

Claims:

1. A method of recovering recombinant protein expressed by open reading frame 2 of PCV2 comprising the steps of:

- 5 A) cloning said recombinant open reading frame 2 from PCV2 into a transfer vector;
- B) introducing the portion of said transfer vector containing said recombinant open reading frame 2 into a baculovirus;
- C) infecting cells in media with said baculovirus;
- 10 D) causing said baculovirus to express the protein from said open reading frame 2;
- E) separating cells from said baculovirus vector from the supernate; and
- F) recovering said expressed open reading frame 2 protein in said supernate at least five days after infecting the cells with said
- 15 baculovirus.

2. The method of claim 1, wherein said method further including the step of amplifying said open reading frame 2 from a strain of PCV2 prior to cloning said open reading frame into said transfer vector.

20 3. The method of claim 1 or 2, wherein said recombinant open reading frame 2 further comprising a sequence selected from the group consisting of a 5' Kozak's sequence, a 3' EcoR1 site, and combinations thereof.

25 4. The method of claim 3, wherein said 5' Kozak's sequence comprising SEQ ID NO: 1.

5. The method of claim 3, wherein said 3' EcoR1 site comprising SEQ ID NO: 2.

6. The method of anyone of claims 1 to 5, wherein said PCV2 open reading frame
5 comprising SEQ ID NO: 4.

7. The method of anyone of claims 1 to 5, wherein said recombinant protein comprising
SEQ ID No: 6.

8. The method of anyone of claims 1 to 6, wherein said media comprising serum-free
10 insect cell media.

9. The method of anyone of claims 1 to 8, further comprising the steps of:

- i) prior to step A, cloning said amplified open reading frame 2 into a first vector;
- 15 ii) excising said open reading frame 2 from said first vector; and
- iii) using said excised open reading frame 2 in step A.

10. The method of anyone of claims 1 to 9, wherein said cells comprising SF+ cells.

11. The method of anyone of claims 1 to 10, wherein said introduced portion comprising
20 SEQ ID NO: 4.

12. The method of anyone of claims 1 to 11, wherein the expressed recombinant open
reading frame 2 protein is secreted by the cells into the surrounding growth media,
25 and

wherein the open reading frame 2 protein is recovered in the supernate, rather than from inside the cells.

13. A method of preparing a composition for invoking an immune response against PCV2, said method comprising the steps of:

- i) introducing a construct into a baculovirus, said construct comprising recombinant DNA from open reading frame 2 of PCV2;
- ii) infecting cells with said baculovirus, said cells being in a growth media;
- iii) causing said baculovirus to express the recombinant open reading frame 2 protein from said open reading frame 2;
- iv) recovering said expressed open reading frame 2 protein in the supernate, wherein said open reading frame 2 protein is recovered at least 5 days after infecting said cells with said baculovirus; and
- v) combining said recovered open reading frame 2 protein with a suitable adjuvant or other pharmaceutically acceptable carrier or excipient.

14. The method of claim 13, wherein said method further including the step of obtaining said construct from a transfer vector.

15. The method of claim 13 or 14, wherein said method further including the step of amplifying said open reading frame 2 from a strain of PCV2 prior to cloning said open reading frame 2 into said transfer vector.

16. The method of anyone of claims 13 to 15, wherein said recombinant open reading frame 2 further comprising a sequence selected from the group consisting of a 5' Kozak's sequence, a 3' EcoR1 site, and combinations thereof.

5 17. The method of claim 16, said 5' Kozak's sequence comprising SEQ ID No: 1.

18. The method of claim 16, said 3' EcoR1 site comprising SEQ ID No: 2.

10 19. The method of anyone of claims 13 to 18, wherein said PCV2 open reading frame 2 comprising SEQ ID NO: 4,

20. The method of anyone of claims 13 to 19, wherein said recombinant open reading frame 2 protein comprising SEQ ID NO: 6.

15 21. The method of anyone of claims 13 to 20, wherein said media comprising serum-free insect cell media.

22. The method of anyone of claims 15 to 21, further comprising the steps of:

- 20
- i) cloning said amplified open reading frame 2 into a first vector;
 - ii) excising said open reading frame 2 from said first vector; and
 - iii) using said excised open reading frame 2 for cloning into said transfer vector.

