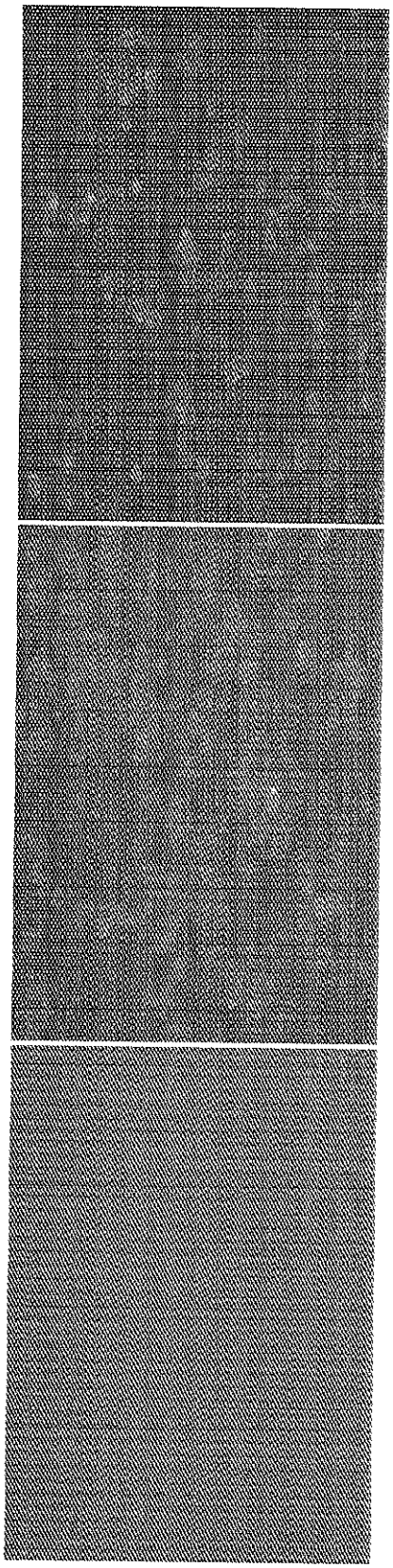
Fig. 1

SF cells infected with ORF2-CSL baculovirus



1a Stained with rabbit anti-CSL serum – Rabbit #13

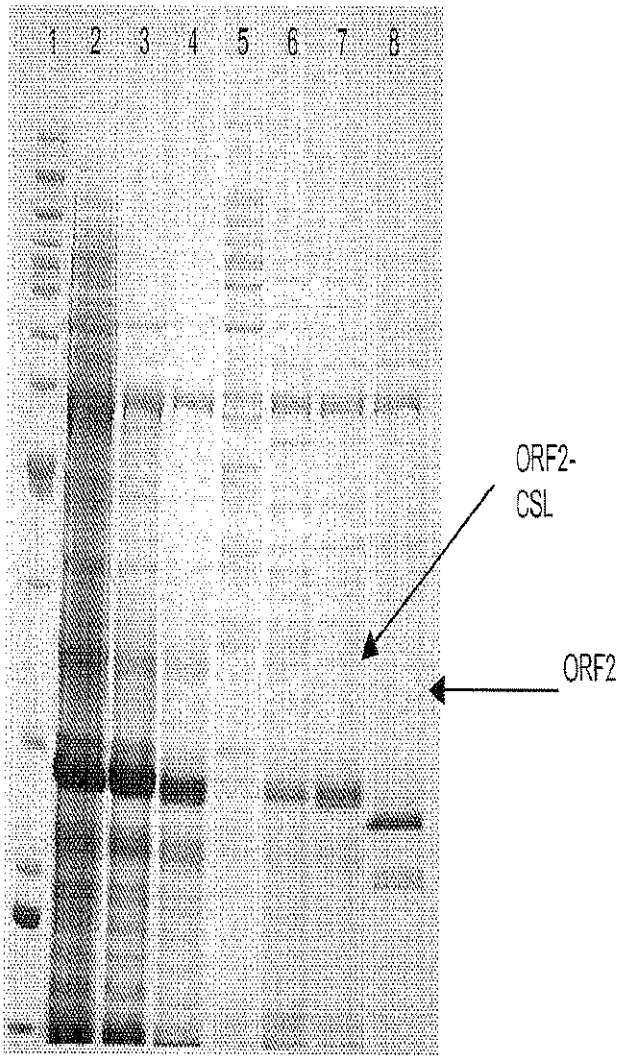
1b Stained with swine anti-PCV2 serum

1c Stained with goat anti-rabbit-FITC

10% Bis-Tris/MOPS gel

1) 10 kDa marker
2) 96 hr $NaHCO_3$ lysate of pellet - 15 µl
3) 120 hr $NaHCO_3$ lysate of pellet - 15 µl
4) 144 hr $NaHCO_3$ lysate of pellet - 15 µl
5) 96 hr supernatant - 20 µl
