CLAIMS

Claim 1. A nitrogen-sensitive expression system comprising:

a transcription factor region comprising an NtcA binding site; and

a core promoter region comprising a RuBisCo promoter or a variant or a functional fragment thereof;

wherein the core promoter region comprises or is operably linked to the transcription factor region.

Claim 2. The expression system of claim 1 further comprising:

a transcribable nucleic acid molecule and a 3' transcription termination nucleic acid molecule;

wherein,

the core promoter region is operably linked to the transcribable nucleic acid molecule; and

the transcribable nucleic acid molecule is operably linked to the 3' transcription termination nucleic acid molecule.

Claim 3. The expression system of any one of claims 1-2, wherein the transcription factor region comprises at least one of: a $GTAN_{11}C$ consensus sequence; a $GTAN_8TAC$ consensus sequence; a $GTAN_8TGC$ consensus sequence; a $GTN_{10}AC$ consensus sequence; a $TGTN_9ACA$ consensus sequence; or a $TGTN_{10}ACA$ consensus sequence.

Claim 4. The expression system of any one of claims 1-3, wherein the transcription factor region comprises at least one of a TAN_3T/A box.

Claim 5. The expression system of any one of claims 1-4, wherein the core promoter region comprises a polymerase binding site of a RuBisCo promoter.

Claim 6. The expression system of any one of claims 1-5, wherein the core promoter region comprises at least about 70% sequence identity to a RuBisCo (*rbcLS*) promoter sequence from *Nostoc* sp. PCC 7120; *Synechocystis* sp. PCC 6803; or Anabaena PCC 7120, or a functional fragment thereof.

Claim 7. The expression system of any one of claims 1-6, comprising an isolated nucleic acid molecule having promoter activity and at least about 70% sequence identity to any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234.

Claim 8. The expression system of any one of claims 1-7, comprising an isolated nucleic acid molecule having promoter activity and

(a) at least 95 contiguous bases of any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234; or

(b) a functional fragment of any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234.

Claim 9. The expression system of any one of claims 1-8, comprising

(a) a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide, wherein the NtcA polypeptide or the NtcB polypeptide is operably linked to the core promoter region of the construct; or

(b) a polynucleotide sequence of SEQ ID NO: 279, encoding an NtcA polypeptide and an NtcB polypeptide, operably linked to the core promoter region.

Claim 10. The expression system of any one of claims 1-9, comprising an:

an isolated nucleic acid molecule of nucleotide positions 177 to 836 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity;

an isolated nucleic acid molecule of nucleotide positions 1 to 352 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity; or

an isolated nucleic acid molecule of nucleotide positions 177 to 352 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity.

Claim 11. The expression system of any one of claims 1-10, wherein the transcribable nucleic acid molecule encodes a polypeptide having: sucrose phosphate synthase activity; sucrose phosphate phosphatase activity; sucrose phosphate synthase and sucrose phosphate phosphatase activity; trehalose phosphate synthase activity; trehalose phosphate phosphatase activity; gluocosylglycerol phosphate synthase activity; gluocosylglycerol phosphate phosphatase activity; mannosylfructose phosphate synthase activity; or mannosylfructose phosphate phosphatase activity.

Claim 12. The expression system of any one of claims 1-10, wherein the transcribable nucleic acid molecule comprises:

SEQ ID NO: 1, or at least 80% sequence identity thereto and encoding a polypeptide having sucrose phosphate synthase and sucrose phosphate phosphatase activity; or

a nucleic acid sequence encoding a polypeptide of SEQ ID NO: 2, or a polypeptide at least 80% similar thereto having sucrose phosphate synthase and sucrose phosphate phosphatase activity.

Claim 13. A method for expressing a transcribable nucleic acid molecule in a host cell comprising:

stably transforming a host cell with an expression system of any one of claims 1-12; and

growing the host cell, or progeny thereof comprising the expression system under conditions whereby the transcribable nucleic acid molecule is expressed;

wherein expression of the transcribable nucleic acid molecule is repressed when nitrate is a predominant nitrogen source and the transcribable nucleic acid molecule is expressed when urea or ammonia is the predominant nitrogen source.

Claim 14. The method of claim 13, wherein

the growth rate of the transformed host organism is no more than about 20% lower than a non-transformed host organism under the same or substantially similar conditions; or

the growth rate of the transformed host organism is more than about a 5% higher growth rate (e.g., more than about a 10%, 15%, or 20% higher growth rate) than a host organism transformed with a non-regulated construct grown under the same or substantially similar conditions.