23. The method of anyone of claims 13 to 22, wherein said cells comprising SF+ cells.

24. The method of anyone of claims 13 to 23, wherein said introduced construct comprising SEQ ID NO: 4.

25. The method of anyone of claims 13 to 24, wherein said recovery step further comprising the step of separating said media from said cells and cell debris.

26. The method of claim 25, wherein said separating step including the step of filtering said cells, cell debris, and growth media through a filter having pores ranging in size from about 0.45 μM to about 1.0 μM .

27. The method of anyone of claims 13 to 26, wherein said method further including the step of inactivating said baculovirus by adding cyclized binary ethylenimine (BEI) and, after the inactivation is completed, a sodium thiosulfate solution is added, prior to combining said recovered open reading frame 2 protein with a suitable adjuvant.

28. The method of anyone of claims 13 to 27, wherein the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media, and wherein the open reading frame 2 protein is recovered in the supernate, rather than from inside the cells.

29. A method of recovering the protein expressed by open reading frame 2 from PCV2, wherein said method including the steps

- i) infecting cells in growth media with a recombinant baculovirus vector containing said open reading frame 2;

- ii) causing said baculovirus vector to express said open reading frame 2 protein;
and
- iii) recovering said expressed open reading frame 2 protein in the supernate,

5 wherein said recovering occurs at least 5 days after the infection of the cells with said baculovirus vector.

30. The method of claim 29, wherein the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media,
10 and wherein the open reading frame 2 protein is recovered in the supernate, rather than from inside the cells.

31. A composition of matter obtained by the method of anyone of claims 12, 28, 30, 41 or 43.

15 32. The composition of claim 31 for use as a medicament.

33. The use of the composition of claim 31 for the preparation of a medicament for the prevention of a PCV2 infection or for the preparation of a medicament for lessening the
20 severity of clinical symptoms associated with PCV2 infection.

34. The composition of claim 31 for use as a vaccine.

35. A method for producing a diagnostic kit for the detection of a PCV2 infection in a sample which comprises

i) producing a recombinant open reading frame 2 protein as recited in anyone of claims 12, 28, 30, 41 or 43;

5 ii) and packaging said recombinant open reading frame 2 protein into a suitable container.

36. The method of claim 35, further comprising the step including an instruction manual with the container that packages the recombinant open reading frame 2 protein.

10 37. Kit including at least one container comprising at least one dose of the composition of claim 31, wherein said composition is immunogenic and one dose comprises at least 2 µg PCV2 open reading frame 2 protein.

15 38. Kit comprising i) a container containing at least one dose of the composition of claim 31, and ii) a container containing an immunogenic composition comprising PRRS antigen.

39. Kit of claim 38, wherein the PRRS antigen is IngelVac PRRS MLV.

20 40. A method of recovering recombinant protein expressed by open reading frame 2 of PCV2 comprising the steps of:

A) cloning said recombinant open reading frame 2 from PCV2 into a transfer vector;

- B) introducing the portion of said transfer vector containing said recombinant open reading frame 2 into a baculovirus;
- C) infecting cells in media with said baculovirus;
- D) causing said baculovirus to express the protein from said open reading frame 2 for at least 5 days;
- E) separating cells from said baculovirus vector from the supernate; and
- F) recovering said expressed open reading frame 2 protein in said supernate.

41. The method of claim 40, wherein in step D) the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media.

42. A method of preparing a composition for invoking an immune response against PCV2, said method comprising the steps of:

- i) introducing a construct into a baculovirus, said construct comprising recombinant DNA from open reading frame 2 of PCV2;
- ii) infecting cells with said transfected baculovirus, said cells being in a growth media;
- iii) causing said baculovirus to express the recombinant protein from said open reading frame 2 for at least 5 days;
- iv) recovering said expressed open reading frame 2 protein in the supernate; and
- v) combining said recovered protein with a suitable adjuvant or other pharmaceutically acceptable carrier or excipient.