6) 120 hr supernatant - 20 µl
7) 144 hr supernatant - 20 µl
8) ORF2 Lot001 - 20 µl

Fig. 2 2/7



Spot Blot Results

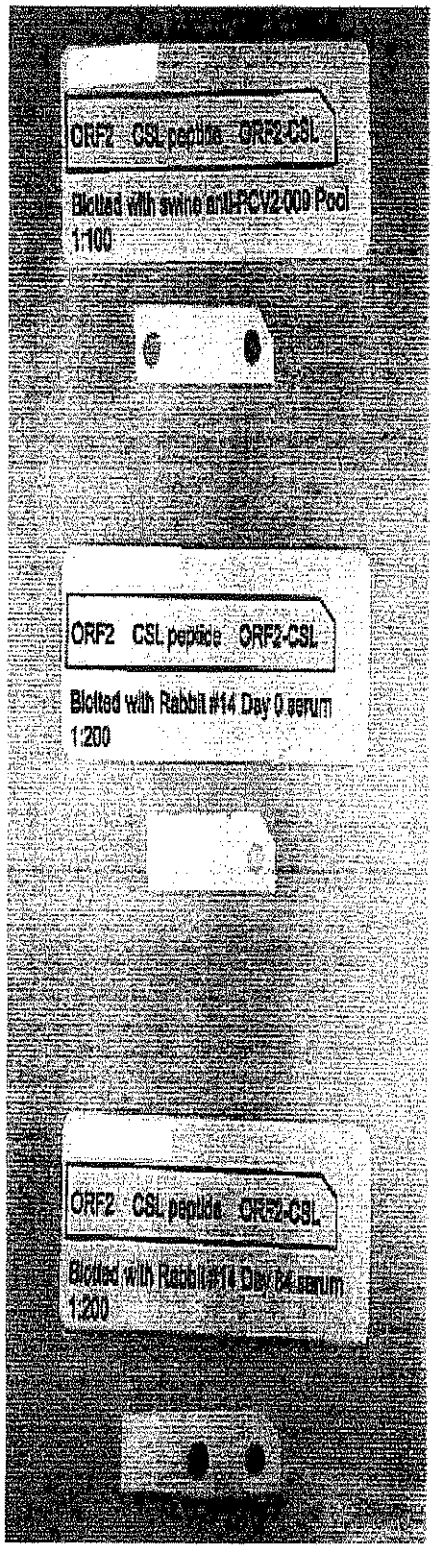


Fig. 3 3/7

Fig. 4a

Western blot – Primary Ab Rabbit#14 serum 1:200

Fig. 4b

Coomassie stained blot Total protein profiles

10% Bis-Tris/MOPS gel

1) 10 kDa marker
2) Prestained Marker
3) 144 hr $NaHCO_3$ lysate of pellet - 15 µl
4) 144 hr supernatant - 20 µl
5) ORF2 Lot001 - 20 µl

