFLVLLPLVSSAAENTDNALSTDTHSNDNVLSTD SRSNENALTNENTLLTDTHS NENALTTDPLTKENTHSYKDSKSLSSDAFNKTIYVVKTGSDGDEGSEANPYATLKK SISQLDDSDNAVIVIGPGNYTGENN SALEINLDHKHDGSLSIIGDSNGGTVDGENL NPHIISEDIVTLINITFTHGKNMGSARSNGNLTDNCIFTENYATNLAAALYVDKHS PLTVMNSKFLENRAKQCADIFYSQNSEILLNNLFEGSTAEYSYAYSPSVSLQTGKSL VKGNTFKNLTGAYYK GALYIAYNNGINIANITDNTFNCNYTGTIDGAILFFQNAAYLK NNKFIDCHSSTAFLYSNTEFNAYLSFEDAIEDGTTFELKANVTDDMGKNVKNKAVIF YLNGENVGSASSDNNGVAMISIKKLENGEVVISGTQSYSEINPFGVNVKNATARVN YDHSLEVWVSTDGDDGSGNGSEDNPFKTLRKALDYGTASAVNLTVHVKNGIYNG DDNRDLSYSTLGKITIVGESYSNVVIDGENITKSIFAFSSTLDVTLNLTINCPSTLINA YTL SMDNIVINSGTIRAQTGNNGVTIDNLRVINGTDQAITGYNLRLTNSRFENCDDG LTHTGLIWLSTNNKVTYLENNTFNNTIAGSAGGAAAYIQSDLSINNTFDSNWIT ESRGENVAYAGGRHIISINDKFINNEVPKYVAQYRSIGNEECHEIIVENITFINNKASGN GAGLATTGAIVKGGKFINNSASNGGAIYLLNHDNTSSYCOMSLEDVIFENNSATCG KDIFIEGSSGNIFTYLNLTIVANDLNVTSLSDNLTVSFHPSPGAIIGGGEISFYLDGE YIGKSTLVNQNASLEYVGFKNNTIYEFTSIYEYASLNDTYIDGIVSTKIPYALENIELY VSDGSGDDENGNGSINPEKSKALSEG YQKSTNITVHILEGTYTGSLSNLRIPPTTV NILLIGEAAKTHSDSSDYFITALKGKCELRIQMTLNRAARDTQSAIYIEEESNVAI DNVTFIGGQGNYGGAINTAGNLSIRNSYFHDNGYADRTL RANA YYGGAICNDGTLII	SEQ ID NO.: 15

Cluster	Description	Sequence	SEQ ID NO.
		DNTIFESDHAGRLSEIANQGTLYMNNKVIDSINAYSINMDLVAIGAYGGQKGEITIE NSQFIVSSKTIINELNDRIYDPTRALTCLGIGSCQHAILINSTFIGEGAVFSPYVFGGINS NNLASGGCTMIPGDLEVYNSTFRNVQAVNIISRTDNVRYHSQRVFEGCLFDNVEYI IAVLNTGNFSEVMHDCVILSDDLAKIGFGSEKTMKMDISNNWWSSNDGSYDNATLG TTNYISNCVVVKLKEISSEIVHPESYLILTLNSSNRTGLLQDAILAFKAYDGENISDYDG ALYPRNFEMSAINATGGGGSGGGGGGSIENADKAIKDFQDNKAPHDKS44YE4NS KLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSSANDAIISKLEKDSIENRRRLAQREI/NKAPM DVKEHLQKQLD	

Cluster	Description	Sequence	SEQ ID NO.
C1.1	mmu_1726, nucleotide	ctgtctcttttcagaagctcagaataaagctcaactttggccaccATGTTCTGCTCTTCTTGGTGCTTCTGCCTCTCGTGCTAGTGCTGCCGAGAAACACAGACAACGCTCIGTCTACGGGACACCCATCTAACGATAACGTGCTTTCTACAGACTCTCGGTCTAACGAGAACGCTTGACCAACGAGAACACCTCTCACCGACACCCACTCTAACGAGAACGCTTGACGACCGACCTCTGACCAAGGAGAACACCCACTCTACAGGACTCAGAAAAAAGTCTCTCTTGTACGGCTTCAACAAAGACCAATCTACGTGAACAAGACTGTTCTGACGAGGGGACGGGTCCGAAGCGAACCCCTTACGGGACCTTIGAAAAAGAGTATTTCTCAGCTCGACGATTCTGACAACGCCGTCACTCTACATCGGGCTGGCAACTACACCGGGAGAACAAATTCAGCCTTGGAGATCAACCTGGACCAACAAGGACCAACGCGGTCTCCAGCATCATCGGGGACTCAACCGGGGAGCGGTCTCGACGGGAGAACCTCAACCTATCATCTCAATCTCCGAGGACTCTATCGTGACCTTGATCAACATCACGTTACCCACGGCAAGAACAAACATGGGCAGTGCCATCCGGTCTCTGGCAACCTTACGATTGACAACCTGCTTACCCGAGAACTATGCCACGAACCTTGGCGGCCCTCTACGTGGACAAGCACTCCCTCTGACGGTCACTAACTCTAAATTCCTCGAGAACCGCGGAAGCAGTGGCCGACATCTACTTCAGTCAAGAACTCTGAGATCATCTCTCAACAACCTTTTCGAGGGCTCTACAGCGGAGTACTCTTACGCCTACTCTCTCTGTCTCTTTCAGACCGGGAAGTCTCTGGTGAAGGGGAATACCTTCAAGAACCTGACCCGGGCGTACTACAAGGGCGCCCTGTACATAGCGTACAACAACGGCATCAATATCGCCAACATCAGGGACAACACCTTTCATCAACTGCAACTACACCGGCACCGGAGCCATCTTGTTCTTCCAGAACGCCCTACCTGAAGAACAAAGTTTCATCGACTGCCACTCATACCGCATTCCTGTACTCAACACGGGAGTTCAACGCCCTACCTGTCTTTCGAGGATGCCGAGATCGATGGCACCCACTTTTCTGAAGGCCAACGTGACCGACGACATGGGTAAACAAGGTCAAGAACGCCAAGGTCACTTCTACCTGAACGGGAGAACGTTGGCAGTGCCCTCTTCTGACAACAACCGCGTCGCCATGATCTCTATCAAGAAACTGCTGGAGAACGGAGAGTATGTCACTCTTGGAACTCAGTCTCTCTCAGAGATCAACCCCTTCGGCGTGAACGTGAAGAACGCCACCGCCCGAGTCAATTACGACCATAGCAGTTTGGAGGTCTGGGTGAGCACCGACGGCGACGACGCGTTCTGGCAACGGCTCTGAGGACAACCCCTTCAAGACCTTGGGAAGGCCCTTGACTACGGACGGCCTCTGCCGTGAACCTCACCGGTGACGTAAGAACGGCATCTACAAATGCCGATGACAACGAGACCTGTCTTACTCCACGCTGGGAAGATTACCATAGTTGGCGAGAGTTACAGCAACGTTGTGTGATCGACGGTGAGAACATCACCAAGTCTAT	SEQ ID NO.: 16

Cluster	Description	Sequence	SEQ ID NO.
		ATTGCAATCTCTCCACCCTGGATGTGACCTTGATCAACCTCACCCCTCATCAAC TGTCTTAGCACCCCTTATCAACGCCCTACACCCCTGTCTATGATGGACAATATCGICA TAAACTCTGGACCATCCGCGCCAGACCCGCAACAATGGGTGACTATCGATA ACCTGCGGTGATTAAAGGCAAGGACCAAGCGATAAACGGTTATAACCTCCGCC TGACCAACTCTCGCTTCGAGAACTGCGACGGCCTTACACACACCGGGCTGATCT GGCTGCTACCAACAACAACAAGTGAATTACCTGGAGATAAACAACCTTCTTCA ACAAACGATCGCGGCGAGTGCAGCGCGGGCGCGCTACTACATCCAGTCCG ACCTTATCAGTATTAAACAATACGTTTCGACTCTAACTGGATCACCGAGATCGAG GCGAGAACGTGGCTACGCGGGCGGACGGCACATCATAAAGCATCAACGATAAAG TTCATAAACAACGAGGTGCCATAATACGTCGCACAGTACCGATCAATCGGGAAC GAGAGTGTGAGATCATCGTGGAAAACATCACTTCATCAACAACAAGGCTCC GGGAACGGCGCGGTGGCCACCAACCGGGGCCATCGTCAAGGGGGGAAGTT CATTAAACAACCTGCTCTGGGAAATGGTGGGCCATCTACCTGTGAACACAGA CAACACGTCTTCGTACTGCCAGATGTCCTGGAAGACGTGATCTTCGAGAACAA CTCTGCCACCTGGGCAAGGACATCTTCATCGAGGGCTCATCTGGGAACAACAT CTTCACCTACCTGAACAACCTGACGATCGTGCTAACGACCTGAACGTGACGTC ACTCTCAGACAACCTCAACGCTCTCTGTGTTCCACCCTAGCGGCGCCATCATCGGG GGCGGGGAGATACTTTCTACCTGGACGGCGAGTACATAGGAAGTCTACTCTC GTCAACCAGAACGCAATCCCTCGAGTACGTGGGGTTCAAGAACAACTATCTAC GAATTCACGTCTATCTACGAGTACGGGTCTCTCAACGACACGTATATCGACGGC ATCGTCTCTACGAAGATCCCTTACGCTCTCGAGAACATGAGCTGTACGTGTCAG ACGGCAGCGCGACGACGAGAACGGCAACGGGAGTATCTTAACCTTTCAAGT CCATCTCTAAGGCCCTGTCTGAGGGTTATCAGAACTCCACCAACATCACAGTGC ACATCTTGGAAAGGTACATACACAGGCTCTCTGAACTCAAACCTCCGCATCCCTAC CACTGTCAACATCTCTGTGATCGGCGAGGGCGCCCAAGACAATCATCTCTGA CTCTTCAAGCGACTACTTCATCACAGCCCTGAAGGGGAAGTGGAGCTGCGGAT CTCTCAGATGACCCCTGAACCGCGCCCGCCGGACACTCAGTCTGCTATCTACATC GAGGAGGAGAGTAACGTGGCAATCGACAACGTCACTTTCATAGGTGGCCAGGG CAACTACGGGGGAGCCATCAACACCGCTGGGAACCTCAGCATACGCAACAGCTA CTTCCACGACAATGGCTACGCGGACCGGACTCTCGGGGCCAATGCTTACTACGG AGGGCAATCTGCAACGACGGCACCTGATCATCGACAACACGATCTTCGAGAG	

Cluster	Description	Sequence	SEQ ID NO.
		CGACCACGCAGGTAGACTGTCTGAGATCGCTAACCAAGGCACCTCTGTACATGAA CAACAGCAAGGTGATTGACTCTATCAAGCCCTATCTATCAACATGGACCTTGTG GCCATCGGGCGTACGGGGCCAAAAGGGGAGATCACCATCGAGAAATAGTCA ATTCAATCGTCAGCTTAAGACCATCAACGAGCTGAACGATTAACGACCC TACCCGGCACTACGTGCTGGGAATCGGCTTTGCCAGCACGCGATTCTCATC AACTTACCTTCAATTGGAGAGGGGCCGTCTTCTCTCTTACGTCTTCGGAGGA TAAATTCAAAACAACCTGGCTCTGGGGGTTCACCAATGATTCCTGGGGAOCTCG AGGTGTACAACTCTACGTTTAGGAACGTTCAAGCCGTGAACATAATCTACTCTCG CACTGACAACTGAGTACCACTCTCAGCGAGTCTTTGAGGGTTGCCCTGTTCCGAC AACGTTGAGTACATTATCGCCGTTCTGAAACACAGGCATTTCTCCGTTGAGATGC ACGACTGCGTGATCCTCAGTGACGATCTGGCCAAAGATCGGCTTCGGGAGCGAGA AGACCATGAAGATGGACATCTCTAACAACCTGGTGGTCTTCCAACGACGGCTCTT ACGACAACGCTACCTCGGGACCACTATACTCCAACCTGCGTGTGAAGC TTAAGGAGATCTTAGTGAGACCGTGCACTGAGTCTTACCTGATACCTGACGCT CAACTCTTCTAATAGAACCGGACTTCTGACGACGCGGATCTGGCCCTTAAAGGCC TACGACGGCGAGAACATCTCTGACTACGACGGCGGCGGCTCTGTGGGGGGGCTCT GAGATGTCTGCCATCAACGCTACAGCGGGCGGCGGCTCTGTGGGGGGGCTCT GGCGGGGAGGGTCTATCGAAACCGCGACAAGGCAATCAAGGACTTCCAGGA CAACAAGGCCCTCAGACAATCTGCCGCTACGAGGCCAACTCTAAGCTGCC TAAGGACCTGCGCGACAAGAACAACTCGGTTTGTGGAAGAGGTGTCTATCGAGAA GGCCATCGTGAGGACGACGAGCGCGTGAAGTCAGCCACGACGCGATTCTAA GCTGAACGAGAAGGACAGCATCGAGAACCGGGCGCTGGCCACGCGGAGGTGA ACAAGGCGCTATGGACGCTAAGGAGCACTTGCAGAAAGCAGCTCGA Ctaatgatagac cagcccaagaacacccgaatggagtctctaagctacataalaaccaacttacactttacaaaatgtgtcccccaaaatgtagccatcgg tatctgtctctaataaaaagaagaagttttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C1.2	mmu_1726, amino acid	MFVFLVLLPLVSSAALDDLEGTIINKAINPFEGVENSQYIEAIVDNQKVNLTVHDS LSIGNAYILAENISINYNETQINVTVLEENGKRGADGGNVSLKLHDKTYISEIINGTAIF DIDVLPKGDYLLNYSLNRPKVYHSSISNSSLTVIPEKINAYANASNIVGVEDAIVIAYL DKDAGGNVTLGEEIQKVNDDGTATFIISNLAKGDYTYQLSYSGDEKYDNETFHVFSV NLKDAISIVENDTLDLFVGSNETIVATISPIGLAVNYSCSNESVAMVDENGVVSAVG AGMAVITLTVGDDVTYSKNSSSVTVIVSKIPTHIEIVNDTISLEVTDSLDASLNPEEG GNLNYAISDDLIAKIDNGQITALSEGSAIITVSFDGNDKYTKAQNKSIKIIVNLKEASV SAFSNIDLLVGENDTLSPKTSPEGLDVRYESNDTSVVLVDNGQAIAVGEGNATITLT VGGDGVYAEENTTTVKVSVSRIATMIEIKDTIELKVNEESPIGAILEPQVGNLTYSISD ESIAKVENGKIIALAEGNASLSISFAGDERYIGTNASVEIRVKNKINTILTETIDITTYKE EGYLIALTKDSQNNPISGAVLTVDLDGKNYTTDSNGQIKIATNLPDITYTARISFAG NENYSSSNGNASVTVKRIGTKLNFNDMNTTAFDSNIEGRIGEYFYFQLVDCDGNPLA NKKVIIGFNGVKYNROTNETGWAKIQINLKVANSTFAIAFLGDDNYSGSFNFVIR VSQQTPKLTASSKTYKSSAKTKTLTATLKSSSGKAIAGKKISFRLNGKVYTTANTNSL GVAIVKVS LNKKGTYSFTAQFSGDSTYKVKTKAKLTIKGGGGGGGGGGGGGSIE NADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSAN DAISKLNKDSIENRRRLAQREVNK4PMDVKEHLQKQLD	SEQ ID NO.: 17

Cluster	Description	Sequence	SEQ ID NO.
C1.2	mir_1726, nucleotide	ctgtctcttttcagagctcagataaagcgtcaacttggccaccATGTTTGTCTTCTTGGTCTCTCCCTTTGGTTAGCAGTGC1GCCCTGGATGACCTCGAAGGAACAATATCAATAAAGGCCATCAATCCTTTTGAGGGGCTCGAGAAATTCGGTACTATATCGAGGCCATTGTGGATAATCAGAAAGGTGAACCTTGACAGTGCAATGATAGCCTGTCAATCGGCAATGCCTACATCTGGCAGAGATAATCTCAATCAATTAACAAGAGACTCAGATCAACGTGACCGTGTGGAAAGAAACGGCAAGAGGGCGGACGGGGTAAATGTGAGCCTGAAGCTCCATGACAAGACCTACATCAGTGAATTAATAACGGGACCGCCATATTCGACATTGACGTGCTGAAGGGGACTACTTGTCTCAACTATTCTTTGAACCGACCTAAGGTACCAAGTATATCCAATTCCTCAAAACCTTACCCTTATACCTTTCAAAATCAATGCATACGCCAACGCCAGCAACATTAAGAATGGGCGAGGATGCCATTGTGATTGCTTATCTGGACAAGGACGCAAGGAGGAACTTACACTCGGCGAGGAAATCCAAGAAAGTTAATGATGTTACGGCAACATTTATAATTAGCAATTGGCTAAAGGCGACTACACCTACCAAGCTGAGTTATTCGGCGACGAGAGTACGACAATGAGACCTTTCAATGCTCATCTCAGTCAATCTGAAGACGCTTCAATCAGCGTGGAGAAATGACACATTGGACCTTTTGTGGGATCTAACGAGACTATCGTCTACTATCTCTCCTATCGGCTTGCAAGTACAGCTGCTCCAATGAATCCGTGGCGATGGTTGACGAAAATGGCGTGGTGAAGCGCAGTGGGTGGGGAATGGCAGTATTACCTTGACA GTGGGAGACGATGTGACCTACTCCAAGAACAGCAGCTCTGTCACTGATGATGTCAGCAAGATCCCTACCAATATCGAAATTTGTAATGACACCAATTTCTCTTGAGGTCA CCGATTCACTGGACAGCATTGCATCTCTCAATCCTGAGGAGGGAGGTAACCTTAATTATGCTATCTCCGACGATCTGATTGCAAAATGACAAATGGGCAGATAACGGCCCTGTGAGGGGAGTGCAATCATCACCGTCAGCTTTGACGGGAATGACAAATATACCAAGGCCCAACAAGTCCATTAAATATCTGTGAACCTCAAGAGGCCTCAGTTAGTGCTTTCTCAAAACATCGACCTGCTCGTGGGAGAGAACGATACCCCTGTC CCCTAAGACGAGCCCTGAGGGCCTGGATGTCCGGTACGAGAGTAATGATACTAGTGTGTGTGGTGCACAACGGCCAAGCAATCGCCGTGGCGAAGGCAACGCCACGATTACACTACGGTGGGAGGTGATGGGGTCTACGCCGAAACACGACGACTGTAAAGTGAAGCTTATGTCGGATAGCTACGATGATCGAGATCGAAAAGGATACCATCGAACTGAAGTGAACGAAGAATCCCCCTATCGGTGCGATCCTGGAACTGTATGGGAACTTACCTACAGTATCTCTGTGATGATCTATTGCCAAGGTGGAGAAATGGCAAGATCATCGGCTTCCAGAGGGCAATGCCTCCCTTTCATCTCAGCCGGC	SEQ ID NO.: 18

Cluster	Description	Sequence	SEQ ID NO.
		GACGAACGGTATATTGGGACAAATGCTTCCGTGGAGATACGAGTGAATAAGATC AACACAAATCCTGACCGAAACTGACATTAACAAACATATAAAGAGGAAGGTAC CTGATGGCCACGCTGAAGGACTCACAAAATAAACCCTATATCCGGTGCCGTGCTC ACAGTGGACCTCGACGGTATCAAGAACTATACCAACGGATAGCAACGGCCAGATT AAGATCGCCACTAACAACTGATTCTTGACACTTACACCGCCAGGATTTCCCTTTG CCGGGAATGAAAAATTATTTCTCCAGCAACGGAAACGCAAGCGTCAACAGTGAAGC GCATAGGCACCAAACTGAACCTTCAACGATATGAACACAACCTGCTTTGACACAGCA ACATCGAGGGCAGGATCGGGAGTATTTCTACTTCCAGCTGGTGGAATGTGATG GAAACCTCTTGGCCAAACAAGAAAGGTTAATAATAGGATTCAAACGGAGTGAAGTACA ACCGGCAGACAAATGAGACTGGCTGGCGAAGATTCAAGATCAATTTTGAAATATG CCAAACAGTTACACATTGGCTATCGCTTTTCTGGCGGATGATAAATACTACAGTGGCAG TTTTAACTTCGGGTCATCAGGGTCTCTCAGCAGACTCCTAAACTGACCCGACG TCTAAACATATAAAAGTAGCGCTAAGACCAAGACACTGACAGCGACCCCTGAA GTCTCAAGCGGTAAGGCAATTGCTGGCAAGAAATCTCTTCAGACTCAATGG GAAAGTCTATACCGCCAAACACTAATTTCTCTGGGGTCCCACTGTCAAAGTGTC CCTTAATAAAAGGTACTACTCTCTTCACTGCGCAGTTCTCTGGAGACTCTACC TACAAAAGGTGACTAAAACCGCTAAGCTGACCATTAAGGGCGCGGAGGATC CGGGGAGGGGTAGTGGAGGGGTGGTGGCTCCATTGAGAACGCCGATAAGGCCA TCAAGGATTTCCAGGATAACAAAGCCCCACGATACGATAAGTCTGCCCCCTTATGAGG CTAACTCTAAGCTGCCTAAGACCTCAGAGACAAAGAATAACAGATTTGTTGAGA AAGTGTCAATCGAGAAGGCTATAGTCCGCCACGATGAAAGAGTTAAGAGCGCTA ACGACGCAATCAGCAAACTTAACGAAAAAGACAGCATCGAGAACCCCGGGCTC GCCCAGCGCGAAGTGAAACAAGGCTCCTATGGATGTCAAGGAGCACCTCCAAAAA GCAGCTGGA Ttaatgataaccagccitcaagaacacccgaatggagtcctcttaagctacataaataccaacttacactttacaaa atgtgtcccccaaaatgtagccattcgtatctgtctcctaataaaaaagaaagtftcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C1.3	WP_042693 350.1, amino acid	MFVFLVLLPLVSSAAFDNSNDTIGNNIALDGSDEMILNADESNGNIIASASYNKTIYV NYTGDDSGAGSQNSPYASLNKGISSVNASDNNAVYLSAGRFTGENNTDLSINLAHKN YNGSLTIIGASNGQTILDNGNETCFKFSISADSIVTLNIHFTHGKAEFGSAIRTSGNLTI DNCTFDSNYAFTLAAYAEDEMEDVKITNSVFKNKYGFEGYADLYCTGTESITIDIINS TFINSTTENLYSLPFSIQNTNANIIGNKFENMTGLYESGTFFELRYGYGKRKVIDNIFV NCNYIGNRKGGIYISNTYLNKNNQFINCTSTNALICSVTEFNAYLKFKNLTVNGTEFK LICEVTDDLNSVSTHGKVHFFIDGKGIGSDNANNGLASITVTKLLDNGDYTLSGTY YYSENPFENVVKNAITHVDFNHDPLELWVSDNGNDTTGNGSKNPNPKTLRHALNY GFENTIDLTIHMKDGIYTGNDNKDLSYNSIGKLTIFGESYRNTIIDAAYNNTIFTGKY LNVTLTNMTLRNTSGNVNAYILKICDSFIEHVDFLNGGYSDTSKIIFNNLTYTNSGGI YIYNAEYNSKFKNCNNTKNNCLLQHSLENNIIHENTTIINTVNGQHGSSIVYISGN SILINNNTVISQNA MYIFQSYANKLTSANETFIGNNVSSYIAYYDFENGNAEIIIF ENITEKNNYAKIDGSGLVIKTGRIKGAKFINNTALNNGGAIIHLPHYGNSPFPKCILED VFFENNANNGKDIFIEKANNGYKNGQLDNITITFNNLITHNLQDIVTANISHPSGAVI GGGMIKFYLNCSYMGVAEVVNGVAKLNYLGFTKDGNYTLSGSYNYETNNTIYNNA TVNVMLSPLKENITLYVSDSRGDDENGNGSYENPYKTIENTALNKGCKQSKVIFIKVL EGNYTDKFNNTIIFDSLNTIITIGEGIDKTIITGNNTKNNWFISVLHAGNGFLKLVNMT ISEINYNKNNMNSQKSAVVIEDGANVMIDSVKFTKNRFGNGGAINNKGLNIVNSIF YNNGDSSYGGSIYNTGITHIDNSSFIANHAKFCADIYNEGILNYNSTIQDSMRNNGWT GTPLVIGGLGNIHSISKIERTGKTPLELINPGDTYADNPGETISIGTTGSIIILNTTIDGH DAKYTGPSIYSTNNA AISWHVSSNIKIYNTSFLNLDLSILNNQRGNITINSSFIKNVSNLI KSTSLYNLTVINSYFADGTISTEKYSNSNICLNNNWVWGSNSKPTYKVANVDTNPET WLILTLNQTNQSVLSKNIIILAFKVSDGENITDYGQLYPRQFTMSSINGTGGGSGG GGSGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFEVKV SIEKAIVRHDERVKSANDAISKLENEKDSIENRRRLAQREVNKAPMDVKEHLQKQL D	SEQ ID NO.: 19

Cluster	Description	Sequence	SEQ ID NO.
C1.3	WP_042693350.1, nucleotide	ctgtgtcttttgcagaagctcagaataaacgtcaactttggccaccATGTTCTGTTCTCTGTTCTGTTCTCTTGGTCTCTCTCTGCGCGTTTCGACAACTCTAACGACACACCTAACGGGAACAACAATCGCACTTGACGGCAGTGACGAGATGCTCAATGCCGACGAGAGTAACGGGAACATCTGGCTTCGGCTCTTACAACAAGAACCATCTACGTGAACCTACACGGGAGACGACTCTGTGGGTGGCTCTCAGAACTCTCTTACGCCAGCTGAACAAGGGCATCTCTAGTGAACGGCTTCAGACAACGCCGTGATCTACCTGTACGGCGCGTTACCGGGCGAGAATAACACCGACCTCTCTATCAAACTGGCCCATAAAGAACTATAACGGGTCTCTTACGATCATCGGGCGCTCTAACGGCCAGACGATACTCGACGGGAACGGGGAGACCTGCATCTTCAAGTCTATCTCTGCCGACTCTATCTGTGACCCCTTATCAACATCACATTACGCCACGGCAAGGCCGAGTTCCGGCTCTGCCATCCGGACATCTGGGAACCTGACGATAGACAACCTGCACATTCCGACTCTAACTACGCCACACCCCTGGCCGCATCTACGGGAGGACATGGAGGACGTTAAGATCACCAACTCAGTCTTCAAAAACAACCTACGGCTTCGAGGGTACGCCGACCTGTACTGCACCGGCAACCGAGTCTATCACGATCGACATTATCAACTCAACTTTTATCAACTCTACGACCGAGAACCTGTACTCTCTCCCTTCTCTATCCAAAACACAACGCCAACATATTCGGAACAACAGTTCGAGAACAATGACCGGTCTCTACGAGAGTGGGACCTTCGAGCTGCGGTACGGGTACGGAAAAAGGAAGTGATCGACAACAATCTTCGTGAACCTGCAACTACATCGGCAACCGAAAAGCGGCATCATCTACATCTCTAACACCTACCTGAAGAATAATCAATTCATCAACTGCACCGAACGAAACGCTTGATCTGTTGTCACCGAGTTCAACGCGTACCTGAAGTTCAAGAACTTGACCGTGACGGGACCGGACCGAGTTCAAGCTGATCTGTAGGTTACCGATGACCTGAACAACCTGTGTCTCCACCCATGGCAAGGTGCACTTCTTCATCGACGGAAAGGGCATCGGGAGCGACAACGGCGACTACACACTCTCCGGCACTATATACCGTGACCAAGTTGTCTGGACAACGGCGACTACACACTCTCCGGCACTCTATTACTCCGAGAACCTTTTCGAGGTCAACGTGAAGAACGCCACGATCCACGTGGACTTCAACCACGACCTCTGGAGCTTTGGGTGTCTAACGACGGTAACGACACACGGGCAACGGCTCTAAAATAAACCTTTTCAAGACACTTCGGCAACGCACTGAACTACGGCTTIGAGAACACCATCGACCTCACCATCCACATGAAGGACGGGATCTACACAGGCAATGACAATAAGGACCTGTCTACAGCAACATCGGAAGCTGACGATCTTCGGGGAGTCTACAGGAACACCATTTATCGACGGCGAGTACAACAACACTATCTTACCTTCGGCAAGTACCTCAACGTGACCCCTCACGAACATGACCCCTGGCAACACAAGTGGCAACGTCAATTAACGCCCTACATTCTGAAGATCTGCGACTCATTCATT	SEQ ID NO.: 20

Cluster	Description	Sequence	SEQ ID NO.
		GAAACGTCGACTTCTCTAACGGTGGCTACTCTGACACCTCCAAGATCATCTTCA ACAACTGACCTACACCAATTCTGGAGGAATTTACATCTATAACGCCGAGATCT ACAACTCTAAGTTCAAGAAGTGCACAATAACGAAGAACAATTGCTGCTGCAGA TCATCAGCCTCGAGAAACAATCATATCCACATCGAGAACACTACGATCATCAACA ACAGGTGAACGGGCAGCACGGCTCTTCTATCTGTTACATCTCTGGCAACTCTAT CCTTATCAACAACAATAACATCAATAACACGGTGATAAGTCAAGAACGCCATGTA CATCTTCAGAGCTACGGGAACAAGCTGACGCTGCGCAACGAGACGTTTCATCGG GAAACAATGTCTCATCTACATCGCATATTACGACTTCAACGGGAACAACAACGCAGA GATCATCTTTGAGAACATCACCTTCAAAAAAACAATAATGCCAAGATCGACGGGTC TGGGCTCGTCAATCAAGACCGGGCGCATCAAGGGGGCTAAGTTTCATCAATAAACAC TGCTCTGAACAATGGCGGGCCATCATATCACTGCTCACTACGGTAACCTCCT TTCCCTAAGTGCAATCTTGGAGGACGTGTTCTTCGAGAACACAACGCGAACAAAC GGAAGGACATATTATAGAGAAGCGGAACAACGGCTACAAGAAGGCCAGTT GGACAACATCACCATCACCTTCAATAACCTCATAAACCAACACCTGCAGGACAT CGTCACCGCCAACATCTCCACCCCTAGTGGCGAGTTATCGGGGTGGCATGAT AAAGTTCTACTTGAACGGGAGTTACATGGCGTGGCGGAGTGGTGAACCGCGT GGCCAAGCTGAACCTACCTCGGATTCACCAAGGACGGCACTACACCTGTCCGG CTCTTACAACCTACGAACAACAACAACAGATCTACAACAACGCCACCGTTAACGT CATGCTCAGCCCTCTGAAGGAGAACATCACCTGTACGTGCTTGACTCACGGGG AGACGACGAGAACGGGAACGGGAGTTACGAGAACCCCTTACAAGACTATCGAGA ACGCCCTGAATAAGGGCTGCAAGCAGTCTAAGGTGATCTTCAAGGTCTTIG AGGGCAACTACACTGACAAGTTTAACAACAACATCACCCCTGTTCGACTCTCTGA ATATCACTATCATCGGGGAAGGCATAGACAAGACTATCATCCCGGCAACAACA CAAGAACAACCTGGTTCATCAGCGTTCTGCACGGGGCAACGGCTTCCTCAAGC TGGTGAACATGACAATCTCTGAGATCAACTACAACATAAAGAACATGAACCTCTC AGAAGAGCGCCGTGTCATCGAGGATGGGGCCAACTGATGATCGACTCTGTCA AGTTACGAAGAAGACCGGGCTTCAACGGGGGGCCCATCAACAATAAGGGAAAG CTGAACATTGTCAACTCCATCTTCTACAACAACGGGGACTCTTACACGGAGGCT CTATTTACAACACGGGGATCACTATAATTGACAACCTCTTCTTCTATCGCTAAACA CGCGAAGTTCTGCGCCGACATCTACAACGAGGGCATACTCAACATCTACAAATAG TACCATCCAGGATTCTATGAGAACCAACGGGTGGACCGGCCCTCTCTGTGAT	

Cluster	Description	Sequence	SEQ ID NO.
		CGGGGGCCCTGGGGAACATATCTATCATCAACTCTAAGATCTTCAGAACCGGCAA GACCCCTCAGCTCAACAACCCCTGGGTGATACCTACGCCGACAAACCCCTGGCTTC ACTATTCTATCGGACAACCGGCTCTATCATCTCTGATCAACACGACCATCGATG GGCAGACGCCAAGTATACCGGGCTTCTATTATCTACGAAACACGCCGCCA TCTCTTGGCACGTGCTTCTTAACATAAAGATTTACAAACACCTCTTCTTAAACCTG GACTCCATCTTGAAACAACGAGGTAAACATCAACATCAACAGAGTTTCATT AAGAACGTCTCAAACTGATAAAGAGTACCTCACTTTACAACCTGACGGTCAIC AACTCTTACTTCGGGACGGGACGATATCTACTGAGAAGTACTCTAACTCTAAC ATCTGCCCTCAACAACAACCTGGTGGGGAGTAACCTTAAGCTTACCTACAAGGTG GCCAACGTGGACACCAACCCCTGAGACCTGGCTGATCTCAACCTCAACCCAGACC AATCAGTCTGTCTCTCTAAGAACATCATCTCTGGCTTTTAAGGTGTCAGACGGCG AGAACATCAACCGACTACACCGGCCAGCTGTACCTCGGCAGTTTCAACAATGAGCT CAATCAATGGTACCGGTGGCGGGGTCTTCTGGGGGGGTGGCTCTGGCGGGGTG GCTCTATCGAGAACGACACAAGCGATCAAGGACTTTCAAGGACAACAAGGCC CCTCAGACAAGTCAAGCCGCTACGAGGCGAACTCTAAGTCGCTAAGGATCTG CGGGACAAGAACAAACCGCTTCTGTGGAGAAAGTGAGCATCGAGAAGGCCATCGT GGCCACGACGAAACGGGTGAAGTCTGCAACGACGCCATCAGCAAGTTGAACG AGAAGGATTCTATCGAGAACCGGGCGCTGGCGCAGCGCGAAGTCAACAAGGCC CCTATGGACGTCAAGGAACACCTGCAGAACGAGCTGGACiaatgatagaccagcctcaaac accogaatggagctctctaaataataccaactttacactttacaaaatgtgtcccccataatgtgoccatctgtatctgctcctaata aaaagaaagtgtttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C1.4	WP_042693 350.1, amino acid	MFVFLVLLPLVSSAALKSANGNITNHIINLINISKNATYYVEATVDNQTVNLTONIT HNVTIIAKDIIFDYGTITINVNVLFDNLPAADSEIITLNLNNKKYTAKIKNGTATFNIDII PHGTYTLKYIINATKLHGEVLNSSILTVNRINANINATSHSVIVGNDVKVEINLPKDLT GTINVLNNETYPEIHNKAIVIPNLAQGEVIKITYSGDNKYLPTNTTVNIKVLGIN ITAPDVEKYKGEKLIQIFIKDSEGNAIIGQNIQIKLNGKNYATATTNNEGASIELDLNI GKYSATHFNDDKKINASITIKSTIHAPNMIRGYNSGLDYQTLLNVVDGTPLANTHITLKI GDKKYSLTITDANGVAKLNKKIAIGTYNIIJINPTNLEKQIKTLKISRNNNKNNLAGD YLSGINYKIRIIDDNKGTAGTGKIVKITINKKHTTYLPIKMDTQHGSGGGSGGGGGG GSIENADKAIAKDFQDNKAPHDKS44YEANSKLPKDLRDKNNRFEKVSIEKAIVRHDERV KSANDAIISKLNEKDSIENRRLAQRVYNKAPMDIYKEHLQKQLD	SEQ ID NO.: 21
C1.4	WP_042693 350.1, nucleotide	ctgtcttttgcagaagctagaataaagctcaacttggccaccATGTTCTGCTTCCTGGTGTGCTCCCT CTTGTCTCTCCGGCGCCCTGAAGTCCGCCAAACGGGAACATAAACCAACACACATC ACCAACCTGATCAACACATTTCAAAAGAACGCCACTATCTACTAGCTCGAGGCAACG GTTGACAACCAAGACCGGTGAACCTTACTCAAAACATTACCCACAACGTCACCATC ATGCCAAGGACATTATCTTCGACTACGGAACCAACGACCATCAACGTCATATGTG TTGTCGATAACCTGCCTGCCGACACAGCGAAACCATCAACGCTCAACCTGAACAAT AAGAAGTATACAGCCAAGACTAAGAACGGCACCGCAACCATTCATATATAGACAT AATTCCTCATGGCACTTACACCTTAAGTACATCATCAACGCTACCAACCTCCAC GGTAGGTGCTGAACCTCAAGTATTTCTGACGGTGAACAGGATAAACGCAACACATA AACGCAACCAAGTCACTCCGTGATCGTTGGCAACGATGTGAAGGTGAGATCAAC CTGCCTAAGGACCTGACTGGCACAAITTAACGTGGTGTCAACACGAGACATAC CCTGTTGAGATCATTAACAACAAGGCCATCGTTATAATCCCTAACCTTGGCCAGG GTGAGTACATCGCCAAGATCACTACAGTGGGAGACAAATAAGTACCTTCCTACAA ACACACCGTGAACATCAAGGTGCTCGGATCAACATCACCGCACCTGACGTCG AGAAATACTACAAGGTTCTGAGAAGCTGCAGATCTTCAATCAAGGACAGCGAGG GCAACGCGATCATCGGTGAGACATACAGATCAAGCTGAACGGCAAGAACTAC ACGGCCACCAACCAACAACGAGGGAATCGCGTCAATAGAGTTGGACCTGAAATATT GGCAAGTACAGCGCCACGATTATCTTCAACGACAGAAGATCAATGCCAGCATC ACCATCAAGTCAACCATCCACGGCCTAACATGATACGGGGGTACAACCTCTGGA CTGGATTACCAAGACTACCTGTCTCAACGTGGACGGGACCCCTCTCGCCAAACACC CACATAACACTGAAGATCGGGGACAAAGAAGTACAGTCTTACTACGGACGCTAAC	SEQ ID NO.: 22

Cluster	Description	Sequence	SEQ ID NO.
C2.1	mmu_1923, MtrE = WP_012956 721.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter ruminantiu m]. fragment 1, amino acid	GGGGTGGCCAAACTGAACAAAAAATCGCCATTGGCAGGTACAAACATCCTGATC ATCAATCCTACCAACCTGGAGAAACAGAGAACGCTCAAGATCATATCACGA ATCAACAACAACAAGAACCTGGCGGGGACTATTGTAGTGGGATCAACTACAAG ATCCGAATCATCGACGACAACGGGAAAGACAGCGGGACCGGCAAGATCGTGAA GATCACCATCAATAAAAGCACACCACTTATCTTCTATCAAAATGGACACACA GCACGGCGGGGTGGTCCGGAGGGGGGGTCTGGCGGAGGTGGCAGCATTG AAAACGCCGACAAGGTATCAAGGATTTCCAGGACAAACAAGGCACCTCACGAC AAGTCCGCCGCATACGAGGCTAACTCTAAGCTCCCTAAGGACCTCCGCGACAAG AACAAATCGCTTTGTGGAGAAAGGTGTCAATCGAGAAAGGTATCGTGCGCCACGAC GAGAGGCTCAAAATCTGCTAACGATGCCATCAGCAAGCTGAACGAGAAAGGACAG TATCGAGAATCGGGCGGTGGCCAGAGAGAAAGTCAACAAGGCCCTATGGACGT TAAGGAGCATTTGCAAAAGCAGCTTGACraatgatagaccagcctcaagaacacccgaatggagtctcta agctacataataaccacttacactttacaaaatggtgtcccccacaaatgagccattgctctcctaataaaaagaagtttctcaca ttct MFVFLVLLPLVSSAAAEDESIGSQSNPNQVQLAPQMGLHRMINKAASGEPGG GGSGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLDRDKNNRPFVEKY SIEKAIVRHDERIVKSANDAIKLNKESIENRRLLAQREYNKAPMDVKEHLQKQLD	SEQ ID NO. 23