43. The method of claim 42, wherein in step iii) the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media, and wherein in step iv) said expressed open reading frame 2 protein is recovered in the supernate of said cell culture

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Claims:

1. A method of recovering the protein expressed by open reading frame 2 from PCV2, wherein said method including the steps:

- 5 i) infecting cells in growth media with a recombinant baculovirus vector containing said open reading frame 2;
- ii) causing said baculovirus vector to express said open reading frame 2 protein; and
- iii) recovering said expressed open reading frame 2 protein in the supernate,

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wherein said recovering occurs at least 5 days after the infection of the cells with said baculovirus vector.

2. A method of recovering recombinant protein expressed by open reading frame 2 of PCV2 comprising the steps of:

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- A) cloning said recombinant open reading frame 2 from PCV2 into a transfer vector;
- B) introducing the portion of said transfer vector containing said recombinant open reading frame 2 into a baculovirus;
- 20 C) infecting cells in media with said baculovirus;
- D) causing said baculovirus to express the protein from said open reading frame 2;
- E) separating cells from said baculovirus vector from the supernate; and

F) recovering said expressed open reading frame 2 protein in said supernate at least five days after infecting the cells with said baculovirus.

5 3. The method of claim 2, wherein said method further including the step of amplifying said open reading frame 2 from a strain of PCV2 prior to cloning said open reading frame into said transfer vector.

10 4. The method of claim 2 or 3, wherein said recombinant open reading frame 2 further comprising a sequence selected from the group consisting of a 5' Kozak's sequence, a 3' EcoR1 site, and combinations thereof.

5. The method of claim 4, wherein said 5' Kozak's sequence comprising SEQ ID NO: 1.

15 6. The method of claim 4, wherein said 3' EcoR1 site comprising SEQ ID NO: 2.

7. The method of anyone of claims 2 to 6, wherein said PCV2 open reading frame comprising SEQ ID NO: 4.

20 8. The method of anyone of claims 2 to 6, wherein said recombinant protein comprising SEQ ID No: 6.

9. The method of anyone of claims 2 to 7, wherein said media comprising serum-free insect cell media.

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10. The method of anyone of claims 2 to 9, further comprising the steps of:
- i) prior to step A, cloning said amplified open reading frame 2 into a first vector;
 - ii) excising said open reading frame 2 from said first vector; and
 - iii) using said excised open reading frame 2 in step A.

11. The method of anyone of claims 2 to 10, wherein said cells comprising SF+ cells.

12. The method of anyone of claims 2 to 11, wherein said introduced portion comprising
SEQ ID NO: 4.

13. The method of anyone of claims 1 to 12, wherein the expressed recombinant open
reading frame 2 protein is secreted by the cells into the surrounding growth media,
and
wherein the open reading frame 2 protein is recovered in the supernate, rather than
from inside the cells.

14. A method of preparing a composition for invoking an immune response against
PCV2, said method comprising the steps of:

- i) introducing a construct into a baculovirus, said construct comprising
recombinant DNA from open reading frame 2 of PCV2;
- ii) infecting cells with said baculovirus, said cells being in a growth media;
- iii) causing said baculovirus to express the recombinant open reading frame 2
protein from said open reading frame 2;
- iv) recovering said expressed open reading frame 2 protein in the supernate,
wherein said open reading frame 2 protein is recovered at least 5 days after

infecting said cells with said baculovirus; and

- v) combining said recovered open reading frame 2 protein with a suitable adjuvant or other pharmaceutically acceptable carrier or excipient.

5 15. The method of claim 14, wherein said method further including the step of obtaining said construct from a transfer vector.

16. The method of claim 14 or 15, wherein said method further including the step of amplifying said open reading frame 2 from a strain of PCV2 prior to cloning said open
10 reading frame 2 into said transfer vector.

17. The method of anyone of claims 14 to 16, wherein said recombinant open reading frame 2 further comprising a sequence selected from the group consisting of a 5' Kozak's sequence, a 3' EcoR1 site, and combinations thereof.

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18. The method of claim 17, said 5' Kozak's sequence comprising SEQ ID No: 1.

19. The method of claim 17, said 3' EcoR1 site comprising SEQ ID No: 2.

20 20. The method of anyone of claims 14 to 19, wherein said PCV2 open reading frame 2 comprising SEQ ID NO: 4.

