

Claims

What we claim is:

1. A process or method of characterizing a strain of IBV comprising the steps of:
 - a) generating an IBV cDNA from the IBV strain;
 - b) exposing the cDNA to a primer pair comprising a forward primer and a reverse primer in a real-time polymerase chain reaction (PCR) to yield an amplicon, wherein the primer pair is specific to the S1 gene of IBV genome;
 - c) performing high resolution melt (HRM) curve analysis on a double-stranded product comprising the amplicon immediately after the real-time PCR; and
 - d) analyzing and comparing the HRM curve thereby characterizing the IBV strain.
2. The process or method of claim 1, wherein the primer pair comprises a forward primer having the sequence as set forth in SEQ ID NO:29 and a reverse primer having the sequence as set forth in SEQ ID NO:30.
3. The method of claim 1 or 2, wherein the IBV strain is selected from the group consisting of 793/B strain, Massachusetts strain, QX-like strain, IBNC90 strain, D274 strain, Iowa strain, Arkansas strain, Holland 52 strain, Connecticut 46 strain, Beaudette US strain, California strain, Jilin strain, Holte strain, HK strain, D41 strain, DE072 strain, Spain/92/35 strain, and Egypt/F/03 strain.
4. The process or method of claim 1 further comprising the step of identifying a novel strain of IBV wherein the S1 sequence of the IBV strain does not align with one or more IBV S1 sequences with about 95% sequence identity.
5. The process or method of claim 4 further comprising the step of isolating the novel strain of IBV.
6. The process or method of any one of claims 1-5, wherein the process provides a means for differentiation between infected and vaccinated (DIVA) animals.
7. The process or method of claim 6, wherein the process further comprises the step of identifying whether the IBV strain is a vaccine-type strain or a wild-type strain that infected the animals.
8. The process or method of any one of claims 1-7, wherein the real-time PCR comprises the steps of:

- a) initial denaturation at 95°C for 3 seconds;
 - b) denaturation at 95°C for 1 minute;
 - c) annealing at 55°C for 1 minute;
 - d) extension at 72°C for 1 minute;
 - e) repeating steps b)-d) 45 times.
9. A kit for detecting an IBV strain comprising:
 - a) a primer pair comprising a forward primer having the sequence as set forth in SEQ ID NO:29 and a reverse primer having the sequence as set forth in SEQ ID NO:30;
 - b) an instruction describing the parameters and conditions to perform real-time PCR.
 10. An isolated polynucleotide or primer for HRM analysis having the sequence as set forth in SEQ ID NO:29 or SEQ ID NO:30.
 11. A process or method of characterizing a strain of CSFV comprising the steps of:
 - a) generating a CSFV cDNA from the CSFV strain;
 - b) exposing the cDNA to a primer pair comprising a forward primer and a reverse primer in a real-time polymerase chain reaction (PCR) to yield an amplicon, wherein the primer pair is specific to the NS5B or 3'NTR region of CSFV genome;
 - c) performing high resolution melt (HRM) curve analysis on a double-stranded product comprising the amplicon immediately after the real-time PCR; and
 - d) analyzing and comparing the HRM curve thereby characterizing the CSFV strain.
 12. The process or method of claim 11, wherein the primer pair comprises a forward primer having the sequence as set forth in SEQ ID NO:8 and a reverse primer having the sequence as set forth in SEQ ID NO:9.
 13. The process or method of claim 11 or 12, wherein the CSFV strain is selected from the group consisting of, ALD strain, Chinese strain (C strain), GPE strain (Japanese strain), C/HVRI strain (from China, genotype 1.1), Riems strain (from China), Alfort 187 strain, Ames strain, Margarita strain, Baker strain, New York strain, Purdue 115 strain, Paderborn strain, Spreeda strain, Oregon strain, Singer strain, Osloss strain, Moredun strain, and Frijters strain.
 14. The process or method of claim 11 further comprising the step of identifying a novel strain of CSFV wherein the NS5B or 3'NTR sequence of the CSFV strain does not align with one or more IBV S1 sequences with about 95% sequence identity.

15. The process or method of claim 14 further comprising the step of isolating the novel strain of CSFV.
16. The process or method of any one of claims 11-15, wherein the process provides a means for differentiation between infected and vaccinated (DIVA) animals.
17. The process or method of claim 16 further comprising the step of identifying whether the CSFV strain is a vaccine-type strain or a wild-type strain that infected the animals.
18. The process or method of any one of claims 11-17, wherein the real-time PCR comprises the steps of:
 - a) initial denaturation at 95°C for 3 seconds;
 - b) denaturation at 95°C for 1 minute;
 - c) annealing at 55°C for 1 minute;
 - d) extension at 72°C for 1 minute;
 - e) repeating steps b)-d) 45 times.
19. A kit for detecting an CSFV strain comprising:
 - a) A primer pair comprising a forward primer having the sequence as set forth in SEQ ID NO:8 and a reverse primer having the sequence as set forth in SEQ ID NO:9;
 - b) an instruction describing the parameters and conditions to perform real-time PCR.
20. An isolated polynucleotide or primer for HRM analysis having the sequence as set forth in SEQ ID NO:8 or SEQ ID NO:9.
21. A process or method of characterizing a strain of NDV comprising the steps of:
 - a) generating an NDV cDNA from the IBV strain;
 - b) exposing the cDNA to a primer pair comprising a forward primer and a reverse primer in a real-time polymerase chain reaction (PCR) to yield an amplicon, wherein the primer pair is specific to the F, NP, P, M, HN or L gene of NDV genome;
 - c) performing high resolution melt (HRM) curve analysis on a double-stranded product comprising the amplicon immediately after the real-time PCR; and
 - d) analyzing and comparing the HRM curve thereby characterizing the NDV strain.
22. The process or method of claim 21, wherein the primer pair comprises a forward primer having the sequence as set forth in SEQ ID NO:17 and a reverse primer having the sequence as set forth in SEQ ID NO:18, or a forward primer having the sequence as set