Claim 15. The method of any one of claims 13-14, wherein

(a) the host cell endogenously expresses an NtcA polypeptide or an NtcB polypeptide under a nitrogen control system;

(b) the host cell is engineered to express an NtcA polypeptide or an NtcB polypeptide under a nitrogen control system;

(c) the expression system comprises a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide and the NtcA polypeptide or the NtcB polypeptide is operably linked to the core promoter region of the construct.

Claim 16. The method of any one of claims 13-15, wherein the host cell is a photosynthetic microorganism.

Claim 17. The method of any one of claims 13-16, wherein the host cell is a photosynthetic microorganism selected from the group consisting of: *Spirulina maximum*, *Spirulina platensis*, *Dunaliella salina*, *Botrycoccus braunii*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Serenastrum capricomutum*, *Scenedesmus auadricauda*, *Porphyridium cruentum*, *Scenedesmus acutus*, *Dunaliella* sp., *Scenedesmus obliquus*, *Anabaenopsis*, *Aulosira*, *Cylindrospermum*, *Synechoccus* sp., *Synechocystis* sp., and *Tolypothrix*.

Claim 18. An expression cassette comprising:

(a) a transcription factor region comprising an NtcA binding site;

(b) a core promoter region comprising a RuBisCo promoter or a variant or a functional fragment thereof;

(c) a transcribable nucleic acid molecule;

(d) a 3' transcription termination nucleic acid molecule; and

(e) optionally, a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide;

wherein,

the core promoter region comprises or is operably linked to the transcription factor region;

the core promoter region is operably linked to the transcribable nucleic acid molecule;

the transcribable nucleic acid molecule is operably linked to the 3' transcription termination nucleic acid molecule;

elements (a)-(d) are positioned in relation to each other such that expression of the expression cassette in a host organism results in production of a polypeptide sequence encoded by the transcribable nucleic acid molecule; and

element (e) when present is positioned such that expression of the expression cassette in the host organism results in production of the NtcA polypeptide or the NtcB polypeptide.

Claim 19. A transgenic host cell:

comprising the expression system of any one of claims 1-12;

produced by the method of any one of claims 13-17, or the progeny thereof, comprising the expression system; or

comprising the expression cassette of claim 18;

wherein

expression of the polypeptide sequence encoded by the transcribable nucleic acid molecule is repressed in the presence of nitrate; and

expression of the polypeptide sequence encoded by the transcribable nucleic acid molecule is induced in the presence of urea or ammonia.

Claim 20. A kit comprising the expression system of any one of claims 1-13 or the expression cassette of claim 18 and at least one reagent for introducing the expression cassette into a host cell and, optionally, instructions for introducing the expression cassette into a host cell.

CLAIMS

1. A nitrogen-sensitive expression system comprising:

a transcription factor region comprising an NtcA binding site; and

a core promoter region comprising a RuBisCo promoter or a variant or a functional fragment thereof;

wherein the core promoter region comprises or is operably linked to the transcription factor region.

2. The expression system of claim 1 further comprising:

a transcribable nucleic acid molecule and a 3' transcription termination nucleic acid molecule;

wherein,

the core promoter region is operably linked to the transcribable nucleic acid molecule; and

the transcribable nucleic acid molecule is operably linked to the 3' transcription termination nucleic acid molecule.

3. The expression system of any one of claims 1-2, wherein the transcription factor region comprises at least one of: a $GTAN_{11}C$ consensus sequence; a $GTAN_8TAC$ consensus sequence; a $GTAN_8TGC$ consensus sequence; a $GTN_{10}AC$ consensus sequence; a $TGTN_9ACA$ consensus sequence; a $TGTN_{10}ACA$ consensus sequence; or a TAN_3T/A box.

4. The expression system of any one of claims 1-3, wherein the core promoter region comprises a polymerase binding site of a RuBisCo promoter.

5. The expression system of any one of claims 1-4, wherein the core promoter region comprises at least about 70% sequence identity to a RuBisCo (*rbcLS*) promoter sequence from *Nostoc* sp. PCC 7120; *Synechocystis* sp. PCC 6803; or Anabaena PCC 7120, or a functional fragment thereof.

6. The expression system of any one of claims 1-5, comprising an isolated nucleic acid molecule having promoter activity and at least about 70% sequence identity to any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234.

7. The expression system of any one of claims 1-6, comprising an isolated nucleic acid molecule having promoter activity and

(a) at least 95 contiguous bases of any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234; or

(b) a functional fragment of any one of SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, or SEQ ID NO: 234.

8. The expression system of any one of claims 1-7, comprising

(a) a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide, wherein the NtcA polypeptide or the NtcB polypeptide is operably linked to the core promoter region of the construct; or

(b) a polynucleotide sequence of SEQ ID NO: 279, encoding an NtcA polypeptide and an NtcB polypeptide, operably linked to the core promoter region.