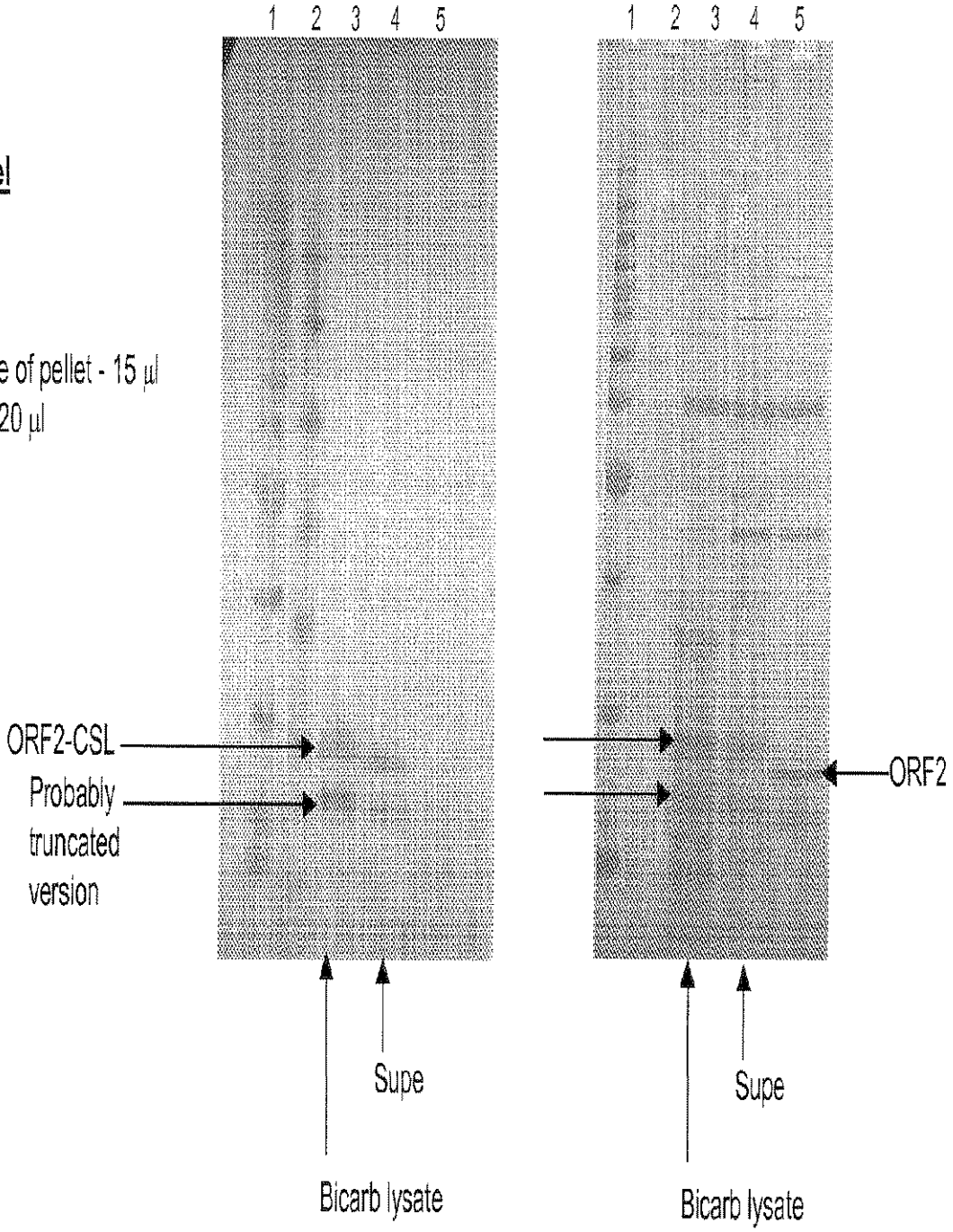


Figure 5. Alignment comparing PCV2 ORF2 with AscI restriction enzyme site to unmodified PCV2 ORF2.

```
            M  T  Y  P  R  R  R  Y  R  R  R  R  H  R  P  R  S  H  L  G  Q  I  L  R  R  R  P  W  L  V  H  P  R  H   34
>PCV2 AscI ATGACGTATCCAAGGAGGCGTTACCGCAGAAGAAGACACCGCCCCCGCAGCCATCTTGGCCAGATCCTCCGCCGCCGCCCCTGGCTCGTCCACCCCCGCCACC 103
            M  T  Y  P  R  R  R  Y  R  R  R  R  H  R  P  R  S  H  L  G  Q  I  L  R  R  R  P  W  L  V  H  P  R  H   34
>PCV2 ORF2 ATGACGTATCCAAGGAGGCGTTACCGCAGAAGAAGACACCGCCCCCGCAGCCATCTTGGCCAGATCCTCCGCCGCCGCCCCTGGCTCGTCCACCCCCGCCACC 103

            R  W  R  A  R  R  K  N  G  I  F  N  T  R  L  S  R  T  F  G  Y  T  V  K  A  T  T  V  T  T  P  S  W  A  V  69
                  *AscI
>PCV2 AscI GCTGGCGCGCCCGAAGGAAAAATGGCATCTTCAACACCCGCCTCTCCCGCACCTTCGGATATACTGTCAAGGCTACCACAGTCACAACGCCCTCCTGGGCGGT 206
            R  Y  R  W  R  R  K  N  G  I  F  N  T  R  L  S  R  T  F  G  Y  T  V  K  A  T  T  V  T  T  P  S  W  A  V  69
>PCV2 ORF2 GCTACCGTTGGAGAAGGAAAAATGGCATCTTCAACACCCGCCTCTCCCGCACCTTCGGATATACTGTCAAGGCTACCACAGTCACAACGCCCTCCTGGGCGGT 206

              D  M  M  R  F  N  I  D  D  F  V  P  P  G  G  G  T  N  K  I  S  I  P  F  E  Y  Y  R  I  R  K  V  K  V  103
>PCV2 AscI GGACATGATGAGATTTAATATTGACGACTTTGTTCCCCCGGGAGGGGGGACCAACAAAATCTCTATACCCTTTGAATACTACAGAATAAGAAAGGTTAAGGTT 309
              D  M  M  R  F  N  I  D  D  F  V  P  P  G  G  G  T  N  K  I  S  I  P  F  E  Y  Y  R  I  R  K  V  K  V  103
>PCV2 ORF2 GGACATGATGAGATTTAATATTGACGACTTTGTTCCCCCGGGAGGGGGGACCAACAAAATCTCTATACCCTTTGAATACTACAGAATAAGAAAGGTTAAGGTT 309

            E  F  W  P  C  S  P  I  T  Q  G  D  R  G  V  G  S  T  A  V  I  L  D  D  N  F  V  T  K  A  T  A  L  T   137