forth in SEQ ID NO:31 and a reverse primer having the sequence as set forth in SEQ ID NO:32.

23. The process or method of claim 21 or 22, wherein the NDV strain is selected from the group consisting of, Avinew strain, LaSota strain, MVP-Mukteswar strain, ND-B1 strain, Korea Dalguban strain, Herts 33 strain, Essex'70 strain, 135/93 strain, 617/83 strain, 34/90 strain, Beaudette C strain, D26 strain, MC110 strain, and 1154/98 strain.
24. The process or method of claim 21 further comprising the step of identifying a novel strain of NDV wherein the F, NP, P, M, HN or L sequence of the NDV strain does not align with one or more NDV F, NP, P, M, HN or L sequences with about 95% sequence identity.
25. The process or method of claim 24 further comprising the step of isolating the novel strain of NDV.
26. The process or method of any one of claims 21-25, wherein the process provides a means for differentiation between infected and vaccinated (DIVA) animals.
27. The process or method of claim 26, wherein the process further comprises the step of identifying whether the NDV strain is a vaccine-type strain or a wild-type strain that infected the animals.
28. The process or method of any one of claims 21-27, wherein the real-time PCR comprises the steps of:
 - a) initial denaturation at 95°C for 3 seconds;
 - b) denaturation at 95°C for 1 minute;
 - c) annealing at 60°C for 1 minute;
 - d) extension at 72°C for 1 minute;
 - e) repeating steps b)-d) 45 times.
29. A kit for detecting an NDV strain comprising:
 - a) a primer pair comprising a forward primer having the sequence as set forth in SEQ ID NO:17 and a reverse primer having the sequence as set forth in SEQ ID NO:18; or a primer pair comprising a forward primer having the sequence as set forth in SEQ ID NO:31 and a reverse primer having the sequence as set forth in SEQ ID NO:32; and
 - b) an instruction describing the parameters and conditions to perform real-time PCR.

30. An isolated polynucleotide or primer for HRM analysis having the sequence as set forth in SEQ ID NO:17, 18, 31, or 32.

What we claim is:

1. A process or method of characterizing a strain of NDV comprising the steps of:
 - a) generating an NDV cDNA from the NDV strains isolated from an animal(s);
 - b) exposing the cDNA to a primer pair comprising a forward primer and a reverse primer in a real-time polymerase chain reaction (PCR) to yield an amplicon, wherein the primer pair is specific to the F, NP, P, M, HN or L gene of NDV genome;
 - c) performing high resolution melt (HRM) curve analysis on a double-stranded product comprising the amplicon immediately after the real-time PCR; and
 - d) analyzing and comparing the HRM curves thereby characterizing the NDV strain, wherein the primer pair comprises a forward primer having the sequence as set forth in SEQ ID NO:17 and a reverse primer having the sequence as set forth in SEQ ID NO:18, or a forward primer having the sequence as set forth in SEQ ID NO:31 and a reverse primer having the sequence as set forth in SEQ ID NO:32.; wherein a saturating dye suitable for HRM is used.
2. The process or method of claim 1, wherein the NDV strain is selected from the group consisting of, Avinew strain, LaSota strain, MVP-Mukteswar strain, ND-B1 strain, Korea Dalguban strain, Herts 33 strain, Essex'70 strain, 135/93 strain, 617/83 strain, 34/90 strain, Beaudette C strain, D26 strain, MC110 strain, and 1154/98 strain.
3. The process or method of any one of claims 1 or 2 further comprising the steps of sequencing the amplicon and identifying a novel strain of NDV wherein the F, NP, P, M, HN or L sequence of the NDV strain does not align with one or more known NDV F, NP, P, M, HN or L sequences with at least 95% sequence identity.
4. The process or method of claim 3 further comprising the step of isolating the novel strain of NDV.
5. The process or method of any one of claims 1-4, wherein the process provides a means for differentiation between infected and vaccinated (DIVA) animals.

6. The process or method of claim 5, wherein the process further comprises the step of identifying whether the NDV strain is a vaccine-type strain or a wild-type strain that infected the animals.
7. The process or method of any one of claims 1 to 6, wherein the real-time PCR comprises the steps of:
 - a) initial denaturation at 95°C for 3 seconds;
 - b) denaturation at 95°C for 1 minute;
 - c) annealing at 60°C for 1 minute;
 - d) extension at 72°C for 1 minute;
 - e) repeating steps b)-d) 45 times.