Cluster	Description	Sequence	SEQ ID NO.
C2.1	mtu_1923, MtrE = WP_012956 721.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter ruminantiu m], fragment 1, nucleotide	ctgtctcttttgcagaagctcagaataaaacgtcaactttggccaccGCCGAGGATCTCGAGTCAGACATCGG AAGCCAGTCAAAACCCCTAACTCTCAGGTCCAGCTGGCCCCCTCAGATGGGGCATCT TCACCCGATGATCAACAAGCCGCCAGTGGCGAGCCTGGCGGCGGGGAGTG GTGGCGGGGGTCTGTGTGAGGGGGCAGCATAGAGAACGCCGACAAAGGCTATC AAGGACTTCCAGGACAACAAGGCTCTCACGACAAGTCCGACGATACGAGGCC AACTCAAAGCTGCCTAAGGATCTTCGGGACAAGAATAAACGGTTCTGTGGAGAAA GTGTCCATTGAAAAGGCGATCGTCCGCCACGACGAGCGAGTGAAGTCCGCCAAT GACGCCATCTCTAAGCTGAACGAGAAGGACAGTATCGAGAACAGACGGCTGGC GCAAAGGGAAGTGAAACAAGGCCCTATGGACGTTAAGGAGCACCTCCAGAAAGC AGTTGGACtaatgatagaccagcctcaagaacacccgaatggagctctaagctacataatccaacttacacttacaatgt tgtcccccaaatgtagccattcgtctccttaataaaaagaaggtttctcacatct	SEQ ID NO.: 24
C2.2	mtu_1923, MtrE = WP_012956 721.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter ruminantiu m],	MFVFLVLLPLVSSAASHMGRIVGQSQEQPLFMDVLTQSLGPGGGGSGGGSGGG GSIENADKA/KDFQDNKAPHDKSA4YEANSKLPKDLRDKNNRFTVEKVSIEK4IIVRHDERV KSANDAISKLENEKDSIENRRLAQREVNKAPMDI/KEHLQKQLD	SEQ ID NO.: 25

Cluster	Description	Sequence	SEQ ID NO.
	fragment 2, amino acid		
C2.2	mrn_1923, MtrE = WP_012956.721.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter ruminantium], fragment 2, nucleotide	cttgcttttttcagaagctcagaataaaacgtcaactttggccaccATGTTTGTCCTCCTGGTGTCTGCTCCCTTTGGTGAGTTCAGCCGCCCTCCACATGCGGCGCATCGTGGGCGCAGTCACAAATTCGAGCAGCCTCTGTTTCATGGACGTGCTACCCAGTCCCTTGGCCCTGGGGGAGGGGGCAGCGGGGGCGGATCTGGTGGCGGCGGAGCATCGAGAATGCAGACAAAGCCATAAAGGACTTCCAGGACAACAAGCCCTCAGACAAAGTCTGCCGCATACGAGCGAAGTCTAAGCTGCCTAAGGACCTCAGAGACAAGAACAACCGGTTCGTGGAGAAAGTTCAGATCGAGAAGGCCATCGTGGCGCACGACGAGCGAGTGAAGAGTGCCCAACGATGCCATTCTAAGCTGAACGAGAAGGACTCTATCGAAACACAGGCGCCTGGCGCAGCGGGAGGTTAACAAAGGCTCTATGGACGTCAAGGAGCACCTCCAGAAAGCAGCTGGACtaatgatagaccagcctcaagacacccgaatggagctctaaagctacataataccaacttacatttacaaaatgtgtcccccaaaatgtagccattcgtatctctcctataaaaaaagaagaagtcttcacattct	SEQ ID NO.: 26
C2.3	mrn_1923, MtrE = WP_012956.721.1 tetrahydromethanopterin S-methyltransferase	MFVFLVLLPLVSSAASSTGDDVHYGAESYQKFEFGGTPVAIQGDIVTNAPMGAKNSMDVVNFCAKGGGGGGGGGGGGSENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAI/RHDERVKSANDAISKLINEKDSIENRRRLAQREVNKAPMDIVKEHLQKQLD	SEQ ID NO.: 27

Cluster	Description	Sequence	SEQ ID NO.
	subunit E [Methanobrevibacter ruminantium], fragment 3, amino acid		
C2.3	mrn_1923, MrE = WP_012956 721.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter ruminantium], fragment 3, nucleotide	ctgtcttttgcagaagctcagaataaacgctcaacttggccaccATGTTGCTCTTCCTGGTGTGCTGCCT CTGGTCTCTTCTGCCCGCAGTAGCACGGGCGATGTGCACTACGGTGGGAGTCT GAGTACCAAAAGTTTCGAGTTCGGGGGGGACCCCTGTGCTATTTCAGGGGGAC ATCGTGACCAACGCACCTATGGGGGCGAAGAACAGATGGACGTGGTCAATTTT TGGCCCAAGGGCGGGCGGTCAGGGGGCGGAGCGGTGGCGGAGGATC TATAGAGAACGCCGACAAAGGCCATCAAGGACTTCCAGGACAAACAAGGCCCTC ACGACAAGTCCGACGCTACGAGGCCAACTTAAGCTCCCTAAAGACCTTCGCG ACAAAACAACAGGTTCTGTTGAGAAAGTGTCTATCGAGAAAGCCATCGTGAGAC ACGACGAGCGGTGAAGTCTGCCAACGACGCGATCTCCAAAGTCAACGAGAAAG GACTCAATCGAGAACCGGGCGACTGGCCAGCGGAAAGTGAAACAAGGCTCCTAT GGACGTCAAGGAGCACCTGCAGAAAGCAGCTCGA Ctaatagatagaccagcctcaagaacacccgaat ggagtcctaaagctacataataaccaacttacacttacaataaagtgtcccccataatgtgccattgtagctccttaataaaagaaa gtttcttcacattct	SEQ ID NO.: 28
C2.4	WP_0040329 19.1 MULTISPECIES: tetrahydromethanopterin S-methyltransferase subunit	MFVFLVLLPLVSSAASSTGDDVHYGAESEYQKFEFGGTPVAIQGDIVTKAPIGAKNS MDVVNFCAKFGGGGGGGGGGGGGGSIENADK4IKDFQDNK4PHDKS4AYEANSKLP KDI/RDKNNRFVEXV/SIEKAI/RHDERVKSAND4ISKI/NEKDSIENRRLAQREVNKAPMDIV KEHLQKQLD	SEQ ID NO.: 29

Cluster	Description	Sequence	SEQ ID NO.
	E [Methanobrevibacter] fragment 1, amino acid		
C2.4	WP_004032919.1 MULTISPECIES: tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter] fragment 1, nucleotide	cttgcttttttcagaagctcagaataaaacgtcaactttggccaccATGTTCTGTTCTTGTCTGCTGCTCCTCTGGTGTCTAGCGCGCCAGCTCTACGGGAGACGTGCAATTACGGTGCTGAGTCCGAATACCAGAAAGTTTGAGTTCGGAGGCGGCACCCCTGTCGCCATCCAGGGGACATCGTGACCAAGCACCTATCGGCGCTAAACAGATGGACGTCGTGAACCTTCGCGCCAAAGTTCGGAGGGGGGGGCTCCGGGGGGGTGGCAGTGGCGGGGGGGGTAGTATAGAGATGCCGACAAGGCCATCAAGGACTTCCAGGACAACAAGGCGCTCACGACAAGTCAGCGGCTACGAGGCAACTCCAAAGCTGCCCTAAAGATCTCCGGGATAAGAACACACAGATTCTGTGAGAGGTGTCATAGAGAAGGCAATC GTGAGGCACGACGAGCGAGTCAAGTCTGCCAACGACGCGATTAGCAAACTTAATGAGAAGGACTCAATCGAAAACCGCGCGCTGGCCCCAGCGCGAGGTGAACAAGGC CCCTATGGACGTC AAGGAGCACTTIGCAA AAGCAGCTCGACtaatgataagaccagcctcaagaa caccgaatggagctctaagctacataataaccacttacactttaaaaaatgttgcctcccaaaalgttagccattcgtatctgctctaataa aaaaagaagttcttcacattct	SEQ ID NO.: 30
C2.5	WP_081738309.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter oralis]	MFVFLVLLPLVSSAAATSHMGRIVGQSQFEQPLFMDVLTQSLGPIAGHGGGGSGGGGSGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIIRH DERVKSANDAISKLNEKDSIENRRLAQREIVNK4PMDVKEHLQKQLD	SEQ ID NO.: 31

Cluster	Description	Sequence	SEQ ID NO.
	fragment 1, amino acid		
C2.5	WP_081738.309.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter oralis], fragment 1, nucleotide	ctgtctcttttgcagaagcagataaaacgctcaactttggccaccATGTTCTGTTTTCTGGTGTGCTTCCTCTGGTTAGTCCGGCCGACGCTCTCACATGGGGCGCATTTGGGGCCAAATCTCAGTTCCGAGCAGCCCTCTGTTCATGGACGTGTTGACCCAGAGTCTCGGCCCTATCGCCGGACATGGCGGGCGGGGCGAGCGGTGGGGCGGGTCAAGGGGTGGCGGATCTATCGAGAACGCAGACAAGGCCATCAAGGACTTCCAGGACAATAAGGCGCCTCAGCACAAAGTCAGCCGCTTACGAGGCAACACAGTAAGCTCCCTAAGGACCTGCGGGACAAAAACAACCGCTTCGTGAGAAAGGTGTCATCGAAGGCCATAGTCAGACACGATGAGCGGGTCAAGTCAGCTAACGACCGCATCTCCAAAGCTGAACGAGAAAGGACAGTATCGAGAACCGGAGGCTGGCCAGCGAGAAAGTGAAACAAGGCCCTTATGGACGTGAAAGAGCACCTTCAGAAAGCAGCTCGATaatgatatagaccagctcaagaacacccgaatggagctctaaagctacataataaccaacttacatttacaataatgtgtcccccaaaatgtagccattcgtctcctataataaaagaaagtcttcacattc	SEQ ID NO.: 32
C2.6	WP_081738.309.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter oralis], fragment 2, amino acid	MFVFLVLLPLVSSAASSTGDRVHYGAESYQKFEFGGTPVAIQGDIVTKAPIGAKNSMDVVNFCAKFGGGGGGGGGGGGGSENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKLNKEDSIENRRRLAQRLEYNKAPMDVKEHLQKQLD	SEQ ID NO.: 33

Cluster	Description	Sequence	SEQ ID NO.
		CTTACGAGGCCAACTCCAAAGTTGCCCTAAAGACCTGCGGGACAAGAACAACCGGTTCGTGGAGAAAGGTCCTATTGAGAAAGCCATCGTGGGCACGACGAGCGGTCAAGAGCGCGAACGACGCGATCTCTAAGCTGAACGAGAGGACAGTATAGAGAACCGAAGACTGGCACAGCGCGAGGTGAACAAGGCCCTATGGACGTGAAGGAGCATCTGCAGAAAGCAGCTGGA Ctaatgatagaccagcctcaagaacacccgaatggagctctaagctacataaccaacttacatttacaaaatgtgtcccccataatgtagccattcgtatctgctcctaataaaaagaagttttcacattct	
C3.2	WP_011954 372.1 MULTISPE CIES: signal peptidase I [Methanobr evibacter], a mino acid	MFVFLVLLPLVSSAALNVVVS GSMPEPVFYRGDIVAVEKADFLGHEFDPSDVRVGDIVVYDATWYNPEPVIHRVINITQINGTTYMIKGDHNSHPDPYYATADQINERVLTDWGHPIVIPYIGNISLWLRGLGGGSGGGSGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIVRHDERVKSANDAISKLINEKDSIENRRRLAQREYNKAPMDVKEHLQKQLD	SEQ ID NO.: 37
C3.2	WP_011954 372.1 MULTISPE CIES: signal peptidase I [Methanobr evibacter], nucleotide	ctgtctttttgcagaagctcagaataaaagctcaactttggccaccATGTTCTGTGTTCTCGTTTTTGCTGCCTCTGGTTTCTTCTGCGCCCTGAACGTGGTCTGTGTCAGGATCTATGGAGCCTGTGTCTACCGCGGCGACATTGTGGCCGTGGAGAAAGCTGACTTCTCTGGGCATCCATGAGTTCGATCCTAGCGATGTCAGGTGGGGACATCGTGGTCTATGACGCCACGTGGTACAAACGAGCCTGTGATACACAGAGTCAACATAACCCAGATCAACGGAACAACTTACTACATGATCAAGGGCGACCACAATTACACCCCTGACCCCTTACTACGCGACCGCCGACCAGATCAACGAGCGGGTCTTGACCTGGGACGGTCAACCTATCGTGATCCCTTACATCGGGAACATTTCTTTTGGCTTCGAGGGCTGGGGGTGGTGGCTCAGGGGGGGCGCAGTGGGGCGGAGGCTCCATCGAGAACGCTGACAAAGGCCATTAAAGGACTTTCAGGACAACAAAGGCCCTCAAGACAAGAGCGCAGCGTACGAGGCAAACTCCAAATCCCTAAAGGACCTCAGAGACAAGAAATAACCCGCTTCGTGAGAAAGTTAGTATCGAAAGGCAATAGTGGGACGACGAAACGAGTCAAGAGTGCCAAACGATGCCATCAGCAAGCTGAACGAGAGGACTCCATCGAGAAACAGGGCGCTGGCGCAGCGGAGGTGAACAAGGCCCTATGGACGTGAAGGAGCACCTGC	SEQ ID NO.: 38

Cluster	Description	Sequence	SEQ ID NO.
		AAAAGCAGCTCGACtaatgatagaccagcctcaagaacacccgaatggagtcftaagctacataataccaacttacactttacaataatgtttcccccaaaatgtagccattcgtatctgctccttaataaaaaagaaagtttcctcacattct	
C3.3	WP_042694 712.1 signal peptidase I [Methanobr evibacter oralis], amino acid	MFVFLVLLPLVSSAALNVVVGSGSMEPAFYRGDIVVIEKSDFLGIHEFNPTDVKVGD VVVYDAAWYDQPVVHRVINITQINGSTYYVVIKGDNNDSPPYYVSPNQINERVVTFG DNLCVIPYVGYLSLWRLGLGGGSGGGGGSGSIENADKAIKDFQDNK4PHDKS44 YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLEKDSIENRRLAQREV NKA PMDVKEHLQKQLD	SEQ ID NO.: 39
C3.3	WP_042694 712.1 signal peptidase I [Methanobr evibacter oralis], nucleotide	ctgtctcttttcagagctcagaataaaagcctcaactttggccaccAIGTTCTGCTCTCTCTGCTGCTTCTCCCT CTGGTCAGCTCAGCTGCATTGAACGTCTGCTGCTCAGGGAGCATGGAACTGCG TTCTACCGCGGAGACATTGTTGTGATCGAGAAAGTCCGATTTTTTGGGCATCCACG AGTTCAACCTACCGACGTGAAGGTCTGGTGACGTGGTGGTACGACGACGCGT GGTATGACCAGCCTGTGATCCACCGGGTGATAAACAATCACACAGATCAACGGCA GTACCTACTACGTTATCAAGGGGACAAATAACGACTCTCTGACCCCTTACTACGT TAGCCCTAACCAATCAACGAGAGAGTGGTCAAGTTCCGGGACAACTGTGCGT GATCCCTTACGTGGGTACTGTCTCTGCTGAGGGGACTTGGCGGGGTTGG TTCTGGAGGGCGGGAGTGGGGCGGGGGCTCTATCGAGAACGCCGATAAAG CCATAAAGGACTTCCAGGACATAAAGCCCTCACGATAAAGTCTGGGGCTTAAG AGGCCAACTCTAACTCCCTAAGGACCTGCGGGACAAGAACAAACCGATTCTGTGG AGAAGGTCAAGTATCGAGAAAGCCATTGTCCGCCACGACGAGAGGGTGAAGTCC GCCAATGACGCCATATCTAAGCTGAACGAGAAAGGACTCAATCGAAAACCGACG GCTGCCCCAGCGGAGGTGAACAAGGCCCTATGGACGTTAAAGAGCATCTGCA GAAGCAGCTTGA Ctaatgatagaccagcctcaagaacacccgaatggagtcftaagctacataataccaacttacattt acaaaatgtttcccccaaatgtagccattcgtatctgctccttaataaaaaagaaagtttcctcacattct	SEQ ID NO.: 40

Cluster	Description	Sequence	SEQ ID NO.
C4.1	mu_2047 homology region, -500 and +500 aa, amino acid	MFVFLVLLPLVSSAARGNNFESIQEYIDNEGSSFNSIENNLVLKYDNDKANLKNEL LYILYLLGHGCSYESANFTGVQASGIFDRWYSFDEYVLTFFNESRNRITIGILESTM IQSQQLDLVNEITERLESKGYNPIYCPAGNAEQLNIMVKYWTSAENISGFLENPQ DFDIYVDGHSMAVYGVGGENFTNATKFFEDANVPFRAVHSEYTTNEQWELSPVGLS TTKSDKWWHVITAESQGFDAVYGGVDSYISNRTGAHLTFVPVHENIELLTDRVDA WVDLKYTPNEDKNISIVYNYPPGKQKONIGASYLDAITSVYNMMLYTLKDEGYLTLDL PNNVSELEDDMMIACGINVANWAPGEVEKLANRSGVALLPVDEYLEWFDLDDIVK VQITEGPVAYIGQMVRRAVLINYYTDEVEITMVNDWYNQIKALLPENQTVAAATNLDK LVNSLKL YANASSDGDENASLYYDEFLRYVYDEFKSLNVSGLNGWGEAPGNIMLVN RNGTDYFVIPGLTFGNVFIGPEPQRGWAEADENLYHCTAVAPTHQYLAAYYMQIR QSNAMVFVGRHATHEWLPKGEVLLSYNDYGSIVVGKVPQVYFYTTDGLAEAIQAK RRGFVLLSHLDSPKSYTHLYGNLTVLAATLLEEDNNHHIESDSKDNQAITYQVIK DNQITYQVINQELDNLTIRAIKDLVIANNYYLTIGFTAEEELNNTDMFSLSSILNAFL KNTQNTLYPLGLHAIGQKWTDDELANTVAIVSHDFEYGGKKTNLFDQLSLYYIGE KYSNLTPLKRDYILNRSVDVCKALIYWDTEVSDTIGIGSPEFIESLNIKKYIDL YNQ CISLELEEMVSALNGGYVPVNIGGESVTPQVLP TGANMYQDQSSSELPTQKAWDYA KTL SLL TLADLNDTTEKIIMGIWCVETARDDGALVSTVLYLLGMEPVWHNSSAGF DEEGIPTGKKVEDLPNVIALENLTRPDGWAKKRIDVTVITSGLFRDLYSSQARLMDN AYRMALACSYTYTIVNNKTIMDSEYGPQVYDALRSIMRSISFKGGGGGGGGGGGGG GSIENADKAHKDFQDNKAPHDKSA4YEANSKLPKDLRDKNNRFTKVSIEKAIIRHDERV KSANDAIKSLNEKDSIENRRLAORELVNKA PMDI KEHLQKQLD	SEQ ID NO.: 41

Cluster	Description	Sequence	SEQ ID NO.
C4.1	mnu_2047 homology region, -500 and +500 aa, nucleotide	ctgtctcttttgcagaagctcagaataaacgtcaacttggccaccATGTTCTGCTCTTCCTGGTGTCTTGCCT CTGGTGTCTAGTGCCTGGCGGGGCAACAACTTCGAGTCTATCCAGGAGTACATC GACAACGAGGGCAGTTCTCTCAACTCTATCTTCAACAACCTGGTGTCTTATAAGG ACATCAACGACAAGGCCAACCTGAAGAATGAGTTGCTGTACATCCTACCTCC TGGGACACGGTTGTCTATACGAGAGTGCCAACTTCAACGGGTCCAGGCCTCTG GGATCTTCGGGACCGCTGGTACTCTTTTCGACGAATACGTCTCTACCTTCTTCA TGAGTCTCGGAACCGGACGATCGGGATCTGGAGTCCACGATGTACATCCAGTC TCAGCAGCTGGACCTCGTGAACGAGATCACAGAGCGACTGGAGTCTAAGGGCTA CAACGTTATCCCTATCTACTGTCTGCTGCAAGGGAACGGGAGCAGCTGAACATCAT GGTGAAGTACTGGACCTCTGCTCTCAAAATATACTGGATTCTCTCGAGAATCCT CAAGACTTCGACATATACGTGGACGGCATCATATCTATGGTGGCTACGGCGTG GGGGGGAGAACTTTACCAACGGGACTAAGTTCTTCGAAAGACGCAAAACGTTCTCT ATCTCAGGGCCGTGCATAGTGAATACATAACGAACGAGCAGTGGAACTCTCT CCTGTGGCCTGTCCACAACCAAGTCTGACAAGTGTGGCAGCTGACCTCTTACATTT GAGTCTCAAGGCATCTTCGACGCCACCTAGCTGGCGGGCTGACCTCTTACATTT CTAACGAAACGGCGCTATCATCTTGACCTTTGTCCCTGTGCACGAGAACAATTGA GCTGTGACCGACCGCTGACGCGATGGTGGACCTGAAGTACACACCTAACGA GGACAAGACATCTCTATCTGTACTACAACCTACCTCTGTTTATAACATGCTGTACACCTG AGGGCCTCATACCTTGACGCCATCACCTCTGTTTATAACATGCTGTACACCTG AAGGATGAGGGATACCTACCTGACCGACCTGCCTAACAACTGTCTGAGCTGGAG GACATGATGATCGCCTGGGCATCAATGTGGCCAACTGGGCCCTGGGGAGGTT GAAAAATTGGCCAACTGGTCTGGCGTGGCGCTCCTGGCTGTGGACGAGTACCTG GAGTGGTTCGACTCACTGGACGATATCGTGAAGGTGCAGATCACTGAGGGCCCT GTTGCCATACATCGGGCAGATGGTCCGAGGGCGGTGCTCATCAACTACACCGAC GAGTCGAGACCATGGTCAACGATTGGTACAACGAGATCAAGGCTCTGTGCCT GAGAACGAGACCGTGGCGCCACCAACATCTCGGACAACCTGTTAACTCCCTT AAGCTGACGCCAACGCATCTCTGACGGCGACGAGAACGCCAGCCTCTACTAC GACGAGTCTCTGGGTACTACGACGAGTCAAGTCTCTGAACGTGTCTGGACTG AACGGGTGGGGCAGGCACTGGGAACATCATGCTGTGTCAACAGAACCGCAC GGACTACTTCGTGATCCCTGGCCTGACATTCGGGAACGTGTTCATCGGACCTGAA CCTCAGAGGGGCTGGAGGGCCGACATCGAGAACTTGTACCACCTGCACCGGGTG	SEQ ID NO. SEQ ID NO.: 42

Cluster	Description	Sequence	SEQ ID NO.
		GCGCCTACCCACCACTGCTGGCGGCTACTATTACATGCAGACCCGCCAGTCTA ACGCCAATGGTGTCTGGGGCGGACGCCACCCACGAGTGGCTGGCAAGG AGGCTCTCTGTCTTACAAAGACTACGGCTCTATTGTGTGGCAAGGTGCTCA GGTCTACTTTTACATCACCGACGGCTCGCGGAGGCTATCCAGGCCAAAGCGCG CGGCTTCGCCGTGCTCATCTCACACCTGGACTCTCCTAAGTCTTACACGCACCTG TACGGCAACCTGACGGTGTGGCACTCTCTCGAGGAGTACGACAACAATCAT ATCATATTGAGTCTGATCTTGACAAGAACAACCAAGGCCATCACATAACCAAGTT ATCAAGGACAACCCAGACAATCACGTACCAAGTCAATTAACAGGAGTTGGAGGA CAACCTGACGGCGCCATCAAGGACCTGGTCAACGCCAACAACCTACTACCTGAC TATCGGCTTACCGCGGAGGAGCTGAACAACACCGACATGTTCTCACTTCTTCA ACTCTGAACGCTTTCCTGAAGATAACCCAGAACACCTCTACCTCTCGGCTGC ACGCCATCGGGCAGAAAGTGGACCGATGAGGACCTGGCCAAACACCGTGGCGATA ATCGTCAGCCACGACTTCGAGTACGGGGGCAAGAGACTCAAACTTGACCCCTCTGAAGCGGG CTGTCTTTGTACTATTACGGGGAGAGTACTCAAACTTGACCCCTCTGAAGCGGG ACTACATACTCAACCGCTCTGTCTGACGTGTGCAAGGCCCTTATCTACTGGGACAC GGAGACTGTGTCGACACGATTGGCATCGGTAGCCCTGAGTTCACTGAGTCTCT GAAATCGCCAAGAAATATATTGACCTGTACAACCAAGTGCATCAGCCTCGAGCT GGAAGAGATGGTCTCCGCACTCAACGGGGGTTATGTGCTGTGAACATCGGGGG CGAGAGTGTACTGTCCCTCAGGTGCTGCTACCGGGGCCAACATGTACCCAGGA CCAGTCTTCTGAGTTGCCCTACGCAGAAAGGCTTGGGACTACGCCAAAACCCCTCAG CCTGCTGACACTGGCGGACCTGAATGATACCAACCGAGAAATCATCATGGGCAT CTGGTGGTGGAGACCGCGCGGACGACGAGCTCTCGTGAGTACCGTCTTGT CCTGCTCGGCATGGAGCCTGTCTGGCAAACTCCTCTTCTGCGCGCTTCGACGAA GAGGGTATCCCTACCGGGAAGAAGTGGAGGACCTCCCTAACGTGATAGCCCTT GAGAACCTGACCGCCCTGACGGTTGGGCAAGAAGAGAAATCGACGTGACGGT GATCACCTCTGTCTGTTCAGGGACTTGTACTCTCTCAGGCACGGCTGATGGAT AAGCCCTACAGAAATGGCCCTCGCCTGCAGTTATTACACCATCGTCAACAACAAG ACCATCATGGACTCTGAGTACGGCCCTCAGGTGTACGACCGCTCTTCGACGTATCA TGGCTCTATCAGCTTTAAGGGGGGCGGAGGGGGCTCAGGGGGTGGGGGCAGC GGGGTGGGGGATCTATCGAGAACGCCGACAAAGGCCATCAAGGACTTCCAGGA CAACAAGGCCCTCACGACAAGTCTGCCGCGTACGAGGCCAACAGTACGTGCC	

Cluster	Description	Sequence	SEQ ID NO.
		TAAGGACCTTCGGGACAAAAACAACCGATTTCGTGGAGAAGGTCAGCATTGAGA AGGCCATTCGTGCGCCACGACGAGCGGGTGAAAGTCTGCCAACGACGCGATCTCTA AGCTGAACGAGAGAAGGACAGCATCGAGAACGGCGACTCGCTCAGAGAGAAAGTG AACAAAGGCCCTATGGATGTGAAGGAGCACCTCCAGAACGAGCTCGACtaatgataga ccagcccaagaacaccgaatggagctctaaagctacataataccaactfiacattcaaaaatggtcccccaaaatgtagccattc gtatctgctcctaataaaaagaaagtcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C4.2	WP_011954 718.1 that aligns to C4.1 (use EMBOSS Needle), up to 1,482 amino acids in total, nucleotide	ctftgtcttttgcagaagctcagaataaagctcaactftggccaccAIGTTCTGTGTCTCTGGTCTGCTGCGCT CTGGTGTCTCTGCGCTAGAGGACAGAACTACGACAICTACTACGAGTACATC ACCAACGGCGACGCAAGCTTGTCAATGCCGAGTTCAACAAGGCCGTGCTTTAC AAGAACTATAACAAACAAGGAGAACAGATCAACGAGATCTCTGTGGGCTCTGAA CATTACCGGTACGAGTGCAGTAACTACTCAGACCCCTCGTTCTCTAAGACCTACGA GTACGGCATCTCCGCGAGCAGTACATGACCCCTCAGGAGTACAAGAAGAAGTA CTTCGACTCTTCTCGGCTTACACTGTCTGGGCTTCTCGAATCTAATATGTACGTGT CTAACGGGCAGCTGCAGCCTTACTACGCCCTCATCAAGACCTCGAGGGCCAAAG GCTGCAACGTTATCCCTGTGGTGGCGGAGGAGGCTCTGAGAACCAAGCTCAAAG TCATGTGAACTACTTACGAACGCGCCTTCTTACGAGGCTTACCTCAACAATCC TCTGAAGTACACCAATAATGTGAACGCCATCATCTCTATGCCCTGCCCTACGGCATA GGTGGCAACCTTTTCGACAACACCAAGAACTCTTCGAGACCCCGGGGTGCTCT GTGTCCGGGCCGTCCGACTATGTGTCTAACGAGGAGTGGGAGCTCTCT GCCACCGGGCTGCCAACCAGTCTGACAAGTGGTGGCAGCTCGCCATCGGC GAGGCCAGGGCATCATCGAAGCGACCTTCGTGGGAGGTGTGAACCCACGAGATC TCTTCC AAGACGGGCGCAATTGAGCGGTTCAAGGCCCATGAGAAAGAACATA GACCTGTTCACGAACGGATCGTCTCTGGATCAATCTGCAGTACACCGTGAAC AGCGACAAGAAGATCTCTGGTGTACTTCAACTACCTCTCTGGGAAGCAGAAC ATTGGCTCTAGTACCTCGACTCTATCACTCTCGTCTACAACTGCTTTACGAGC TTAAGTCTCAGGGTTACAACGTGGGGAAGCTGCCATAACCGTGAAGGAGTTGG AGGACATGATGATTAATCCGGGATAAAGCTTGCCACATGGGCCCTGGGGAAC TCGAGAAGCTGAGTAACGAGCTTAACATCGTTCTCTCTGCCCTGTGCCGAGTACG AGAAGTGGTCACTCTTGGAGCTTATCTCAAGGTCAGGTGATCGAGGGAC CTGTGCCCTACATCGGCGAGCTGGCCCGGAACGCCATCGCTATCAACTACACCT CTCTATGAAGGACATCATCTGTACTGTGTACACGGGATGAAGTCTCTTCTGCC TGAGAATAACCGAGTCCGGGTGATGCTCTCTGGACAAGATCGTGGCTGCTCT CAACAAGTACCTGCAGTCTGGGAACGACTCCGATTACCAAGAGTACCTGTCTCT GAAGTCTCAGTGGAAAGGCTTCAACATTCCTGGGTGACCGGGTGGGCAAGGC CCCTGGCAACATAATGACTGTCAACCAAGAACGGCGTGGCTACTTTGTGATCCCT GGCTTGAAGTTTGGGAACATCTTCAATCGCCCTGAGCCTCAGAGAGGCTGGGAG GCTAAGTCTGATTTGCTGTACCCTCTCTTCGCGGTGGCTCTACTACCACTATCC	SEQ ID NO.: 44

Cluster	Description	Sequence	SEQ ID NO.
		TTGCGGCATATTACTACATGCAAAAGGAGTACAGTAGTGCCATGGTCTTCATCG GCCGCCACGCCACGCACGAGTGGCTGCCCTGGGAAGGAGGTGCTCCCTGAGTACCA CCGACTAIGGCTCTATCGTGGTGGGATGTCCTCAGATCTACTTCTACATCTC TGACGGGCTGGGGAAAGGTCTTGAGGGCCAAAGCGACGCGGGTTCGCCGTGATGAT CACGCACCTGACTTCTCTCTGGCCTATACATCAGTACGGCAACCTGACCGCC ATCGCCAAACCTCATTAACAAGTACGAGAACACGACAGACAAGACGCAAGAGGA TACCATCGGCTTAACATCAAGCTGCTGATCGAAAAAACAACCTACATCCAGTC TATGGGGCTGACTCAGGAGGAGTTTGAGAAAGTGAACCTTAACGAGGTGTGAA GGCCGCCGACAAAGTTCCTCTCGAAGTCCAGAACACGCTGTACCCCTCTGGGCTTG CACCGGATAGCCAGAACTGGACCGCTCACCGACATCAGCCGCAAGGTTGTGGCC GCGCTCTCAGGAGTTCACTACGACGGGATCACCACTATTTTCGACGAA GTTGGGAAATACCTGTCTCTAAGAAGTACTCAGAGCTGAAGCCCTGGAGCGA GACAAAGTCTTAACAACCTCTGAGCAGATAGTGGCGGCCCTCATTTCTCAAAC AGCACCGGTGGCCAACTGCTCGGCTCTGACACACCTTCTCTGATCGCCGCC ATGAACCTACGCCGCTACTACATCTCTCTGATCTACGCACTATCAACAACGAGC TGACCTCTTTCATTAATGGGCTGAACGGCAAGTACATCCCTGTGTCAGTGACGG AGACGTTATCAATATCAACTCACTGCTACGGCTACGGCGGGAACCTTCTCCACGACCA GAGCCAGGAGTCCCTACCGAGGAGGCTATAACTACCGGAAGACCTCACACT GCTGACCTCTCTCTTGAACGAGAAACCCAGAGATCGCGATGGGAATCTG GTGCTCGAGACAGCACGGGACACAGCGCCCTCATCTCTGTGGTCTTACCT GTTGGGAATGCAGCCTGTGTACAGTCACTCTCTCTGCGCGGGAAGACGGA GGACGGGCACTCCGTGGCAAGAAAGACCAAGATAATGCCTAAGTTCGTGGGCT GAAGGACCTGGTCCGGCTGAGGGCTGGGCCAAGAACGCAATCGACATCGTGGT GATCACCTCTGGCAACTTCCCGGACCTGTATAGTACCCAGGTCACTCTCTCGAC AACGCTTCCGGTGGCTGGCCCGGTCACTACCTGACCATCATCAACAACAAG ACCTGTATGGAGAGCAAGTACGGGAAGACATGAAGGAGGCGCTGGACAAGGT TATGGAGGGCATCGGTACTACGGCGGGGTGGAGGCAAGTGGTGGCGGGGGCTC TGGCGGGGGGGCTCTATCGAGAACGCTGACAAGGCCATCAAGGACTTCCAGGA CAACAAGGCCCTCAGACAATCTGCCGCTACGAGGCCAACTCTAAGCTCCC TAAGGACTTGAGAGACAAGAACACCCGCTCTGTGGAGAAGGTGTCAATCGAGA AGGCCATCGTGAGGCACGACGAGAGGGTCAAGAGTGCAACCGATGCAATATCT	

Cluster	Description	Sequence	SEQ ID NO.
		AAGCTGAATGAGAAAGGACTCTATCGAGAACAGGCGACTCGCCCAAGCGCGAGGT CAACAAAGGCACCTATGGACGTGAAGGAGCACCTGCAGAAGCAATTGGACtaagata gaccagcctcaagaaacaccggaatggagctctaaagctacataataaccacttaacaaaatgtagccat tcgtatctgctcctaataaaaagaaagttttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C5.1	mr_1383 staphylococcal nuclease domain-containing protein = WP_012956.182.1 thermonuclease family protein [Methanobrevibacter ruminantium], amino acid	MFVFLVLLPLVSSAAVTGTGFSDHPFSKYSSQSNSDILNKYNNITDCHSEIKGICTYV ADGDTIIDVEGVGRVRFVGVNTPERGVTAYICSKRFVQKFCNLKEVSLDVEDDSKRND RYGRITLAVVIVDGNLNMELKEGLAEIMYIPSEFYPYDWSSDSTTSSSYTSGSSSS NSGSSSSSFTSGSTVSAPYVGSANSHKPHYSTCKWGKKISDKNRVTFNSRSDAIS QGYAPCKACQPGGGSGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLP KDLRDKNNRFVTKV/SIEKAIIVRHDERV/KSANDAISKLNKDKSIENRRLAQREYNKAPMDIV KEHLQKQLD	SEQ ID NO.: 45
C5.1	mr_1383 staphylococcal nuclease domain-containing protein = WP_012956.182.1 thermonuclease family protein [Methanobrevibacter ruminantium]	ctgtgttttgcagaagctcagaataaagctcaacttggccaccATGTTCGTCTTCCTTGTGCTGCTGCCT TTGGTCAGTCCCGCTTACACCGGGACCGGCTTCTCCACGACATCCCTTTCT CCAAAGTACAGTAGCCAGICTAACTCTGATACTCTGAACAAGTACAACAACACAG ACTGCCACAGCGAAATCAAAGGGATCTGCACCTACGTGCGCGACGGAGACACCA TAGATGTTGAGGGGGTGGACGCGTCCGGTTGTGGGAGTGAACACCCCTGAGA GAGGTGTCACCGGTACATCTGCAGTAAGCGGTTCTGTCAGAAAGTTCTGCCTTA ACAAAGGAGGTGTCACTCGACGTTGACGACTCCAAGCGAAACGACCGCTACGGCA GGACGCTGGCGTTGTGATCTGTGACGGTAAGAACTTAAACGAGATGTTGCTCA AGGAGGTCTGGCAGAGATCATGTACATCCCTCTTCGGAGTTCTACCTTATGA CTGGAGTCCGACTACACAGTCTAGTAGCTACACTTCTGGCTCTTCTTCTTCA AACTCTGGCGGCTCATATAGTTCACTTTCATTCACGAGCGGCAATACCGTGTCCG CCCCTTACGTGGGTCTGCAAACTCACACAAGTTCCACTACAGCACTTGCAAGT GGGGAAGAAGATCTCTGACAGAAGACCGGGTGACATTCAACTCAAGATCAGAC GCAATCAGTCAGGATACCGGCTTGTAGGCTTGCCAACTGGGGGAGGCGGC TCTGGCGGGGGGTAGCGGGGGGGGGGAGTAGATAAGAAAACGCCGACAAAGC	SEQ ID NO.: 46

Cluster	Description	Sequence	SEQ ID NO.
	m], nucleotide	TATCAAGGATTTCCAGGACAACAAGGCCCTCATGACAAAGTCTGCCGCCTACGAGGCCAATAGCAAGCTGCCTAAGGACCTCCGAGACAAAATAAACAGGTTTGTCGAAGGTCTCTATCGAAAGGCCATTGTGCGGACGACGAGCGGTGAAGTCCGC GAACGACGCTATATCTAAATTGAACGAGAAAGGACTCCAATTGAGAACCGGCGCCTGGCCAGCGAGAGGTTAACAAAGGCCCTATGACGTGAAAGAGCACCTCCAGAAGCAGCTGGAATaatagatagaaccagcctcaagaacacccgaatggagctctaaagctacataataaccaacttiacatttaca aaatgtgtcccccacaaatgiagccattgtatgtctcctaataaaaagaagtgttcacattct	
C5.2	WP_063720.174.1 thermonucle ase family protein [Methanobr evibacter oralis], amino acid	MFVFLVLLPLVSSAAAYTGTFSHDPLSKYSDSSYKNILDKYNDTECEAEVSGICTR VVDGDTIYVDGVGVKRVFVGVNTPENGVEGDDVSKYFVQKLCMNQEVGLDIDDSK QQDKYGRTLAVVIIDDKNLNEMLLKEGLAEIMYPPSEFDPSWSNSGSTDNEHAHS KSTDTSSDSGKYVASMNSDKFHKPSCRWAEKIYEQNKISFNSRESAINNGYQPCKV CNPGGGGGGGGGGGSIENADKAIKDFQDNK4PHDKS4AYEANSKLPKDLRDKNNR FVEKVSIIEKAIIVRHDERVKSANDAISKLNKESINRRRLAQRVFNKAPMDVKEHLQKQLD	SEQ ID NO.: 47
C5.2	WP_063720.174.1 thermonucle ase family protein [Methanobr evibacter oralis], nucleotide	ctgttttttttcagaagctcagaataaaacgtcaactttggccaccATGTTTCGTGTTCTTGTCTGCTCCCT TTGGTGAGTTCTGCCGCTACACGGGCACCGGCTTAGCCATGACATCCCTCTGA GTAAAGTACTCCGACTCTCTCTACAAGAACAATCTTGATAAGTACAAACGACACAG AGTGTGAGGCAGAGGTGTCGGGATCTGCACAGAGTGGTGGACGGTGACACTA TCTATGTGGACGGGTGCGTAAGGTGCGCTTCGTGCGGTGAACACACCTGAGA ACGGCGTCGAGGGTGGCGACGTTAGTAAGTATTTGTCCAGAAGCTGTGCATGA ACAGGAGGTGGGCTTGATATCGACGACTCTAAGCAGCAAGCAAGCAAGTACGGG AGGACCTGGCCGTGGTATCATCGACGACAAAGAACCTCAACGAGATGCTGCTC AAGGAGGGGCTCGCCGAGATCATGTACATCCCTCTCTGAGTTCGACCCCTTACT CCTGGAGCAACGGGCTACGGACATAAACGAGCACGCTCACTCTAAGTCAACCG ATACCTCCAGCGACTCATCTGTGTAATATACGTCCGCGAGTATGAATTCAGACAAGT	SEQ ID NO.: 48