>PCV2 AscI GAATTCTGGCCCTGCTCCCCCATCACCCAGGGTGATAGGGGAGTGGGCTCCACTGCTGTTATTCTAGATGATAACTTTGTAACAAAGGCCACAGCCCTAACCT 412
            E  F  W  P  C  S  P  I  T  Q  G  D  R  G  V  G  S  T  A  V  I  L  D  D  N  F  V  T  K  A  T  A  L  T   137
>PCV2 ORF2 GAATTCTGGCCCTGCTCCCCCATCACCCAGGGTGATAGGGGAGTGGGCTCCACTGCTGTTATTCTAGATGATAACTTTGTAACAAAGGCCACAGCCCTAACCT 412

            Y  D  P  Y  V  N  Y  S  S  R  H  T  I  P  Q  P  F  S  Y  H  S  R  Y  F  T  P  K  P  V  L  D  S  T  I  D 172
>PCV2 AscI ATGACCCATATGTAAACTACTCCTCCCGCCATACAATCCCCCAACCCTTCTCCTACCACTCCCGTTACTTCACACCCAAACCTGTTCTTGACTCCACTATTGA 515
            Y  D  P  Y  V  N  Y  S  S  R  H  T  I  P  Q  P  F  S  Y  H  S  R  Y  F  T  P  K  P  V  L  D  S  T  I  D 172
>PCV2 ORF2 ATGACCCATATGTAAACTACTCCTCCCGCCATACAATCCCCCAACCCTTCTCCTACCACTCCCGTTACTTCACACCCAAACCTGTTCTTGACTCCACTATTGA 515

              Y  F  Q  P  N  N  K  R  N  Q  L  W  L  R  L  Q  T  S  R  N  V  D  H  V  G  L  G  T  A  F  E  N  S  K  206
>PCV2 AscI TTACTTCCAACCAAATAACAAAAGGAATCAGCTTTGGCTGAGGCTACAAACCTCTAGAAATGTGGACCACGTAGGCCTCGGCACTGCGTTCGAAAACAGTAAA 618
              Y  F  Q  P  N  N  K  R  N  Q  L  W  L  R  L  Q  T  S  R  N  V  D  H  V  G  L  G  T  A  F  E  N  S  K  206
>PCV2 ORF2 TTACTTCCAACCAAATAACAAAAGGAATCAGCTTTGGCTGAGGCTACAAACCTCTAGAAATGTGGACCACGTAGGCCTCGGCACTGCGTTCGAAAACAGTAAA 618

            Y  D  Q  D  Y  N  I  R  V  T  M  Y  V  Q  F  R  E  F  N  L  K  D  P  P  L  E  P  *                    233
>PCV2 AscI TACGACCAGGACTACAATATCCGTGTAACCATGTATGTACAATTCAGAGAATTTAATCTTAAAGACCCCCCACTTGAACCCTAA                    702
            Y  D  Q  D  Y  N  I  R  V  T  M  Y  V  Q  F  R  E  F  N  L  K  D  P  P  L  E  P  *                    233
>PCV2 ORF2 TACGACCAGGACTACAATATCCGTGTAACCATGTATGTACAATTCAGAGAATTTAATCTTAAAGACCCCCCACTTGAACCCTAA                    702
```