9. The expression system of any one of claims 1-8, comprising an:

an isolated nucleic acid molecule of nucleotide positions 177 to 836 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity;

an isolated nucleic acid molecule of nucleotide positions 1 to 352 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity; or

an isolated nucleic acid molecule of nucleotide positions 177 to 352 of SEQ ID NO: 234, or at least about 70% sequence identity thereto having promoter activity.

10. The expression system of any one of claims 1-9, wherein the transcribable nucleic acid molecule encodes a polypeptide having: sucrose phosphate synthase activity; sucrose phosphate phosphatase activity; sucrose phosphate synthase and sucrose phosphate phosphatase activity; trehalose phosphate synthase activity; trehalose phosphate phosphatase activity; gluocosylglycerol phosphate synthase activity; gluocosylglycerol phosphate

phosphatase activity; mannosylfructose phosphate synthase activity; or mannosylfructose phosphate phosphatase activity.

11. The expression system of any one of claims 1-10 wherein the transcribable nucleic acid molecule comprises:

SEQ ID NO: 1, or at least 80% sequence identity thereto and encoding a polypeptide having sucrose phosphate synthase and sucrose phosphate phosphatase activity; or

a nucleic acid sequence encoding a polypeptide of SEQ ID NO: 2, or a polypeptide at least 80% similar thereto having sucrose phosphate synthase and sucrose phosphate phosphatase activity.

12. A method for expressing a transcribable nucleic acid molecule in a host cell comprising:

stably transforming a host cell with an expression system of any one of claims 1-11; and

growing the host cell, or progeny thereof comprising the expression system under conditions whereby the transcribable nucleic acid molecule is expressed;

wherein expression of the transcribable nucleic acid molecule is repressed when nitrate is a predominant nitrogen source and the transcribable nucleic acid molecule is expressed when urea or ammonia is the predominant nitrogen source.

13. The method of claim 12, wherein

the growth rate of the transformed host organism is no more than about 20% lower than a non-transformed host organism under the same or substantially similar conditions; or

the growth rate of the transformed host organism is more than about a 5% higher growth rate (e.g., more than about a 10%, 15%, or 20% higher growth rate) than a host organism transformed with a non-regulated construct grown under the same or substantially similar conditions.

14. The method of any one of claims 12-13, wherein

(a) the host cell endogenously expresses an NtcA polypeptide or an NtcB polypeptide under a nitrogen control system;

(b) the host cell is engineered to express an NtcA polypeptide or an NtcB polypeptide under a nitrogen control system;

(c) the expression system comprises a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide and the NtcA polypeptide or the NtcB polypeptide is operably linked to the core promoter region of the construct.

15. The method of any one of claims 12-14, wherein the host cell is a photosynthetic microorganism.

16. The method of any one of claims 12-15, wherein the host cell is a photosynthetic microorganism selected from the group consisting of: *Spirulina maximum*, *Spirulina platensis*, *Dunaliella salina*, *Botrycoccus braunii*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Serenastrum capricomutum*, *Scenedesmus auadricauda*, *Porphyridium cruentum*, *Scenedesmus acutus*, *Dunaliella* sp., *Scenedesmus obliquus*, *Anabaenopsis*, *Aulosira*, *Cylindrospermum*, *Synechoccus* sp., *Synechocystis* sp., and *Tolypothrix*.

17. An expression cassette comprising:

(a) a transcription factor region comprising an NtcA binding site;

(b) a core promoter region comprising a RuBisCo promoter or a variant or a functional fragment thereof;

(c) a transcribable nucleic acid molecule;

(d) a 3' transcription termination nucleic acid molecule; and

(e) optionally, a polynucleotide sequence encoding an NtcA polypeptide or an NtcB polypeptide;

wherein,

the core promoter region comprises or is operably linked to the transcription factor region;

the core promoter region is operably linked to the transcribable nucleic acid molecule;

the transcribable nucleic acid molecule is operably linked to the 3' transcription termination nucleic acid molecule;

elements (a)-(d) are positioned in relation to each other such that expression of the expression cassette in a host organism results in production of a polypeptide sequence encoded by the transcribable nucleic acid molecule; and

element (e) when present is positioned such that expression of the expression cassette in the host organism results in production of the NtcA polypeptide or the NtcB polypeptide.

18. A transgenic host cell:

comprising the expression system of any one of claims 1-11;

produced by the method of any one of claims 12-16, or the progeny thereof, comprising the expression system; or

comprising the expression cassette of claim 17;

wherein

expression of the polypeptide sequence encoded by the transcribable nucleic acid molecule is repressed in the presence of nitrate; and

expression of the polypeptide sequence encoded by the transcribable nucleic acid molecule is induced in the presence of urea or ammonia.