Cluster	Description	Sequence	SEQ ID NO.
		TCCACAAACCTTCTTGCAAGTGGCGGAGAGATCTACGAACAGACAAAGATAA GTTTCAACTCTCGGGAGTCAGCCATCAACAATGGATACCAAGCTTGCAAGGTTT GCAACCTTGGCGGAGGGGATCCGGCGGGGATCAGCGGGGGGGGAGC ATCGAGAACCGGACAAAGCAATTAAAGGACTTCCAGGACAAATAAGGCCCTCA CGACAAAAGTGCCGCTTACGAAGCAACAGCAAGCTGCTAAGGACCTGCGGG ACAAAGAACACACCGATTCTGTGGAGAAAGGTGAGCATTGAGAAAGCGATCTTCGG CACGATGAGCGCGTGAAGTCTGCCAACGACGCTATATCTAAATTGAACGAAAG GACTCAATCGAGAAATAGACGATTGGCGCAGCGCGAGGTCAACAAGGCCCTATG GATGTCAAGGAGCACCTGCAGAAACAGCTGGACtaagtagaccagcctcaagaacaccgaatg gagtcctaaagctacataataaccacttaactttacaaaatgtgtcccccaaaatgagccattcgtatcgtcctcctaataaaaagaag ttcttcacattct	
C6.1	mrn_1923, MtrE = WP_012956 721.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter ruminantiu m], amino acid	MFVFLVLLPLVSSAAEDLESIGSQSNPNPNSQVQLAPQM ¹ GH ² LHRMINKAASGEPVA YGCWCGISGAIAALAMGMGIPIV ³ AIAMGSTV ⁴ AALVHAIYTVT ⁵ SHMGRIVGQSQFEQ PLFMDVLTQSLGPIAAHGFIASFGIVGIA ⁶ YLMTLPLDGLGHPFPL ⁷ LLAVLWGIIIGAI GSSTGDVHYGAESYQKFDYGGGTPVAIQGDIVTKAPLGA ⁸ KN ⁹ SIDVGNFCAKYGGP LTGFCFGLIVFSFWITVVF ¹⁰ GALGGQIVGIVIVILLIAAN ¹¹ YLLEKSTRAKFGPYEEGGG GSGGGGGGGGSIENADKA ¹² IKD ¹³ QDNKA ¹⁴ PHDKSAAYEANSKI ¹⁵ PKD ¹⁶ L ¹⁷ RDKN ¹⁸ NR ¹⁹ VEK ²⁰ TSI EKAI ²¹ /RHDERI ²² /KSANDAISK ²³ LN ²⁴ EK ²⁵ NIENRR ²⁶ LAQRE ²⁷ YNKAPMDVKEHLQKQLD	SEQ ID NO.: 49

Cluster	Description	Sequence	SEQ ID NO.
C6.1	nu_1923, MtrE = WP_012956 721.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter ruminantiu m], nucleotide	ctgtctttttcagaagctcagaataaagcctcaactttggccaccAIGTTCGTTTTCTTCGTGCTCTTGCCCT CTGGTCTCTCAGACGCCGAGGACCTGGAAAGCGACATCGGCTCCAGAGT AACCCTAACTACAGGTGACGTGGCCCCCAGATGGGCCACCTGCACCGGATG ATCAACAAGCCGCCCTCTGGGAGCCCTGTCGCGTACGGCTGCTGTTGCGGGATC TCTGAGCCATCGGGCTCTGGCCATGGGCATGGCATATCCCTATCTGGCC ATCGCCATGGGCTCTACCGTGGCCGCCCTGGTGCAGCCATATACACGGTACCT CTCACATGGCAGGATCGTGGCCAGAGCCAGTTCGAGCAGCCCTCTGTTTCATGG ACGTGCTTACGCAGAGTCTCGGCCCTATCGCCGCCCATGGGTTCATTGCGTCTTT CGGCATCTGGTATCGCTACCTTATGACCCCTGCCTCTGGACGGCTCGGGCAC CCTTTCCCTCTCTCTGCTGGCCGIGCTCTGGGGAATTACCATAGGGCTATAG GGTCTCTACCGGCAGGTGCACTACGGGGCTGAGTCTGAGTACCAGAAAGTTCTG ACTACGGCGAGGTACGCTGTGGCCATCCAGGGTGACATCGTTACCAAAGCAC CTCTTGGGCCCAAGAAACAGTATCGACGTGGCAACTTCTGGCCCAAGTACGGCG GGCTCTCACTGGGTTTTGCTTCGGGCTCATCGTCTTCGTCTTCTCGGATCACA GTGGTCTTCGGTGCATCGGGGGCCAGATCGTGGGATTGTCATCGTTATCTCTGC TCATCGGGCCAACTACTGTTGGAAAAGTCAACCGAGCGAAGTTCGGCCCTT ATGAGGAGGAGCGGGGATCTGGCGGGGGGGGTGAGCGGTGGGGGGTCTG ATCGAGAACGCCGACAAAGCGATCAAGGACTTCCAAAGACAACAAAGGCCCTCA CGCAAGTCTGCCGTACGAGGCTAAACAGCAAGCTCCCTAAGGACCTGCGCGA TAAGAACAACAGATTGTGGAGAAGGTGTCTATCGAGAAGGCCATCGTGGCGCA CGACGAGCGGTGAAGTCTCGAACGACGCCATCTCCAAGCTGAACGAGAAGG ACTCTATCGAGAACTCGGGGTTGGCCACGCGGAGGTGAACAAGGCACCTATGG ACGTCAAGGAGCACCTGCAGAAACAGCTCGAATaatgatagaccagcctcaagacaccgaatgga gtcttaagctacataataaccaacttacaaaatgtgtcccccaaatgtagccattctgtctcttaataaaaaaagt cttcacattct	SEQ ID NO.: 50
C6.2	WP_004032 919.1 MULTISPE CIES: tetrahydrom ethanopterin	MFVFLVLLPLVSSAAEDLESIGSQSNPNQVQLAPQMGLHRMINKAASGEPVA YGVWCGVAGSIAYVLMILGFVPIISIAMGSCVAAFVHAIVTVTSHMGRIVGQSQFEQ PLFMDVLTSQSGPIVGHGFTSFVIGISYLMIPNLNGTTLHFVPLPLAVLWGLALGAI GSSTGDVHYGAESEYQKFEFGGTPVAIQGGDIVTNAPMGAKNSMDVVNFCAKFGG PLTGFCFGLVVFSSFWNTVVFGIYGGGLVVGFIIVILLIIMNDRLEVFARNRYGPYEED	SEQ ID NO.: 51

135

Cluster	Description	Sequence	SEQ ID NO.
		cgaaatggagctctctaaagctacataataacaaactacacattacaataatgtgtgcoccccaaaaatgtagccattcgtatctgctccttaataaaa agaagtttctcacattct	
C6.3	WP_081738 309.1 tetrahydrom ethanopterin S- methyltransf erase subunit E [Methanobr evibacter oralis], amino acid	MFVFLVLLPLVSSAAAEEDLESIGSQSNPNSQVQLAPQM ^{GH} LHRMINKAASGEPVA YGVWCGVAGAJAYLIHFGIEPIV ^{GI} AGASVAAFVHSIYTVTSHMGRIV ^{GQ} SQFEQPL FMDVLTQSLGPIAGHGFTSFCIVGISYLMTIPLNGTALHVFPLPL ^{AML} WGIALGAIG SSIGDVHYGAESEYQKFEFGGTPVAIQGDIVTKAPIGAKNSMDVVNFC ^{AK} FGGGL TGFCFGLVVFESFWNTVVF ^{GI} YGGIIVGLIIVLLGV ^{MN} DRLEVFAREK YGPYEEGG GSGSGGGGGG ^{SI} ENADKA ^{IK} DFQDNKAPHDKSAAYEANSKLPKDL RD KNNR ^{FE} EKV SIEKAIIVRHDERVK ^{SA} ND ^{AI} SKLNEKDSIENRR ^{LA} QRFYNKAPMDV ^{KE} HIQK ^{QL} D	SEQ ID NO.: 53

Cluster	Description	Sequence	SEQ ID NO.
C6.3	WP_081738.309.1 tetrahydromethanopterin S-methyltransferase subunit E [Methanobrevibacter oralis], nucleotide	ctgtctcttttcagaagctcagaataaagctcaactttggccaccATGTTCTGTTGTTCTCTGTCCTCTGCGCTCTGGTGCTTCCGACGCGCCGAGACTTGGAGAGCGACATCGGCAGTCAGTCAACCTAACTCTCAAGTGCAGCTGGCCCTCAGATGGCCACCTCCACCGCATGATCAATAAGCCGCTTCTGGCGAGCCTGTTGCCACGGCGTCTGGTGGGGTCCGCGGCCATCGCTTACATCCTGTATACACTTCGGCAATTTTCTCTATCGTGGGCA TCGCCATCGGCCGAAGGTGGCCGCTTCTGTTCACTCTATCTACACCGTTACGAG TCACATGGGTAGGATCGTCGGACAAATCACAGTTCGAGCAGCCTCTGTTCATGGA CGTGCTTACTCAGTCTCTGGCCCTATCGCCGGCACGGCTTCATCACCTCTTTC TGCATCGTGGGCATCAGTCACTCATGACCATCCCTCTGAATGGCACAGCCCTGC ACGTCTTCCCTCTGCTCTTGGCGATGCTCTGGGGATTCGCCCTTGGGGCCAT CGGGTCTCTACCGGAGACGTCCACTACGGGGCCGAGTCCGAGTACCAGAAAGTT CGAGTTCGGTGGTGGACACCTGTGGCAATCCAGGGCGACATCGTGACCAAGGC CCTATCGGAGCGAAGAACTCAATGGATGGTGAATCTCTGTGCTAAGTTCTGG CGGCCCTCTGACGGGGTCTGCTTCGGGCTCGTCGTTTTTTCAGTTTTTGGAAACA CCGTGTCTTCGGCATCTACGGGGGATCATAGTCGGTCTTATCATAGTGGTGCT GCTGGCGTTATGAATGACCGGCTTGAAGTTCGCTCGAGAGAAGTAAGGCC CTTACGAGGAGGGGTGGGGAAGTGGAGGGGAGGTTCGGCGGGGAGGTA GCATAGAGAACGCCGATAAGGCCATAAAGACTTCCAGGACAACAAGGCCCT CATGACAAAAGCGCTGCGTACGAGGCAAACTCAAAATTCCTAAGGATCTTCGC GACAAGAACACAGGTTCTGTCGAGAAGGTGTCAATCGAAAAGGCCATTGTGCG GCACGACGACGCGTCAAGAGTGCGAACGACGCCATTTCGAAGTTGAACGAGA AGGACTCTATTGAGAAACGACGCGTGGCACAGAGAGAGGTGAACAAGGCCCT ATGGACGTGAAGGAACATTTCAGAAAGCAGCTGGACtaatgatagaccagcctcaagaacccc gaatggagtctctaagctacalaatlaccacacttacactttacaaaalgtgtcccccaaaatgtgccaatcgtatctgtccttaataaaaa gaaagtcttcacattct	SEQ ID NO.: 54
C7.1	WP_011953.874.1 before TM and short C-term loop, amino acid	MFVFLVLLPLVSSAAATTVELTSDNIMGTNDDADMLNSIKTYIEEISNGKINIVVDS QSPGEGTTRAIEADSNVSVFAAVDPGNFLVLSKYSTATTDKQHFVNTGDDYDLDT AESLRRAWDDNNYSKTIFAGINPGTFLNDGGISYIQPLKEYHDAGSDGINQNDDV NKYIAQEVNNINNNYNNKHYDNNLVITHKLAPSNMAHGSQSLLESDNEMINGTYN SYSAPQLLYLTSSYLNGNLEPNPDYKAPDSPLKYSILTKDSYSIYDIYKIMGGIVKN VMDENGOABNVINVEGATISVVDIQVNEAKITANHTDGSMDDEDEVEVHEKVNDSI	SEQ ID NO.: 55

Cluster	Description	Sequence	SEQ ID NO.
C7.1	WP_011953874.1 before TM and short C-term loop, nucleotide	GGGSGGGSGGGSGSIENADK4IKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV/SIEKAIIVRHDERVKSAND4ISKLENEKDSIENRRRLAQRV/NKAPMDV/KEHLQKQLD ctgtcttttgcagaagctcagaataaacctcaacttggccaccATGTTTGTGTTCTGTTGTTGCTCCCTCTGGTGTCTAGCGCGCGCCGACACGACCGTGTCTTACCTCCGACAAACATCATGGGCACGAACGACGACGCCGACATGCTGAATAGCAATCAAGACCTACATCGAGGAAATTTCAAACGGGAAGATCAACAGTCAATTGTGGACTCCAGTCACTGACCTGGACCTGGGAGGGAACCCGCGCATCGAAGCCGACAGCAACGTGAGTGTCTGTTTTTCGCCGCCGTGGACCCCTGGCAACTTCCCTGGTGTCTGCTAAAGTACTCTACCGCCACCAACCGACAAGCAGATCATCTTCGTGAACACGGCGCACTATGACCTCGACACTGCCGAGTCTCTGCGCGGGCCCTGGGACGACAACCTACAGCAAAACCACTCTTCGCAAGGGATAAACAACTCTGGCACATTCCTGAACGACGGGGGCATATCTTACATCCAGCCTCTGAAGGAGTACCACGACGCCGGCTCTGACGGCATTATCAACAGAACCAACGACGACGTGAACAAGTACATCGCCAGGAGATCGTCAACAATAATCAACAACCAACACCAAGCACTACGACAACAACTTGTTCATCACCCACAAGCTGGCTCTAGCAACATGGCCACGGCTCTCAGTCCCTCTCTGGAGTCTAACGACAACGAGATGAATGGGACCTACAACTCTTACTCTGCGCTCAGTTGTTGTACTTACTTCTGAGTTACCTGAAACGGAAACGGCTGGAGAACCTTGGAGACTATAAGGCGCTGACAGTCTCTGAAAGTACAGTATCTCAAGAAAGGACTCTTACTCTATCTACGATTACATCAAGATGGCGCGCATCGTCAAGAACTACATGGATGAGAACGGCCAAAGCCCTAACTACATCAACTACGAGGGGCTACATCTCTTATTACGACCTCCAGTACAACCTTCGCGGAAGATCAACGCAAAACCAACAGACGGGTCTCACATGGACTTCGACCGCGGAGTACCAATTTGACAAAGGTCAACGACTCTATCGCGGTGTTGATCAGGCGGGGGGCTTCGGGGGGCGCGGCTCTATCGAGAACGGGACAAGGCCATCAAAAGACTTCAGGACAACAAAGCCCTCACGATAAGTCTGCCCTACGAGGCTAACTTAAGCTCCCTAAGGACCTCCGCGACAAGAACAAACCGATTCTGTCGAGAAAGTGTCTATCGAGAAAGGCCATAGTGGCGGACGATGAGCGGGTGAAGTCTGCAAAATGACGCTATCTCAAAGCTCAACGAGAAAGGACTCAATCGAGAATAGGAGACTGGCGCAGCGGAGGTGAACAAAGGCCCTATGGACGTTAAGGAGCACTGCAAGAGCAAGCTGGACtaatgatagaccgctc	SEQ ID NO.: 56

Cluster	Description	Sequence	SEQ ID NO.
		aagaacacccgaatggagtcctaaagctacataataaccacactttacacaaatggtgtcccccaaaatgtagccattcgtatctgctcctaataaaaagaagttcttcacattct	
C7.2	WP_042694 202.1 before TM and short C-term loop, amino acid	MFVFLVLLPLVSSAAAQTVFITSDNIIDHDIDVRMLNSIKEHIEELSNGELQVIVDNQ APGPGEGYRSIQVTSQVCVNIASDAGNYLQLANYTVYNDKHIFVNSGSYDLDNSS NYLRAWDDNYSNQYFAGVHDPGTLLKNSGVTYIQPLKEYPDAGDGDNIDKYDEE MNKYIAQEVVNQVKNNYNYESKILSDSLVYHHIEPSIMASASKELVKSNDTEMNQTY GGYSAPQLLYQTSSYLNGNGLDNPKNFTAPESPMKFSLLTKGSYTINDYIKMGGIVK TYMDENGQAPDYINYEYAYISYYDLVYNFAKITQNHNTQHMGGFDSEYEFEKTNDS GGGGSGGGSGGGSGGSIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVE KYSIEKAI/RHDER/VKSANDAISKLNKDKSIENRRLAQREYNKAPMDI/KEHLQQLD	SEQ ID NO.: 57

Cluster	Description	Sequence	SEQ ID NO.
C7.2	WP_042694202.1 before TM and short C-term loop, nucleotide	ctgtctcttttgcagaagctcagaataaaccgtcaactttggccaccATGTTCTGTTGTTTCTCTGCTTCTGCCT CTCGTGICAAAGTGGCCGCCAGACAGACAGTGTTCATCATCCTCCGACAAACATAATC GACCAACGATACCGACGTTAGGATGTTGAACAGTATCAAGGAACACATCGAGGA GCTGTCAAACGGGGAGCTGCAGGTTATCTGTGGATAACCAAGGCCCTCGGGCCCTGG GGAGGGTTAACCGAAGTATCCAAAGTCACCTTCGGACATCTGCTCAATATCGCTGCT AGCGACGCTGGCAACTATCTGCAACTCGCCAACTATACTGTCTACAACGACAAAG CATATACTTCGTCAACTCAGGCAGCTACGACCTGGACAACTCTTCCAAATTACC TGAGACGAGCATGGGATGACAACCTACTTAACCAAGTACTTTGCAAGGAGTGCACG ACCTGGCACATTGCTGAAGAACTCAGGTGTCACCTACATCCAGCCTCTGAAAAG AGTACCTGACCGCGGGACGGAGACAATCATCGACAAGTACGACGAGGAGATG AACAAGTACATCGCTCAGGAAAGTTGTGAACCAAGGTGAAGAACTACAACATACGA GTCTAAGATCCTGTCTGACAGTCTGATCGTGAAGAGTAAACGACACGGAGATGAATGGA ATGGCCTCTGCACTTAAGGAGTTGTCGAAGAGTCTGTGACCAAGACTAGCTCTTACCTTA ACCTACGGGGCTACTCAGCCCCCTCAGCTCTGTACCAAGACTAGCTCTTACCTTA ATGCCAACGGCCTTGACAACCCCTAAGAACTTCAACCGCCCTGAGTCACTATGA AGTTCTCTCTGACGAAAGGAAAGTACACCATCAATGACTACATCAAGATGG GCGGATAGTGAAAACGTATATGGACGAGAACGGCCAGGCGCTGACTACATC AACTATGAGGGGCCCTACATCAGTTACTATGATCTGGTGTACAACCTCGCCAAAG ATCACCCAGAACCAACCAACCCAGCACATGGGCTTCGACTCTGAGTACGAG TTCGAGAAACAAACGACTCTGGGGGTGGTGGCTCTGGTGGGGCGGGAAGCGG GGCGGGCGGCTCTATCGAGAACCGGACAAAGGCCATTAAAGACTTCCAAAGATA ACAAGGCCCTCAGCAAGATCCGACGCTACGAGCGGAATTTCTAAGCTGCCTA AGGACTTGCGCGACAAGAACACCCGCTTCGTGGAGAAAGGTGTCCATAGAAAAG GCCATCGTGGGCAATGACGACGGGTGAAGTCTGCGAACGATGCAATTTCCAAAG CTTAACGAGAGGACTCTATTGAGAACCGGGCGCTCGCCAGAGGGAGGTTAAC AAGCGCCTATGGACGTCAAGGAACACCTCCAGAAACAGCTTGACtaagtagaccag cctcaagaacacccgaatggagctcttaagctacataataccaacttaacattgtcccccaaatgtagccattcgtatc tgtctctaataaaaagaagtttcttcacattct	SEQ ID NO.: 58

Cluster	Description	Sequence	SEQ ID NO.
C7.3	ABQ87089.1 adhesin-like protein [Methanobrevibacter smithii ATCC 35061], amino acid	MFVFLVLLPLVSSAANDLSTLGENTTVPNHIVGDAPEVDPVPDIPDPDFVDPDNP DIIDDQNDSDIVNLTFINIDEYFVDGILGVEHSNTKFVLTQNFNDNLGLLKIEANNVTIL GNNFTLQNVAFLINGKDVTLANFTLVNDFEFDKADGAAILTLANNTHIRDCVINYN VPRDSEGYGISA VGRRIAPISGLEVINCIINFEGHNYKANTYNYALKVSNCPNALIAN NSIYTQLPLRDVNFAGVADLNSNYVASVGEIYSNNLTFIGNIVASIVNKRPGSPPT LDGHIADSNDCLVKNNTLYMEDFLTTPGLNNLYGIDVWRVNSLTLDSSNNIALTTG GMLSAGTAYPIQITGPSKKINITNNDLYSISNGPNIGIYSQNY YGDTQLYIAHNKINVT GLAGNDSWALVAGIEVQDSNDTIINTTIEVHSV AEVKDNDNMYGISYSQSTKGNHT FVVKNNTVTSDAKY AISLSAENSVIDNLLISTRKDAKASYDAFNTKGKFYNTSYYN NRVVNAFDYYAEIYNHIVDGGSEFNYYTPTNVNNTNKVDGSKIKPWFPDPNRPPL LPKPGDSSVIVTPDDGDDKNPNVPPDRPDGDAGYVDVPDL SGDDGGSKNPSNGTSS TDKNSNKLGVSLLDALINFLNSNTIDTGDSTRSNSYGGTVRTNSSTSSDSPSLDGNPSP ASSTKSSSGSNAKSAGSSAGDDSKSVKAYEIDKNIMKSNPNTGGGGSGGGSGGGG SIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFFLEKTSIEKAIIVRHDERIK SANDAI SKLNEKDSIENRRLLAOREV NK4PMDI/KEHLQKQLD	SEQ ID NO.: 59

Cluster	Description	Sequence	SEQ ID NO.
C7.3	ABQ87089.1 adhesin-like protein [Methanobrevibacter smithii ATCC 35061], nucleotide	ctgtctcttttgcagaagctcagaataaacgctcaactttggccaccATGTTCTGTTCTCTGGTCTGTTCGCTCTCTGTTTCCAGTGGCCAAACGACCTCAGCACGCTGGGTGAAACACGACCGTCCCTAAACATCATCATCGTCTGGAGACGCCCTGAGGTGCTGATGTGCTGACATCCCTGACATCCCTGATTTCCCTGTGGATCTCTGATAATCCTGACATCGACGACCAAGAAACGACAGTGACACAGTTAACCTGACGATCTTTAATATATAGACGAGTACTTTGTTGACGGCACTCTGGAGTGGAGCACAGTAATACAAAGTTCTGCTTACGAGAACTTCGACAACTCTGGCTGTGTGAAAATAGAGGCGAACAACGTCACTATCTACTGGGAAACAACTTCACCCCTGTGTGAAACGACTTCGACTTCAAGGACGCCGACGGAGCCGCAACCAACTTCACCCCTGTGTGAAACGACTTCGACTTCAAGGACGCCGACGGAGCCGCAATCCTGACGCTCGCAACAACACACACATACGCGATTGCGTGATCAATTACAACGTGCCTAGGGATAGCGAGGGGTACGGCATCTCCGCTGTGGCAGCGGAATCGGCCCTATCAGTGTCTGGAGGTCAATAAATGCAATCAATTAACCTTCGAGGGTCACAACTATAAGGCAAAACACTTACAACTACGCTTTGAAAAGTCAAACTGTCTTAACGCCCCTCATCGCTAACAACTCTATCTATACCCAGCTCCCTTTGAGAGACGTGAACCTTCGGTGCGGCGCGAATTTGAACCTCAACTACGCTGGCTCTGTCTGCGGATCGAGTATTCAAATAACCTCACCTTCATCGGTAACATTTGGCCCTCAATCGTGAACAAAGCGGCCTGGATCCCCTTCCCTACACTGGACGGGAATCATCATCGCAGACAGCAACGAC TGCTGTGTGAAGAACAACTCTCTCTACATGGAGGACTTTCTCACCTTTCTCTGGGTGAACAACTACCTGTATGGCATCGACGTCTGGCGGGTGAATTCCTTACACTGGACAGTAACAACATCGCGATTCTGACCAACCGGGGGAATGTTGTCCGCCGCAACCGCTTACCTTACTCTATCAGCAACGGGCTTCAAAAGATAAACAATTACCAACAACGACGGAGACACTCAACTGTATCTCGCTCACATAAATAACAACGTCAACCGGCTTGCCGGCAACGACTCTTGGGCTCTGTGGCCGGTATCGAGGTCCAGGACTCAAAACGACACCATTTATAACAACACTATTGAGGTCACTCCGTCGCAGAGGTCAAGGACAACGACAACATGTACGGTATCTCATCTCTCAGTCAACTAAAGTAAACCATACGTTCTGTCATAAAGAACACACAGTTACGTCTGACGCCAAGTACGCTATCAGCCTTATATCCGCCGAGAACTCTGTGATTTGTCGACAACCTGCTCATCAGTACAAGAAAAGACGCCAAGGCATCCTATGACGCCCTTCAACACCAAGGGGAAGTTCTACAATACCA GTTATTACAATAACCGAGTCGTGAATGCCTTCGACTACTACGCCGAGATCTACAATCACGTCGACGGGAGCGAGTTAACTATACTACCTTACCAACGTTAACA	SEQ ID NO.: 60

Cluster	Description	Sequence	SEQ ID NO.
		ACCTGACCAATAAAGTGGACGGCTCCAAGATAAAGCCTTGGTCCCTGATTTCCTAAACAGGAACCTCTTCTTCCTAAGCCTGGGATGGAGCAGCGTGATAGTCACCCTGACGATGGCGATGACAAGAACCTAACGTGCTGACCGGCTGACGGCGACGAGGGTACGTGACGTCTCTGACCTGAGTGGGACGACGGGGCTCAAAGAACCTTCAAAATGGCACCTCTCCACCGACAAGAAATTCCAACAAAGCTGGGCGTGAGTCTTTGGACGCTCTGATAAACTTTCTTAACCTCAAAACACCGATACAGGGGACAGCCGACGCAATAGTTACGGCGGAACCTGTTAGAACCAATAGTTCTACCTTCTAGCGACTCCCTTACCTTGAACGGAAATCCTAGCCCTGCGTCTTCTACGAAAGTCCTCTAGCGGACGCAACGCAAGCTGCGCGGTCTACAGCGGCGACGACTCTAAAGTCAGTGAAGGCTACGAGATCGACAAGAACATTAAGAAAGTAACCCCTAACACTGTGGAGGGGTTACGGGGGGGGGTAGTGTGGGGGGGTAGTATCGAAAAACGCTGACAAGGCAATCAAGGACTTCCAGGACAATAAGCACCTCACGACAAAATCTGCTGCTACGAGGCAAACTCCAAGTTGCCCTAAGGATTTGCCGACACAAAACAAACCGTTCTGTGAAAAGGTGAGTATCGAGAGAGGCGATAGTTCGACACGACGAGCGGGTTAAAGAGTCCGAATGATGCAATCTCAAAGCTCAACGAAAAGGACAGCATTGAAAACCGAAGGTTGGCACAGAGAGAGGTGAATAAGGCTCTATGGACGTGAAGGAGCAATTTGCAAAAGCAGTTGGAATaatgatagaccagctcaagacacccgaatggagctctaatcatataataccaacttacacttacaanaatgtgtcccccaaaatgtagccattcgtatctgctcctataaaaaagaaagtttcttcacattct	
C7.4	WP_052331828.1 hypothetical protein [Methanobrevibacter]	MFVFLVLLPLVSSAASNLIESLSTNNNGSEAIYIPNSDLNLLSGNGNDVETPDPLI VNDTFYVTSDDIEDYFPNRLQLESKYYNKTLLFTGNFENVGKLHIDVNNVTLKGVGSY LKNTVFDLRADNITLDSFNMDLDSFEDNDGAAIEFIANNIVLSNLRINYIVPRNVEA YAIYGIGQPYRSIKNFKMFNSHNFEGHNDYV/NK YNYAVKL VDCVDSVMENNSLVT SLPLRNVVFGAMGASLDSDFVLSVGVENCHNFTFIGNSHIANVNNRPFTYPTLDCFL ISKCDNSSLNNSIYMTDFLTFPGVENLYLGIDVYNLNLTIAYNNISIITGGKLAAG TAYPIQITGPISAVNITYNDIYTFNNGPNIGIYSQNYGYNTSLSIHNRINVTGLAGSHE	SEQ ID NO.: 61

Cluster	Description	Sequence	SEQ ID NO.
	oralis], amino acid,	WALVAGIETQDSNSTIQNTTIEVHSVAPVDIGDNIYGISYRQKTSNGHNTYNIQNNTVFSDGFYSVYLLSSVNSNVINNLLVSYNDKVKAAGLNGFNYNFSSHIGINFYNNKVVINAFDYFANKYNNIDGGEGFNYPNTNINSINIDSSVIAIPSNHYNYPNPLIPGNSNNQGNPNQGSNQNNQGSNSNGSSQGGSGNGVNDNNSGEGNSSGNKLSLRDLLANFFNSNSDKGVNRSNYNSNANHEITSNNTDQTPSTEGEDALMSDVKSVESTSESPSASDSASKKAYELDDLVKENKLFIPSGGGSGGGGGGSIENADK4IKDFQDNKAPHDK.SAAYEANSKLPKDLDRDKNNRFVTEKVSIEKAIVRHDERVKSANDAKISKLEKDSIENRRRLAQREVNKAPMDVKEHLQKQLD	
C7.4	WP_052331828.1 hypothetical protein [Methanobrevibacter oralis], nucleotide	ctgtctttttgcagaagcagcaataaagcctcaactttggccaccATGTTGCTGTTTCTGCTTCTGCTCTGCTGCTGCTGCTGCTAGCAACAACTTGATTGAGTCCCTGTCAACAAACAACAATGGTAGCGAGGCCATTATATCCCTAACAGCGATCTTAACCTGTTGTCTGGCAA TGGGAACGATGTCGAACACCTGACATCCCTGACCTCATTTGTCAACGACACCTTCTACGTGACGCTGACGATATCGAGGATTACTTCCCTAACAGCGAGCTCGAGTCTAAATACTATAACAAAACCTTGATCTTTACAGGTAACTTTGAGAAATGTCGGGAAGCTTATTGACGTTAATAACGTCACCTCTGAAGGGGTGGGTCTATCTGAAGA ACACAGCTTTGACCTGGAGCGGACAAATATTACACTGGACAGCTTCAACATGGATCTGGATTCTGAATTCGAGGACAAACGATGGAGCTGCCATCGAAATTTATCGCAAATAAATTGTGCTGAGCAACCTTAGAAATTAACACTACATTTGTTCTCGCAACGTCGAAGCTTACGCCATATACGGAATAGGTCAACCTTACAGATCCATTAAACACTTCAA GATGTTCAACTCAATCAATTAACCTTTGAGGGTCAATAATGACTATGTGAACAAGTACAACTATGCAGTGAAGTTGGTGGACTGTGTGGATTCAAGTTATGGAGAAATAACAGCCTGGTCAACCAAGCTTCCCTCAGAAACGTGGTGTGGTGGCCATGGGCGCTTCAC TGGATAGCGACTTCGTCCTTAGCGTGGGCGTCGAGAACTGCCACAACCTTCACTCCCATAGGGAAATTCATAGCAAAACGTGAATAACAGGCCCTGGGTTTACATAATCC TACCTTGGATTGTTTCTTGATCTCAAAGTGGGATAATAGCAGTATCCTCAACAATAGCAATTTATGACGGACTTCTGACCTTCCCTGGGGTGGGAAATTTATTTGTATG GCATCGACGTGTACAATCTCAACAACCTTACTATCGCTTACAATAACATTTCAAT TATTACAACAGGGGCAAACTGGCGCGCGGACCGCATACCCCTATACAGATCAC GGGGCTATCAGCGCTGTGAATATTACATAACAACGATATATATACCTTCAGTAAT GGACCTAACATCGGCAATTTATTTCCAGAAATTACTACGGGAACACCGCCTCAGC ATCACTCATAATAGAAATCAACGTCACCGGGCTGGCCGGAAAGTCAATGAGTGGCC	SEQ ID NO.: 62

Cluster	Description	Sequence	SEQ ID NO.
		CTGGTCGCCGGCATCGAGACACAGGATAGTAATAGCACCATCCAGAATAACACT ATCGAGGTTCACAGCGTGGCTCCTGTGGACATAGGTGATAACATCTACGGTATC TCATACCGCCAGAAAACCTCTGGTAATCACACCTACACATCCAAAACAACACC GTGTTTTCAGACGGCTTCTACTCCGTGTATCTGCTCAGTAGTGTGAACAGCAACG TGATTAAATAACCTGCTCGTCAGCTATAACGACAAGGTGAAAAGCAGGACTGAACG GGTTTAATTACAAACGAAATTCCTCTCCACATCGGAATTAACTTTTACAAACAACAA AGTGATCAACGCATTCGACTACTTTGGGAAACAAGTACAATAACATCGACGGGG CGAGGGCTTTAACTACACTAATCCTACTAACATCAATTCAATCAAGTAACAATATT GATGGTCAATCCGTTATCGCCATACCTTCTAATAATCACTATAATPACAATCCTC TTATCCCTGGCAACTCTAACAAATCAGGGCAATCCTAACAAACCAAGGAAGTAATC AACAGAATAATCAGGGAAACAGCAATGGAAATAGCTCTCAGGGAGGATCCGGA AACGGGTGAATGACAATAAATCTCGGGGAGGGGAATCTCTTGCCAATAAGCT GTCCTTGGCGGACCTCTGGCTAACTTCTTTAACAGTAATTCCGATAAGGGAATA GTGAACCGGAGCAACTATAATTCAAAATGCGAAACCAATGAATAAATTCCAACAAC ACTGACCAGACGCCTAGTACGGAAGGCGAGGATGCCCTGATGAGCGATGTGAA GTCCGTTGAGTCCACCTCCGAGTCTCTAGCGCCTCTGACAGCGCTCCAAGAAA GCATATGAGCTGGATGATCTGGTCAAGAGAAATAAGCTTTCATCCCTTCCGGA GGCGTGGTGTAGTGGGGCGGAGGCAGTGGGGCGGGGGCTCCATCGAGAAATGC AGACAAAGCGATAAAGGACTTTCAGGATAATAAAGCTCCTCAGCAGCAAAAGTG CGGCTTACGAAGCCAAATCCAAGCTGCCTAAGGATTTGCGAGATAAGAACATA GGTTCGTGGAAAAGGTGCAATCGAAAGGCCATCGTTCCGATGACGAACGGG TGAAGAGTGCCCAACGACGCTATTTCCAAACTGAACGAAAAGGACTCTATTGAAA ACAGGCGCTTCGCACAGCGGGAGGTCAATAAGGCCCTATGGACGTGAAGGAG CACCTGCGAGAACGAGCTTGAC ¹ aatgataagaccgctcaagaacacccgaatggagtcctaaagclacataa ccaacttacactttacaaaatgtgtcccccaaaatgtagccatcgtatcgtctctaataaaagaaagtcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C8.1	mnau0493 = ADC46344.1, adhesin-like protein [Methanobrevibacter ruminantium], amino acid	MFVFLVLLPLVSSAAIDVDTNLDLDDGSSNSDLSSSLDSSSSDDVSSGSSEVSSS DESLDGNLSDGNVSSSDESVGADNLSDGNVSSSDESVGADNLSDDESSDALSEEL PKTETVIKADPINYNVYASVKGLTINLTDAGLALSNKTLTVKVSALNKTSLNLTJNSK GQAIFKLSASVGSYDVFIISFTGDESYPASNASSKITIKKSSTKIKLSNIHGVLTIISNYVS VTLLDSAGKPIKSKSVTIQVNKAKYNVKTDSKGIKVKVANKIGTYSVNAKFSGDK NYYASSNSSKLTITKMKVYIKAPSVKYVYMTNSSAPYLTIINLTNVKGSPLAKKKVSVK IGKKTYILKTNSQGIKFKFTKKVSSYNCKINFKATSNFYGASVNSKMTIQKMPTSL KAPSVSINSTNYGKVLISLKDGGKALKNTTVTVNVTELKKVFTLKTNASGVATFSF NGEKTENLKIKYAGNKNYAASSVSSKINVKQIKVKLSDVIGASRVLIDYVNRTKDLP SNVQYNNYNFTVTQLTYLASKAVKNINKNYGDIVLISVPKSYKSSGEIYDVTYKK DFVKIANSVVGSSYNKKNKEYVSYSIYKVPFKVYSISFAKVLNFGNKKLPNYSLF TLADFAKVKDNGGYNFYLTITDNIAGKKSDLNMLKSLAKTLKSMGYNAVIVGIGPDI HNVAARYGCTGNNSVLLACFGGVGVGCIIEWAGDLGDLNGHSFVNSYQGAHVLG LWFTKPYGASVSLNKKVGIADWDADYGFPLNTPAKYMKSHNISYIETGTVANACKLL SEGKMGGPQLISGGGGGGGGGGGGSENADKAIKDFQDNKAPHDKSAAYEANSKLP KDLRDKNNRFVEKVSIEKAIIRHIDERVKSNADAIKSLNEKDSIENRRLAQRETNKAPMDV KEHLQKQLD	SEQ ID NO.: 63