PCV2 AscI = PCV2 ORF2 with AscI site added.
PCV2 ORF2 = unmodified PCV2 ORF2.

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Figure 6. Alignment indicating insertion of the M2ae1 region into the PCV2 AscI coding region.

```
          M  T  Y  P  R  R  R  Y  R  R  R  R  H  R  P  R  S  H  L  G  Q  I  L  R  R  R  P  W  L  V  H  P  R  H   34
>PCV2 AscI ATGACGTATCCAAGGAGGCGTTACCGCAGAAGAAGACACCGCCCCCGCAGCCATCTTGGCCAGATCCTCCGCCGCCGCCCCTGGCTCGTCCACCCCCGCCACC 103
          M  T  Y  P  R  R  R  Y  R  R  R  R  H  R  P  R  S  H  L  G  Q  I  L  R  R  R  P  W  L  V  H  P  R  H   34
>int M2ae1 ATGACGTATCCAAGGAGGCGTTACCGCAGAAGAAGACACCGCCCCCGCAGCCATCTTGGCCAGATCCTCCGCCGCCGCCCCTGGCTCGTCCACCCCCGCCACC 103

          R                                                                                                       
                                                                                                  W  R  A  R  R  K  N 42
                                                                                                    *AscI
>PCV2 AscI GCT-----------------------------------------------------------------------------------GGCGCGCCCGAAGGAAAAA 125
          R  W  R  A  M  S  L  L  T  E  V  E  T  P  I  R  N  E  W  G  C  R  C  N  D  S  S  D  R  R  A  R  R  K  N 69
                *AscI                                                                                 *AscI
>int M2ae1 GCTGGCGCGCCCATGAGCCTGCTGACCGAGGTGGAGACCCCCATCCGCAACGAGTGGGGCTGCCGCTGCAACGATAGCAGCGATCGGCGCGCCCGAAGGAAAAA 206