21. The method of anyone of claims 14 to 20, wherein said recombinant open reading frame 2 protein comprising SEQ ID NO: 6.

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22. The method of anyone of claims 14 to 21, wherein said media comprising serum-free insect cell media.

23. The method of anyone of claims 16 to 22, further comprising the steps of:

- i) cloning said amplified open reading frame 2 into a first vector;
- ii) excising said open reading frame 2 from said first vector; and
- iii) using said excised open reading frame 2 for cloning into said transfer vector.

24. The method of anyone of claims 14 to 23, wherein said cells comprising SF+ cells.

25. The method of anyone of claims 14 to 24, wherein said introduced construct comprising SEQ ID NO: 4.

26. The method of anyone of claims 14 to 25, wherein said recovery step further comprising the step of separating said media from said cells and cell debris.

27. The method of claim 26, wherein said separating step including the step of filtering said cells, cell debris, and growth media through a filter having pores ranging in size from about 0.45 μ M to about 1.0 μ M.

28. The method of anyone of claims 14 to 27, wherein said method further including the step of inactivating said baculovirus by adding cyclized binary ethylenimine (BEI) and, after the inactivation is completed, a sodium thiosulfate solution is added, prior to combining said recovered open reading frame 2 protein with a suitable adjuvant.

29. The method of anyone of claims 14 to 28, wherein the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media, and wherein the open reading frame 2 protein is recovered in the supernate, rather than from inside the cells.

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30. A composition of matter obtained by the method of anyone of claims 13, 29, 40 or 42.

31. The composition of claim 30 for use as a medicament.

10 32. The use of the composition of claim 30 for the preparation of a medicament for the prevention of a PCV2 infection or for the preparation of a medicament for lessening the severity of clinical symptoms associated with PCV2 infection.

33. The composition of claim 30 for use as a vaccine.

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34. A method for producing a diagnostic kit for the detection of a PCV2 infection in a sample which comprises

- 20 i) producing a recombinant open reading frame 2 protein as recited in anyone of claims 13, 29, 40 or 42;
- ii) and packaging said recombinant open reading frame 2 protein into a suitable container.

35. The method of claim 34, further comprising the step including an instruction manual
25 with the container that packages the recombinant open reading frame 2 protein.

36. Kit including at least one container comprising at least one dose of the composition of claim 30, wherein said composition is immunogenic and one dose comprises at least 2 µg PCV2 open reading frame 2 protein.

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37. Kit comprising i) a container containing at least one dose of the composition of claim 30, and ii) a container containing an immunogenic composition comprising PRRS antigen.

38. Kit of claim 37, wherein the PRRS antigen is IngelVac PRRS MLV.

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39. A method of recovering recombinant protein expressed by open reading frame 2 of PCV2 comprising the steps of:

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A) cloning said recombinant open reading frame 2 from PCV2 into a transfer vector;

B) introducing the portion of said transfer vector containing said recombinant open reading frame 2 into a baculovirus;

C) infecting cells in media with said baculovirus;

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D) causing said baculovirus to express the protein from said open reading frame 2 for at least 5 days;

E) separating cells from said baculovirus vector from the supernate; and

F) recovering said expressed open reading frame 2 protein in said supernate.

40. The method of claim 39, wherein in step D) the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media.

41. A method of preparing a composition for invoking an immune response against PCV2, said method comprising the steps of:

- i) introducing a construct into a baculovirus, said construct comprising recombinant DNA from open reading frame 2 of PCV2;
- ii) infecting cells with said transfected baculovirus, said cells being in a growth media;
- 10 iii) causing said baculovirus to express the recombinant protein from said open reading frame 2 for at least 5 days;
- iv) recovering said expressed open reading frame 2 protein in the supernate; and
- v) combining said recovered protein with a suitable adjuvant or other pharmaceutically acceptable carrier or excipient.

15 42. The method of claim 41, wherein in step iii) the expressed recombinant open reading frame 2 protein is secreted by the cells into the surrounding growth media, and wherein in step iv) said expressed open reading frame 2 protein is recovered in the supernate of said cell culture.