Cluster	Description	Sequence	SEQ ID NO.
C8.1	mn0493 = ADC46344.1, adhesin-like protein [Methanobrevibacter ruminantium], nucleotide	ctgtctcttttgcagaagctcagaataaagctcaactttggccaccATGTTCTGCTCTCTCTGGTCTCTCTCCCTCTGGTGTCTCTGACGCTATCGACGCTGATACAAATGACAACCTTGGACGACGGGAGCTCATCTAACTCCGACTTGATTAGCTCCAGTCTCTGGACTCTTCTTCAAGTGATGACGTCTCTCCGGTCCAGTGAAGTTAGCTCTAGTGACGAGTCACTGGACGGTAACAATCTTAGCGACGGTAATGTGTCTTTCATCAGATGAGAGCGTGGGAGCAGACAACTTGTCTGATGGCAATGTCTCTTGACGCCCTGTCTGAGGAGTGCCTAAACATCTCTGATGACGAGTCTCTCTGACGCCCTGTCTGAGGAGTGCCTAAAGGTGAGACCGTTATAAAGGCTGATCTCTATAAATTACAATTACGCACTCTGTAAAGGTCTTACAATCAATCTGACTGATTCGGCTGGCTCGCCCTCAGCAACAACAACTCTGACCGTGAAAGTCTCCGCACTGAATAAGACACAGCAACCTGACTACAACAGTAAGGGTCAAGGCCATCTTCAAACTGAGCGCAAGTGTGGATCATACGATGTGTTCATCTCTTTACGGGGACGAAAGCTAGCTCTCTTCAACGCATCTCTCAAAAGATAACCAATAAGAAGAGCTCCACCAAGATCAAGCTTTTCAAAACATCCATGGGTATCTTACCATCTCAAACTATGTCTGTGACCCCTTTTGGACAGCGCCGGAAGCCTATTAAATCCAAGAGTGTGACGATCCAGGTGAATAAAGCCCAAGTATAACGTGAAGACGACTCTAAGGGCATCGCGAAAGTCAAGGTGCGCAATAAGATAGGACATATAGCGTCAACGCCAAGTTCAGCGGGGATAAAATTACTACGCAATCCAGCAACTCTTCAAAAGCTGACGATAACGAAATGAAGTGTACATTAAGCCCTTCCGTCAATACTACATGACCAACAGTTCTGCCCCCTTATCTGACTATCAACCTCACTAATGTTAAGGGCTCCCTCTCGCCAAGAAGAAGGTCTCTGTCAAGATTGGGAAGAAACGTATACCCGTGAAGACAACAGCCAAGGAATTGCTAATTTAATTTACAAGAAGAGTGCTCTCTACAACCTGTAAGATCACTTTAAGGCCACCAGCACTTCTATGGAGCATCAGTCAATTCAAAATGACGATCCAAAGAATGCGCTACATCACTGAAGGCCCTTCCGTGAGCATTAACAGTACCACTATGGCAAGTTCTGATAAGTCTGAAGGATGGGAAGGGCAAGGCCCTCAAGAACACGACTGTGACCGTGAATGTACCCGAGCTGA AAAAGGTGTTTACACTCAAGACCAACGCTAGCGCGTCGCGACTTTTGTAGTTTAAACGGTGAAAAAATTTTAACTTGAAGATTAAAGTACGCCGGCAACAAGAATTACCGTGTCTTCCGTTAGCAGTAAATCAATGTGAAGCAGATAAAGGTGAAGCTGAGCGACGTGATCGCGCGTCCCGAGTGTGATTGACTACGTGAATAGGACTAAGACCTGCCTAGTAATGTTCAGTATAATAATTACAATTTACAGTGACACAGTTGACCTATCTGGCTAGCAAGCGGTCAAGATAATCAACAAGAACTACGGGATATCGTTCTG	SEQ ID NO.: 64

Cluster	Description	Sequence	SEQ ID NO.
		TGATTAGTGTGCTAAATCTCTATAAAAGCTCTGGCGAGATTTATGATACCGTCTA CAAGAAGGACTTTGGAAGATTGCTAACTCCGIGGTGGCTCCCTCATACAAATTAT AAAACAAAGAATAACGTGCTCTATAGTATTTACAAAGTGCCCTTTCAAAGTGTA TCCATCTCATTCGCTAAGGTCCTGAAATTTTACGGAACAATAAAGAAGCTCCCTA ATTACAGTTTGTTCACACTCGCTGATTTGCGCAAAGTTAAAGATAACGGAGGCTA TAATTTCTACCTTACACAGACAAATATCGCGGTAAAGTCCGATCTGAACAT GCTCAATCTCGGCAAGACCCCTGAAGAGTATGGGATACAAACGCTGIGATTGT TGGAAATTGGCCCTGACATCCACAACGTGGCTTATAGATATGGGTGCACAGGAAA TAATTCCTGTTCTCCCTGCTGCTTCGGAGGGGTGGATGTCGGCTGCATCGAGGAA TGGCCGGGACCTTGGCGACCTGAACGGCCATAGCTTCGTCATTCATATACCA GGTGCACGTGCTGGCCCTTTGGTTCACTAAACCTTACGGTGCTAGTGTGTCCTC TCAATAAAAAGGTGGGATCGCTGGGATGCCGACTATGGATTTCCCTTTGAACA CCCTGCCAAGTATATGAAAAGTCATAACATCAGCTACATCGAGACTGGCACCG TGGCCAACGCTGTAACTCTTGAGCGAAGGCAAGATGGGAGGACCTCAACTGA TATCCGGGGCGGAGGAGCGGTGGCGGGGTAGCGGTGGGGCGGCAGCATC GAGAACGCCGACAAAGGCCATCAAGGATTTCCAGGACAAACAAGCACCTCACGA TAAGAGCGCAGCTACGAGGCGAACAGCAAACTCCCTAAGGATTTGAGAGATA AGAACACAGGTTTGTTGGAGAAAGTGAGTATTGAAAAGCAATCGTGGCCAC GATGAACGGGTGAAAGCGCCACACGCGCTATCAGCAAGCTGAATGAAAAAGA CTCCATTGAGAACCGGGCCTGGCGCAGCGGGAAGTCAACAAAGCCCCATGGA CGTGAAAGGACCTCCAGAAACAGCTGGACtaatgataagaccagcctcaagacacccgaatggag tctctaagctacataataccaacttacactttcaaaaatgtgtcccccaaaatgtagccattcgtatctgctccttaataaaaagaaagtttc ttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C8.2	miru0824 = ADC46675, adhesin-like protein with transglutaminase domain [Methanobrevibacter ruminantium M1], amino acid	MFVFLVLLPLVSSAAQSLDNINYANDGFDSDEMNCDLHKDSQSLKSNALSNKK ITNTIVKLIDMKKAESNDVKQTGAAKASNTKSTSKTTKINATKSNITKSTATKITA NSSSTKKATQNTTINTQTLAKSSSSYMAVVEKNAKLQEPITISKKKYKSP EYLVLVS KAVSNISKTKV EIKDKLITNYSNTDCKSVNGTINKTEYVQAKKTVSFIEKNHRAPN WIASSKGNIPRNQJL VFSKCLDQYNKSGKLPPSIKLNLDLNMKQKIDSSKKVNST STKKNTSSTKTNTS AKKTNTTSTKKTNPTATSTNNKSLVESTLDSIKSILNNIENK LNPTNKVLSTGTGKKNTVTVNSSKVN VQISSSTVNVKISAKDNTNSGKNTNSGSAK KTNTTSTKKTNTTSTKKIDTNSTKKTNTTSTKNTTSSAKKTNTTSTKNTTSSAKKTN TTSTKNTTSSAKKVNTSSSKTNTSAKNNTSTTAKSSNSKYLSTSVLNDKYLGESLK KYLA VGNKNCQVTNKAIKTLANTLSKLSKSDYKKGEKIFNWVRDNIGYEKYRNTKK GALKTLQTRGNCVDHAHLIVALSRAAGLPARYVNANNCKFSSGYVSGHVWAQV LVGNTWV VADATSNRNKFGVKNWNVNSYKLVGKYSISFSGGGSGGGSGGGG SIENADKAIKDFODNKAPHDKSAAYEANSKLPKDLRDKNRREYKTSIEKAIVRHDERIK SANDAISKLNKEDSIENRRLAORETNKAPMDV/KEHLOKOLD	SEQ ID NO.: 65

Cluster	Description	Sequence	SEQ ID NO.
C8.2	mru0824 = ADC46675.1, adhesin-like protein with transglutaminase domain [Methanobrevibacter ruminantium M1], nucleotide	ctgtctcttttgcagaagctcagaataaagctcaacttggccaccATGTTTGTCTTCTCGTGTGCTCCCTCTGGTCTCCTCTGCAGCCAGAGCCCTGGATTAACATTAACCTACGCCAACGACGGCTTCGATTCAGATGAATGATTAATTGCGATTGACAAAGGATAGTTCACAGAAAGTCCCTTAAGAGCAACGCTCTGTCTCCAAACAAAGAAACGACAAATACCGTGAAACTCACGGATATGAAGAAAGCAGAGTCAACGACGTGAAGCAGACCGGGGGGCCAAAGCCAGCAATACAAAGAGCACATCCAAAGTCTACAAACGAAACCAACGCAACCAAAAGCAACACAAAGAAAGTACTGCCACCAAAACGACCGCTAACTCTTCCTCCAACAAAAGCTACCCAAAACACTACCTACTATCAATACTCAGACTCTCGCCAAAGTCCAGTTCCTCTATATGGCCTATGTGGAGAAACGCGAAAGTCCAGGAACCTATTACCAATTAGCAAGAAGAAGTACAAATCTCTGAATACCTGTATCTGTGTGAGCAAAGCGGTGAGTAACATCAGCAAAACCAAGGTGAGATAAAGGACAAAGCTGATAACAACTACAGTAATACAGACTGTAAAGTCAGTTAATGGCACCATCAACAAAACCGAGTACGTCCAGGTCGGAAGAGACCGTCTCTTTTCATAGAGAAAGAACCATCGCGCCCTAACTGGATCGCAAGCAGCAAAAGGAAACATCCCTCGCAACCAATTGATCTCGTGTTCAGTAAGTGTCTTGACCAGTACAAATAAAAGCGGAAAGCTTCCTAGCTCTATCAAGCTGAACGATCTGGACCTCAATAAGATGAAGCAGAAATAGACAGCAGTAAAGAGTGAACTCCACTTCTACTAAGAAACAAATACCTCTTCACTAAGACACAGCACAAAGCGCTAAGAAGACTAATACTACCAAGTACCAAGAGACAAACCTACGGCCACATCTACCAATAATAACAATCACTGGTGGAAAGTACGTTGGACTCTATAAAGTCCATTCTTAATAATATTGAGAACAAAGCTGAACCCCTACTAATAAAAGTCTCTCAACAACAGGGACCAAGAAGAACACTGTGACTGTGAATTCATCCAAAGTGAAATGTTTCAGATCTCTCTCTCTCAACAGTTAACGTGAAAATCTCAGCCAAAGACAAACCAATTTCTGGAAAACACCAACTCCGGATCCGCCAAAGAGACCAATACAAACCAGCACCAAGAAGACGACACGACAAAGTACCAAGAGATCGATACAAACTCCACTAAAAGACGACACTACTAGCACTAAGAAATACACCTCTTCAGCAAAAACCAATACCAACAGTACGAAGAACACACCTCCAGTGCAGAAAGAGACAAATACCACGAGTACAAAGAAATAATACATCCAGCGCCAAAGAAAGTGAACACTTCTCCAGTAAACCAATACCAGTGCACAGAACACACAGCCAGCAACCCGCAAAAAGCTCTAGCAACAGCAAAATATCTGAGTACCTCCGTTCTGATGACAAATACCTCGGGGAGTCTCTGAAGAAAATACCTTGCTGTGGGAAGAACTGCCAGGTACCAACAAGGCTATCAAGACGCTGGCCAAATACACTGACGCAAGTTGAAGTCAGACTATAAG	SEQ ID NO.: 66

Cluster	Description	Sequence	SEQ ID NO.
		AAGGGTGAAGAAGATTTTCAATTTGGTCCGAGATAACATTTGGCTACGAGAAAGTAC AGGAACACTAAAAAGGGCGCACTTAAGACTCTGCAAAACAAGGGCGGTAAATTG CGTGACCAACGCCACCTTATTGTGCCCCCTGTCAGGGCTCGGGCTGCTGCTGCT CGGTACGTGAATGCCAATAAATGTAAATTTTCAATCCGGCTATGTGCTGCTCACG TGTGGCCCAAGTGTCTGTCGGCAATACCTGGGTGGTGGCCGACGCAACCCAGCA ACCGGAACAAATTTGGGGTGTGAAAAAAGTGAATGTGAATCATATAAGCTCG TCGGAAGTACTCTCAATCTCATTCGGTGGCGGTGCGAGTGGGGGAGGCGGCA GCGGAGGAGGGGGAGCATCGAAATGCAAGACAAGGCTATCAAGGACTTCCAG GACAACAAAGCCCTCATGATAAATCTGCGGCTATGAGGCTAATAGCAAACTG CCTAAGGACCTGCGGGATAAGAACAAACAGATTGTGGAAAAAGTGAGCATCGA GAAGGCTATCGTGGCGACGAGAAAGAGTCAAAATCTGCTAACGATGCTATTAG CAAGTTGAATGAAAAAGATTCTATCGAGAACCGAAGACTGGCACAGCGCGAGG TCAACAAGGCCCTATGGACGTTAAGGAGCATCTGCAGAAACAACCTGGATaatgat agaccagcctcaagaaacccgaatggagctctaaagctacataataccaacttacaaatgtgtcccccaaatgtagcc attcgtatcgtcctctaataaaagaagtttcttcacattct	
C8.3	mrul604 = ADC47454. I, adhesin- like protein with transglutami nase domain [Methanobr evibacter	MFVFLVLLPLVSSAAASNASDNLDDLITSDSNSLDLVSTNSDILSSDSGVSSDDSSND ASGDVLGSDVSSNESNNQSQSTLDSNNQSQGLSDSNSTLLDSQSNQSNSESSDSS DSSETVIKNATISIVSSKTVVRGNSLNTILKDNASTLLSNKTVFTFNGKTYNKTNA KGIASLTLTATPKKYLVKIAFVGDEL YEASSKSVNVTL SKTPTSISNSGKSIVRGKLY KLTLKDAKGKALS GKKISISFNGKKYTKTTNSNGQVNLJINVNVGKTYKMTYKFAG DSNYLSSSGSVSIKVKMGTSHGSGSSIVKGSYTVTLKNANGAVLSNQKIAFTLSGK TYNRTTNAGKQASLKIGLSSGKTYNLT YKYVAGNSYVGGSSGKVS LFKVTPPTMKNS GKTIVSGETYKVTLKDDAGKSLANKKVSIFNNKTYAKTTNSNGQASLTIKGTGFRS YPLSYKFA GDSKYGPSSGSLCLRVKATSLKGSASSIVQKSYTVTLKDSNSTPLAN	SEQ ID NO.: 67

Cluster	Description	Sequence	SEQ ID NO.
	ruminantium M11, amino acid	QTIVFTLDITKKYNRTTNAKGQASLKIGLAAGKTYNLAYKYSGTSYNYNGSSGSVKLK VKFPISLJNSGKSVMGTYGYNIVLKDSSNLSVSNKTIISGFNGKTYDEIHDANGTIVT LLIDANVPKTYKMTYKFAGDSYDYGASSTGNLTVKFKNAFTISQIISASSLSKSYVLK NKKVPATVSVNGVSLNLTSTYTLMAKATISINSNKTSGSILLVPVDSNYTNNGSRINA NLYKANYIDLAKKVISSAEANKLVPNSVSTNIGLVSHDLYSFLGAKALVFFNSDHYL PNYLILSSDDVGEKHSVIPSNARGNASQFKAGLNEAETLTAQAIAKYLVASGHDAT NSEIKALAAKLVSGKTSLWDKANAIPTFARDNITYSVYADSKKGAAAGTLLSSKSGNC CDHSNLIVSLCRAANITAREFSHAQGCFTSSGLVAGHVWAQIYIDGVVWTADATSRR NSLGNIVNWNTNHYNTLKQYDHLSPFGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG PHDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKATVRHDERVKSANDAISKLEKDSIENR RLAOREVNAKPMDIKEHLOKOLD	

Cluster	Description	Sequence	SEQ ID NO.
C8.3	mnrl604 = ADC47454.1, adhesin-like protein with transglutaminase domain [Methanobrevibacter ruminantium M1], nucleotide	ctgtctcttttgcagaagctcagaataaacgtcaacttggccaccATGTTCTGTTTTCCTGGTCTGCTCCCTCTGGTCTCTCCGACGCTCAAAACGCCCTCTGACAAATCTGACGACCTCACATCTCTGACTCAAACTCTCTGGAGCTCGTGTCTACCTTAACCTTGACATCTCTTCTTCTGACTCTGGCGTGAGTTCCGATGACAGCTCTAACGACGCCCTCTGGCGACGTGCTGGGAGTGACGTTTTCTAGTAACGAGAGCAACAACCACTCTCAGTCTACGCTGGATTCGAACAACCAAGTCTCAGAGCGGGCTTGACAGTGACAACTCTACACTGCTCGACTCCAGTCTAAACAAGTCTAACTCTGAGTCTTCTGACTCTTCAGACTCTTCTGAGACCCGTGATCAAGAACGCCACGTCTCACTCTGTCTGATTCAGTTCAAGAAGCGTGTGCGGGGAACTCTTTGAAATCAGCTTAAAGGACAAACGCTCTACCTGCTCTCTCTAACAAGACTGTACCTTCACGTTTAAACGGCAAGACCTACAAAGACCAACGAAACGCCCAAGGGGATCGCCTCTCTCACTCTGACGGCCACTCTTAAGAAAGTACCTGGTTAAGATCGCCTTCGTGGGACGAGCTCTACGAGGCACTCTTCTAAGTCTGTGAACGTCACGCTCTTAAGACCCCTACCTCTATTAGCAACTCTGGGAAATCTATCTGTCGCGGAAAGTTGATAAAGCTGACGCTGAAAGACGCCAAGGGGAAGGCCCTGTCTGGTAAAGATCTCTATCAGCTTCAACGGCAAGAGTACACAAGACCAACAACCTCTATGCCAGGTGAACCTGACAAATCAATGTCAATGTGGGCAAGACCTACAAAGATGACCTACAAGTTCGCCGGCGACTCCAAATTAATCTGTCTTCTATCAGGCTCAGTGTCTATCAAGTCAAGATGGGCACCTCCATCATCGGCTCTGGCTCGTCAATCGTGAAGGGCAAGTCTTACACCGTGACTCTCAAGAACCGGAATGGCGCGGTGCTGTCTAACCAAGAAATCGCAATTCACCTCAGTGGCAAGACGTACAATAGGACAACCAACGCTAAGGGCAAGCGTCTCTCAAGATCGGCTGAGTAGCGGCAAGACCTACAACTCACATACAAGTACGCCGGGAACCTTACTACGGCGGCTCTTCTGGGAAAGTCTCTTTGTCTGTGAAGACGCTACGACCATGAAGAACAAGCGGCAAGACGATCGTGTCAAGGGAGCGTACAAGGTGACCTCAAGGACGCTGACGGCAAGTCTCTGGCGGAACAAGAGGTCTCCATCATTCAACAATAAGACATAGCCCAAGACCAACTCTAACGGCAGGCAAGTCTTACCAATTAAGGGACCTTCGCCGCTTACCTCTGTCTACAAGTTCGCCGGCGACTCTAAGTACGGTCTAGCTCTGGATCTCTGTGCGCTGCGGTGAAGAAGCGACCTCTCTGAAGGGGTCTGCTCTAGTATCGTCCAGGGGAAGTCTTACACCTTGAAGGACTCCAATCTACCCCTCTGGCTAACAGACCATCGTGTTCACACTGGACACGAAGAAGTACAACGGGACCCGCTAAGACCTTCTGTGAAGATCGGCTGCGGCTAAGACGTACACTTGGCT	SEQ ID NO.: 68

Cluster	Description	Sequence	SEQ ID NO.
		ACAAAGTACTCTGGCACCTCTTATTACAACGGCAGCTCTTGGGAGCGTGAAGCTCA AGGTGAAGTTCCTACCTCTCTGACCAACAGCGGAAAGTCTGTGATGAACGGGA CCGGGTACAACATCGTGTGAAGGACTCCAAGTCTAACCTGGTGAGTAACAAGA CCATATCTATAGGTTTCAACGGGAAGACCTACGACGAGATCACAGATGCCAACG GCACGGTCACCTTCTCATCGACGCTAACGTCCTTACGCGAATCTGTGAACCTGA ACAAAGTTCGCGGTGACTCTGACTACGGCGCTCTAGCGGAATCTGTGAACCTGA CGGTCAAAATTAAAGAAAGCCTTCAACCATCTCACAGATCATCTCTGCAAGCTCTAG TCTCAAAGTCTTACGTGCTCAAAGAAACAAAAGGTGCTGCGACTGTGTCTGTCAA CGCGGTGAGTCTCAATCTGACCAAGTTTCAACCTACCTGATGGCGAAGGCCACCAT CTCGATTAACTCAAAACAAGACCAAGCGGTCTATCTCTGCTGCTGCTGCTGCTCT AACTACACCAACAACGGCTCAAAGAATCAATGCCAACCTGTACAAGGCCAACTAC ATAGACCTGGCCAAAGAAAGTATCTCTTCTGCCGAGGCCAACAAAGCTGGTGCCT AACTCTGTTTCCACCAACATAGGGCTGGTTTCCACGACCTGTACTCTTTCGGAC TCGCCAAGGCTTGGTGTCTTCAACAGCGACCACTACCTGCCTAACTATCTTAT CCTGTCTAGCGATGACGTGGGGAGAAAGCACAGTACCTGATCCCTTCTTAAAGC CCGAGGCAACCGCTCTCAGTTCAAGGCCGTCTGAACGAGGACAGAGACCTTAC GGCTGCCCAGATAGCCAAAGTATCTGGTGGCGTCTGGCCACGATGCAACGAACTC TGAGATCAAGCGCTCGCGGCCAAGTTGGTGTCTGGCAAGACCTCTCTGTGGGA CAAGGCCAAACGCCATCTTCACTTGGCTAGAGACACATCAGTACAGTTACTA CGCTGACTCTAAGAAGGGAGCCCGCGGACCTCTCTTCTAAGTCAGGCAACTG CTGCGACCACTCTAACTTGATAGTGTCCTGTGCCGCGGCCAACATCACTGCG CGGTTTAGTCACGCACAGGATGCACTTCTCATCTGGCTTGGTGGCGGCCACG TGTGGGCTCAGATTACATTGACGGCTGTGGTACACCGCCGACGCAACATCCC GCCGAAACTCACTTGGAAACATCGTTAACTGGAACACCAACCACTACAACACCC TCAAGCAGTACGACCACTGTCTTTCGGCGGGGGGGGTACGGGGAGGGGGG TCAGCGCGCGTGGTCTATCGAGAACGCCGACAAAGGCCATCAAGGACTTCCAG GACAAACAAGCCCCTCACGACAAGAGTGCCGCTACGAGGCGAACTCCAAAGCT GCCTAAGGACCTCCGGGACAAGAACACCCGTTCTGTGGAGAAAGGTCTCTATCGA GAAAGCGATCGTCCGGCACGACGAGCGGTGAAGTCCGCGAACGACGCCATCT CTAAACTCAACGAGAAGGACAGCATCGAGAACCCGACGGCTGGCCCCAGCGGGAA GTGAACAAGGCCCCATATGGATGTGAAGGAACATCTGCAGAACGCTGGA	Ctaatg

Cluster	Description	Sequence	SEQ ID NO.
		atagaccagcctcaagaacacccggaatggggtctctaaagctacataataaccacacttacacttiacaaaatgttgcccccaaaatgtagc cattcgtatctgctcctataaaaaagaaagtcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
C8.4	mmu1499 = ADC47349.1, adhesin-like protein with transglutaminase domain [Methanobrevibacter ruminantium M1], amino acid	MFVFLVLLPLVSSAAIDIDEASSSDLSDDSSISNDYLVANSGLDSDSVASSASSIAAD DSDLNNASSNVNFENEVLSTNNNEDIIESEIVKDSKNQLSSSLQASIKTKTTLKGS GSSVYRGNPYYVTLTDSNGKVLASQKVTFENILGKNYTRTIDSKGVASININLAKGK YNIACLYAGTENYASSKLSVALJVNLMSTKINTGGSTVKKGNAYSVTLTDGNGKAL SSQKVTLNIIIGKNYTRTIDSKGVASIAINLAAGKKFTLTASYAGSANYLSSKVSATV TVQKGDTSIKPSGTSIVKGNYSFTLVDSGKGLANQKVAIKISGKYSRTTNSNGV ASIAINLAAGKKYSIVCSYAGSSNYKASSSTVSLSVTNPSTNSKTFIAKIEAAATNLK AYVNKNKAVPTTVSVGGTNLKISEFSYLMKAIIVNLNSNNTNAITLPSGIYNGASAS NSLNAVYKAQYVDLSKR VYNYIDKNKVPAA YGTVYNANGASLGNAGFNLYTFAF AKILDFHIKTNKYLPNYCSFDSVFKASNGSSSSSSSTNSSSSTNSSSSSSTNSGSS TPAVTVKATSLKAASTSVIRGDDYSVTLTDSGNALANQKITFALSSSSYTRTTNSKG VASLTLNLAGGKYSIITSYAGTSAYKASKLTNTVTISNSSRFFELNDIETAAENVKTY VTKNKALPNTVTVAGTQLTLSQFSYVMAKAHNINASNSNYISLKSVAASNSTGDYL DITVYRAQYMNLTNRVISFVESDKITPTFATVYNSNGKSVGKAFFKLYTFAFAKILA FYKTNNYLPITYCTFQSSAIGVVPDVATNVTINSKINANMNQFVGLNEKNTVSNLSA YLVGTGQSTHTTNIKNVA AQLTKGLNSTATKALAIYNFVRDDISYYSYSDSRKGADG TLSSGSGNCVDQASLVVALCRAAGIPARYSHAQGCFTSSGLVTGHVWVAQILVDGV WYSADATSVRNSLGNIVNWNTNSYHSMIKQYAAVPEFGGGGGGGGGGGGSIENAD KAIKDFODNKA PHDKS44YEANSKL PKDLRDKNNRFEKVSIEK41VRHDERVKSANDAI SKLNEKDSIENRRLAQREVNKAPMDVKEHLQKQLD	SEQ ID NO.: 69

Cluster	Description	Sequence	SEQ ID NO.
C8.4	mnrl499 = ADC47349.1, adhesin-like protein with transglutaminase domain [Methanobrevibacter ruminantium M1]. nucleotide	ctgtctcttttgcagaagctcagaataaacgtcaacttggccaccATGTTCTGTTGTTCTTGTCCCTTCTGCCTCTCGTTTCTAGCGCCGCGGATCGACATCGATGAGGCCCTCTCTCTCCCTCTGACCTCA GCGACTCTCTATCTCTAACGACTACCTGGTGGCTAACAGCGGGGACGACTCAG TTGCCAGCTCTTCCGCCCTCTAGCTCTATCGCGGACGACGACTCTGACCTGTCTAA CAAGCCTCTTCTTCTAACGTCAACTTCGAGAACGAGTCTCTGCCACCAACAAT AACGAGGATACCGAGTCTGAGATCGTCAAGGACTCTAAGAATCAAGCTGTCTCT TCTAGCCTCCAGGCTCTACAAAGACCAAGACCCTCAAGGGCTCCGGTTCT TCTGTGTACCGGGGAACCTTACTACGTTACTCTCACTGACTCTAACGGCAAGG TGCTGGCCAGTCAAGAAAGGTGACGTTCAATATACTGGGCAAGAACTACACCAAGGA CCACCGACAGTAAAGCGGTGGCAAGTATCAACATACTGGCCAAAGGGCAAG TACAACATCGCTTGCCCTCTACGCCGGAACGGAGAACTACGCATCTTCTAAGCTGT CTGTTGCCCTGACTGTGAACCTCATGTCTACGAAATTAACACGGGGGCTCTAC CGTGAAGAAAGGGGAACGCTACTCCGTGACCTGACTGACGGAACGGGAAGG CTCTGTCTTCTCAGAAAGTCAACCTTAACATCTTGGGAAGAACTACACCAAGAAC TACGGACTCTAAGGGGTTGCTTCTATCGCGATAAACCTGGCCGCCGTTAAGAA GTTCAACCTCACCGCCTCTTATGCCGGCTCTGCCAACTACTAGCTCTAAGGTG TCCGGACCGTGACCGTGACAGAGGGCGACACATCTATTAGCCTAGTGGGACC AGCATCTGTGAAGGGAACCTCTATTCTTTTCACTGTGGGACGGCTCTGGGAAG GGCTTGGCGAATCAAAGGTGCGGATCAAGATCTCTGGCAATCTCTATAGCCGG ACCACCAACTCTAACGGGTGGCTCAATTGGCATCAATTGGCGGCGGCAAG AAGTACTCTATCGTCTCTTACGGGGTCTTAGTAACTATAAGGCTTCTTCTTCA ACCGTGTCTGTGTGACCAACCCCTTCAACCAACAGCAAAACCTTCTCTATC GCCAAGATCGAGGCCGCCGCCACCAACCTGAAGGCTTACGTGAACAAGAACAA GGCCGTGCTACCAAGTGTCTGTGGCGGCCACCAACCTGAAGATCTCTGAATT CTCTTACTTGATGTCTAAGGCCATGTCAACCTGAACCTTAACAACAACAACGCG ATAAACGCTCCCTCTCGCATCTACAACGGGCCCTACGCTCTAAGCTCTCAACG CCACCGTGTACAAGGCCAGTACGTGGACTTGAGCAAGCGAGTCTACAACCTACA TCGACAAGAACAAGGTCCCTGGGGCTACGGCACCGTCTACACGCCAACGGCG CTCTCTTGGCAACGCCGCTTCAACCTGTACACCTTCGGTTCGCCAAGATCTT GGACTTCCACAAGACCACAAGTACCTCCCTAACTACGCTCTTTCGACTCTTCT GTCTTCAAGGCCCTCAACGGCTCTAGCTCTAGTAACTCATCTTCTTCTACCACT	SEQ ID NO.: 70

Cluster	Description	Sequence	SEQ ID NO.
		CATCTTCATCTACGAACATCATCTTCCGGCTCTTCTAACAGTAGTGGGTCTGGGAGTTCTACGCCGTGCCGTACCGTGAAGGCGACCTCTTTGAAGGCCGCCAGTACCTCCGTGATCCGGGGCGATGACTACTCAGTGACGCTCAGGGACTCTTCTGGGAACGCACTGGGGAACCAAGAAAGATTACGTTTGCCCTCTCATCTAGTAGTTACACCCCGACCACCAACTCTAAGGGCGTTGCCAGCTCAGCTGAACCTGGCCGCTGGCAAGTATAGCATCACCACTTCATACGCCGGAACATCTGCCTACAAGGCTCTAAGCTGACAAACACCGTCACGATCTTAATTCTTCACTCTCGCTTCTTCTGAAACGACATTGAGACCGCCGCCGAGAACGTGAAGACGTACGTACCAAGAAACAGGCTCTCCCTAACACGGTGACGGTCCCGCACCCAGCTGACCTGTCTCAGTTCTCTCTACGTGATGGCAAAGCGGATCCATAACATCAACGCTAGTAACCTTAACATACTCTGAAAGTCTGTGGCCAGTTCAACACAGTACCGGGGACTACCTCGACACGACCGGTGTACAGGGCCAGTACATGAACCTGACCAACCGCGTCAATCTCTTTCGTGGAGTCCGATAAGATCACCCCTACCTTTGCCACCGTCTACAACTCAAAACGGAAGTCCGTGGGAAGGCCGAGTTCAAGTTGTACACATTGCTTTTCGCCAAGATCCTGGCCTTCTACAAAACCAATAACTACCTGCTACGTACTGCACGTTTCAGAGCTCTGCCATCGCGGTGGTGCCTGACGTGGCCACGAACGTGACCATCAACAGTAAGATCAACGCCAACATGAACCAAGTTCAAGTCGGTCTGAACGAGAAGAACACTGTGTCTAACCTGTCTGCATACCTGGTGGTACCGGCCAGTCTACTATCAACAATAACATCAAGAACGTGCGCCCACTTGCTGACCAAGGACTCAACTCTACAGCCACTAAGGCCCTGGCTATCTACAACTTCGTGCGAGACGACATCTCTTACTCTACTACTCTGACAGCCGCAAGGGAGCCGACCGCACCTGTCTCCGGTCCGGGAACCTGCGTCGATCAGGCATCTTTGGTGGTGGCCCTGTGCCGGCAGCCGGATCCCTGACGCTACTCAACGCCGAGGGGTGTACCTTCTAGCGGCTTGTACGGGCCACGTGTGGGCCAAATCCTGGTGGACGGAGTCTGGTACTCTGCAGACGCCACCTCAGTGGGAACTCTTTGGGCAACATAGTGAACCTGGAATACCAACTTTACCCTCAATGAAGCAGTACGCCCGGTGCCTTTCGGTGGCGGGGTCTGGAGGGGGCGTCTGGCGGGGGGGCTCTATCGAGAACCGGACAAAGCGGATCAAGGACTTCCAGGACAAACAGCGCCTCACGACAAAGATGCCGATACGAAGCCACAGCAAGCTCCCTAAGGACCTTCGCGACAAGAATAATCGGTTCGCGAGAAGGTGTCTATCGAAGAGGCCATCTGTGCGCCACGACGAGAGATTAAATCTGCCAACGACGCCATCTCTAAGCTGAACGAGAAGGACAGTATCGAGAACC	

Cluster	Description	Sequence	SEQ ID NO.
		AGGAGCACCTGCAGAAAGCAGCTCGACtaatgataagaccagcctcaagaacaccccgaatggagctctaaagc tacataataccaacttacactttacaaaatgttgcccccacaaatgtagccatcgtatctgctcciaataaaaaggaaagtcttctcacattct	

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.1	swapped ssp for PPa_v2 without SIP sites, mmu0493 = ADC46344. 1, adhesin- like protein [Methanobr evibacter ruminantiu m], amino acid	MREPSIFTAVLFAASSALAMPLSGGGSGSMPLNTTTEDETAQIPAEAVIGYL DLEGDFDVAVLPEFSNTNNGLLFINFTIASIAAKEEGVSAADVDTNDNLDDGSSS NSDLISSSSLDSSDDVSSGSSEVSSSDESLDGNLSDGNVSSDES VGADNLS DGN VSSDES VGADNLS DDESSDALSEELPKTETVIKADPINYNYASVKGLTINLTDSAG LALS NKTLTVKVSALNKTSNLTNSKQQAIFKLSASVGSYDVFI SFTGDES YAPSNAS SKITIKKSS TKIKLSNIHGYLTISNYVSVTLTDSAGKPIKSVTIQV NKAKYNVKTDS KGI AKVKVANKIGTYSVNAKFSGDKNY YASSNSSKLITTKMKVYIKAPSVKY YMTN SSAPYL TINLTNVKGSPLAKKKVSVKIGKKTYTLK TNSQGI AKFKFTKKVSSYNCKIN FKATSNFYGASVNSKMTIQKMPTSLKAPSVSINSTNYGKVLISLKDGGKALKNTTV TVNVTELKKVFTLKTNASGVATFSFNGEKTENLKIKYAGNKNY AASSVSSKINVKOI KVKLSDVIGASRVLDYVNR TKDLPSNVQYNNYNFTVTQLT YLASKAVKNINNKNY GDIVLISVPKSYKSSGEIYD TVYKKDFVKIANSVVGSSYNYKNKEYVSYSIYKVPFKV YSISFAKVLN FYGNKKLPNYSLFTLADFAKVKDNGGYNFYLT TDNIAGKKSDLNM LKSLAK TLKSMGYNAVIVGIGPDIHNVAYRYGCTGNNSVLLACFGGVDVGCIEEWA GDLGDLNGHSFVNSYQGAHV LGLWFTKPYGASVSLNKKVGIADADYGFPLNTPA KYMKSHNISYIETGTVANACKLLSEGKMGGPQLISGGGGSGGGSGGGSGIENADKA IKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFEKTSIEKAHVRHDERVKSANDAI SKL NEKDSIENRRLAQREVNKAPMDI/KEHLQKQOLD	SEQ ID NO.: 71

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.1	swapped ssp for PPa_v2 without SIP sites, mmu0493 = ADC46344. 1, adhesin- like protein [Methanobr evibacter ruminantiu m], nucleotide	ctgtctcttttgcagaagctcagaataaacgctcaactttggccaccATGCGCTTCCCTTCTAATTTTCACCGCC GTGCTCTTCGCCCGCTCTTCAGCACTCGCAATGCCCTCTGCTGCGGGCGGGTCTG GCGGCAGCGGCTCTATGCGCTCTGAACACCACTACCGAGGACGAGACGGCGCAGA TACCTGCGGAGGCGGTGATCGGTACCTCGATCTGAGGGCGGACTTCGACGTGG CCGTGCTCCCTTTCAGTAACTCAACCAACAAACGGGCTCCTGTTCAACAACACAC CATCGTAGTATCGCCGCAAGAGGAGGAGGGGCTTTCAGCCGCCATCGACGTCGA CACCAACGACAACCTGGATGACGGCTCTTCTTCTAATTCGACCTGATCAGCTCC TCTTCTTTGGACAGCTCTAGCAGTGACGACGCTGTCACGGGTCAAGTGAAGTG TCATCCTCTGACGAGTCTCTGGACGGCAATAACTTGAGCGACGGGAAACGCTGCT TCATCGGACGAGAGCGTTGGGCGCGACAACCTTCTGACGACGAGTCATCTTCTGAC AGTGACGAGTCTGTGGGCGCGACAACCTTCTGACGACGAGTCATCTTCTGAC GCCCTTCTGAGGAACCTGCCTAAGACCGGAGACTGTGATCAAGGCCGACCCCTATA AACTACAACCTACGCTCGTGAAGGGTCTGACCAATAAACTTGACCGGACTCTGCG GGCTGGCACTGTCTAACAGACCTGACCGTGAAGGTGTCTGCCCTTAATAAG ACCTCTAACCTGACAACGAACTCCAGGGCGAGGCCATCTTCAAGCTTCTGCT CCGTTGGGCTTACGACGCTTCTATCTCTTCCCGGAGACGAGAGCTACGCCCC TAGTAACGCACTCTTAAGATCACCATCAAGAGTCAAGTCTAGTACTAAGATCAAACT TTCTAACATCCATGGGTACCTGACCATCAGTAACTATGTGTCTGTGACTCTGTG GACTCTGCCGGAAGCCTATCAAGTCCAAATCCGTGACCATCCAAAGTGAACAAG GCGAAGTACAACGTCAAGACGGACTCTAAGGGGATCGCCAAAGGTGAAGGTGGC CAACAAGATCGGGACCTACTCTGTGAACGCTAAGTTCAGCGGGGACAAGAACTA CTACGGCTTCTAACTCTAGTAAGCTCACCATCAGAAAGATGAAGGTATACATC AAAGCCCTTCTGTTAAGTACTACATGACAAACTCTTCTGCCCCCTTACCTGACGA TCAACCTGACGAACGTGAAGGATCTCTCTGCGCAAGAAAGAGTGAAGGTGAGCGTGA AGATAGGGAAGAAGAACCTACACGTGAAGACCAACTCTCAGGGAAATCGCCAAAG TTCAAGTTTACGAAGAAGGTGTCTTCTTCAACTGCAAGATCAACTTCAAGGCC ACCTCTAATTTCTACGGCGCTCCGTGAACCTTAAGATGACCATCCAGAAAGATGC CTACAAGTCTGAAGGCCCTTCTGTGTCTATCACTCAACAACCTACGGGAAGG TCCTGATCTCACTGAAGGACGGCAAGGGGAAGGCCCTCAAGAACACGACAGTC ACGGTCAACGTGACCGGAGTTGAAGAAGGTCTTCAACCTGAAGACAACGCCCTCT GGTGTGGCTACCTTCTCTTTAACGGGGAAGAAGACCTTCAACCTCAAGATTAACT	SEQ ID NO.: 72