          G  I  F  N  T  R  L  S  R  T  F  G  Y  T  V  K  A  T  T  V  T  T  P  S  W  A  V  D  M  M  R  F  N  I  76
>PCV2 AscI TGGCATCTTCAACACCCGCCTCTCCCGCACCTTCGGATATACTGTCAAGGCTACCACAGTCACAACGCCCTCCTGGGCGGTGGACATGATGAGATTTAATATT 228
          G  I  F  N  T  R  L  S  R  T  F  G  Y  T  V  K  A  T  T  V  T  T  P  S  W  A  V  D  M  M  R  F  N  I  103
>int M2ae1 TGGCATCTTCAACACCCGCCTCTCCCGCACCTTCGGATATACTGTCAAGGCTACCACAGTCACAACGCCCTCCTGGGCGGTGGACATGATGAGATTTAATATT 309

          D  D  F  V  P  P  G  G  G  T  N  K  I  S  I  P  F  E  Y  Y  R  I  R  K  V  K  V  E  F  W  P  C  S  P  110
>PCV2 AscI GACGACTTTGTTCCCCCGGGAGGGGGGACCAACAAAATCTCTATACCCTTTGAATACTACAGAATAAGAAAGGTTAAGGTTGAATTCTGGCCCTGCTCCCCCA 331
          D  D  F  V  P  P  G  G  G  T  N  K  I  S  I  P  F  E  Y  Y  R  I  R  K  V  K  V  E  F  W  P  C  S  P  137
>int M2ae1 GACGACTTTGTTCCCCCGGGAGGGGGGACCAACAAAATCTCTATACCCTTTGAATACTACAGAATAAGAAAGGTTAAGGTTGAATTCTGGCCCTGCTCCCCCA 412

          I  T  Q  G  D  R  G  V  G  S  T  A  V  I  L  D  D  N  F  V  T  K  A  T  A  L  T  Y  D  P  Y  V  N  Y  S 145
>PCV2 AscI TCACCCAGGGTGATAGGGGAGTGGGCTCCACTGCTGTTATTCTAGATGATAACTTTGTAACAAAGGCCACAGCCCTAACCTATGACCCATATGTAAACTACTC 434
          I  T  Q  G  D  R  G  V  G  S  T  A  V  I  L  D  D  N  F  V  T  K  A  T  A  L  T  Y  D  P  Y  V  N  Y  S 172
>int M2ae1 TCACCCAGGGTGATAGGGGAGTGGGCTCCACTGCTGTTATTCTAGATGATAACTTTGTAACAAAGGCCACAGCCCTAACCTATGACCCATATGTAAACTACTC 515

          S  R  H  T  I  P  Q  P  F  S  Y  H  S  R  Y  F  T  P  K  P  V  L  D  S  T  I  D  Y  F  Q  P  N  N  K  179
>PCV2 AscI CTCCCGCCATACAATCCCCCAACCCTTCTCCTACCACTCCCGTTACTTCACACCCAAACCTGTTCTTGACTCCACTATTGATTACTTCCAACCAAATAACAAA 537