Cluster	Description	Sequence	SEQ ID NO.
		ATGCGGGAACAAAACTACGCGGCCTCTTCTGTCTCTTCTAAAGATCAACGTTA AGCAGATTAAAGGTCAAACTCTCTGACGTCAATCGGCGCTCTCGGGTGTCTCATCG ACTACGTCAACCGGACCAAGGATCTGCCCTTCAAAAGTGTGAGTACAACTACA ACTTCAACCGTGACGCACTACCTATCTGGCTTCTAAGGCCGTGAAGAACATCA ACAAACAAGAACTACGGGACATCTGCTCATATCTGTCCCTAAGAGTTACAAGT CATCTGGCGAGATCTACGACACGGGTGTACAAGAAGGACTTCGTGAAGATCGCTA ACTCCGTGGTGGGCTCTTTACAACTACAAGAAACAAGGAGTATGTTTCTTACTC AATCTACAAGGTGCCCTTCAAGGTCTACTCTATTCTTTCGCCAAGGTGCTGAAT TTTTACGGCAACAACAAAAGCTGCCTAACTACTCCCTCTTCAACCCTCGCCGACT TCGCGAAAGTGAAGGATAACGGCGGTTACAACTTCACTGACCAACCGACAATA TCGCCGGAAGAAGTCAGACCTGAACATGCTGAAGTCCCTCGCCAAGACCCCTGA AGTCTATGGGTACAATGCCGTCAATTGGGCACTCGGCTGACATCCACAACG TCGCTACAGATACGGGTGCACTGGGAACAACCTGTCTCTCTCGCGTCTTCGG CGGAGTCGACGTGGCTGCATCGAGGAGTGGCGCGGACCTGGGCGACCTGA ACGGCACTCTTTCGTGAACCTTATCAGGGCGCGCACGTCTCTGGGCTCTGGTT CACTAAGCCTTACGGCGCTCCGTCACTGTAACAAGAAAGTTGGCATCGCCTG GGACGCGGACTACGGCTTCCCTCTCAACACGCTGCAAGTACATGAAGAGTCA CAACATCAGCTACATCGAGACCGGTACCTGGCAACCGCATGCAAGCTGCTCAG CGAGGGGAAGATGGCGGCCCTCAGCTTATCAGCGCGCGCGGCTCTGGCG GGGGGGCAGCGGTGGTGGGGTCTATAGAGATGCGGACAAAGCCATCAAG GATTTCAGGACAACAAGGCTCTCACGACAAGTCAGCCGCTTACGAGGCCAAC TCTAAGTTGCCTAAGACCTGCGGACAAAGAACACCCGCTTCGTGGAGAAAGTG TCTATTGAGAAAGCCATCTGTGCGGACGACGAGCGGTGAAGTCTGCCAACGAC GCCATCTCCAAGCTGAACGAGAAGGACAGTATCGAGAACAGGCGGTGGCCCCA GCGAGAGGTCAATAAGGCTCTATGGACGTCAAGGAGCACCTTCAGAAGCAGCT GGAC ¹ aatgataaccagcctcaagaaacacccgaatggagtctctaaagctacataataaccacttacacttacaataaagtgtgcc ccaaaatgtagccattgtatctgctcttaataaaagaagtttcttccactct	

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.2	swapped ssp for PPa_v2 without SIP sites, mnu0824 = ADC46675. 1, adhesin- like protein with transglutami nase domain [Methanobr evibacter ruminantiu m M1], amino acid	MREPSIFTAVLFAASSALAMPLSGGGSGSGMPLNTTTEDETAQIPAEAVIGYL DLEGDFDVAVLPEFSNSTNNGLLFINFTIASIAAKEEGVSAAQSLDNINYANDGFDS DEMINDLHKDSSQKSLKSNAISNKKTTINTVKLTDMKKAESNDVKQTGAAKASNT KSTSKSTTKTNATKSNFTTKSTATKTTANSSSTKKATQNTTTTNTQTLAKSSSSVMAY VEKNAKLQEPITISKKKYKSPEYLYLVSKAVSNISKTKVEIKDKLIJTNYSNTDCKSVN GTINKTEYVQVAKKTVSFIEKNHRAPNWIASSKGNIPRNQLILVFSKCLDQYNKSGK LPSSIKLNDLNLNKMKQKIDSSKKVNSTSTKKINTSTSTKTNSTSAKKTNTTSTKKTNP TATSTNNKSLVESTLDSIKSILNNIENKLNPTNKVLSTTGTKKNTVTVNSSKVNVOI SSSSTVNVKISAKDNTNSGKNTNSGSAKKTNTTSTKKTNTTSTKKIDTNSIKKTNNTS TKNNTSSAKKTNNTSTKNNTSSAKKTNNTSTKNNTSSAKKVTNTSSSKTNTSAKNNTS TTAKSSNSKYLSTSVLNDKYLGESLKKYLA VGKNCQVTNKAIKTLANTLTSLKLS DYKKGEKIFNWVRDNIGYEKYRNTKKGALKTLQIRGGNCVDHAHLIVALSRAAGL PARYVNAANCKFSSGYVSGHVWAQVLVGNTWVVADATSNRNKFGVVKNWNVNS YKLVGKYSSISFSGGGGGGGGGGGGSIENADKAIKDFQDNKAPHDKSA4YEANSKLP KDLRDKNNRFVEKVSIEKAIIRHDERVKSANDAISKLNEKDSIENRRRLAQREIYNKAPMDIV KEHLQKQLD	SEQ ID NO. SEQ ID NO.: 73

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.2	swapped ssp for PPa_v2 without SIP sites, mnu0824 = ADC46675. 1, adhesin- like protein with transglutami- nase domain [Methanobr evibacter ruminantiu m M1], nucleotide	ctgtctcttttgcagaagctcagataaaacgtcaactttggccaccATGAGGTTCCCTAGCATTTTACCGC GGTCTCTTTTGCCGCTTCTAGCGCTCTCGCAATGCCTCTCTCCGGGGGGAAGC GGAGGGTCAGGCTCTATGCGCTTTGAACACACCACAGAAAGACGAGACTGCGCAA ATACCTGCGGAGGCCGTGATCGGCTACCTGGATCTGGAAGGGGATTTCGACGTG GCAGTGTGCTCTTTTAGCAACTCTACCAACAATGGACTTCTGTTCATTAAACACAA CAATCGCCTCCAATCGCAGCTAAGGAGGAAGGGGTGTCAGTGCCTCAGAGTCTGG ATAACATCAATTATGCCAACGACGGATTGACTCTGACGAAATGATCAACTGCG ACCTCCACAAGGACTCCAGTCAGAAAAGCCTCAAGTCTAACGCACTGTCCACAACA AGAAAGCGACCAATACCGTCAAGCTGACAGATATGAAAAAGCTGAGTCAAAAC GACGTGAAGCAAAACAGGCGCAGCTAAGGCCAGCAATACTAAAGTACTTCTAA GAGTACTACAAGACCAACGCTACTAAATCTAATACCACAAAAGTCAACTGCCAC CAAGACTACCGCAAAATTTCTCAAGCACAAAGAAAGCCACGCAAAATACCACTAC AATCAACACCCAGACGCTGGCCAAAGTCTCATCCAGCTACATGGCATATGTGGA GAAGAATGCCAAGTTGCAAGAGCCTATCACCAATTTCTAAGAAAAATATAAGTC TCCTGAGTATCTGACCTGGTGTCTAAAGCCGTGAGCAACATCAGCAAAACAAA GGTGAGATCAAGGACAAATTGATTACCAATTACTCCAACACGGAATTGCAAAAG CGTGAACGGCACAATCAATAAACAAGAAATACGTGACGTCGCCAAAAAAACCG TGAGTTTCATAGAGAAGATCATCGGGCTCCTAATTGGATCGCCTCAAGCAAGG GGAACATACCTAGAAATCAGCTTATTTCTGGTCTTTAGCAAAATGTCGATCAGTA CAACAATCAGGTAACTCCCTTCAAGTATAAACTGAACGATTGGACCTGAA CAAGATGAAGCAGAGATCGACTCCTCCAAGAGGTTAATTCACCTAGCACAAA GAAGACCAACACCTCCAGCACTAAACAACTCCACCTCAGCAAAAGAGACTA ACACGACATCCACAAAAGACTAATCCTACCGCCACGAGCAAAATAATA AGTCCCTCGTCGAGAGCACTCTGGATAGTATCAAGAGCATTTTGAATAATATTG AGAACAAAGCTTAATCTACGAAACAAAGTCTCTCCACAAACCGGAACCAAGAAA ACACTGTTACCGTTAACAGTTCTAAAGTGAATGTTAGATTAGCAGCTCTAGCAC CGTGAATGTCAAGATCAGTGCCAAAGGATAACACCAACAGCGGGAAGAACACCA ACTCCGGCAGCGCCAAAAGACCAACACTACGACACTAAGAAACCAATACC ACCTCAACAACAAAGATTGATACAAATTCACGAAATAACCAACACCCACGAG TACAAAAAATAACACGTCCAGCGCCAAAGAAAGACTAATACGACCTCAACCAAAA ACAACTACTCCAGTGCCAAAGAAAGACGAAACAACTTCCACTAAGAACACACAA	SEQ ID NO. SEQ ID NO.: 74

Cluster	Description	Sequence	SEQ ID NO.
		GTTCCGCAAAAAAGTGAACACTTCTTCCAGTAAACCAACACGTCGCGAAAGA ACAAATACATCTACGACGGCTAAGTCATCCAGCAATTCCAAGTACCTCAGCACCT CCGTCTCAACGACAAAGTACCTGGCGAGAGTTGAAGAAATATCTGGCCGTTG GAAAGAAATTGTCAGGTGACCAATAAGGCTATTAAACACTTGCCAACACCCCTGA CTTCCAAACTCAAGAGCGATTACAAAAAGGGGAAAAAGATCTTTAACTGGGTCC GGGACAAACATAGGCTACGAAAGTATAGAAACACCAAAAAAGGGAGCCCTGAAG ACCTTCAGACACGGGGGGCAATTGTGTCGATCATGCACACTTGATAGTGGCG CTGAGTCGAGCCGCTGGTCTGCCAGGTATGTC AATGCCAACAACTGC AAA TTTTCAAAGTGGCTACGTGCTGTTGGGCAATGTGTGGCTCAGGTCTGGTCGGTAACA CTTGGTTGTGGCCGACCAACCAAGCAACCGCAATAAGTTCCGGAGTGGTCAAAA ACTGGAATGTGAACCTTACAAGCTGGTGGCAAGTATTCAAGCATCAGTTTCG GTGGTGGCGTTCTGGGGAGCGGGCTCTGGTGGCGGCGGAAGCATCGAAAAAC GCTGACAAAGCGATCAAGGACTTCCAGGATAACAAGCGCCTCACGATAAGTCT GCTGCATACGAGGCTAATTCTAACTGCCTAAGGACCTTCGGGACAAAGAATAAC AGATTCTGTTGAAAAGGTGTCATCGAGAGAGGCCATTGTGAGGCACGACGACGC GTTAAGAGCGCCAAATGATCGGATCAGTAACTGAACGAAAAAGATTCCATTGAG AACCGACGCTGGCTCAGCGGGAGGTGAACAAGCACCTATGGACGTCAAGGA GCACCTCCAGAAAGCAGCTGGAC ¹ taatgatagaccagcccaagaacacccgaatggagctctaaagctacataa taccacattacactttacaaaatgtgtcccccacaaatgtagccattctgtctcctaataaaaagaagtttcttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.3	swapped ssp for PPa_v2 without SIP sites, mmu.1604 = ADC47454. 1, adhesin- like protein with transglutami- nase domain [Methanobr evibacter ruminantiu m M1], amino acid	MRFPSIFTAVLFAASSALAMPLSGGGSGGSMPLNTTTEDETAQIPAEAVIGYL DLEGDFDVAVLPEFSNTNNGLLFINFTIASIAAKEGVSAASNASDNLDDLTISDSN SLDLVSTNSDILSSDSGVSSDDSSNDASGDVLSGVSSNESNNQSQTLDSSNNQSQS GLDSDNSTLDSQSNQNSSESSDSSDSETVIKNATISVSSKTVVRGNSLNITLKDN ASTLLSNKTVTFIFNGKTYNKTINAKGIASLTLTATPKKYLVKIAFVGDLEYEASSK SVNVTLSKTPTSISNSGKSVIRGKLYKLTLDKAKGKALSGKKISIFNGKKYTKTTNS NGQVNLITNVNVGKTYKMYKFAGDSNYLSSSGSVSIKVKMGTSIIHSGSSIVKGS YTVTLKNANGAVLSNQKIAFTLSGKTYNRTTNAGQASLKIGLSSGKTYNLTYYKA GNSYYGGSSGKVSLEVKTPPTTMKNSGKTIVSGETKYVTLKDADGKSLANKKVSITF NNKTYAKTTNSNGQASLTIGTIFGRSYPLSYKFAGDSKYGPSSGSLCLRVKKATSLK GSASSIVQGKSYTVTLKDSNSTPLANQIVFTLDTKKYNRTTNAGQASLKIGLAAG KTYNLAAYKYSGTSYNGSGSVKLVKFPTSLTNSGKSVMNGTGYNIVLKDSKSNL VSNKTISIGFNGKTYDEHTDANGTVTLIDANVPKTYKMTYKFAGDSYDGASSGTVN LTVKFKNAFTISQIISASSSLKSYVLKNNKVPATVSVNGVSLNLTSTYLMKATISIN SNKTSGSILLVPVDSNYTNGSRINANLYKANYIDLAKKVISSAEANKLVPNSVSTNI GLVSHDLYSFGLAKALVFFNSDHYLPNYLILSSDDVGEKHSTVIPSNAARGNASQFKA GLNEAETLTAQAQIAKYLVASGHDATAENSEIKALAAKLVSQKTSWDKANAIFTFARD NITYSYVADSKKGAAGTILSSKSGNCCDHSNLIVSLCRAANITARFSHAQGCFTSSGL VAGHVWAQIYIDGVWYTADATSRRLNSLGNIVNWNINHYNTLKQYDHLSTFGGGGS GGGGSGGGGSIENADK4IKDFQDNK4PHDKS44YEANSKI PKDLRDKNNRFVEKVSIEK AIVRHDERVK SANDAISKI NEKDSIENRRLAQREVNKAPMDYKEHLOKQLD	SEQ ID NO.: 75

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.3	swapped ssp for PPa_v2 without SIP sites, mmu1604 = ADC47454. 1, adhesin- like protein with transglutami- nase domain [Methanobr evibacter ruminantiu m M1], nucleotide	ctgtgtcttttgcagaagctcagaataaacgctcaactttggccaccATGCGCTTCCCTAGTATCTTCAACCGCC GTGC'TG'ITCGCT'GCGCT'CTTCT'GCGCT'GCGCATGCCCT'CTGCT'GCGGAGGCTCTG GGGGCTCAGGTTCTATGCTCTTCAACACGACGACGAGGAGCAGACCGCCAGAG TACCTGCCGAGGCCGTGATCGGGTACCTCGACCTCGAAGGTGACTTTGACGTCG CGGTGCTCCCTTTCTCTAACTCTTACAAAACACGGCTTGTCTGTTTCATCAACACCCAC GATCGCGTCAATCGCGGCAAGGAGGAGGGGCTCTCTGCCGCTCCCAATGCTC CGATAACCTGGACGATTGACCAATTAGCGACAGTAACCTCCCTGGATCTTGTGAG CACTCTAACTCTGACATACTGTCTTCTGACTCTGGCGTCTCTTCTGACGACTCTA GTAAAGACGCCAGTGGAGACGTCTCTGGCGAGTGTGTCATCTAAACGAGTCCA ACAA'CCAGTCCAGTCTACCTCGACTCTAACAAACCAAGTCTCAAGTCTGGTCTCGA CTCTGACAACTTCTACCTCTCTGACTCAACAGTCTAACAAACAGAGCAACTCTGAG TCTTCTGACTCTAGGACTCTTTCAGAGACTGTGATCAAGAAATGCAACCTCTATCT CTGTGAGCTCTAAGACCTGTCTGGGGGAACTCCCTGAAACATCACCTGAAAGG ACAAACGCTTCAACCTGTCTTCCAAACAAACCGTCACTTACGTTTCAATGGAAA GACTTACAAACAAGACACCAACCGCAAGGGATCGCTCTCTGACCTGACTGC CACCTTAAGAGTACCTTGTGAAGATCGCGTTCGTCGGGACGAGCTGTACGA GGCGTCTAGCAAGTCTGTGAACGTCACTCCCTTTCTAAGACGCTTACAAGCATATCT AACTCTGGCAAAAGCATCGTGCAGGGAAGCTGTACAAGTCAAGCTCAAGGAC GCCAAGGGAAAGCCCTGTCTGGAAAGAAAGATCTCTATCTCTTCAACGGCAAG AAGTACACCAAGACCAACCAAGTAACGGTCAAGTTAACCTGACGATCAACGTG AACGTGGGCAAGACATATAAGATGACCTACAAGTCAAGATGGGACCTCTATTATCG CTGTATCTAGTGGCAGGTGTCTATCAAGGTCAAAATGGGACCTCTATTATCG GGTCTGGGTCTTCTATCGTTAAGGGTAAGTCAATACCGTCACTCAAGAAACG CCAAACGGGGCCGTGTCTAACAGCAAGGGCCAGGCTCCCTGAAGATCGGGCTGT CGTACAACAGAACGACCAACCGCAAGGGTACAAAGTACCGCGGGAACCTTACTACG CTTCTGGGAAGACCTACAACCTGACGTACAAGTACCGCGGGAACCTTACTACG GAGGGAGTCTGGCAAGGTGTCTGTCTGTCAGAACCCCTACCCACAATGAAGA ACTCTGGGAAGACCATCGTCTCTGGCGAGACCTACAAGTGAACCTCAAGGACG CCGACGGCAAGAGCTTGCAAAACAAGAGGTGTCAATCACTTCAACAATAAGA CCTACGCCAAGACGACCAACAGTAACGGGACGGCTCTCTGACGATTAAAGGAA CCTTCGGACGCTCTTATCCTCTGTCTTACAAGTTGGGGGACAGCAAGTACGG	SEQ ID NO. SEQ ID NO.: 76

Cluster	Description	Sequence	SEQ ID NO.
		GCCTTCCAGTGGCAGCCTGTGTCTGCGGTCAAGAAGGCGACGAGCCTCAAGGG GTCGTGCTCTCTATCGIGCAGGGCAAGTCTTACACCGTGACTCTGAAGGACTCA AACAGTACGCTCTCGCCAAACCAAGACCATAGTGTTCACCTCTTGACACCAAGAAG TACAACAGGACCAACGCCAAGGCCAGGCTCTTTGAAGATCGGGCTGGCC GCCGGAAGACCTACAACCTGGCTACAAAGTACTCTGGACCTCTTACTACAAAC GGCTCAAGTGGGTCAAGTGAAGCTGAAGTTAAAGTTCCCTACCTCTCTCAACCAACT CCGGAAGTCTGTGATGAACGGCACCGGTACAACAATGTCCTCAAGGACTCTA AGAGCAACCTGGTTTCTAATAAAGACCATCTCCATCGGCTTCAACGGCAAGACGT ACGACGAGATCACCGATGCGAACGGCACCGTTACCTCTCTCATCGACGCTAAACG TCCCTAAGACGTACAAGATGACTTACAAGTTCCGGGGGACTCCGACTACGGGG CGTCTAGCGGGACAGTGAACCTCACTGTGAAATTCAGAAGCGCTTACGATCA GTCAGATCATTAGTGCCAGCTCTTCTGTGAAGTCTTACGTGCTCAAGAACAAGAA GGTGCTGCCACGGTGTGAGTCAATGGCGTGTCTCTGAACCTGACCACTTTCACC TACCTGATGGCGAAGCAACCATCTCCATTAACCTTAACAAGACCTCTGGCTCTA TACTGCTCGTGTGCTGACTCAAACTACACCAACAACGGCTCTCGGATCAACG CCAACTGTACAAGGCCAATTACATCGACCTGGCCAGAAGGTCACTCTTCTGTC TGAGGCCAACAAATTGGTGCTAACAGCGTGTCCACAACAATCGGGCTGGTGTCT TCACGACTTGACTCTCGGACTCGCCAAAGCGTTGGTCTTCTTCAACAGTGAC CACTACTTGCTAATACTACCTGATCTTCTTCTGACGACGTCGGGGAGAAACACT CTACGGTGATCCCTTCTAACGCTCGGGCAACGCATCTCAGTTCAAGCGGGCTT CAACGAGGCCGAGACTCTACAGCCGCAACAATCGCCAAATATCTGGTGCGGTCT TGGGCACGACGCCACGAATCTGAGATCAAGGCCCTGGCCGCCAAGCTGGTGTCT AGGCAAGACCTCTCTGTGGGACAAAGGCAACGCCATTTTCACTTTGCTCGCGA CAACATCACCTACAGTTATTACGCCGACAGTAAGAAGGCCGCGCTGGCACCTT GAGTCAAAATCTGGCAACTGTGTGCGAACCTCTAACCTTATCGTGTCTCTGTGC CGGCCGCCAACATAACGCCCGGTCTCTACGCCACAGGGGTGCACATTTCTCTT CTGGGTGGTGGCCGCCACGTCTGGGCACAGATCTACATCGACGGCGTGTGT ACACTGCCGACGCCACGTCTAGGCGCACTCCCTTGGTAACATCGTGAATTGGA ACACCAACCACTATAACACTCTGAAGCAGTACGACCACTGTCTTTCGGCGGCG GCGGTTCTGGCGGAGGTGTTCTGGCGGCGGGGCTCTATCGAAACGCCGACA AGGCCATCAAGACTCCAGGATAACAAGGCACCTCAGCAAGTCAAGCGGCT	

Cluster	Description	Sequence	SEQ ID NO.
		ACGAGGGCTAACTCTAAGCTGCCCTAAGGACCTCCGGACAAGAACAACCGCTTCG TTGAGAAAGGTGTCCTATCGAGAAAGGCTATCGTTAGACACGACGAGCGGGTGAAGT CTGCTAACGACGCGATCTCAAAGCTGAACGAGAGGACTCTATCGAAAAACCGGC GGCTGGCCCAAGCGAGAGGTGAACAAGGCCCTATGGACGTGAAGGAGCATCTC CAGAAAGCAGCTGGACtaatgataagaccagcctcaagaacacccgaatggagtccttaagctacataataaccaacttaca ctttacaaatgtgtcccccataatgtagccattcgtatctgctccttaataaaaaagaaggttttcacattct	

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.4	swapped ssp for PPa_v2 without SIP sites, mmu1499 = ADC47349. 1, adhesin- like protein with transglutami nase domain [Methanobr evibacter ruminantiu m M1, amino acid	MREPSIFTAVLFAASSALAMPLSGGGSGGSMPLNTTTEDETAQIPAEAVIGYL DLEGDFDVAVLPEFSNTNNGLLFINFTIASIAAKEGVSAIDIDEASSSDLSDDSI SNDYLVANSGLDSSVASSASSIAADSDLSNASSNNFENEVLSTNNNEDTESEI VKDSKNQLSSSLQASTKTKTLKGGSSVYRGPNPYVLTDSNGKVLASQKVTFNI LGKNYTRTIDSKGVASININLAKGKYNIACLYAGTENYASSKLSVALTVNLMSTKIN TGGSTVKKGNAYSVTLIDGNGKALSSQKVTLNIGKNYTRTIDSKGVASIAINLAA GKKFTLTASYAGSANYLSSKVSATVTVQKGDTSIKPSGTSIVKGNYSFTILVDGSGK GLANQKVAIKISGKSYSTRTTNSNGVASIAINLAAGKKYSIVCSYAGSSNYKASSSTVS LSVTNPSTNSKTFSIAKIEAATNLKAYVNKNKAVPTTVSVGGTNLKISEFSYLMASK AIVNLNSNNTNAITLPSGIYNGASNSLNATVYKAQYVDLSKRVVNYIDKNKVP AYGTVYNANGASLGNAGFNLYTFATAKILDFHKTNKYLPNYCSFDSVFKASNGSS SSNSSSTNSSSTNSSSGSSNSGSSSTPAVTVKATSLKAAASTSVIRGDDYSVTLTD SSGNALANQKITFALSSSSYTRTTNSKGVASLTNLNLAGKYSITTSYAGTSA YKASKL TNTVTISNSSRRFFLNDIETA AENVKTYVTKNKALPNTVTVAGTQLTILSQFSYVMAK AIHNINASNSNYISLKSVAASNSTGDYLDTTVYRAQYMNLNTRVISFVESDKITPTFA TVYNSNGKSVGKA EFKLYTFATAKILAFYKTNNYLPTYCTFQSSAIGVVPDVATNVT INSKINANMNQFKVGLNEKNTVSNLSAYLVGTGQSTHTTNKNVAAQLTKGLNSTAT KALAIYNEVRDDISYYSYSDSRKGADGTLSSSGGNCVDQASLVVALCRAAGIPARYS HAQGCTFSSGLVTGHVWAQILVDGVWYSADATSVRNSLGNIVNWNNTNSYHSMKQ YAAVPFEGGGGGGGGGGSIENADKAHKDFQDNKAPHDKS44YEANSKLPKDLRDK NNRFVEKTSIEKAIVRHDERVKSANDAI SKLNEKDSIENRRRLAQREVNKAPMDVKEHLQK QLD	SEQ ID NO.: 77

Cluster	Description	Sequence	SEQ ID NO.
PPa2delS 1P-C8.4	swapped ssp for PPa_v2 without SIP sites, mnu1499 = ADC47349. 1, adhesin- like protein with transglutami nase domain [Methanobr evibacter ruminantiu m M1, nucleotide	ctgtctcttttgcagaagctcagaataaacgctcaactttggccaccATGCGCTTCCCTTCAATCTTCACAGCC GTCTGTTCGCGGCCCTCTCTGCCCCCTGCTATGCCCTTGTCTGGCGGGCTCTGG CGGGTCCGGCTCAATGCTCTCAACACGACACAGACAGAGAGAGAGAGAGAGAGAGAGAGAT CCCTGCGAGGGGTTATCGGATATCTGGACCTGGAGGGAGATTTCGACGTGGC GGTGTGCTCTTTCAGCAACAGCACTAACACACGGGCTCTCTGTCAATCAACACCCAC CATCGCTCTATTGACGCAAGGAGGAGGGGCTGTCTGCCGCTAGACATCGA CGAGGCTCTCTCTCTGTGATCTCTGTGACTCTTCTATCTCTAACGACTACTTGG TGGCCAACTCTGGCGACGACTCCGTGCGGTCTTCTTCTGCGAGTTCTAGTATCGC CGCAGACGACTCAGACCTCAGTAACAACGGTCTCTTCTTAACGTCAACTTCGAG AACGAGGTCTCTCTACAACAACAATGAGGACACCGAGAGTGAATCGTCAA GGACAGTAAGAACCAGCTGAGCTCTTCCAGCCTGCAAGCTAGCAAGAGACCAA AACTACCCCTTAAGGGCTCCGGGTCTTCTGTGTACCGGGTAACCTTACTACGGIG ACTCTGACTGACTTAACGGCAAGGTGCTGCGCAGCCAGAGAGTCACTTCAAC ATCTTGGGGAAGAACTACACTAGAACGACCGACTCTAAGGGCGTGGCCAGTATC AACATCAACCTTGCCAAAGGCAAGTACAACATTTGCTGCTTCACTGCGGGGACG GAGAACTAGCCTCTCTAAACTCTCAGTGGCTCTACCGTGAACCTGATGAGC ACTAAGATCAATACGGGGGTCTTACCGTCAAGAGGGCAACGCTACTCTGTG ACCTTGACAGACGGCAACGGCAAGGCAATTTGTCTAGCCAGAGTCACTTGAAC ATTCTGGGGAAGAACTACACCCGGGACCCAGACAGTAAGGGCGTGGCGTCAATC GCCATAAACCTGGCGGCGGTAAAGAGTTCACTCTGACCCGCTCTACGCGCGG TCTGCCAACTACCTGTCTTCCAGGTGTCAAGCCACCGTTACGGTGCAGAAAGGGC GACACATCTATCAAGCCTTCTGGCAACCTCAATCGTCAAGGGGAATTCATCTCTT TCACGCTTGTGGACGGCTCTGGGAAGGGCTGGCCAAACCAAGGTGCGCATCA AGATCAGCGGGAAGTCTTACTCCCGGACCCAGCAATTCAAACGGGTGGCTTCCA TCGCCATCAACCTGGCCCGCGGGAAGAGTACTCAATCGTGTGAGTTACGGG GCTCTTCTAACTACAAGGCTCTTCAAGCACCGTGTCTGTCTGTGACGAACCC TAGTACCAACTCTAAGACATTTAGTATCGGAAGATCGAGGGCCGAGCGAACCA CCTGAAGGCTTACGTGAACAAGAATAAGGCAGTGGCTACCAAGTGTGTGGG GGCACCAACCTGAAGATATCTGAGTCTCTTACCTGATGATAAGGCCATCGT CAACCTGAACCTCAACAACACCAACCGGATCAGCTGCTTCTGGAACTCAAA CGGGGCTCTGCTCTAACTCTCTCAATGCCACCGTGTACAGGCCAGTACGTC	SEQ ID NO. SEQ ID NO.: 78

Cluster	Description	Sequence	SEQ ID NO.
		GACCTGTCTAAGAGGGTCTATAAATACTACATCGACAAAGAACAAGGTGGCTGCCGCC TACGGCACAGTGTACAAACGCCAACGGGCCCTCTCGGGCAACGCAGGCTTCAAC CTGTACACGTTCGCCCTCGCAAGATCCTGGACTTCCACAAAGACTAATAAATATT TGCCTAATTACTGTCTTTTCGATTCTTCAGTGTTCAAGGCCTCTAACGGCTCTTCA TCTTCAAACTCTTCTAGCAGTACCAAACTCTTCTTCTTACCAAACTCTAGTTCTGG GAGTCCAACTCTTCTGGATCTGGCTCTCTACCCCTGCCGTGCCGTGAAGGCC ACCACTTTAAGGCAGCTTCTACCTCTGTCTATCCGGGGCAGCAGTACTCAGTGA CCCTGACCGACAGCAGCGGGAACGCCCTGGCCAAACCAGAAATTACGTTTGGCTC TGCTTCTTCTTCTACACAGGACAAAGAACTTAAGGGGTTGCCCTCTCTGAC CCTCAACCTTGGGGCGCAAGTATTCTCATCACCACTTACGCCGGGACCTCA GGGTACAAGGCCAGTAACTGACCAACACCGTCAACCATCAGCAACTCTCTTCT CGATTCTTCTCAACGACATCGAGACCGCAGCCGAGAACGTGAACACGTACGTC ACCAAGAAACAAGCGTTGCCCTAACACCGTACTGTGCGGGACCCAGCTGACG CTCTCTCAGTTCTTATGTGATGGCCAAAGCGATCCACAAACATCAACGCGTCTA ACTTAACATACTCCCTGAAGTCTGTGGCTTCACTAACTCCACCGGAGACTA CCTGGACACCACTGTTTACCGCGCCAGTACATGAACCTGACCAACCGAGTGAT CAGCTTCGTTGAGTCTGACAAGATCACCCCTACCTTCGCAACGGTCTACAACCTCT AACGGAAAGTCTGTCGGCAAGGCCGAGTTCAAACCTCTATACGTTCCGCTTCGCC AAGATTCTCGGTTCTACAGACGAACAACCTACCTTCTACCTACTGCACATTC AGAGTTCTGCCATCGGGCTGCTGCTGACGTGCGCCACCAACGTGACCATCAACT CTAAGATCAACGCCAACATGAACCAAGTTCAAGTGGGTCTCAACGAAAAGAAC ACCGTCTCTAACTTTCTGCTACCTGGTGGGACCGGCCAGTCTACCATCACAA CCAAATATTAAAGATGTGGCCGCCAGCTCACGAAGGGCTCAACAGTACCGCCA CCAAAGGCCCTCGCCATATACAACTTCGTCCGCGACGACATCTTTACAGTTACTA CTCTGACTCTCGGAAGGTGCCGACGGTACCCCTTCTTCTGGGTCTGGCAACTGT GTGGACCAAGCCCTACTGGTTGTGGCCCTGTGCCGGGCCCGGGAATACCTGCC CGCTACTCTACGCCAGGGCTGCACCTTCTCTTCTGGGTGGTCAACGGGCCACG TGTGGGCCAGATCCTGTGGACGGCGTTTGGTACTCTGCAGACGCTACGAGCG TGCGGAACAGCTTGGGAACATCGTGAACCTGGAACACTAACTTACCACCTTA TGAAACAGTACGCCCGCTGCTTTTGGTGGAGGGGTTCCGGAGCGCGCGGT CTGGGGCGGTGGCAGCATCGAAGACGTGACAGGCCATCAAGGACTTCCAG	

Cluster	Description	Sequence	SEQ ID NO.
		GACAACAAGGCACCTCACGATAAAGTCTGCAGCCTACGAGGCCAACTCTAAACTC CCTAAGGACCTCCCGGACAAAACAACCGCTTCGTGGAGAAGGTCAGTATCGAG AAGGCAATCGTTCCGCCACGACGAGCGGGTGAAGTCAGCCAACGACGCGATATCT AAGCTGAACGAGAAAGGACTCTATTCGAAAACCGGCGCCTGGCTCAGAGAGAGGT GAACAAGGCCCTATGGATGTGAAGGAGCATCTGCAGAAAGCAGCTGGAClaaigala gaccagcccaagacacccgaatggagtcctctaaagctacataataccacacttacatfiacaaaatggtcccccaaaatgagccat tcgtatctgcctcctaataaaaagaagttttcacattct	

[illegible]

Cluster	Description	Sequence	SEQ ID NO.
		AGGCACCTCAGACAAAGTCTGCCGCATATGAAGCGAACTCCAAACTTCCTAAGG ACCTGAGAGACAAAATAATCGGTTTGTCGAGAAGGTTTCCATCGAGAAGGCTA TAGTGAGACATGATGAACGAGTTAAAAGCGCCAACGATGCTATCAGTAAAGCTGA ACGAGAAGGATAGCATCGAGAAATAGACGCCCTTGCGCAGCGGAGGTCAACAAA GCCCCATATGATGTGAAGGAGCACCTGCAGAAACAGCTGGACtaatgatagaccagccica agaacacccgaatggagictaagtacataataaccaactacacttcaaaaatgtgtccccaaaatgtagccattcgtatcgtc ctaataaaaagaagttttcacatc	
C9.2	M2.WP...01 1954062.1, Methanobre vibacter smithii 2173, amino acid	MFVFLVLLPLVSSAAYQTANQVVPGTSDAMWDSMVWINENTADNIVVASWWDF GYLFEIAADRRQVIFDGGSGSGNSRAFWLQGAMTTDNMDLSAGIFRMLGTSGENATN TLTDYTGSPGKATDILIDILPKNAQDAKNAALINTYGLTTEQANTIPLTHPDNPRPVIF VASSDMLQKAGWWSYFGAWDFDKQNSTNFNYVPSQQVTVEPGSTGRLALINESG LEYDAVITRGTGNNTTTGHTEA VYSNNGSLLKINGSEFNPLNVSRIIMVIEGNQLVKN ESIAGAPSDSNYTLFLMGENNVTPIIMHNKLADSMFTRL YLLGGMGQNVFSMVHM ENGVS LWQVNYNNTAAGGTSTPAASGATDGNIGTTS PDRA GGGGSGGGGGGGSI ENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSA NDAISKLINEKDSIENRRLLAQREYNKAPMDYKEHLQKQLD	SEQ ID NO.: 81

Cluster	Description	Sequence	SEQ ID NO.
C9.2	M2_WP_01 1954062.1, Methanobre vibacter smithii 2173, nucleotide	ctgtctctttttgcagaagctcagaataaaacgtcaactttggccaccATGTTTGTGTTCTCTGGTCTCTTCTGCCT CTCGTCAAGTAGCGCTGCCATATCAAACTGCCAAATCAGGIGGTTCCTGGCACTCCTG ACGCTATGTGGGATTCTATGGTGTGATTAAAGGAGAACACGCGCCGACAAACACGG TGGTGGCCTCATGGTGGGATTTCCGGTACCTGTTTGAGATTGCCGCCGACCGACA GGTTATTTTCGATGGGGCTCCAGAGCGGAAATTCAGGGCTTTTTCGGCTCGGG CAGGCTATGACCACGGACAATATGGACCTCTCAGCCGGCATTTTCCGGATGCTC GGTACTTCTGGGAAACGCCACTAACACCTGACAGATTATACCGGCTCACCT GGTAAGGCGACTGATATATACTTATCGACATTCTGCCTAATAATGCAAGACGCC AAGAAATGCCCTTGATCAACACATATGGCTGACCAAGAGCAGGCAATAACATC ATTCTTTGACACACCTTGATAATCTAGGCTGTCTCTTCGTCGCCAGCTCCG ACATGCTTCAAAAGGAGGCTGGTGGTCACTTCGGCGCTTGGGATTTTGACA AACAAAATAGTACGAATTTTAATTACTATGTGCTTCTCAGCAGGTTACCGTGGA GCCTGGAAGCACGGGGCTTGGCACTGATAAATGAAAGTGGCTCGAGTATGA TGCAGTCAATCACTAGGGTACCGGGAAACACACACTACCGGCCATACTGAGGC AGTGTACTCCAACAACGGCAGCTGCTGAAAATCAACGGGTCCGAATTTAAACCC TCTGAACGCTCCCGAATAATGGTGAATGAGGGTAACCAAGCTGGTGAAGAACGA AAGCATCGGGAGCACCTAGCGACAGTAATTATACACTGTTCTTGTATGGGTGA AAACAACGTGTACACACCTATCATCATGCAATACAACTTCGGGACAGCATGTT CACCCGCTGTACCTGTGGGGGGATGGGACAGAACGTTTCTCTATGTTCCAC ATGGAAAACGGAGTCTCACTGTGGCAGGTGAACATAATAACAGCCCGCGT GGCACCTCCACACCTGGGCTTCGGGAGCAACCGATGGCAATATTGGCACCACT TCACCTGATCGCGCTGGTGGGGAGGCTCTGGGGGAGGAGGATCCGGAGGGGG CGGGTCCATAGAGAAATGCTGATAAGGCTATTAGGACTTTCAGGACAAACAAGC CCCTCATGACAAGTCTGCTGCATACGAAGCGAACAGCAAGCTCCCTAAGGATCT TAGAGACAAGAATAACCGGTTCTGTGGAAAAGTTAGTATCGAAAAGGCCATCGT GCGCCACGACGAGCGGGTCAAGAGCGCCAACGACGCTATCAGCAAACTCAATG AGAAGGACAGTATCGAGAACAGACGGCTCGCCCCAGAGAGGTTGAACAAGGCC CCTATGGATGTGAAAAGAGCACCTTCAGAAAACAGTTGGACaatatagaccagcctcaagac acccgaatggagtcctctaaagctacataataaccaacttacacitttaaaaaatggttcccccaaaatgtagccattgtatctgctcctaata aaaagaaaagtcttcttcacattct	SEQ ID NO.: 82

Cluster	Description	Sequence	SEQ ID NO.
C9.3A	M3 WP_04 2693613.1, Methanobre vibacter oralis 66851, amino acid	MFVFLVLLPLVSSAAAYITSENVIPGTSDPMWNSMEWINEHTSNDTIVTSWWDFGYL FEIAADQRQVTFDGGSQIGSRAFWLQQAUTTDNLELSAGIFRMLDTSGERAVDALIN YTGDTGKTTKILIDILPMTKQNAQKTLIDKYDLSTDQAKEIVGYTHPSKPRPVIFVAS SDMLQKAGWWSYFGAWDFKNQSSSENYVYVPTQVKVESGSSGKLSLIVDGGMT VNAVITRIGIGNNTTSAYTEAVYTHNGSQIYVVKTVYNPLNISNLIVVEDGYLMKNE SVGNVKDANYTLFLMGEKNKYTPILISNKLANSMTKLYLLGGAGQDIFTNVHTEE GVMLWQVNFNNTVAGKGGGSGGGGGGSIENADKAIKDFQDNK4PHDKS44YE ANSKLPKDLRDKNNRFVEKVSIEK41VRHDERIKSANDAIKLEKDSIENRRLAQREYNK APMDI'KEHLQKQLD	SEQ ID NO.: 83
C9.3A	M3 WP_04 2693613.1, Methanobre vibacter oralis 66851, nucleotide	ctgtctttttgcagaagcagataaagcctaactttggccaccATGTTGCTCTTCTGGTGCTGCTTCCT TTGGTGAGTAGCGCCGCTTATATACATCTCTGAGAACGTTATTCCTGGTACATCTG ACCCTATGTGGAATTCATGGAGTGGATCAACGAACATACATAGTAAACGACACTG TTATCACCTCTTGGTGGGACTTTGGGTACCTCTTCGAGATCGCGGCAGACAGACA AGTGACCTTCGATGGCGGAGCCAAACAGGAGCAGGGCATTCCTGGTGGGCCA GGCCATGACTACTGATAACCTCGAGCTCAGCGCAGGTATCTTTCGCATGCTCGAT ACGAGTGGGAACGGGCCGTGGACGCCCTGATAAACTATACGGGAGACACCCGG CAAAACAACCAAGATCTTGATTGACATCCTGCCTATGACGAACACAGAACGCCCA GAAACTCTGATTGATAAGTACGATCTGAGTACAGATCAGGCCAAAGAGATTGT CGGTACACACATCTAGCAAGCCTAGGCTGTATATTTGTCGCTTCTAGCGAC ATGCTGCAGAGGCGGGTGGTGGTCACTCTTGGAGCCTGGGATTTCAAAAAC CAGAGTTCAGAAAATACAACTACTATGTGCTACAGAACAAAGTCAAGGTGGAG TCCGGATCATCTGGCAAGCTTTCCTGATCGTTGATGGCGCATGACCGTGAACG CTGTATCACCCCGGCCACCGCAACAATACCAACAGTGCCTACACTGAGGCTG TGATACCCATAATGGGTCCAGATCTATGTGAATAAGACCGTTTATAATCCTCT GAATATCTCAAATCTTATTTGCTGGAGGACGGTACCTGATGAAGAATGAGAG CGTGGTAACGTGAAGGACGCTAATTACACCTGTCTTGTGATGGCGGAAAGAA CAATATACGCTTACTGATCTCCAAACAAGTGGCCAACTCTATGTTACCAAG CTGTACCTCCTCGGAGGAGTGGCCAGGACATTTTCAAAAATGTCCACACAGAG GAAAGGAGTGATGCTCTGGCAGGTGAACCTTCAACAACACGGTGGCAGGAAAGG GGCGGAGGCTCTGTGGAGGGGGGAGCGGGGTGGGGATCAATTGAGAACG CCGACAAGGCGATTAGGATTTTCAGGATAATAAGCACCTCAGGATAAATCCG	SEQ ID NO.: 84