          S  R  H  T  I  P  Q  P  F  S  Y  H  S  R  Y  F  T  P  K  P  V  L  D  S  T  I  D  Y  F  Q  P  N  N  K  206
>int M2ae1 CTCCCGCCATACAATCCCCCAACCCTTCTCCTACCACTCCCGTTACTTCACACCCAAACCTGTTCTTGACTCCACTATTGATTACTTCCAACCAAATAACAAA 618

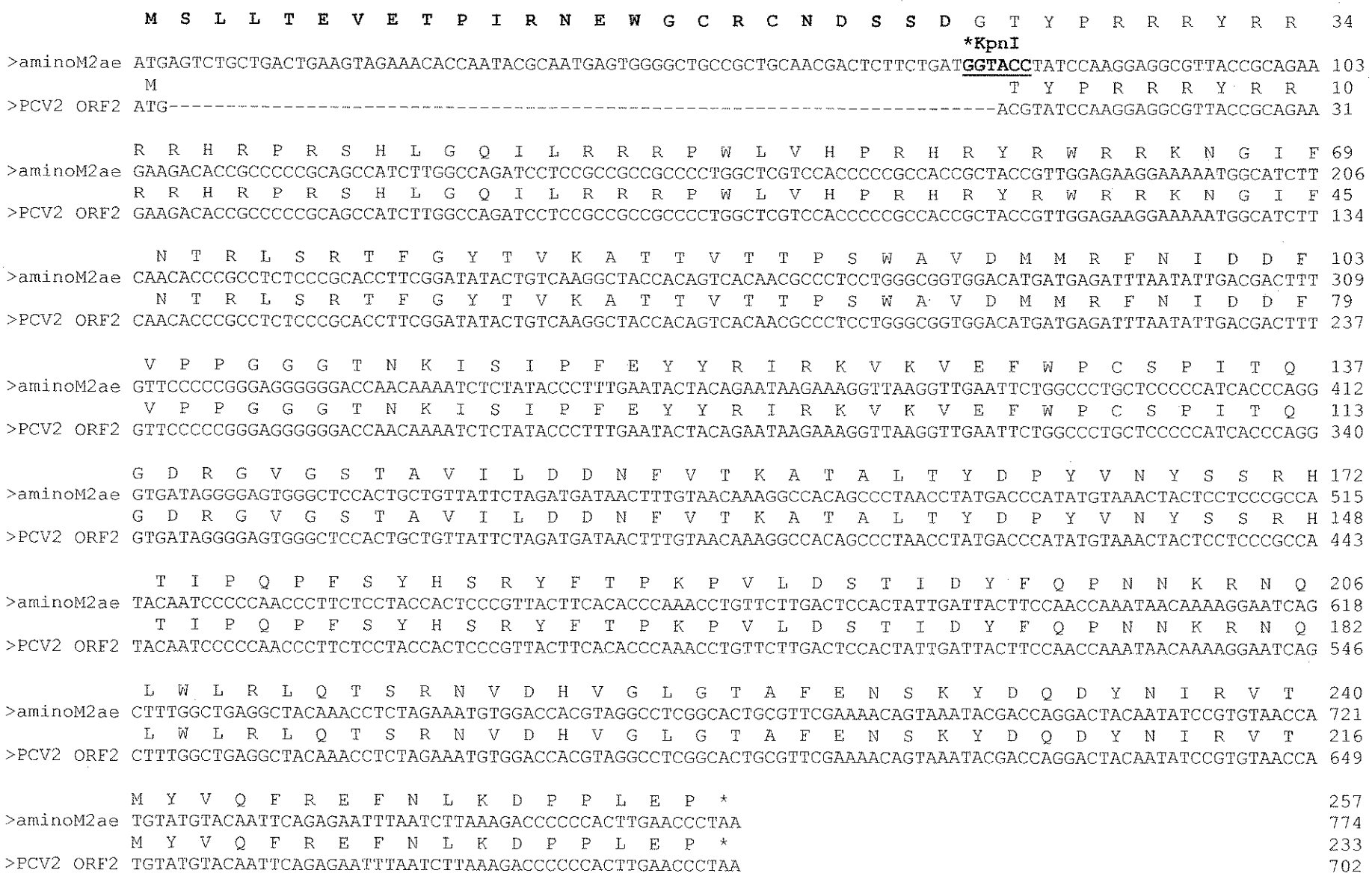
          R  N  Q  L  W  L  R  L  Q  T  S  R  N  V  D  H  V  G  L  G  T  A  F  E  N  S  K  Y  D  Q  D  Y  N  I  213
>PCV2 AscI AGGAATCAGCTTTGGCTGAGGCTACAAACCTCTAGAAATGTGGACCACGTAGGCCTCGGCACTGCGTTCGAAAACAGTAAATACGACCAGGACTACAATATCC 640
          R  N  Q  L  W  L  R  L  Q  T  S  R  N  V  D  H  V  G  L  G  T  A  F  E  N  S  K  Y  D  Q  D  Y  N  I  240
>int M2ae1 AGGAATCAGCTTTGGCTGAGGCTACAAACCTCTAGAAATGTGGACCACGTAGGCCTCGGCACTGCGTTCGAAAACAGTAAATACGACCAGGACTACAATATCC 721

          R  V  T  M  Y  V  Q  F  R  E  F  N  L  K  D  P  P  L  E  P  *                                          241
>PCV2 AscI GTGTAACCATGTATGTACAATTCAGAGAATTTAATCTTAAAGACCCCCCACTTGAACCCTAA                                          702
          R  V  T  M  Y  V  Q  F  R  E  F  N  L  K  D  P  P  L  E  P  *                                          260
>int M2ae1 GTGTAACCATGTATGTACAATTCAGAGAATTTAATCTTAAAGACCCCCCACTTGAACCCTAA                                          783
```

PCV2 AscI = PCV2 ORF2 with AscI site added.
Int M2ae1 = M2ae1 inserted into AscI site of PCV2 AscI coding region.

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Figure 7. Alignment comparing Amino M2ae1 PCV2 ORF2 to unmodified PCV2 ORF2.



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