Cluster	Description	Sequence	SEQ ID NO.
C9.3B	M3_WP_04 2693055.1, Methanobrevibacter oralis 66851, amino acid	CTGCTTACGAGGCCAACAGCAAGCTTCTCTAAAGACCTCCGAGATAAAAACAATC GGTTTGTCGAAAAGGTCCTCATCGAAAAAGCCATCGTCGGCAGCAGAGAGAG TCAAAGTCGCGAACGACGCCATAAGCAAGCTGAATGAGAAAGACTCCATTGAA AATCGCAGACTGGCACAGAGGGAAGTGAACAGGCCCTATGGATGTGTGAAGGA GCACCTTCAGAAAGCAATTGGAClaatgalagaccagcccaagaacacccgaatggagcctctaaagctacataat accaacttacactttacaaaatgtgtgtcccccataatgtgccattcgtatcgtctcctaataaaaagaagttcttcacattct	SEQ ID NO.: 85
C9.3B	M3_WP_04 2693055.1, Methanobrevibacter oralis 66851, amino acid	MFVFLVLLPLVSSAASLIDTIDTINRYDHYTDTTMEINNFNDNTIVYSIEHEHFRFH EQLKGTNEFLVPKNRNLFPNDYNLSVMPIDKIVNENPHKDIYILRIQKSNENFGEG IKVNKSAIYICPSFVKLEKINGGGGGGGGGSGGGGSIENADK4IKDFQDNKAPHDKSAA YEANSKLPKDLRDKNRRFVEKVSIEKAI/RHDER/VKSANDAISKLEKDSIENRRLAQREV NKAPMDVKEHLQKQLD	SEQ ID NO.: 86

Cluster	Description	Sequence	SEQ ID NO.
C9_4A	M4_WP_04 2706503.1, Methanomic robium mobile 2205, amino acid	AAAGCGCGCTACGAAGCAAACTCCAAGCTGCCTAAGGATCTGCGAGATAAG AACAAATAGATTCTGAAAAGGTGAGCATCGAGAAAGCAATCTGCGGCAATGA TGAACGGGTGAAGAGCGCTAATGATGCCATCTCCAAACTCAATGAGAAGGATAG TATTGAGAACCGCCGCTGGCACAGAGGGAGGTTAACAAAGGCCCTATGGACGT GAAGGAACACTTGCAGAAAGCAGCTGGACtaagatagaccgcctcaagaacacccgaatggagctcta agctacataataaccaacttacactttacaaaatgttgcccccaaaatgagccattgctatctgctcttaataaaaagaaagtcttcacata ttct MFVFLVLLPLVSSAAAYFMGYTTYTKNDWRGFSQQLSSIINEGDYVVVMPDYITK PLNYYSNSTDGTIEVGASSAATLEQINAKRLNSTGNPAAYYIITWDISAANPSGDAL DWISKNAQFIGQNMGIYVFRSAGGGGGGGGGGSIENADKAIKDFQDNKAPHDK SAAYEANSKIPKDLRDKNNRFYEKVSIEKAIVRHDERVKSANDAISKLINEKDSIENRRIAQ REVNKAPMDI/KEHLQKQLD	SEQ ID NO.: 87
C9_4A	M4_WP_04 2706503.1, Methanomic robium mobile 2205, nucleotide	ctgtcttttgcagaagctcagaataaagcicaactttggccaccATGTTCTGTGTTCTCTGTTCTTCTGCCT TTGGTCAGCTCCGCCGCCCTATTTCTATGGTTACTACACCACCTACACCAAAAACG ATTGGCGCGGTTTCAGTGGGCAACTGTCTATCCATCACAAAAGAGGGAGACTACG TGGTGGTTATGCTGTACTATATCATAAGCCCTTTAACTATTATTATTCTAACAG CACAGCGCACGATTGAAGTGGGCGCTTCTAGCGCAGCCACTCTCGAGCAGAT CAATGCAAAAAGGCTCAACTCTACAGGGAATCTGCGCGTACTACATCATTTAC CTGGGACATTTCCGCCGCTAACCTTAGTGGGATGCCCTCGATTGGATCAGTAA GAATGCACAGTTTATAGGACAAAATATGGGCAATTCAGTGTTCGGAGCGCCGG AGGGGTGTTAGCGCGCGGAGGTTCCGGCGGAGGCTCAATCGAGAAATG CTGATAAGCGGATCAAGGACTTTCAGGACAAATAAGCTCTCTCATGATAAGTCAG CAGCTACGAGGCCAACTCCAACCTGCTAAGGATTTGAGGGACAGAACAATC GCTTTGTCGAAAAGGTGAGCAATTGAGAAAGGCCATAGTCAGACACGATGAACGG GTTAATCCGCAACACGCGGATAAGTAACTGAATGAAAAGGATTCTATCGAG AACCGAAGACTCGCTCAGCGGGAAGTGAACAAGCTCTATGGACGTCAAAGGA GCACTGCAGAACGCTGGACtaagatagaccgcctcaagaacacccgaatggagctcttaagctacataa taccacacttacactttacaaaatgttgcccccaaaatgtagccattgctatctgctcttaataaaaagaaagtcttcacattct	SEQ ID NO.: 88

Cluster	Description	Sequence	SEQ ID NO.
C9.4B	M4 WP_05 2358913.1, Methanomic robium mobile 2205, amino acid	MFVFLVLLPLVSSAAHTSFTTGDIADPIVQVQNSEELREVFPYMDNTTKIAVSNNYVR WPFVWYYQGDYPKRISYSDPGYNKDIPENYDLIIHVDGEKVDSEGFKEKHTYKK CYWIDVNTLIQSYGKPIDIFNANDRALLVEDLKRVIHYFTRDAGLGSINIDVFVKKD RAGGGSGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKIPKDI DRDKNNRF YEKY/SIEKAIH/RHDERVKSANDAISKLENEKDSIENRRRLAQR EV NKAPMDI/KEHLQKQLD	SEQ ID NO.: 89
	M4 WP_05 2358913.1, Methanomic robium mobile 2205, nucleotide	ctgttctttttgcagaagctcagaataaacgctcaacttggccaccATGTTTGTTCTTCCTGGTTCCTGCTGCCT TTGGTGAGCAGCGCCGCTCACACCTCTTTACACAGAGAGACATAGCCGACCCCT ATAGTGCAGGTCCAGAACTCCGAGGAGCTGAGAGAGGTGTTCTCTTACATGGAT AACACTACCAAGATTGCAGTGTCCAAATTACGTGCGGTGGCCTTTCGTGTGTACT ATCAAGGAGACTATCCTAAACGAATCTCTTACTATTCGACCCCTGGCTGACAAATAA GGATATCGGGCTGA AA ACTATGATCTCATATTATCGTCCATGACGGGGA AAAA AGT GGACAGCATCGAGGGGTTTGAGAAACATACGTACAAGAAGTGCTATTGGATTGA CGTCAACACTCTCATCCAGAGTTACGGCA AA CCCTATTGACATCTTCAACCGCA AAA CGACAGGGCCCTGCTGGTGAAGATCTTAAACGGGTGATTCACACTACTTTCAC AAGGACCCAGGGCTCGGATCAATCAACATCGACGTTTTCTGTC AAA AAAGGACCCG GGCTGGAGCGGTGGCTCTGGCGGTGGAGGGTCAGGCGGTGGCGGCAGCATCG AGATTGCTGATAAGGCCATTAAAGGACTTCCAGGACAATAAAGCCCTCACGATA AGAGCGCTCGGTATGAAGCGAACTCTAAGCTCCCTAAAGATCTGCCGACACAAGA ACAAACAGATTGTGGAAAGGTCTCCATCGAGAAAGCAATAGTGAGGCAATGATG AGCGAGTGAAGAGTGCACACGATGCCATTTCAAGCTTAATGAA AAA AGATAGTA TCGAGAAATCGCCGCTTGGCTCAAAGAGAGGTTAATAAGGCCCTATGGATGTTA AGGAACACCTTCAGAAAGCAGCTGGACtaatgatagaccagcctcaagaacaccgaatggagctcttaagc tacataataccaacttacactttacaaaatgtgtgtcccccacaaatgtagccatctgatactgctcttaataaagaaggtttcttcacattct	SEQ ID NO.: 90
C9.4C	M4 WP_04 2705279.1, Methanomic robium mobile	MFVFLVLLPLVSSAAFSATHDIALGNAMKQGGMTPDWEEALSWMGENTPDTGID YYAIYTRDSYENPEEAYGIMTWWDYGHWTIFISKRAPNSNPFQRGVAGPNGAAAYF IQQDEAAASNAILDNLDRYVITDAEMDTAKFWAMCTWYNSSAGTGQYQQTFAMIN PDNSISTGNLYSEKYNTMISRLHNLDGS ME PTSVYYVEYVDGSLYSIPIPIVTDAR EMSYADAKAAVDAAYNANARAGYGAGIYSLSTAPSGEVP AL EHYRLIHESPPFSA ATTLKYVKVFEYVKGAKVKGDGHI DL ESDQGRKFTYSQ RS TDGYFTVPYSTTGNN	SEQ ID NO.: 91

Cluster	Description	Sequence	SEQ ID NO.
	2205, amino acid	YGTKVLGDYRIRGTQQTFKVSEDAVMNGLSVNGGGSGGGSGGGSIENADK4IK DFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSAND4ISKLN EKDSIENRRRLAQREYNKAPMDVKEHLQKQLD	
C9.4C	M4.WP_04 2705279.1, Methanomic robium mobile 2205, nucleotide	ctgtcttttgcagagctcagaataaacgtcaacttggccaccATGTTTIGTGTTCCTCGIGCTCTTGCCCT CTGGTCCTCTTCAGAGCTTTTAGCGCAACACACAGACATTCGCCCTGGGCAATGCTA TGAAACAGGGAGGCATGACACCTGACTGGGAGGAGGCCCTCTCTGGATGGG GAGAACACTCCTGATACGGGAATCGATTACTACGCCATATACACAGGGACTCC TACGAAAACCCCTGAAGAGGCCCTATGGGATTATGACATGGTGGACTACGGTCAC TGGATCACCTTTCATCAGCAACCGGCACCTAACTCTAACCCCTTTTCAAAGAGGC GTCGGGGACCTAATGGCGCTGCCGCTTACTTTATCCAGCAAGATGAAAGCTGCC AGCAACGCCATCTGGACAACCTTGACACTAGATATGTGATTACTGATGCGGAG ATGGACACGGCCAACTTCTGGGCCATGTGCACCTGGTACAATAGCAGTGCCGGA ACAGGCCAGTATCAGCAGAACCTTTGGCCATGATAAACCTTGATAATAGCATCAGT ACGGGGAATTTGTATTCGGAGAAATATTACACACAGATGATAAGCCGCCTGCAT AACCTGGATGTAGCAATGGTCGAGCCTACAAGTGTTTACTATGTGGAGTACGTG GATGGCTCCCTGTACAGTATCCCTATCCCTATCTCACAGATGCTAGGGAGATGT CATATGCGGATGCCAAAGCCGCTGTGGACGCTTACAAACGCCAACGCGAGGGCCG GATATGGAGCCGGTATCTATAGCCTGTCTCTGACCGCACCTTCCGGCGAAGTTCC TGCACTTGAACATTACGGCTCATTCATGAGAGCCCTTCTCACCTTTCTCAGCG GCAACCCCTGAAATATGTTAAAGTGTTCGAGTACGTTAAGGGCGCCAAAGTGT AAGGGGATGGATCATTTGAGATTGACCTTGAAAGTGACCAAGGTGCAAGTTT ACCTATTCTCAGAGATCCACTGACGGCTACTTACCGTGCCTTACTCAACCCACAG GGAACAAATTACGGCACTAAGGTGTGGGGACTACAGGATACGAGGGACCCAA CAGACCTTCAAGGTGTCTGAAGATGCCGTCTGAGGGCGTGGTAGTATTGAAAACGC GGGGCGGAAGTGGGGAGCGGGTCTGGAGGGCGTGGTAGTATTGAAAACGC TGACAAGGCCATTAAGGACTTCCAGGATAATAAGGCTCCTCAGCATAAATCCGC GGCATACGAAGCTAATTCAAACCTGCCTAAGGACCTCCGCGACAAGAATAACCG CTTCGTGGAGAAAGTGAGCATCGAGAAAGCTATTGTGCGGCACGACGAGAGAG TTAAATCAGCAATGACGCAATCTCCAAGCTGAACGAGAAGGACAGCATCGAG AACCGGGCGCTTGCCCGAGCGGAAGTCAATAAGGCCCTTATGGACGTGAAGGA	SEQ ID NO.: 92

Cluster	Description	Sequence	SEQ ID NO.
		ACACCTGCAGAAAGCAGCTCGATTaattagatagaccagcctcaagaacaccccggaatggagctctaaagctacataa taccacitacactttacaaaatgttgcctcccccacaaatgtagccattgctatcgtctccttaataaagaagaagtttcttcacattct	
C9.5	M5.WP...01 1405971.1, Methanosph aera stadtmanae 2317, amino acid	MFVFLVLLPLVSSAAADHLASSQAVGSTNDDMYNLTWTWIKANTSQDTVLASWWD FGHLFTAVADRQVVFDDGGSQNNMRA YWIGNAL TSTDEAKSAGILRMLANSGEDAS NTLDLYTNNTTEKTVEILNAILPMDRTEANSAL TGTVGLSQQEANSVLDLTHPAKVKP VNILSSDMLSKAAWWSYFGSWDFKNQNSHYSYYPQSQNIENINGREFTLGMDNG VIGVSSPTINETNGSTMFAVVDQSKLNKSLNMSSTLEDKQRMSKELSDGTGNTLLKP HKLIIVVENNQLTEKIVNNNSNMIMAIHQNDGSYFTVLFDSHLEESLFTKLYLKSGL NVTRFNMTHSEPGISVWDVSEYANTTANSTNSSTNGTINVQNTSTGGGSGGGGS GGGGSINADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIVRHD ERVKSANDAISKLNKEDSIENRRRLAQRLETNKAPMDVKEHLQKQLD	SEQ ID NO.: 93

[illegible]

Cluster	Description	Sequence	SEQ ID NO.
		<i>HDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSANDAIKSLNEKDSIENRR LAQREYNKAPMDVKEHLQKQLD</i>	
C7.B.4	MBQ22264 34.1 /Methanobr evibacter sp. adhesin variant, amino acid	<i>MFVFLVLLPLVSSAAASKTVFITSNDNIIDTDLQMLNSIKNYIEEISGGELQVIVDNQ APSPGEGWRAIEVTSVSICLAACDAGNFQLAGASTNSDKQIILVNTGDYDLDNQT NFLRRACDDNYSDAYLAGLRDPGTFLKNSGVYGGGGSGGGGSIENADKAIAK DFQDNKAPHDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSANDAIKSLN EKDSIENRR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 100
C7.B.5	MBQ26354 81.1 /Methanobr evibacter sp. adhesin variant, amino acid	<i>MFVFLVLLPLVSSAATKTVELTSDNVIDHDIDLKVLNSLKNYIEEISGGELQVIVDNQ APGPGEGWRAISVTSVSIALAAADAGNFLQLATSTVDINKQIILINIGDYDLDNNSN YLRRAWDDNYSNESLAGIRDPGTFLKNAAGIYVLPVKKEFPNNAHDGILSNYDEEMN RNLAEQIVKLINAHENDTKVLSLSLIVTNKLSPKGMANASKLLVNSDDKEMKGPYG SYSAPQLLYQTSAYLNGDGDIPKEYSEPNPMGISFLVKDTYSIYDYMNMAIVKN YMDENGQAPDSIEYEGAHIGIYDLYNFQKITQNHITDAKHMGESEYHFDKVNDSI LLHIFPFVILFVLLIAVYFLKRRIRFGGGGGGGGGSIENADKAIAKDFQDNKAP HDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSANDAIKSLNEKDSIENRR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 101
C7.B.6	WP_296783 736.1 /Methanobr evibacter sp. adhesin variant, amino acid	<i>MFVFLVLLPLVSSAAAKTVFITSNDNIHDSDVKLLNSLKSYYEELS GGELQVIVDNQ SPAPGEGWRSIEVTSVSDVSLAASDAGNYLQLASSTVNSNKQIVFNIGNYDLDNHT NFLRRAWDDNYSNETLAGMHDPTGLKNAAGIYVQPAKEFPNNVNDGVISNYDEE MNKQJAEIVDIINTHGNDEKTLSDGLVTHNIIKPSIMAKASQELIKSNDKEMKGTYG NYSAPQLLYQTSSYLNGNGLDIPKSYDEPENPMGISFMAKDTYSVYDYFKMGIVR EYMDQNGRAPDSIEYDGAQISYVYDLYNFQKITQSHITDVKHMGESEYHFDKVNDS ILLHIFPFILFVLFVLAFLKRRIRFGGGGGGGGGSIENADKAIAKDFQDNKAP HDKSAAYEANSKLPKDLRDKNRRFVEKVSIEKAIIRHDERVKSANDAIKSLNEKDSIENRR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 102
C7.B.7	MDO58103 90.1 /Methanobr evibacter sp.	<i>MFVFLVLLPLVSSAAAKTVFITSNDNIHDNDIKMLNSLKQYIEEISGGELQVIVDNQ APAPGEGWRAIEVTSVSICLAASDAGNYLQLATATTNSDKQIVFNTGSYDLDNH SNYLRRAWDDNYSNESLAGLHDPGTFLKNAAGIYVQPSODFPDNKADGYLAKYDES MNKQLAEIVDIINKITHENDTTILSDGLITHNIIKPSIMANASKELIKSGDKEMDGTGYN</i>	SEQ ID NO.: 103

Cluster	Description	Sequence	SEQ ID NO.
	adhesin variant, amino acid	YSAPQLLYQTSSYLNGNGLDVPKAYDAPENPMGISFMAKDTYSIYDYFKMGGIVRDYMDQNGRAPDSIEYEGAHISYYDLMYNFAKIQNHNTGKNNMGFESEYHFEDKVNDSILLHIFPFAILFILFLAYLLYKRLRRFGGGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKI PKDLRDKNRRFEKVSIEKAIIRHDERVKSANDAIISKINFEKDSIENRR LAQREVNKAPMDI KEHLQKOLD	
C7.B.8	MBR16107 80.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAATKTVFTSDNIHDDTDIEMLSLKNYIEEISGGELQVIIDNQAPGEGWRAINVTSDVCINLAAADAGNYLQLAKATVYSDKQIVLINTGDDYDLNNTNYLRRAWDDNYSNESLAGIRDPGTFLKNAGIYLYQPVKEFPDHAHDGYLDYDEE MNRKLAEEIVEIVNKHGNDTKILSDSLIVTNKIPPTGMANASKMLVNSEDKEMKGPYGSYSAQQLLYQTSAYLNGDGDIPKEYTEPDHPMGLSFLVRDSYSCYDYMHMAGLDKNYMVGGGGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKI PKDLRDKNRRFEKVSIEKAIIRHDERVKSANDAIISKINFEKDSIENRR LAQREVNKAPMDI KEHLQKOLD	SEQ ID NO.: 104
C7.B.9	MBQ22270 26.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAADNIHDDTDIEMLSLKNYIEEISGGELQVIIDNQAPSPGEGWRAIEVTSVSICLAACDAGNFQIOLAGASTNSDKQIHLVNTGDDYDLNHTNFLRAWDDNYSDAYLAGLRDPGTFLKNSGVVYIQPVKEFPNANEGYIDRYDDEMKNQIAQEIFDIVNGHNDTRIFSDELVSNNIVNPGVMMAKSSQALNSGDNEMKGTGNYTAAQLLYQTSYSLNGNGLDVPKTFSEPDKPMGISFLTRDSYSIYDNFRMGGLVREYMDQN GKAPDSIEYEGAHIGYYDLYNF AKITQNHDTAKHMGFDESEYHFEDKVND SILLHIFP FVHILLVLFVAYWFYKRLRRYGGGGGGGGGGGSIENADKAIKDFQDNKAPHDKS AAYEANSKI PKDLRDKNRRFEKVSIEKAIIRHDERVKSANDAIISKINFEKDSIENRR LAQREVNKAPMDI KEHLQKOLD	SEQ ID NO.: 105
C7.B.10	MBR16105 30.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAAEEMNKQIAQEIIVDINVTNTHENDTKILSDGLITHNIKPAVMANASKALIKSNDTEMKGTGNYTGPQLLYQTSSYLNGNGLDIPKAYDEPEDPMGISFLAKDTYSVYDYFKMGGIVREYMDANGRAPDSIEYQGAHIGYYDLYNF AKITQNHDTTAHMGFESEYHFEDRVNDSILLHIFPILFLVFLFIYFLFKRIRRFGGGGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKI PKDLRDKNRRFEKVSIEKAIIRHDERVKSANDAIISKINFEKDSIENRR LAQREVNKAPMDI KEHLQKOLD	SEQ ID NO.: 106
C7.B.11	ADC47454. 1	MFVFLVLLPLVSSAASNASDNLDDLTISDSNSLDELVTSSNSDILSSDSGVSSDDSSNDASGDVILGSDVSSNESNNQSQSTLDSNNQSQGLSDSDNSTLLDSQSNNSQSNSESSDSS	SEQ ID NO.: 107

Cluster	Description	Sequence	SEQ ID NO.
	/Methanobr evibacter ruminantiu m M1. adhesin-like protein with transglutami nase domain variant, amino acid	DSSEITVKNATSISSVSKTVVRGNSLNTILKDNASTLLSNKTVTFTFNGKTYNKTNA KGIASLTLTATPKKYLVKIAFVGDELYEASSKSVNVLTKPTISISNGKSIVRGKLY KLTLKDAKGKALSGKKISFNGKKYTKTTNSNGQVNLJINYNVGKTYKMTYKFA DSNYLSSSGSVSIKVKMGTSHGSGSSIVKGSYTVTLKNANGAVLSNQKIAFTLSGK TYNRTTNAKGQASLKIGLNSGKTYNLTGYAGNSYGGSSGKVSFLVKPTTMMKNS GKTIVSGETYKVTLKADGKSLANKKVSHFNNTKYAKTTNSNGQASLTIKGTGFRS YPLSYKFAGDSKYGPSSGSLCLRVKKATSLKGSASSIVQKGSYTVTLKDSNSTPLAN QTIVFTLDTKKYNRTTNAKGQASLKIGLAAGKTYNLA KYSGTSY YNGSSGSVKLK VKFPTSLTNSGKSVMNGTGYNIIVLKDSKSNL VSNKTISIGFNGKTYDEITDANGTVT LLIDANVPKTYKMTYKFAGDSKYGPSSGSLCLRVKKATSLKGSASSIVQKGSYTVTLKDSNSTPLAN NKKVPATVSVNGVSNLNTSFTYLMKATISINSNKTSGSILLVPVDSNYTNNNGSRINA NLYKANYIDLAKKVISSAEANKLVPSVSTNIGLVSHDLVSFGLAKALVFFNSDHYL PNYLILSSDDVGEKHSTVPSNARGNASQFKAGLNEAETLTAQAIAKYL VASGHDAT NSEIKALAAKL VSGKTSLWDKANAITFARDNITYSY YADSKKGAAGTILSSKSGNC CDHSNLIVSLCRAANITARFSHAQGCFTSSGLVAGHVWAQIYIDGVWYTADATSR NSLGNIVNWNTNHYNTLKQYDHLSEFGGGGGGGGGGSIENADKA/KDFQDNKA PHDKS44YEANSKLPKDLRDKNNRFEKVSIEK4IIVRHDERVKSAND4ISKLEKDSIENR RLAOREVINKAPMDVKEHLOKOLD	
C7 B 12	WP_292782 159.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAASKTVHTSDNIHQENDVKVLNSIKEIIEISDGELQVIVDNEA PAPGEGYRAIQVTSDISICLSAADGGNYLQLAEASADSDKQIILINIGSYDLNNSNYL RRAWDDNYSNETFMGIHDPGTFLKNAGIYYIQPVVEEFPDNFDDGYLAKYDEEMN KQIAQEIVDIANEHANDTTLMSDNLVVRNTISPAEMAEASKALLNSNDTEMNGEY NYTAPQLLYQTSSYLNGLDMPRSFDA PENPMGISFMVKDSY SIYDYMKMAGIVR NYMDENGQAPDSIEYEGAHIGYYDL VYNFAKITQNHTDAEHMDFEQYKFDKVND SILLKAFPFILILFILFLAYLGYQKIRRFGGGGGGGGGGGSIENADKA/KDFQDNKA PHDKS44YEANSKLPKDLRDKNNRFEKVSIEK4IIVRHDERVKSAND4ISKLEKDSIENR RLAOREVINKAPMDVKEHLOKOLD	SEQ ID NO.: 108
C7 B 13	MBQ68132 41.1 /Methanobr evibacter sp.	MFVFLVLLPLVSSAAMDIDEVGSDDDISDSGVVLESGADAGAYGSSNADVDSANQ LSSNDTAGNVNSESEVLSTDTNENSLESDIIDKDSKNVLSSSLQAAAKKKTVIKSGS TSITRGSSYYITLIDSGNGLVSNQKVTFESIKGKTYNKTITNSKGVAASKISLAKGKYDF VCAYAGTGAYYSKLSVSLTVNAMATNIRTSNRSIKRGKSYITLTDANSNLLANEK	SEQ ID NO.: 109

Cluster	Description	Sequence	SEQ ID NO.
	Ig-like domain repeat protein variant, amino acid	VSENI SGHVYNRJTTSKGVASLTMTNTAAKYSLSVCSYGGSSYYKASSVALTLTVLKGDTHIKISTDTVKKGNAVVMITLLDVRNKLVSQKVSFTIKGKTYNLTITNSNGTAKLIINLAAGKYPLVCSYDGSSENFASKASENLTIIDTVKTFISIADIEFAATNLKAYVLKNKVLPSITVTGGASLKISEFSYLMAKAVTNLNSNNKNTITJTGISNGDSATVVLNAKVYKNQYVNVSKRVYSYIDSNKVPATYATVYSSSGANVGKAGFNLYTFAFAKILAFHKTENYLPNYCTFESSALKASTAAAGSSSTSSANNQSKLKTSLKAQSTSTRGDSYSVTLKDGSGNALPNQKITFTVSNKQYSDTTDSKGIAVLGADLLSGKYSITASFAGSSAYKSSKLSNTVTVKNSSTRFELDDIENAAVNVKNYVSKNKALPTTIVTVA NTKLTIAQFSYIMAKAVHNINAGNKKYISLISISNCKSSGNYLDTTVYKAQYMNLTNRVISFTESNKVPPVYATVYGTNGKSVGNSEFNLYTFAFAKILAFHKTNNYLPNYCTFQSSAIGVKKPSSTTTTVVNVNGPIKANSSQFKTGLNEKNTVGNLSAYLVDSGYSKITSSIQNLANQLTKNLNSTASKALAIYNYVRDEISYSYIANSRYGASGTLVSGSGNCVDQANLIVALCRASGIEARYAHAKGCTFSSGLVTGHVWAQILVNGVWYSADATSVRNSLGNIVNWNTNSYYSLKQYTAIPFEGGGGGGGGGGGGSIENADKAIKDFQDNKAPHDKS44YEANSKLPKDLRDKNRNFVEKTSIEKAIVRHDERVKSANDAISKLENEKDSIENRRLAQREIVNKAPMDVKEHLQKOLD	
C7.B.14	WP_292788.318.1 /Methanobrevibacter sp. pseudomure in-binding repeat-containing protein variant, amino acid	MFVFLVLLPLVSSAAMDIDSSSIDDSNDLSGASVSSNSNENLASDSNSNNLESDNAGSSNVNSDYEILNTDNTVEDSDLEHNSNIANTKAMLGASSQSDSAVLQANAKVKTTLKGSSSIYRGNYVYTLTLTDSKGVLSGQKLSYNINGKTYTLTITDSKGSTYLQINLKEGKYTMVCSYGGSSAYESSRLSVTLVSLKNPNFTVKEIEDAATNVKNFVLKNKRLLPDLAKNVVSYIGSKKVAPNEILVKDASKRSVGNANFNLYTFAFAKILDFHKSKNYLPNYCTFESSAFNQAVSLKATILKGSSNSIYRGNYVYTLTLTDGNGKALSQKRLRYAINGKTYTLTITDSKGSTYLQINLKEGKYPMVCSYAGSKVYKSAKNSVTLTVLKNNSNAFSVNEIETAATNVKNYVVLKNKRLLPNTVKVGSKTLKISEFTYLSKAVSNLNSNNKKDIVLNGISNGGSSSTYSLKFTVYKAQYVDLAKRSA SNIESKKVPANLYSVKDDGSKNKAAGNAYNLYTFAFAKILDFHKSNNLNPNYCTFESSVYAPLKKSTSIKASSNSV NKGDSYSVTLVDNAGNALANQKITFNFSGLYQSQTNSKGVASLKIGASQGTYSVVS SYAGSSACEASKLSSTVTVKDITNRF SISEIEVAAASNVEYINIKNVNRP GTVTVAN KRLTISEFSYLMAKAVYNINAGNTNYITLPSGISGGNSEGDSMDATVYKAQYVDLSKRVVSFEESNKVSPVYAKVYSSSSGSSLGNAGFDLYTYSFAKILDFHKSHKNLPNYCTFDSSSVFKSSVVP	SEQ ID NO.: 110

Cluster	Description	Sequence	SEQ ID NO.
C7.B.15	MBE64998 44.1 /Methanobr evibacter thaueri adhesin variant, amino acid	AGISSDIPYNSNQFKAGLNEKNTESDL SKYLIGTGQSAITSSISNLANQLTKGLKSNEA KQAIYNYVRDEIDYSYANSKYGASGILSAGSGNCVDQASLVVALCRASGIEARY AHAKGCTFSSGLVTGHVWAQILVNGVWSADATSVRNKLGNIQNWNINSYSLN RYAAVPFGGGGGGGGGGSIENADKAIKDFQDNKAPHDKSAAYEANSKI PKDIRD KNNRPVEKTSIEKAIIRHDERIKSANDAISKLENEKDSIENRRLLAQREYNKAPMDVKEHLQ KQLD	SEQ ID NO.: 111
C7.B.16	MBQ66301 39.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAAKTIVFLTSDNIIDHEHDLQVLNSLKRHIEEISGGKLQVIVDNQ APAPGEGWRAIEVTSVSICLAASDAAGNYLQLATASTNSNKQIVFNIGSYDLDNNT NFLRAWDDNYSPESLAGMNDPGTFLKNAGIYVQPTKEFPENANDGVL SNYDEA MNEKIAQEIVDIINKHGNDHKILSDGLVTHNNVKPSVMAKASKELIESNDFSMDGKY GRYTGPOQLLYQTSSYLNGNGLDIPKDYGEPEPNMTTSFLAKDTYSVYDFFKMGIV RAYMDDTGKAPDSIEYEGAHISYYDILYNFAKITQIHTSAKHMGESEYHFDKVN SILLHIFFPILIFVLFLAYLFIKRIIRFSGGGGGGGGGSIENADKAIKDFQDNKAPH DKSAAYEANSKLPKDIRDKNNRFEKTSIEKAIIRHDERIKSANDAISKLENEKDSIENRRLL AQREYNKAPMDVKEHLQKQLD	SEQ ID NO.: 112
C7.B.17	WP_298501 801.1 /Methanobr evibacter sp. adhesin variant, amino acid	MFVFLVLLPLVSSAAKTVITSDNIIDQDNVVKMLNSIKNYIEEISGGELQVIVDNQ APSPGEGYRAIQVTSISICLAAADAGNYLQLQGTSA NSNKQIVLINTGDYDLDNH NYLRAWDDNYSNETEMGMHDPGTLKNSGIYIEPIKEFPDNGKNGYIDRYDEDM NKQIAQEIVDIVNGHNDKTFSDNLVVTNTISPKVMADASKELSSNDTEMGTY GNYTAGQLLYQTSSYLNGNGLDIPKSYDGPHEPMSGISFLVKDTYSIYDYNMAGIVK NYMDENGRAPDSIEYQGAHISYYDLQYNFAKITQNHTDAKHMGEFDEYKFKDKVND SILLHIFFPILIFVLFLAYLGYKKIRRFSGGGGGGGGGSIENADKAIKDFQDNK KAPHDKSAAYEANSKLPKDIRDKNNRFEKTSIEKAIIRHDERIKSANDAISKLENEKDSIE NRRLAQREYNKAPMDVKEHLQKQLD	SEQ ID NO.: 113

Cluster	Description	Sequence	SEQ ID NO.
C7.B.18		<i>PHDKS44YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKSLNEKDSIENR RLAQREYNKAPMDVKEHLQKQLD</i>	
	WP_296862 675.1 /Methanobr evibacter sp. adhesin variant, amino acid	<i>MFVFLVLLPLVSSAAATKTVFITDNIVDHDFDVLITAIKNYVQELSGGELQVVIDN QAPAAAGEGYRSIEVTSASIDLAASDAGNYQLANYSASNSKQIVFVNIQDYLDDNS SNYLRRAWDDNYTNETIAGVHDPGRLLRNSGIFYVQPAKEYPDKYKNGVLNYYDD AMAKQISQEIVDIINTHDNDTKVFSEDLVVKNKISPAGMANASKELLNSGDKKEFNGT FGAYTAPQLLYQTSSYLNGNGLDIPKIFKEPENPMGVSIKAGSYISIDYFKMGGIVR NYMDEHGQAPDSIEYEGAQISYYDLYNFAKITQNHITDSAHMGPENIEYQFEKVNSS FLIDAFPFILVLFILFLAYLVYKRIRRGSGGGSGGGSGGGSIENADKAIKDFQDNKAP HDKS44YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKSLNEKDSIENR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 114
C7.B.19		<i>MFVFLVLLPLVSSAAAKTVFELTSNDIVDHDITDLQVLNSIKSYIEEISGGELQVVIDNE APAAAGEGWRAIAVTSDVSICLAASDAGNYLQLGTASANSKQYIFVNVGDYDLDN HTNFLRAWDDNYSNESLAGMHDPGTFLKNAGVYVYIQPTKEFPQNTDDGIMDRYD EGMNRQIAQEIVDIVNSHGGDSKVLSDSLVTHNIVKPAVMAQASKALVESGDKEMQ GTYGNYTAAQLLYQTSSYLNGNGLDVPKSYDPPSDPLGISFTKDTYSVYDYFNMA GIVREYMDQNGKAPDSIEYEGAQISYYDLYNFAKITQNHITDAEHMGESEYHFEDK VNDSEILLHLFPFVVLVLLIAYRFEKRIIRFGGGSGGGSGGGSIENADKAIKDFQ DNKAPHDKS44YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKSLNEKDSIENR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 115
	MBR69278 38.1 /Methanobr evibacter sp. adhesin variant, amino acid	<i>MFVFLVLLPLVSSAAAKTVFITSNDIHDHDSDLKMLNSLSYIEEISGGSLQVVIDNQ APAAAGEGWRSEVTSVSIDIAASDAGNYVQLASYTANSKQIVFVNIQDYLDDNHT NFLRAWDDNYSNENLAGLHNPGTFLKNAGIYVYQPAKEFPENAKSGILDTNDDDEM YKKMAQEIVDIINTHENDEKVLSDGLISRNIVKPSVMANASKELJNSNDKEMTGTYG NYTAPQLLYMTSSYLNGNGLDVPKSFEEPEPNMGISFARMADKYSVYDYFKMGGIVK EYMDENGRAPDSIEYEGAQISYYDLYNFAKITQNHITDAKHMGFDSYHFEDKVN SILLHIFPFILVLFILFLAYLVYKRIRRGSGGGSGGGSGGGSIENADKAIKDFQDNKAP HDKS44YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKSLNEKDSIENR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 116
C7.B.20		<i>MFVFLVLLPLVSSAAAKTVFITSNDIHDHDSDLKMLNSLSYIEEISGGSLQVVIDNQ APAAAGEGWRSEVTSVSIDIAASDAGNYVQLASYTANSKQIVFVNIQDYLDDNHT NFLRAWDDNYSNENLAGLHNPGTFLKNAGIYVYQPAKEFPENAKSGILDTNDDDEM YKKMAQEIVDIINTHENDEKVLSDGLISRNIVKPSVMANASKELJNSNDKEMTGTYG NYTAPQLLYMTSSYLNGNGLDVPKSFEEPEPNMGISFARMADKYSVYDYFKMGGIVK EYMDENGRAPDSIEYEGAQISYYDLYNFAKITQNHITDAKHMGFDSYHFEDKVN SILLHIFPFILVLFILFLAYLVYKRIRRGSGGGSGGGSGGGSIENADKAIKDFQDNKAP HDKS44YEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIKSLNEKDSIENR LAQREYNKAPMDVKEHLQKQLD</i>	SEQ ID NO.: 117
	WP_303372 282.1	<i>MFVFLVLLPLVSSAAATKTVFITDNIVDHDFDVLITAIKNYVQELSGGELQVVIDN QAPRPGEGYRSIAVTSDVSIDLAASDAGNYQLANYSANSKQIVFVNIQDYLDDNS</i>	

[illegible]

Cluster	Description	Sequence	SEQ ID NO.
	evibacter sp. transglutaminase domain-containing protein variant, amino acid	EKNRRAPNWVSSSKGNIPYNQLILSYSKCLDFENKNSRLPNTVRLDLDLKDINKL NKNKSEAVNASSKTSKQTSNKFVNKTGTGNAKTNANKTNKTTKTASNKEDDSSK NASLVERTLNNIHNILNLDRLDPSKYQADVTKTDEANVKLNTSKINVDINGKSTV NIKVSAKNTTKATAKKTATKSTVKKTASKTVSSNKNSSINEVLRKCLASSKNCQVYK RQIQDLAKLTSLKSDYEKGKKLFTWVRDNIQYKKYRNRTRRGAVKTLQTKKGNC VDQSHLLIALSRASGIPARYVKGGNCKFSNGYVSGHIWAQMYINKKWVWVADTTSH RNTLGNIRNWNTKNYQLCGQFSSINFGGGGGGGGGGSIENADK4IKDFQDNKA PHDKS44YE4NSKLPKDLRDKNRNFTEKVSIEK4IIVRHDERFKSAND4ISKLEKDSIENR RL4OREVYNK4PMDVKEHLQKOLD	
C7.B.25	MDQ58197 61.1 /Methanobr evibacter sp. transglutaminase domain-containing protein variant, amino acid	MASVSASDVNDINDGNDHISLSEDSASSDLANDDLASSADADSIDDDLDLASSNE AYEDFSANPSNGESSYGPQSNALSESGDSNSQTTNTSIESSTSNVVKGENYSVTLKD KDGNVLSGKNHIFTLNGTFRNTNNSNGIASLTNTRVGNLYLVVSFLGDDSVANSFF SQQLTVSKIPTAIENSTGLAVIGRAYSVVLKDKKGNPLSSKAVTLTFNGKTYKRTTN SQGIVSHTLDGKRGNSYNLTYSKFAAGDSSYMASSGSVSLKLMSTKIVGTDARIVQGK VFTVTLKDDASNKLLANKKVTVLHNGKTYNRTSNKSGIVSLTSLQTPGKYYNISYRFA GDSTYTGSSKKLSVFIKTPTKPFVNSGSFVCKGNVYVYVTLKDSNDNVLANKTIVKVTY GTKNYTLTTNSKGKVGVKINSAGKGVYKFTYKFNGNSLYGPSSGSLNLRTKLATSLI GSSATHIKGNPYKVTLKDSGDGAVIANQNIENFESGAKYVRTTNSKGLASLVNPSALK TYNLSYSYAGNSLYNKSSGNVSLAVKLGTHIKNSGTTVANSSYEVTLKDSNNNAL ASKVHIFTLDGKTYRNTTNSKGVASLFISEKNFTTNLTYSKFAAGDSMYVASSGSVKV RVVSDKVFTFNQIVAAASKALRKYVEKNSELPSITVTVNGLKVNITSFAYLMSKSIYNV NNGKKSSVEVVSVSSNYSNSGSLINANLYKAGYLNLSNYLISYTKSNHKNIPNHINT RIGDLSPNLYIFGLSKALDFYSNNSYLPNYLILNSEDVCGKSNAKIHGNSQNKKGGL NEAQSLNATQLAVYLKSSGNDALNDAIRNLAKQLTSGKSTTLAKANAIFTYVVRDNV AYEYVADTKYKATGTLAKRGNCCDHANLVVALCRAANIPARYSHAQGCITFSSGL VTGHVWAQIYIEGVWYSADATSKRNLGNFHIWITNYFNSLKQYVHLPFGGGGGGGG GSGGGGGSIENADK4IKDFQDNKAPHDKS44YE4NSKLPKDLRDKNRNFTEKVSIEK4IIV RHDERFKSAND4ISKLEKDSIENRRL4OREVYNK4PMDVKEHLQKOLD	SEQ ID NO.: 121
C7.B.26	MBQ68131 09.1 /Methanobr	MFVFLVLLPLVSSAAQSLDDLNYGDSIDGLNDSGNPELNDRDSTFKSLQSTVIESNT DKTEKTNNTNESLGAKAISNCEKTDITYEDKSVKTSKAKASNTTQVKENKTSMKITTL AKSASSYFAYVKKNGKLPNPVSISSNKKYQSAQYLYLVSKAVSNISASTVEIKDLSLVN	SEQ ID NO.: 122

Cluster	Description	Sequence	SEQ ID NO.
	evibacter sp. transglutami nase domain- containing protein variant, amino acid	NFSYINYKTANGTLSKKECEELANKTARFIEKNHRAPTWIGSDKGNI PCNQILLFTK CLDYNNNSKLPSVVKLSDVDLDKISGKSNVSSSKASNSSKNAGNSEILLNSTLN AIS SILTDLNNILNPSTTTTHISSNSKTNNTTATIKTNITTVSTKSNTSAKINS SAKNTSA KTNNSKTNNSTKTNNTKVNTTAKTNSSTKTNNTKVNTTAKTNNTAT TSSSSKN SLSSWVDKAITAVGKSLIKFIDSSILNDKYNGESLKLYLSASKNCQSTSSAIKSLAKK LTSSLTSDYDKGEKFENWVRDNI EYQKYNTKKGALNTLNSKSANCVDQAHLVV AL ARAAGLPARYVNANCKFTSGYVS GHVWAQILVGITWVVADTTSSRNSFGIVK NW NVKSYKL VGKYSISIFGGGGGGGGGGGGGGJENADKA IKDFQDNKAPHDKSAAYE AN SKLPKD LRDKNRRFVEKTSIEKANVRHDERVKSAND AISKLENEKDSIENRR LAQR EVNKAP MDVKEHLOKOLD	

[0475] For SEQ ID Nos. 1-94, the below key indicates sequence features: Nucleotide sequences in lower case correspond to the untranslated region (UTR); nucleotide sequences in UPPER CASE correspond to the open reading frame. Amino acid sequences in bold correspond to the signal peptide, amino acid sequences with no mark correspond to the antigen, and amino acid sequences in italics correspond to a linker and the Sbi adjuvant fusion.

EXEMPLIFICATION

Example 1: Description of methods used in Examples described herein

[0476] IVT Template production: For experiments testing RNA vaccine candidates in cattle, constructs defined in this Example, which encode bovine-optimized versions of methanogen-targeted antigens or cytokines, were amplified from respective custom-synthesized plasmids with pUC19 backbones (GenScript). Amplification was carried out at an annealing temperature of 50°C in a 20 µL reaction consisting of 0.25 µM each primer T7-AGG_fwd and 120pA_rev, 1X Herculase II buffer, 25 mM each dNTP, 15 ng plasmid (GeneScript), and 0.4 µL Herculase II enzyme (Agilent). After the PCR program completed, 20U DpnI (NEB) was added to each PCR reaction and incubated at 37°C for 15 minutes to digest plasmid template. DpnI treated PCR product was purified using the DNA Clean & Concentrator-25 kit (Zymo Research) and eluted into 60 µL Nuclease free water.

[0477] The sequences of primers used were as follows:

[0478] T7-AGG_fwd:

[0479] gaattTAATA CGACTCACTA TAAGGcttgt tcttttgca gaagc (SEQ ID NO.: 95)

[0480] 120pA_rev:

[0481] TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT
TTTTTTTTTTT agaatgtgaa gaaactttct ttttattag (SEQ ID NO.: 96)

[0482] In vitro Transcription (IVT) of RNA Vaccine Candidates

[0483] For each vaccine candidate, RNA was synthesized in 22 µL IVT reactions consisting of 200 ng of the respective template, 20 mM MgCl₂, 7.5 mM each NTP, 7.5 mM CleanCap AG (TriLink), 1X HiScribe Transcription Buffer, 2M Betaine, and 2 uL HiScribe polymerase mix (NEB) and incubated at 37°C for 2 hours. For RNA conditions using chemically modified nucleotides, either UTP was substituted by 5-hydroxymethyluridine triphosphate (TriLink) or cytidine was substituted by N4-acetylCTP (Jena BioScience) at the indicated ratio in the IVT mixture.

[0484] All IVT products were cleaned up using Monarch 500 µg RNA Clean Up kit (NEB) and eluted into 83 µL nuclease-free water. Eluted products were then digested in 100 µL reactions consisting of 1X DNase I buffer, 10U of DNase I (RNase-free) (New England Biolabs)

and 100U of CIAP (Promega) at 37°C for 5 minutes to degrade DNA template and remove residual 5' triphosphates on RNA. Treated samples were cleaned up using Monarch 500 ug RNA Clean Up kit (New England Biolabs) and eluted into 100 µL 1 mM sodium citrate, pH 6.5.

[0485] One-Pot In vitro Transcription (IVT) of Multiplexed RNA Vaccine Candidates

For multiplexed RNA Vaccine Candidates, RNA can be synthesized in separate reactions, as in the IVT method above, and then normalized and pooled in a final step, or in a single or “one-pot” reaction. In the latter case, multiplexed RNA was synthesized by mixing 20 mM MgCl₂, 7.5 mM each NTP, 7.5 mM CleanCap AG (TriLink), 1X HiScribe Transcription Buffer, 2M Betaine, 2 uL HiScribe polymerase mix (NEB), and equimolar or predetermined molar ratio amounts of each respective DNA template, to a total of 200 ng DNA, in 22 µL IVT reactions and incubated at 37°C for 2 hours. For RNA conditions using chemically modified nucleotides, either UTP was substituted by 5-hydroxymethyluridine triphosphate (TriLink) or cytidine was substituted by N4-acetylCTP (Jena BioScience) at the indicated ratio in the IVT mixture.

All IVT products were cleaned up using Monarch 500 µg RNA Clean Up kit (NEB) and eluted into 83 µL nuclease-free water. Eluted products were then digested in 100 µL reactions consisting of 1X DNase I buffer, 10U of DNase I (RNase-free) (New England Biolabs) and 100U of CIAP (Promega) at 37°C for 5 minutes to degrade DNA template and remove residual 5' triphosphates on RNA. Treated samples were cleaned up using Monarch 500 ug RNA Clean Up kit (New England Biolabs) and eluted into 100 µL 1 mM sodium citrate, pH 6.5.

[0486] Formulation for In Vivo RNA Experiments

[0487] Formulations of RNA in lipid nanoparticles (RNA-LNPs) were prepared using a microfluidic mixer (Precision Nanosystems, Vancouver, BC). Briefly, GenVoy-ILM lipid mixture (Precision Nanosystems NWW0042) was diluted to 12.5 mM in anhydrous ethanol and combined with an aqueous solution of RNA (0.14 mg/mL) in PNI buffer (Precision Nanosystems NWW0043), using the manufacturer-recommended formulation parameters. Formulations were immediately diluted 30:1 in phosphate-buffered saline (Gibco 10010023), concentrated using Amicon centrifugation filters (MilliporeSigma UFC901008), and adjusted to the appropriate final volume with PBS. Formulations were stored at 4°C for up to 2 days prior to the prime dose and up to 25 days prior to the boost dose.

[0488] RNA Administration Study in Cattle

[0489] Animal experiments were carried out in accordance with the guidelines set forth by Texas A&M University (TAMU, College Station, MA, USA) and were approved by the TAMU Institutional Animal Care and Use (IACUC) committee. Cattle were housed at TAMU McGregor Research Facility, consisting of a breed of Angus crossbred cattle with no more than 0.25 *Bos indicus* composition of *Bos indicus*. Calves were weaned at approximately 205 days of age for a minimum of 4 weeks post weaning before being trained to the GreenFeed Pasture Systems (C-Lock Inc.) and a minimum of 8 weeks before receiving vaccination treatments.

[0490] During some RNA vaccine studies, such as M1 and M2, calves (n=4 or 5 per condition) received two RNA administrations at Day 0 (prime) and Day 21 (boost). RNA injections consisted of 2 mL RNA-LNP formulation (0.5 mg RNA dose per animal) delivered via neck intramuscular injection, unless noted otherwise. Collection of blood, saliva, and rumen fluid samples were taken from each animal on Days -1, 21, 34, and 90 and shipped on dry ice for analysis, unless noted otherwise.

[0491] During other RNA vaccine studies, such as M3, calves (n=10 per condition) received two RNA administrations at Day 0 (prime, 1°), Day 25 (boost, 2°) and Day 104* (boost, 3°). RNA injections consisted of 2 mL RNA-LNP formulation (0.55 mg RNA dose per animal) delivered via neck intramuscular injection, unless noted otherwise. Blood samples were collected on Days -1, 25, 42, 90, 109*, and 122*, and rumen fluid samples were collected on -1, 90, 109*, and 122*. Samples were shipped on dry ice for analysis, unless noted otherwise. (*Only conditions F1 and F3.)

[0492] Blood was collected via jugular venipuncture (8-10mL) and stored on ice for >2hrs to allow for clotting before centrifugation. Serum was collected and stored at -20°C until thawed for assay.

[0493] Saliva was collected using a vacuum into a conical flask. >10mL saliva was harvested per collection and stored at -20°C until thawed for assay.

[0494] Rumen fluid was collected using an esophageal tube and pump to withdraw rumen fluid, and was either immediately snap frozen in liquid nitrogen or placed on ice until being centrifuged at -4°C. The solid fraction of the centrifuged sample (i.e. the pellet) was withdrawn and placed in microtubes and snap frozen in liquid nitrogen prior to being stored at -80°C and shipped on dry ice to CosmosID Inc. (Germantown, MD) for sequencing. The liquid fraction was stored at -20°C until thawed for assay. For Study M3, separate samples were processed for

Volatile Fatty Acid (VFA) analysis. For studies M1 and M2, animals were randomly distributed by body weight (BW) into treatment groups and penned together.

[0495] Enteric methane (CH₄), oxygen (O₂), and carbon dioxide (CO₂) emissions per individual calf was measured via the GreenFeed Pasture System (C-Lock Inc.) on Days -8 to -1, representing a pre-vaccine baseline, and again on Days 22 to 29. Feed intake per individual calf was measured via GrowSafe Feed Intake Systems (GrowSafe Systems, Ltd.) and RFID ear tags for the duration of the acclimation and experiment time, Days -32 to 90.

[0496] For Study M3, animals were monitored for CH₄ emissions and dry matter intake (DMI) for a pre-trial baseline period, and distributed into treatment groups based on initial CH₄/DMI and BW and penned separately. Gas emissions per individual calf were measured continuously from D-22 to the end of the study and a pre-vaccine enteric methane baseline was determined after sorting into final pens and before injection (D-6 to D0). Feed intake per individual calf was measured via GrowSafe Feed Intake Systems (GrowSafe Systems, Ltd.) and RFID ear tags for the duration of the acclimation and experiment time.

[0497] Sequencing and Bioinformatics Analysis

[0498] For DNA extraction, DNA from samples was isolated using the QIAGEN DNeasy PowerSoil Pro Kit, according to the manufacturer's protocol. Non-fractionated samples were allowed to thaw at 4°C for a maximum of 16 hours, and optionally homogenized and/or centrifuged before extraction. Extracted DNA samples were quantified using Qubit 4 fluorometer and Qubit dsDNA HS Assay Kit (ThermoFisher Scientific).

[0499] For library preparation and sequencing, DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and IDT Unique Dual Indexes with total DNA input of 1 ng. Genomic DNA was fragmented using a proportional amount of Illumina Nextera XT fragmentation enzyme. Unique dual indexes were added to each sample followed by 12 cycles of PCR to construct libraries. DNA libraries were purified using AMPure magnetic beads (Beckman Coulter) and eluted in QIAGEN EB buffer. DNA libraries were quantified using Qubit 4 fluorometer and Qubit dsDNA HS Assay Kit. Libraries were then sequenced on an Illumina NextSeq 2000 platform 2x150bp

[0500] For bioinformatics analysis, unassembled sequencing reads were directly analyzed by CosmosID-HUB Microbiome Platform (CosmosID Inc., Germantown, MD) described elsewhere (Ottensen et al., 2016, Ponnusamy et al., 2016, Hasan et al., 2014, Lax et al., 2014) for multi-

kingdom microbiome analysis and profiling of antibiotic resistance and virulence genes and quantification of organisms' relative abundance. Briefly, the system utilizes curated genome databases and a high-performance data-mining algorithm that rapidly disambiguates hundreds of millions of metagenomic sequence reads into the discrete microorganisms engendering the particular sequences.

[0501] ELISA

[0502] ELISA assays measured antigen-specific IgG and IgA response to RNA vaccine formulations. For coating ELISA plates, antigen protein was either reconstituted in H₂O from a lyophilized state (e.g., OVA protein, 2000 ug/mL) or synthesized via an *E. coli*-based cell-free protein expression system (Liberum Biotech) and a plasmid template DNA comprising the sequence of the antigen protein (e.g., mru1499), as well as start/stop codons, an upstream T7 promoter and ribosome binding site, and a downstream T7 terminator. This reaction incubated for 8hrs at 27°C and then was diluted 1:10 in PBS. Nunc ELISA plates (ThermoFisher Scientific) were then coated with an antigen protein solution (50 µL/well) for an overnight incubation at 4°C.

[0503] Plates were washed three times with PBS-Tween 1% (PBST) and blocked (1hr, room temperature) with 200 µL of SuperBlock (Thermo Fisher Scientific). A diluent was created from a 1:2 mixture of SuperBlock (Thermo Fisher Scientific) and H₂O. Serial dilutions of serum samples (range 1:10–1:1010) in diluent were added (90–135 µL/well) to each well and incubated (2hr, room temperature). The plates were washed three times in PBST. For IgG titers, plates were then incubated for 1 h at room temperature with 50 µL/well of HRP-conjugated sheep anti-cow IgG (ab112618, Abcam), 1:3,000 in diluent. For IgA titers, plates were then incubated for 1 h at room temperature with 50 µL/well of HRP-conjugated sheep anti-cow IgA H&L (ab112755, Abcam), 1:1,000 in diluent. The plates were washed three times in PBST and incubated for 10 min at room temperature with SigmaFast OPD Solution (Sigma Aldrich) at 100 µL/well. The reactions were stopped with addition of 50 µL/well of 2M HCl. The absorbance read at 450nm on a GloMax Plate Reader (Promega).

[0504] Endpoint titers are defined here as the reciprocal of the highest analyte dilution that gives a reading above the cutoff. The cutoff is calculated by summing the average of the untreated (UTD) values plus the standard deviation of the untreated (UTD) values. The

geometric mean titer (GMT) and geometric standard deviation (GSD) are taken across condition group.

[0505] Vaccine formulations used in Example 2 through 5.

[0506] Ssp refers to Secpep1. HNpep refers to a PPa2 secretory signal. HNadj refers to an Sbi adjuvant. HNmod1 refers to N4-acetyl CTP. HNmod2 refers to 5-hydroxymethyluridine triphosphate.

[0507] Vaccine formulations used in Example 5.

[0508] Approach for antigen cluster generation.

[0509] One approach utilized to generate multi-component RNA vaccines in Examples 4 and 5 comprises using bioinformatic methods to identify groupings of two or more antigens (e.g., DNA or amino acid sequences) that are related to one another (e.g., share a defined level of sequence identity or structural identity, such as >80%) and are associated with two or more species of methanogen or methanogen associated protein in the rumen (e.g., antigens within a grouping have >80% identity to more than one methanogen genome, collectively).

[0510] In one example, such groupings were generated from a pool of antigens that were 50aa in length and represented all possible 50aa-frames present in a set of open reading frames (ORF) generated from metagenomic data of a rumen. In some instances, this set of ORFs was limited to entries that shared a defined sequence identity (e.g., >80%) to the genome of a methanogen species or a methanogen associated protein. In some instances, “hypothetical proteins” and/or sequences for de novo peptide designs that shared structural similarity to methanogen-associated proteins (e.g., structure identity via AlphaFold or LLM prediction) were included. Alternatively, in some instances, pools of 50aa-frames were generated from protein or genome databases and limited to select methanogen species (e.g., methanogen species most abundant in rumen samples, *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanosphaera stadtmanae*). In some instance, this set of ORFs was limited to entries that were algorithmically predicted to be surface-associated proteins (e.g., >0.5 confidence index). In some instance, the grouping of antigens that were generated consisted of 2, 3, 4, or 5 antigens with >80% identity to each other and the grouping contained antigens that were associated with 2, 3, 4, or 5 different methanogen species. In some instances, the final antigen groupings were defined as the full protein sequences from which the 50aa-frames were derived from.

[0511] Approach for multi-component formulation composition.

[0512] Multi-component RNA vaccines targeted to multiple methanogen proteins and/or proteins associated with multiple species of Methanogen/Archaea, used in Examples 4 and 5 were generated by using algorithmic or rational methods to select combinations of antigens and antigen grouping to provide coverage across multiple species of methane-associated microbes present in the rumen. Vaccine formulations, as shown in Table 3 or Table 5, are described to consist of multiple antigens (e.g., 3, 8, or 5 antigens per formulation), are associated with multiple methane-associated species (e.g., 3 or 5 species per formulation), and are combined at specified proportions with respect to molarity or mass.

[0513] Specifically, in this example, F2 comprises three antigens C3.1 (SEQ ID NO: 35), C3.2 (SEQ ID NO: 37), and C3.3 (SEQ ID NO: 39), generated via an antigen cluster generation workflow described above. The antigens of F2 formulation have regions that share a define level of similarity (e.g., sequence identity) and provide coverage of at least three methane-associated species. Also, the antigens of the F2 formulation are associated with the name/ functionality of “signal peptidase” (ex. BlastP highest match). Thereby, they are characterized as being membrane-bound, enzymes, and/or are critical for converting secretory proteins to their mature forms by cleaving signal peptides from the N-termini proteins during or after that protein’s translocation through the membrane.

[0514] F3 comprises two grouping of two antigens each (C7.1 (SEQ ID NO: 55), C7.2 (SEQ ID NO.: 57), C7.3 (SEQ ID NO.: 59), C7.4 (SEQ ID NO.: 61)), and one grouping of 4 antigens (C8.1 (SEQ ID NO.: 63), C8.2 (SEQ ID NO.: 65), C8.3 (SEQ ID NO.: 67), C8.4 (SEQ ID NO.: 69)), in which the former two groupings were generated via an antigen cluster generation workflow and the later grouping was selected via rational design. The former two antigen groupings each respectively have regions that share a define level of similarity (e.g., sequence identity) and provide coverage of at least two methane-associated species. Also, the antigens of the F2 formulation are associated with the name/functionality of “adhesin”, “adhesin-like”, or “immunoglobulin (Ig)-like” and/or have regions that share a defined level of similarity to adhesin/adhesin-like/Ig-like domain-containing domains (ex. BlastP matched). Thereby, they are characterized as being cell-surface components that facilitate binding to other cells or surfaces. Overall, the F3 Formulation comprises eight antigens that cover at least five methane associated species.

[0515] F4 comprises a grouping of five antigens: C9.1 (SEQ ID NO.: 79), C9.2 (SEQ ID NO.: 81), C9.3A (SEQ ID NO.: 83), C9.4C (SEQ ID NO.: 91), and C9.5 (SEQ ID NO.: 93), generated via a rational design method. The antigens of F4 formulation are associated with the name/ functionality of “archaeal oligosaccharyltransferase” or “AglB” and/or have regions that share a defined level of similarity to oligosaccharyltransferase / AglB proteins (e.g., BlastP matched). Thereby, they are characterized as enzymes critical to N-glycosylation—the covalent attachment of oligosaccharides to target proteins. Overall, the F4 Formulation comprises five antigens that cover at least five methane associated species.

[0516] F1 is a non-methanogen antigen control, ovalbumin (OVA), derived from *Gallus gallus* and intended to generate an immune response that is not specific to methanogens.

[0517] **Table 3** Compositions for various mRNA vaccine formulation.

Formulation Name	Antigen Name	Species Association (per antigen)	Molarity (%)	Mass (%)
F1	Ova_Sbi (Control)	<i>Gallus gallus</i> (Non-methanogen)	100%	100%
F2	C3.1	<i>Methanobrevibacter ruminantium</i>	33%	33%
	C3.2	<i>Methanobrevibacter smithii</i>	33%	33%
	C3.3	<i>Methanobrevibacter oralis</i>	33%	33%
F3	C7.1	<i>Methanobrevibacter smithii</i>	7.5%	5.9%
	C7.2	<i>Methanobrevibacter oralis</i>	7.5%	5.9%
	C7.3	<i>Methanobrevibacter smithii</i>	7.5%	11%
	C7.4	<i>Methanobrevibacter oralis</i>	7.5%	10%
	C8.1	<i>Methanobrevibacter ruminantium</i>	17.5%	16%
	C8.2	<i>Methanobrevibacter ruminantium</i>	17.5%	13%
	C8.3	<i>Methanobrevibacter ruminantium</i>	17.5%	19%
	C8.4	<i>Methanobrevibacter ruminantium</i>	17.5%	19%
F4	C9.1	<i>Methanobrevibacter ruminantium</i>	28.6%	31%
	C9.2	<i>Methanobrevibacter smithii</i>	17.9%	17%
	C9.3A	<i>Methanobrevibacter oralis</i>	17.9%	17%
		<i>Methanomicrobium mobile</i>		

Formulation Name	Antigen Name	Species Association (per antigen)	Molarity (%)	Mass (%)
	C9.4C	<i>Methanosphaera stadtmanae</i>	17.9%	17%
	C9.5		17.9%	17%

[0518] **Table 5: Additional exemplary compositions for various mRNA vaccine formulation.** Formulation name, Antigen name, Species association, Input ratio by molarity (%), and Input ratio by mass (%) are specified per row of table. Formulations comprise of combinations of antigens and antigen groupings derived from the methods described above.

Formulation Name	Antigen Name	Species Association	Protein Name Associations	Molarity (%)	Mass
F3+.A	C7.1	Methanobrevibacter smithii	Adhesin-like protein, Transglutaminase domain-containing protein, Ig-like domain-containing protein, Right-handed parallel beta-helix repeat-containing protein	2.40%	n/a
	C7.2	Methanobrevibacter oralis		2.40%	
	C7.3	Methanobrevibacter smithii		2.40%	
	C7.4	Methanobrevibacter oralis		2.40%	
	C8.1	Methanobrevibacter ruminantium		5.70%	
	C8.2	Methanobrevibacter ruminantium		5.70%	
	C8.3	Methanobrevibacter ruminantium		5.70%	
	C8.4	Methanobrevibacter ruminantium		5.70%	
F3+.B	C7.B.1	Methanobrevibacter sp.	Adhesin	2.60%	n/a

Formulation Name	Antigen Name	Species Association	Protein Name Associations	Molarity (%)	Mass
	C7.B.2	Methanobrevibacter sp. UBA188	Adhesin	2.60%	
	C7.B.3	Methanobrevibacter sp. UBA188	Adhesin	2.60%	
	C7.B.4	Methanobrevibacter sp.	Adhesin, partial	2.60%	
	C7.B.5	Methanobrevibacter sp. uncultured	Adhesin	2.60%	
	C7.B.6	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.7	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.8	Methanobrevibacter sp.	Adhesin, partial	2.60%	
	C7.B.9	Methanobrevibacter sp.	Adhesin, partial	2.60%	
	C7.B.10	Methanobrevibacter sp.	Adhesin, partial	2.60%	
	C7.B.11	Methanobrevibacter ruminantium M1	Adhesin-like protein with Transglutaminase domain adhesin	2.60%	
	C7.B.12	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.13	Methanobrevibacter sp.	Ig-like domain repeat protein	2.60%	
	C7.B.14	Methanobrevibacter sp.	pseudomurein-binding repeat-containing protein	2.60%	

Formulation Name	Antigen Name	Species Association	Protein Name Associations	Molarity (%)	Mass
	C7.B.15	Methanobrevibacter thaueri	Adhesin	2.60%	
	C7.B.16	Methanobrevibacter sp. uncultured	Adhesin	2.60%	
	C7.B.17	Methanobrevibacter sp. uncultured	Adhesin	2.60%	
	C7.B.18	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.19	Methanobrevibacter sp. uncultured	Adhesin	2.60%	
	C7.B.20	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.21	Methanobrevibacter sp.	Adhesin	2.60%	
	C7.B.22	Methanobrevibacter sp. uncultured	Adhesin	2.60%	
	C7.B.23	Methanobrevibacter sp.	Transglutaminase domain-containing protein	2.60%	
	C7.B.24	Methanobrevibacter sp.	Transglutaminase domain-containing protein	2.60%	
	C7.B.25	Methanobrevibacter sp.	Transglutaminase domain-containing protein	2.60%	
	C7.B.26	Methanobrevibacter sp.	Transglutaminase domain-containing protein	2.60%	

Example 2: Generation of antigen-specific antibodies with a multi-component vaccine formulation

[0519] This example describes antigen specific IgG titers generated from the vaccination of cattle with an exemplary vaccine against OVA and mru1499. The methods used in this Example are provided in Example 1 above.

[0520] As shown in **FIGS. 1A-1B**, antigen-specific IgG titers for OVA-IgG and mru1499-IgG (adhesin) were detected by ELISA in the serum or saliva of animals receiving either one dose or two doses of the multi-component RNA vaccine. Further as shown in Table 4, antigen specific IgA titers were also detected from the serum or saliva of vaccinated animals.

[0521] **Table 4: Antigen-specific IgA and IgG titers from serum or saliva, for various RNA vaccine formulation.** RNAExperimental group size, dosing conditions, and RNA vaccine formulations are specified per row of table. ELISA setups are shown in the table below and recorded titers measure IgA or IgG response to an exemplary multi-component RNA vaccine formulation and exemplary RNA vaccine formulations targeting Methanogen proteins and with various secretion signals. ELISAs all used Day 34 Serum or saliva samples.

Experiment	Condition	Formulation	ELISA Coating	ELISA Ab2	Group Size (n =)	GMT	GSD
M1	1 Dose	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgG	5	3E+4	8
M1	2 Dose	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgG	5	3E+7	66
M1	1 Dose	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgA	3	50	2
M1	2 Dose	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgA	2	160	3
M1	1 Dose**	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgG	5	331	15
M1	2 Dose**	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgG	5	3162	4
M1	1 Dose**	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgA	5	833	36
M1	2 Dose**	0.5 mg of 50% OVA_HNadj (85% HNmod1) + 50% mru1499_HNadj (85% HNmod1)	OVA	IgA	5	1821	15

M2.1	F2	0.5 mg of mru1499_HNadj (100% HNmod1)	mru1499	IgG	4	1E+6	1
M2.1	F4	0.5 mg of HNpep_mru1499_HNadj (100% HNmod1)	mru1499	IgG	4	3E+5	4
M2.1	F2	0.5 mg of mru1499_HNadj (100% HNmod1)	mru1499	IgA	4	57	6
M2.1	F4	0.5 mg of HNpep_mru1499_HNadj (100% HNmod1)	mru1499	IgA	4	320	2

*Saliva Sample.

[0522] This data demonstrates that a single component or multi-component RNA vaccine formulations can generate an antigen-specific antibody response in cattle. This data supports the development of a single component or multiplexed vaccination approach (e.g., where two or more antigens are used), to vaccinate an animal, e.g., a ruminant.

Example 3: *In vivo* effect on methanogen abundance and methane emissions in cattle vaccinated against a Methanogen protein

[0523] This Example describes the *in vivo* effect on methanogen abundance and methane emissions in cattle vaccinated with exemplary RNA vaccine formulations targeting Methanogen proteins with various secretion signals. The methods used in this Example are provided in Example 1 above.

[0524] As shown in FIG. 2, calves vaccinated with an exemplary multi-component RNA vaccine showed a decrease in Methanogen species *Methanobrevibacter (Mbb.) ruminantium* M1 in rumen fluid at Days 20, 34 and 90 post-vaccination as compared to control animals which were either left untreated or not dosed with the vaccine formulation. Similarly, FIGS. 3A-3B show a decrease in Methanogen species *Methanobrevibacter (Mbb.) ruminantium* M1 in rumen fluid of calves vaccinated with an exemplary RNA vaccine targeting Methanogen proteins with various secretion signals. The normalized relative abundance of Archaeal species was also generally decreased in rumen fluid of calves vaccinated with RNA vaccine targeting Methanogen proteins (FIG. 3B). Animals vaccinated with RNA vaccine construct having a HNpep secretory signal, the mru1499 antigen, and HNadjuvant had an increase in Archaeal abundance.

[0525] FIG. 4 shows the abundance of methanogen and archaeal species in rumen fluid in cattle vaccinated with exemplary RNA vaccine formulations targeting methanogens, and in some instances including cytokines. The RNA used also included the Ac4C and/or 5hmU modifications, as indicated in the figure. Animals vaccinated with RNA vaccine formulations targeting methanogens (mru1499) and those additionally with cytokines CXCL10 or VIP showed lower methanogen and archaeal species compared to each groups' respective Day 0 levels (apparent as negative delta values). Additionally, these groups showed a decrease in total Archaeal abundance compared to the untreated group. The RNA vaccination formulation with both Ac4C and 5hmU modifications resulted in a decrease in abundance of Methanogen species *Mbb. ruminantium* M1 compared to the untreated group, however, an increase in total Archaeal abundance.

[0526] Additionally, methane emissions based on feed intake were also measured for calves vaccinated with an exemplary RNA vaccine formulation targeting Methanogen proteins with various secretion signals. The data in FIG. 5 shows that calves vaccinated with an exemplary construct targeting mru1499 and having a PPa2 secretory peptide and a Sbi adjuvant had lower methane emissions compared to before the vaccination (compare last two bars on right). FIG. 6 shows reduced methane emissions in animals vaccinated with exemplary RNA vaccine formulations targeting methanogens and/or treated with RNA expressing cytokine (CXCL10, VIP).

[0527] Taken together, the data in this Example demonstrates that vaccination with RNA vaccine formulations targeting methanogens and/or treatment with RNA expressing cytokines expressed by cattle can reduce the abundance of methanogens and/or reduce methane emissions. This data further supports the development of a vaccination approach comprising methanogen antigens and/or ruminal-associated antigens for reducing methanogens in the digestive tract of an animal and/or reducing methane emissions.

Example 4: In vivo effect on methanogen abundance and methane emissions of cattle vaccinated with a multi-component RNA vaccine against multiple Methanogen proteins

[0528] This Example describes the *in vivo* effect on methanogen abundance and methane emissions in cattle vaccinated with exemplary multi-component RNA vaccine formulations

targeting multiple methanogen proteins. The methods used in this Example are provided in Example 1 above.

[0529] As shown in **FIGs. 7A-7B**, calves vaccinated with an exemplary multi-component RNA vaccine formulation targeting multiple Methanogen proteins showed a decrease in Total Archaea at +5d POST and +18d POST, relative to abundance at the PRE time point. From the PRE to +18d POST timepoint, calves vaccinated with the F3 multi-component RNA vaccine formulation achieved a 44% decrease in total archaeal abundance (-32%, normalized to the CNT(OVA) group). In addition, calves vaccinated with an exemplary multi-component RNA vaccine formulation targeting multiple methanogen proteins showed a decrease in each individual archaeal species identified at +5d POST, relative to abundance at the PRE time point and normalized to the CNT(OVA) group. Note, the CNT(OVA) negative control is a vaccine formulation targeted to non-methanogen protein, ovalbumin, to generate a non-methanogen specific immune response.

[0530] Additionally, methane emissions based on feed intake were also measured for calves vaccinated with exemplary multi-component RNA vaccine formulations targeting multiple methanogens. The data in **FIGs. 8A-8B** shows that calves vaccinated with F3 or F4 vaccine formulations (as described in Table 3) had lower methane emissions (CH₄/day) post-injection relative to the control CNT-OVA (**FIG. 8A**, last two bars on right). Further, all tested exemplary multi-component RNA vaccine formulations targeting multiple methanogen proteins demonstrated lower methane emissions per feed intake (CH₄/day per lb feed) post-injection relative to the control CNT-OVA (**FIG. 8B**).

[0531] Taken together, the data in this Example demonstrates that vaccination with multi-component RNA vaccine formulations targeting one or more methanogen antigens and/or one or more methanogen species can reduce the abundance of methanogens and reduce methane emissions. This data further supports the development of a vaccination approach comprising methanogen antigens or ruminal-associated antigens for reducing methanogens in the digestive tract of an animal and reducing methane emissions.

Example 5: In vivo effect on growth efficiency of cattle vaccinated against a Methanogen protein

[0532] This Example describes the *in vivo* effect on growth efficiency, measured via average daily gain (ADG, lb/day) or the average amount of weight an animal gained per day during a defined feeding period, of cattle vaccinated with exemplary RNA vaccine formulations targeting Methanogen proteins with various secretion signals, and in some instances multi-component formulations, cytokines, and the Ac4C and/or 5hmU modifications. The methods used in this Example are provided in Example 1 above.

[0533] As shown in **FIG. 9**, calves vaccinated with an exemplary RNA vaccine targeting methanogen proteins and having either a secpep1 or HNpep secretory signal (F3, F4) had significant increases in average daily gain (ADG) during time period “D0-D20” (a 20-day period post prime injection) and “D20-D90” (70-day period post boost injection) as compared to control animals which were either left untreated or not dosed with the vaccine formulation. For the secpep1 formulation, average ADG normalized to untreated (UTD) increased over 0.8 lbs/day (+53% change from baseline ADG) for the D0-D20 period, and over 1.0 lbs/day (+65% change from baseline ADG) for the D20-D90 period. Additionally, the data showed that exemplary RNA vaccine formulations targeting methanogens and with cytokines and/or the 5hmU modification also increased ADG during time period D0-D20 and D20-D90 as compared to control animals which were either left untreated or not dosed with the vaccine formulation.

[0534] In addition, this Example describes the *in vivo* effect on growth efficiency, measured via average daily gain (ADG, lb/day), of cattle vaccinated with an exemplary multi-component RNA vaccine against multiple methanogen proteins. As shown in **FIGS. 10A-10B**, calves vaccinated with multi-component RNA vaccine against multiple Methanogen proteins had increased ADG during time period “D0-D25” (a 25-day period post prime injection) and “D25-D90” (65-day period post boost injection) as compared to animals in the CNT(OVA) group which were vaccinated with a formulation targeted to non-methanogen protein, ovalbumin, to generate a non-methanogen specific immune response.

[0535] The data in this Example demonstrates that vaccination with RNA vaccine formulations targeting methanogens can increase growth efficiency of cattle, by way of their average daily weight gained. Additionally, the data demonstrates that vaccination with RNA vaccine formulations targeting one or more methanogen antigens and/or one or more methanogen species can increase growth efficiency of cattle relative to a non-methanogen specific vaccine. Without wishing to be bound by any particular theory, in some embodiments,

vaccination with polynucleotides comprising one or more ruminal-associated antigens increases the growth efficiency of an animal (e.g., a ruminant) by reducing one or more microorganisms (e.g., methanogens) in a digestive tract of an animal thus lowering methane emissions. Further without wishing to be bound by any particular theory, since methane produced in the digestive tract of an animal is energy lost (e.g., not absorbed by the body) from food consumed by an animal, reducing methane emissions allows for more energy from the food consumed by the animal to be used for growth, thus increasing growth efficiency of an animal.

[0536] This data further supports the development of a vaccination approach comprising ruminal-associated antigens (e.g., ruminal antigens and/or methanogen antigens) for increasing the growth efficiency of ruminant animals.

NUMBERED EMBODIMENTS

[0537] Embodiment 1. An isolated polynucleotide encoding one or more ruminal-associated antigens, fragments thereof or variants thereof.

[0538] Embodiment 2. The isolated polynucleotide of embodiment 1, wherein the one or more ruminal-associated antigens comprise one or more ruminal antigens and/or one or more methanogen antigens.

[0539] Embodiment 3. The isolated polynucleotide of embodiment 1 or 2, wherein the one or more ruminal-associated antigens comprise one or more ruminal antigens.

[0540] Embodiment 4. The isolated polynucleotide of any one of the preceding embodiments, wherein the one or more ruminal antigens are derived from: a polypeptide that is involved in attachment to fermenting bacteria, or a fragment or variant thereof.

[0541] Embodiment 5. The isolated polynucleotide of any one of the preceding embodiments, wherein the polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 ruminal antigens.

[0542] Embodiment 6. The isolated polynucleotide of embodiment 1 or 2, wherein the one or more ruminal-associated antigens comprise one or more methanogen antigens.

[0543] Embodiment 7. The isolated polynucleotide of embodiment 6, wherein the polynucleotide comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, or at least 20 methanogen antigens.

[0544] Embodiment 8. The isolated polynucleotide of embodiment 6, wherein the polynucleotide comprises about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 20, about 4 to about 20, about 5 to about 20, about 6 to about 20, about 7 to about 20, about 8 to about 20, about 9 to about 20, about 10 to about 20, or about 15 to about 20 methanogen antigens.

[0545] Embodiment 9. The isolated polynucleotide of any one of embodiments 6-8, wherein the one or more methanogen antigens are the same, e.g., having the same sequence.

[0546] Embodiment 10. The isolated polynucleotide of any one of embodiments 6-8, wherein the one or more methanogen antigens are different, e.g., having different sequences.

[0547] Embodiment 11. The isolated polynucleotide of any one of embodiments 1-2 or 6-10, wherein the one or more methanogen antigens is derived from a polypeptide found on the cell surface of a wild-type methanogen, or a fragment or variant thereof.

[0548] Embodiment 12. The isolated polynucleotide of any one of embodiments 1-2 or 6-11, wherein the one or more methanogen antigens is secreted.

[0549] Embodiment 13. The isolated polynucleotide of any one of embodiments 1-2 or 6-12, wherein the one or more methanogen antigens comprise a peptide that is involved in adhesion, attachment, or mobility, or a fragment or variant of a peptide that is involved in adhesion, attachment, mobility.

[0550] Embodiment 14. The isolated polynucleotide of embodiment 12 or 13, wherein the secreted methanogen antigen comprises a signal peptide.

[0551] Embodiment 15. The isolated polynucleotide of embodiment 14, wherein the signal peptide can be predicted using a prediction algorithm and optionally wherein the prediction score is at least 0.5.

[0552] Embodiment 16. The isolated polynucleotide of any one of embodiments 1-2 or 6-15, wherein the one or more methanogen antigens comprise one or more peptides having at least 80% sequence identity to a methanogen protein.

[0553] Embodiment 17. The isolated polynucleotide of any one of embodiments 1-2 or 6-16, wherein the polynucleotide comprises a plurality of methanogen antigens each having at least 80% sequence identity to each other.

[0554] Embodiment 18. The isolated polynucleotide of any one of embodiments 1-2 or 6-17, wherein the polynucleotide comprises a plurality of methanogen antigens wherein each methanogen antigen in the plurality is associated with a different methanogen species.

[0555] Embodiment 19. The isolated polynucleotide of any one of embodiments 1-2 or 6-18, wherein the polynucleotide comprises a plurality of methanogen antigens, and wherein the methanogen antigens in the plurality are associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species.

[0556] Embodiment 20. The isolated polynucleotide of any one of embodiments 1-2 or 6-19, wherein the polynucleotide comprises a plurality of methanogen antigens and wherein each methanogen antigen in the plurality is associated with the same methanogen species.

[0557] Embodiment 21. The isolated polynucleotide of any one of embodiments 18-20, wherein the methanogen species comprises: *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter* sp. UBA188, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, or *Methanosarcina acetivorans*.

[0558] Embodiment 22. The isolated polynucleotide of any one of embodiments 18-21, wherein the methanogen species is or comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, or *Methanobrevibacter oralis*.

[0559] Embodiment 23. The isolated polynucleotide of any one of embodiments 18-21, wherein the methanogen species is or comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile* or *Methanosphaera stadtmanae*.

[0560] Embodiment 24. The isolated polynucleotide of any one of embodiments 1-2 or 6-23, wherein the one or more methanogen antigens comprise one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity.

[0561] Embodiment 25. The isolated polynucleotide of embodiment 24, wherein a polypeptide having signal peptidase activity:

[0562] (i) is membrane bound;

[0563] (ii) has the ability to cleave one or more signal peptides;

[0564] (iii) plays a role in converting a secretory protein to a mature form (e.g., from a non-secretory form to a secretory form); and/or

[0565] (iv) or any combination thereof.

[0566] Embodiment 26. The isolated polynucleotide of any one of embodiments 1-2 or 6-25, wherein the one or more methanogen antigens comprise one or more peptides having: (1) at least

80% sequence identity to an Immunoglobulin (Ig)-like polypeptide, or (2) substantially similar function to an Ig-like domain-containing polypeptide.

[0567] Embodiment 27. The isolated polynucleotide of embodiment 26, wherein an Ig-like domain-containing polypeptide comprises a polypeptide characterized as a cell-surface protein that facilitates binding to other cells and/or cell surfaces.

[0568] Embodiment 28. The isolated polynucleotide of any one of embodiments 1-2 or 6-27, wherein the one or more methanogen antigens comprises an adhesin or fragment or variant thereof.

[0569] Embodiment 29. The isolated polynucleotide of embodiment 28, wherein the one or more methanogen antigens comprises an adhesin protein provided in Table 1 or a sequence with at least 85% identity thereto.

[0570] Embodiment 30. The isolated polynucleotide of any one of embodiments 1-2 or 6-29, wherein the one or more methanogen antigens comprises mru1499, or a fragment or variant thereof.

[0571] Embodiment 31. The isolated polynucleotide of any one of embodiments 1-2 or 6-30, wherein the one or more methanogen antigens comprises an antigen sequence provided in any one of SEQ ID NOs: 2, 6, or 8 or a sequence with at least 85% identity thereto, or is encoded by a sequence provided in any one of SEQ ID NOs: 1, 5, or 7 or a sequence with at least 85% identity thereto.

[0572] Embodiment 32. The isolated polynucleotide of any one of embodiments 1-2 or 6-31, wherein the methanogen antigen comprises a pili protein or fragment or variant thereof.

[0573] Embodiment 33. The isolated polynucleotide of any one of embodiments 1-2 or 6-32, wherein the methanogen antigen comprises a flagellin protein, or fragment or variant thereof.

[0574] Embodiment 34. The isolated polynucleotide of any one of embodiments 1-2 or 6-33, wherein the one or more methanogen antigens comprise one or more peptides having: (1) at least 80% sequence identity to an archaeal oligosaccharyltransferase (AglB) polypeptide, or (2) substantially similar function to an AglB polypeptide.

[0575] Embodiment 35. The isolated polynucleotide of embodiment 34, wherein an AglB polypeptide is characterized as having N-glycosylation activity.

[0576] Embodiment 36. The isolated polynucleotide of any one of the preceding embodiments, wherein the one or more methanogen antigens comprise:

[0577] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;

[0578] (ii) one or more secreted antigens comprising a signal peptide;

[0579] (iii) a plurality of peptides having at least 80% sequence identity to each other;

[0580] (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;

[0581] (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity;

[0582] (vi) or any combination thereof.

[0583] Embodiment 37. The isolated polynucleotide of embodiment 36, wherein the polynucleotide comprises three methanogen antigens.

[0584] Embodiment 38. The isolated polynucleotide of embodiment 37, wherein the three methanogen antigens are associated with at least three different methanogen species.

[0585] Embodiment 39. The isolated polynucleotide of any one of embodiments 1-35, wherein the one or more methanogen antigens comprise:

(i) one or more peptides having at least 80% sequence identity to a methanogen protein;

(ii) one or more secreted antigens comprising a signal peptide;

(iii) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;

(iv) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide;

(v) or any combination thereof.

[0586] Embodiment 40. The isolated polynucleotide of embodiment 39, wherein the polynucleotide comprises five methanogen antigens.

[0587] Embodiment 41. The isolated polynucleotide of embodiment 40, wherein the five methanogen antigens are associated with at least five different methanogen species.

[0588] Embodiment 42. The isolated polynucleotide of any one of embodiments 1-35, wherein the one or more methanogen antigens comprise:

- [0589] (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
- [0590] (ii) one or more secreted antigens comprising a signal peptide;
- [0591] (iii) a plurality of peptides having at least 80% sequence identity to each other;
- [0592] (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
- [0593] (v) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; and/or
- [0594] (vi) any combination thereof.
- [0595] Embodiment 43. The isolated polynucleotide of embodiment 42, wherein the polynucleotide comprises eight methanogen antigens.
- [0596] Embodiment 44. The isolated polynucleotide of embodiment 43, wherein the eight methanogen antigens are associated with at least three different methanogen species.
- [0597] Embodiment 45. The isolated polynucleotide of any one of the preceding embodiments, wherein the one or more ruminal antigens and/or the one or more methanogen antigens are each situated on a separate nucleotide sequence.
- [0598] Embodiment 46. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 2 methanogen antigens and the at least 2 methanogen antigens are situated on separate nucleotide sequences.
- [0599] Embodiment 47. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 3 methanogen antigens and the at least 3 methanogen antigens are situated on separate nucleotide sequences.
- [0600] Embodiment 48. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 4 methanogen antigens and the at least 4 methanogen antigens are situated on separate nucleotide sequences.
- [0601] Embodiment 49. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 5 methanogen antigens and the at least 5 methanogen antigens are situated on separate nucleotide sequences.

[0602] Embodiment 50. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 6 methanogen antigens and the at least 6 methanogen antigens are situated on separate nucleotide sequences.

[0603] Embodiment 51. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 7 methanogen antigens and the at least 7 methanogen antigens are situated on separate nucleotide sequences.

[0604] Embodiment 52. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 8 methanogen antigens and the at least 8 methanogen antigens are situated on separate nucleotide sequences.

[0605] Embodiment 53. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 9 methanogen antigens and the at least 9 methanogen antigens are situated on separate nucleotide sequences.

[0606] Embodiment 54. The isolated polynucleotide of any one of embodiments 1-2 and 6-45, wherein the polynucleotide comprises at least 10 methanogen antigens and the at least 10 methanogen antigens are situated on separate nucleotide sequences.

[0607] Embodiment 55. The isolated polynucleotide of any one of embodiments 1-45, wherein the one or more ruminal antigens and/or the one or more methanogen antigens are situated on the same nucleotide sequence.

[0608] Embodiment 56. The isolated polynucleotide of any one of embodiments 1-2 and 6-55, wherein the one or more methanogen antigens comprises a polypeptide antigen sequence provided in **Table 2**, or a variant and/or fragment thereof.

[0609] Embodiment 57. The isolated polynucleotide of any one of embodiments 1-2 and 6-55, wherein the one or more methanogen antigens comprises a polypeptide antigen sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to an antigen sequence provided in **Table 2**.

[0610] Embodiment 58. The isolated polynucleotide of any one of the preceding embodiments, wherein the polynucleotide comprises a signal peptide.

[0611] Embodiment 59. The isolated polynucleotide of embodiment 58, wherein the signal peptide has at least 85% homology to an archaeal signal peptide.

[0612] Embodiment 60. The isolated polynucleotide of embodiment 58, wherein the signal peptide has at least 85% homology to a bacterial signal peptide.

[0613] Embodiment 61. The isolated polynucleotide of any one of embodiments 58-60, wherein the signal peptide is or comprises a PPA2 signal peptide, or a fragment or variant thereof.

[0614] Embodiment 62. The isolated polynucleotide of any one of embodiments 58-60, wherein the signal peptide is or comprises an SSP signal peptide, or a fragment or variant thereof.

[0615] Embodiment 63. The isolated polynucleotide of embodiment 58, wherein the signal peptide is or comprises a SARS-CoV-2 Spike secretion signal, or a fragment or variant thereof.

[0616] Embodiment 64. The isolated polynucleotide of any one of embodiments 58-63, wherein the signal peptide is situated at the N terminal of the ruminal-associated antigen sequence.

[0617] Embodiment 65. The isolated polynucleotide of embodiment 2, wherein the polynucleotide comprises:

- (i) a first nucleotide sequence encoding one or more ruminal antigens,
- (ii) a second nucleotide sequence encoding one or more methanogen antigens; and/or
- (iii) a third nucleotide sequence encoding a chemokine and/or cytokine.

[0618] Embodiment 66. The isolated polynucleotide of embodiment 65, wherein the first nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 ruminal antigens.

[0619] Embodiment 67. The isolated polynucleotide of embodiment 65 or 66, wherein the second nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 methanogen antigens.

[0620] Embodiment 68. The isolated polynucleotide of any one of embodiments 65-67, wherein the first nucleotide sequence, the second nucleotide sequence and/or the third nucleotide sequence are situated on one polynucleotide.

[0621] Embodiment 69. The isolated polynucleotide of any one of embodiments 65-67, wherein the first nucleotide sequence, the second nucleotide sequence and/or the third nucleotide sequence are situated on different polynucleotides.

[0622] Embodiment 70. The isolated polynucleotide of any one of the preceding embodiments, wherein the polynucleotide comprises a transmembrane domain.

[0623] Embodiment 71. The isolated polynucleotide of any one of the preceding embodiments, further comprising a complement C3d-binding polypeptide from an immunoglobulin-binding protein (Sbi) of *Staphylococcus aureus*.

[0624] Embodiment 72. The isolated polynucleotide of embodiment 71, wherein the complement C3d-binding polypeptide is or comprises:

[0625] (i) domain III of the Sbi of *Staphylococcus aureus*, or a functional fragment or a variant thereof;

[0626] (ii) domain IV of the Sbi of *Staphylococcus aureus*, or a functional fragment or a variant thereof; or

[0627] (iii) both (i) and (ii).

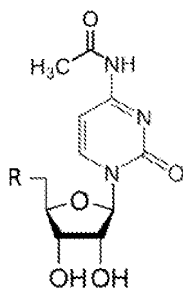
[0628] Embodiment 73. The isolated polynucleotide of any one of the preceding embodiments, wherein the polynucleotide is or comprises DNA.

[0629] Embodiment 74. The isolated polynucleotide of any one of the preceding embodiments wherein the polynucleotide is or comprises RNA.

[0630] Embodiment 75. The isolated polynucleotide of embodiment 74, wherein the RNA comprises a 5' cap.

[0631] Embodiment 76. The isolated polynucleotide of embodiment 74 or 75, wherein the RNA comprises a polyA tail.

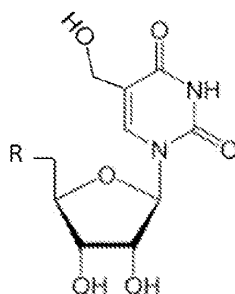
[0632] Embodiment 77. The isolated polynucleotide of any one of embodiments 74-76, wherein the polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

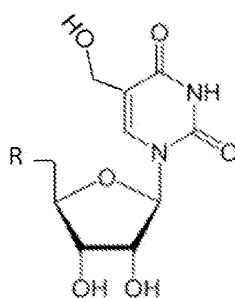
[0633] Embodiment 78. The isolated polynucleotide of embodiment 77, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than N4-acetylcytidine, optionally wherein the nucleoside is chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof.

[0634] Embodiment 79. The isolated polynucleotide of embodiment 78, wherein the nucleoside of the one or more modified ribonucleotides is 5-hydroxymethyluridine, and the modified ribonucleotide and has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

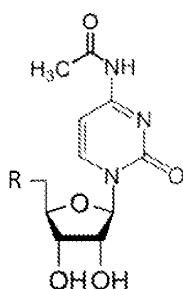
[0635] Embodiment 80. The isolated polynucleotide of any one of embodiments 74-76, wherein the polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0636] Embodiment 81. The isolated polynucleotide of embodiment 80, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than 5-hydroxymethyluridine, wherein the one or more modified ribonucleotides comprises a nucleoside chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof.

[0637] Embodiment 82. The isolated polynucleotide of embodiment 80 or 81, wherein the nucleoside of the one or more modified ribonucleotides is N4-acetylcytidine and the modified ribonucleotide has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

[0638] Embodiment 83. A polypeptide encoded by the polynucleotide of any one of the preceding embodiments.

[0639] Embodiment 84. A composition comprising one or more polyribonucleotides of any one of embodiments 1-82, or a polypeptide of embodiment 83.

[0640] Embodiment 85. The composition of embodiment 84, wherein the composition is a pharmaceutical composition.

[0641] Embodiment 86. The composition of embodiment 84, wherein the composition is an immunogenic composition.

[0642] Embodiment 87. The composition of embodiment 84, wherein the composition is a vaccine composition.

[0643] Embodiment 88. The composition of any one of embodiments 83-87, wherein the composition is formulated for delivery with a carrier.

[0644] Embodiment 89. The composition of 88, wherein the carrier is a lipid nanoparticle, a cationic lipid, a polymeric particle.

- [0645] Embodiment 90. The composition of any one of embodiments 83-87, wherein the composition is formulated for delivery without a carrier.
- [0646] Embodiment 91. A method comprising administering a composition according to any one of embodiments 84-90, to a cell, tissue or an animal.
- [0647] Embodiment 92. The method of embodiment 91, wherein the method is a vaccination method.
- [0648] Embodiment 93. The method of embodiment 90 or 91, wherein the animal is a ruminant.
- [0649] Embodiment 94. The method of any one of embodiments 90-92, wherein the ruminant is a cattle, sheep, goats, buffalo, moose, antelope, caribou, or deer.
- [0650] Embodiment 95. The method of any one of embodiments 90-92, wherein the animal is a domestic animal.
- [0651] Embodiment 96. The method of any one of embodiments 90-95, wherein the composition is characterized in that administration of the composition to the animal reduces methane emissions from the animal as compared to an otherwise comparable animal not administered the composition or administered a different composition.
- [0652] Embodiment 97. The method of embodiment 96, wherein the reduction in methane emissions is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to an animal not administered the composition or administered a different composition.
- [0653] Embodiment 98. The method of embodiment 97, wherein the reduction in methane emissions is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to an otherwise comparable animal not administered the composition or administered a different composition.
- [0654] Embodiment 99. The method of any one of embodiments 90-98, wherein the composition is characterized in that administration of the composition to the animal reduces a population of microorganisms in the animal, as compared to an animal not administered the composition or administered a different composition.

[0655] Embodiment 100. The method of embodiment 99, wherein the microorganisms is a methanogen.

[0656] Embodiment 101. The method of embodiment 100, wherein the methanogen comprises a methanogen from one or more of the following clades: *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Methanothermobacter*, *Candidatus Methanomethylophilus*, *Thermoplasmatales*.

[0657] Embodiment 102. The method of embodiment 100, wherein the methanogen comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter* sp. UBA188, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vannielii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.

[0658] Embodiment 103. The method of any one of embodiments 100-102, wherein the methanogen abundance is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to methanogen abundance in an animal not administered the composition or administered a different composition.

[0659] Embodiment 104. The method of any one of embodiments 100-102, wherein the methanogen abundance is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to abundance of the methanogen in an otherwise comparable animal not administered the composition or administered a different composition.

[0660] Embodiment 105. The method of any one of embodiments 100-104, wherein the composition is characterized in that administration of the composition to the animal reduces the abundance of all or substantially all methanogens (e.g., total methanogen abundance) in the

animal as compared to methanogen abundance in an otherwise comparable animal not administered the composition or administered a different composition.

[0661] Embodiment 106. The method of embodiment 105, wherein total methanogen abundance is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to methanogen abundance in an otherwise comparable animal not administered the composition or administered a different composition.

[0662] Embodiment 107. The method of embodiment 105, wherein total methanogen abundance is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to methanogen abundance in an otherwise comparable animal not administered the composition or administered a different composition.

[0663] Embodiment 108. The method of any one of embodiments 90-107, wherein the composition is characterized in that administration of the composition to the animal increases a growth rate of the animal as compared to the growth rate of an otherwise comparable animal not administered the composition or administered a different composition.

[0664] Embodiment 109. The method of embodiment 108, wherein growth rate is assessed by measuring the weight of the animal at one or more timepoints.

[0665] Embodiment 110. The method of embodiment 109, wherein the weight of the animal is measured daily.

[0666] Embodiment 111. The method of any one of embodiments 108-110, wherein the growth rate of the animal increases over a period of time.

[0667] Embodiment 112. The method of claim 11, wherein the period of time comprises at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months or at least 12 months.

[0668] Embodiment 113. The method of any one of embodiments 108-110, wherein the increase in growth rate comprises a daily increase in weight of the animal.

[0669] Embodiment 114. The method of embodiment 113, wherein the daily increase in weight of the animal is an increase of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to the daily increase in weight of an otherwise comparable animal not administered the composition or administered a different composition.

[0670] Embodiment 115. The method of embodiment 113, wherein the daily increase in weight of the animal is an increase of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to daily increase in weight of an otherwise comparable animal not administered the composition or administered a different composition.

[0671] Embodiment 116. The method of any one of embodiments 91-115, wherein the composition modifies outputs of cellulose fermentation.

[0672] Embodiment 117. The method of any one of embodiments 91-116, wherein the composition is delivered with a carrier.

[0673] Embodiment 118. The method of any one of embodiments 91-117, wherein the composition is delivered without a carrier.

[0674] Embodiment 119. The method of any one of embodiments 91-118, comprising administering one dose of the composition to the animal.

[0675] Embodiment 120. The method of any one of embodiments 91-119, comprising administering a plurality of doses of the composition to the animal.

[0676] Embodiment 121. The method of embodiment 120, wherein the animal is administered a first dose of the composition followed by one or more subsequent doses of the composition.

[0677] Embodiment 122. The method of embodiment 121, wherein the first dose and the one or more subsequent doses of the composition comprise the same methanogen antigens and/or ruminal antigens.

[0678] Embodiment 123. The method of embodiment 121, wherein the first dose and the one or more subsequent doses of the composition comprise different methanogen antigens and/or ruminal antigens.

[0679] Embodiment 124. The method of any one of embodiments 91-123, wherein the one or more methanogen antigens and/or the one or more ruminal antigens are specific to the animal.

[0680] Embodiment 125. The method of embodiment 124, wherein the one or more methanogen antigens and/or the one or more ruminal antigens that are specific to the animal are obtained by a method comprising:

(i) identifying one or more methanogen antigens and/or one or more ruminal antigens that are expressed in the animal; and

(ii) responsive to the identification, selecting one or more methanogen antigens and/or the one or more ruminal antigens to be included in the composition.

[0681] Embodiment 126. The method of embodiment 124 or 125, wherein the one or more methanogen antigens and/or the one or more ruminal antigens that are specific to the animal is different from the one or more methanogen antigens and/or the one or more ruminal antigens that are specific to a different animal.

[0682] Embodiment 127. The method of embodiment 126, wherein the different animal comprises:

[0683] (i) a different species of animal,

[0684] (ii) an animal of a different sex,

[0685] (iii) an animal of a different age, or

[0686] (iv) an animal located in a different geographical location;

[0687] (v) the same animal but at a different timepoint, and/or

[0688] (vi) any combination thereof.

[0689] Embodiment 128. The method of any one of embodiments 120-127, wherein the composition is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or 30 times to the animal.

[0690] Embodiment 129. The method of any one of embodiments 120-128, wherein the composition is administered once every 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months.

[0691] Embodiment 130. The method of any one of embodiments 91-129, wherein the composition is administered in combination with one or more additional agents.

[0692] Embodiment 131. The method of embodiment 130, wherein the one or more additional agents comprises a chemical additive, a biological feed additive.

- [0693] Embodiment 132. The method of any one of embodiments 91-131, wherein the composition is administered in combination with one or more additional compositions.
- [0694] Embodiment 133. The method of embodiment 132, wherein the additional composition immunizes the animal from a disease, e.g., an infectious disease.
- [0695] Embodiment 134. A method of manufacturing a composition comprising one or more polyribonucleotides of any one of embodiments 1-82.
- [0696] Embodiment 135. The method of embodiment 134, wherein the composition is an RNA composition.
- [0697] Embodiment 136. The method of embodiment 134 or 135, wherein:
- [0698] (i) the method is an in vitro transcription reaction method; and/or
- [0699] (ii) the method produces a plurality of polyribonucleotides.
- [0700] Embodiment 137. The isolated polynucleotide of any one of embodiments 1-5 or 58-81, wherein the ruminal antigen comprises an antigen expressed in any one or all of: (a) Rumen ciliates symbiotic with methanogens; (b) Hydrogen-producing organisms which supply methanogens; (c) Taxa associated with low feed efficiency.
- [0701] Embodiment 138. The isolated polynucleotide of embodiment 137, wherein Rumen ciliates symbiotic with methanogens comprises one or more, or any combination of: *Diplodinium dentatum*; *Diploplastron affine*; *Enoploplastron triloricastrum*; *Entodinium simplex*; *Entodinium caudatum*; *Entodinium longinucleatum*; *Epidinium ecaudatum*; *Eremoplastron bovis*; *Endiplodinium maggii*; *Ostracodinium obtusum*; or *Polyplastron multivesiculatum*.
- [0702] Embodiment 139. The isolated polynucleotide of embodiment 137, wherein Hydrogen-producing organisms comprise one or more, or any combination of: *Butyrivibrio proteoclasticus*; *Bacteroides thetaiotaomicron*; *Ruminococcus flavefaciens*; *Ruminococcus albus*.
- [0703] Embodiment 140. The isolated polynucleotide of embodiment 137, wherein taxa associated with low feed efficiency comprise one or more of: *Veillonellaceae*; *Prevotellaceae*; *Lachnospiraceae*; *Succinivibrionaceae*; *Fibrobacteraceae*; *Anaerovibrio*; *Clostridiales*; *Prevotella*; *Ruminococcaceae*.
- [0704] Embodiment 141. The method of any one of embodiments 100-104, wherein the composition is characterized in that administration of the composition to the animal reduces the abundance of at least one methanogen species in the animal as compared to abundance of the

same methanogen species in an otherwise comparable animal not administered the composition or administered a different composition.

[0705] Embodiment 142. The method of embodiment 141, wherein abundance of at least one methanogen species is reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to abundance of the same methanogen species in an otherwise comparable animal not administered the composition or administered a different composition.

[0706] Embodiment 143. The method of embodiment 141, wherein abundance of at least one methanogen species is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or about 90% as compared to abundance of the same methanogen species in an otherwise comparable animal not administered the composition or administered a different composition.

[0707] Embodiment 144. The method of any one of embodiments 91-123, wherein the one or more methanogen antigens and/or the one or more ruminal antigens are present and/or expressed in the animal and one or more additional animals.

[0708] Embodiment 145. The method of embodiment 144, wherein the animal and the one or more additional animals are or comprise:

[0709] (i) a same or different species of animal,

[0710] (ii) an animal of the same or different sex,

[0711] (iii) an animal of substantially the same age or development stage, or

[0712] (iv) an animal located in the same or a substantially similar geographical location; or

[0713] (v) any combination of (i)-(iv).

[0714] Embodiment 146. The method of embodiment 144 or 156, wherein the composition is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 or 30 times to the animal.

[0715] Embodiment 147. The method of any one of embodiments 144-146, wherein the composition is administered once every 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months or 6 months.

[0716] Embodiment 148. The method of any one of embodiments 144-147, wherein the composition is administered in combination with one or more additional agents.

[0717] Embodiment 149. The method of embodiment 148, wherein the one or more additional agents comprises a chemical additive, a biological feed additive.

[0718] Embodiment 150. The method of any one of embodiments 144-149, wherein the composition is administered in combination with one or more additional compositions.

[0719] Embodiment 151. The method of embodiment 150, wherein the additional composition immunizes the animal from a disease, e.g., an infectious disease.

EQUIVALENTS

[0720] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Further, it should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the claims that follow.

CLAIMS

What is claimed is:

1. An isolated polynucleotide encoding one or more ruminal-associated antigens, fragments thereof, variants thereof or variant fragments thereof.
2. The isolated polynucleotide of claim 1, wherein the one or more ruminal-associated antigens comprise one or more ruminal antigens and/or one or more methanogen antigens.
3. The isolated polynucleotide of claim 1 or 2, wherein the one or more ruminal-associated antigens comprise one or more ruminal antigens.
4. The isolated polynucleotide of any one of the preceding claims, wherein the one or more ruminal antigens are derived from a polypeptide that is involved in attachment to fermenting bacteria, or a fragment or variant or variant fragment thereof.
5. The isolated polynucleotide of claim 3 or 4, wherein the one or more ruminal antigens comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 ruminal antigens.
6. The isolated polynucleotide of claim 1 or 2, wherein the one or more ruminal-associated antigens comprise one or more methanogen antigens.
7. The isolated polynucleotide of claim 6, wherein the one or more methanogen antigens comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, or at least 20 methanogen antigens.
8. The isolated polynucleotide of claim 6 or 7, wherein the one or more methanogen antigens are the same.

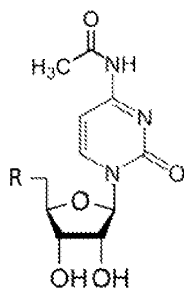
9. The isolated polynucleotide of claim 6 or 7, wherein the one or more methanogen antigens are different.
10. The isolated polynucleotide of any one of claims 6-9, wherein the one or more methanogen antigens comprise:
- (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
 - (ii) one or more secreted antigens comprising a signal peptide;
 - (iii) a plurality of peptides having at least 80% sequence identity to each other;
 - (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
 - (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity;
 - (vi) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide;
 - (vii) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; or
 - (viii) or any combination thereof.
11. The isolated polynucleotide of any one of claims 6-9, wherein the one or more methanogen antigens comprise:
- (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
 - (ii) one or more secreted antigens comprising a signal peptide;
 - (iii) a plurality of peptides having at least 80% sequence identity to each other;
 - (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
 - (v) one or more peptides having at least 80% sequence identity to a polypeptide having signal peptidase activity;
 - (vi) or any combination thereof.

12. The isolated polynucleotide of claim 11, wherein the polynucleotide comprises three methanogen antigens, optionally wherein the three methanogen antigens are associated with at least three different methanogen species.
13. The isolated polynucleotide of any one of claims 6-9, wherein the one or more methanogen antigens comprise:
- (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
 - (ii) one or more secreted antigens comprising a signal peptide;
 - (iii) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
 - (iv) one or more peptides having at least 80% sequence identity to an AglB polypeptide or substantially similar function to an AglB polypeptide; or
 - (v) any combination thereof.
14. The isolated polynucleotide of claim 13, wherein the polynucleotide comprises five methanogen antigens, optionally wherein the five methanogen antigens are associated with at least five different methanogen species.
15. The isolated polynucleotide of any one of claims 6-9, wherein the one or more methanogen antigens comprise:
- (i) one or more peptides having at least 80% sequence identity to a methanogen protein;
 - (ii) one or more secreted antigens comprising a signal peptide;
 - (iii) a plurality of peptides having at least 80% sequence identity to each other;
 - (iv) a plurality of peptides associated with at least 2 different, at least 3 different, at least 4 different or at least 5 different methanogen species;
 - (v) one or more peptides having at least 80% sequence identity to an Ig-like domain-containing polypeptide or substantially similar function to an Ig-like domain-containing polypeptide; or
 - (vi) any combination thereof.

16. The isolated polynucleotide of claim 15, wherein the polynucleotide comprises eight methanogen antigens, optionally wherein the eight methanogen antigens are associated with at least three different methanogen species.
17. The isolated polynucleotide of any one of claims 1-2 or 6-16, wherein the one or more methanogen antigens are derived from a polypeptide found on the cell surface of a wild-type methanogen, or a fragment or variant thereof.
18. The isolated polynucleotide of any one of claims 1-2 or 6-17, wherein the methanogen antigen is secreted.
19. The isolated polynucleotide of any one of claims 1-2 or 6-18, wherein the methanogen antigen comprises a peptide that is involved in adhesion, attachment, or mobility, or a fragment or variant of a peptide that is involved in adhesion, attachment, mobility.
20. The isolated polynucleotide of any one of claims 1-2 or 6-18, wherein the methanogen antigen comprises: an adhesin or fragment or variant thereof; a pili protein or fragment or variant thereof; and/or a flagellin protein, or fragment or variant thereof.
21. The isolated polynucleotide of claim 20, wherein the methanogen antigen comprises an adhesin protein provided in Table 1 or a sequence with at least 85% identity thereto.
22. The isolated polynucleotide of any one of claims 1-2 or 6-21, wherein the methanogen antigen comprises: an antigen provided in **Table 2** or a fragment or a variant or a variant fragment thereof; or a sequence with at least 85% identity to an antigen sequence provided in **Table 2**.
23. The isolated polynucleotide of any one of the preceding claims, wherein the one or more ruminal antigens and/or the one or more methanogen antigens are situated on different polynucleotides.

24. The isolated polynucleotide of any one of claims 1-23, wherein the one or more ruminal antigens and/or the one or more methanogen antigens are situated on one polynucleotide.
25. The isolated polynucleotide of any one of the preceding claims, wherein the polynucleotide comprises a signal peptide or a variant or fragment thereof.
26. The isolated polynucleotide of claim 25, wherein the signal peptide has at least 85% homology to an archaeal signal peptide or to bacterial signal peptide.
27. The isolated polynucleotide of claim 25 or 26, wherein the signal peptide is or comprises:
(i) a PPA2 signal peptide, or a fragment or variant thereof;
(ii) an SSP signal peptide, or a fragment or variant thereof;
(iii) a SARS-CoV-2 Spike secretion signal, or a fragment or variant thereof; or
(iv) any combination of (i)-(iii).
28. The isolated polynucleotide of any one of claims 25-27, wherein the signal peptide is situated at the N terminal of the ruminal-associated antigen sequence.
29. The isolated polynucleotide of any one of the preceding claims, wherein the polynucleotide comprises:
(i) a first nucleotide sequence encoding one or more ruminal antigens,
(ii) a second nucleotide sequence encoding one or more methanogen antigens; and/or
(iii) a third nucleotide sequence encoding a chemokine and/or cytokine.
30. The isolated polynucleotide of claim 29, wherein:
the first nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 ruminal antigens; and
the second nucleotide sequence comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 methanogen antigens.

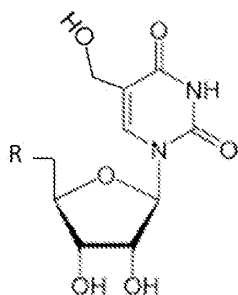
31. The isolated polynucleotide of any one of the preceding claims, wherein the polynucleotide comprises a transmembrane domain.
32. The isolated polynucleotide of any one of the preceding claims, further comprising a complement C3d-binding polypeptide from an immunoglobulin-binding protein (Sbi) of *Staphylococcus aureus*.
33. The isolated polynucleotide of claim 32, wherein the complement C3d-binding polypeptide is or comprises
- (i) domain III of the Sbi of *Staphylococcus aureus*, or a functional fragment or a variant thereof;
 - (ii) domain IV of the Sbi of *Staphylococcus aureus*, or a functional fragment or a variant thereof; or
 - (iii) both (i) and (ii).
34. The isolated polynucleotide of any one of the preceding claims, wherein the polynucleotide is or comprises DNA.
35. The isolated polynucleotide of any one of claims 1-34, wherein the polynucleotide is or comprises RNA.
36. The isolated polynucleotide of claim 35, wherein the polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

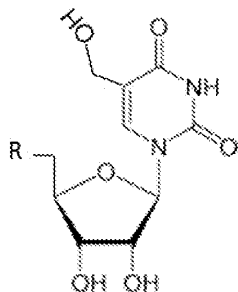
37. The isolated polynucleotide of claim 36, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than N4-acetylcytidine, optionally wherein the nucleoside is chosen from: an adenosine, an inosine a guanosine, a cytidine or a uridine, or any combination thereof.

38. The isolated polynucleotide of claim 36, wherein the nucleoside of the one or more modified ribonucleotides is 5-hydroxymethyluridine, and the modified ribonucleotide has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

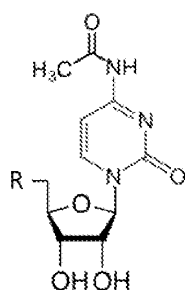
39. The isolated polynucleotide of any one of claims 33-35, wherein the polynucleotide sequence comprises one or more ribonucleotides comprising a nucleoside comprising a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has a structure of



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

40. The isolated polynucleotide of claim 39, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than 5-hydroxymethyluridine, wherein the one or more modified ribonucleotides comprises a nucleoside chosen from: an adenosine, an inosine, a guanosine, a cytidine or a uridine, or any combination thereof.

41. The isolated polynucleotide of claim 39 or 40, wherein the nucleoside of the one or more modified ribonucleotides is N4-acetylcytidine and the modified ribonucleotide has a structure of:



wherein R is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate.

42. A polypeptide encoded by the polynucleotide of any one of the preceding claims.

43. A composition comprising one or more polyribonucleotides of any one of claims 1-41, or a polypeptide of claim 42.

44. The composition of claim 43, wherein the composition is a pharmaceutical composition.
45. The composition of claim 44, wherein the composition is an immunogenic composition and/or a vaccine composition.
46. A method comprising administering a composition according to any one of claims 43-45, to a cell, tissue or an animal.
47. The method of claim 46, wherein the method is a vaccination method, and the animal is a ruminant or a domestic animal.
48. The method of claim 46 or 47, wherein the composition is characterized in that administration of the composition to the animal reduces methane emissions from the animal as compared to an otherwise comparable animal not administered the composition or administered a different composition.
49. The method of claim 48, wherein the reduction in methane emissions is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% as compared to an otherwise comparable animal not administered the composition or administered a different composition.
50. The method of any one of claims 46-49, wherein the composition is characterized in that administration of the composition to the animal reduces a population of microorganisms in the animal, as compared to an otherwise comparable animal not administered the composition or administered a different composition.
51. The method of claim 50, wherein the microorganisms is a methanogen, optionally wherein the methanogen comprises a methanogen from one or more of the following clades: *Methanobrevibacter*, *Methanosphaera*, *Methanobacterium*, *Methanosarcinales*, *Methanomicrobiales*, *Methanothermobacter*, *Candidatus Methanomethylophilus*, *Thermoplasmatales*.

52. The method of claim 51, wherein the methanogen *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter* sp. UBA188, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jannaschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeta thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.

53. The method of any one of claims 46-52, wherein the composition is characterized in that administration of the composition to the animal increases a growth rate of the animal as compared to the growth rate of an otherwise comparable animal not administered the composition or administered a different composition.

54. The method of claim 53, wherein the increase in growth rate comprises a daily increase in weight of the animal, optionally wherein the daily increase in weight of the animal is an increase of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of weight as compared to an otherwise comparable animal not administered the composition or administered a different composition.

55. The method of any one of claims 46-54, comprising administering one dose of the vaccine composition to the animal.

56. The method of any one of claims 46-54, comprising administering a plurality of doses of the vaccine composition to the animal.

57. The method of claim 56, wherein the animal is administered a first dose of the composition followed by one or more subsequent doses of the composition.

58. The method of claim 56, wherein the first dose and the one or more subsequent doses of the composition comprise the same methanogen antigens and/or ruminal antigens.
59. The method of claim 56, wherein the first dose and the one or more subsequent doses of the composition comprise different methanogen antigens and/or ruminal antigens.
60. The method of any one of claims 57-59, wherein the composition is administered at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20 or at least 30 times to the animal.
61. The method of any one of claims 46-60, wherein the composition is administered in combination with one or more additional agents, optionally wherein the one or more additional agents comprises a chemical additive, a biological feed additive.
62. The method of any one of claims 46-61, wherein the composition is administered in combination with one or more additional compositions, optionally wherein the additional composition immunizes the animal from a disease, e.g., an infectious disease.
63. A composition comprising the isolated polynucleotide of any one of claims 1-41, or the pharmaceutical composition of claim 43 or 44, for use in administration to (e.g., vaccination of) an animal.
64. Use of a composition comprising the isolated polynucleotide of any one of claims 1-41, or the pharmaceutical composition of claim 43 or 44, in the preparation of a medicament for administration to (e.g., vaccination of) an animal.
65. The composition of claim 63, or the use of claim 64, wherein the isolated polynucleotide or pharmaceutical composition is administered to the animal.

66. The composition of claim 65, or the use of claim 65, wherein administration of the isolated polynucleotide or pharmaceutical composition results in:
- (i) reduced methane emissions;
 - (ii) reduced abundance of one or more microorganisms (e.g., methanogens) in a digestive tract of the animal; and/or
 - (iii) increased growth rate of the animal,
- as compared to an otherwise comparable animal not administered the composition, or administered a different composition.
67. The composition of claim 65 or 66, or the use of claim 65 or 66, wherein the animal is a ruminant or a domestic animal.
68. The composition of any one of claims 65-67, or the use of any one of claims 65-67, methanogen comprises *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanomicrobium mobile*, *Methanobrevibacter wolinii*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter boviskoreani*, *Methanosphaera stadtmanae*, *Methanosarcina mazei*, *Methanobrevibacter thaueri*, *Methanobrevibacter sp. UBA188*, *Methanosarcina soligelidi*, *Methanothermobacter thermautotrophicus*, *Methanococcus aeolicus*, *Methanocaldococcus jamaerschii*, *Methanococcus voltae*, *Methanococcus vanniellii*, *Methanococcus maripaludis*, *Methanopyrus kandleri*, *Methanocorpusculum labreanum*, *Methanococcoides burtonii*, *Methanosaeete thermophila*, *Methanoregula boonei*, *Methanosphaerula palustris*, *Methanoculleus marisnigri*, *Methanospirillum hungatei*, *Methanosarcina acetivorans*, or any combination thereof.