

Figure 1A

SEQ ID NO	Type	Description
1	primer	XCE2- ^a primer for IBV
2	primer	XCE2- ^b primer for IBV
3	primer	XCE3- primer for IBV
4	primer	BCE1+ primer for IBV
5	primer	DCE1+ primer for IBV
6	primer	MCE1+ primer for IBV
7	DNA	S1 sequence of IB-QX strain amplified by PCR
8	primer	Forward primer for CSFV
9	primer	Reverse primer for CSFV
10	DNA	Sequence of PCR product of CSFV of LBK
11	DNA	Sequence of PCR product of CSFV of VRI
12	DNA	Sequence of PCR product of CSFV of Pestiffa
13	DNA	Sequence of PCR product of CSFV of MVP
14	DNA	Sequence of PCR product of CSFV of QYHC
15	DNA	Sequence of PCR product of CSFV of ZBC
16	DNA	Sequence of PCR product of CSFV of YSC
17	primer	Forward primer 1 for NDV (250bp amplicon)
18	primer	Reverse primer 1 for NDV (250bp amplicon)
19	DNA	NDV strain clone30 (Genbank Accession No. Y18898)
20	DNA	NDV strain Mukteswar (Genbank Accession No. JF950509)
21	DNA	NDV strain B1 (Genbank Accession No. AF309418)
22	DNA	NDV strain VG/GA (Genbank Accession No. EU289028)
23	DNA	NDV strain KBNP-C4152R2L (Genbank Accession No. EU140955)
24	protein	NDV strain clone30 (Genbank Accession No. CAB51325.1)
25	protein	NDV strain Mukteswar (Genbank Accession No. AEL75038)
26	protein	NDV strain B1 (Genbank Accession No. AAG36978.1)
27	protein	NDV strain VG/GA (Genbank Accession No. ABZ80389.1)
28	protein	NDV strain KBNP (Genbank Accession No. ABV60359.1)
29	Primer	Forward primer for IB-QX for IBV
30	Primer	Reverse primer for IB-QX for IBV
31	Primer	Forward primer 2 for NDV (535bp amplicon)
32	Primer	Reverse primer 2 for NDV (535bp amplicon)
33	peptide	F fusion of NDV cleavage site motif 1
34	peptide	F fusion of NDV cleavage site motif 2
35	peptide	F fusion of NDV cleavage site motif 3
36	DNA	M5365 (Genbank Accession No. FJ518780.1)
37	DNA	V9/04 (Genbank Accession No. FJ518779.1)
38	DNA	THA50151 (Genbank Accession No. GQ503613.1)
39	DNA	THA40151 (Genbank Accession No. GQ503612.1)
40	DNA	THA20151 (Genbank Accession No. GQ503610.1)

Figure 1B

SEQ ID NO	Type	Description
41	DNA	THA90151(Genbank Accession No. GQ503617.1)
42	DNA	THA60151(Genbank Accession No. GQ503614.1)
43	DNA	AF193423 (Genbank Accession No. AF193423.1)
44	DNA	AY043312 (Genbank Accession No. AY043312.1)
45	protein	F fusion cleavage motif 4
46	protein	F fusion cleavage motif 5

Figure 2

Sensitivity of real-time PCR assay for IBV

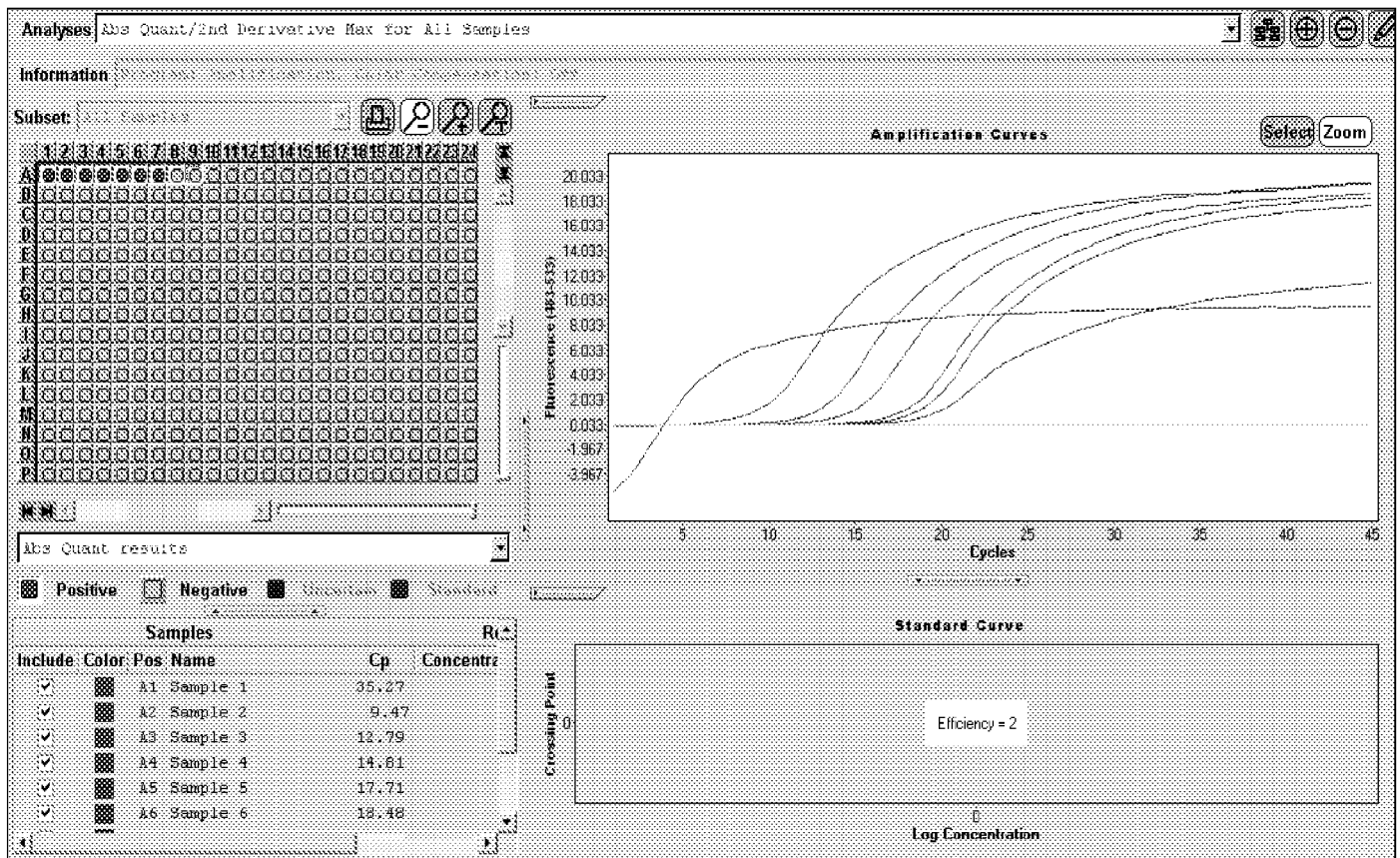


Figure 3

Amplification Curve – IB-QX (Specificity)

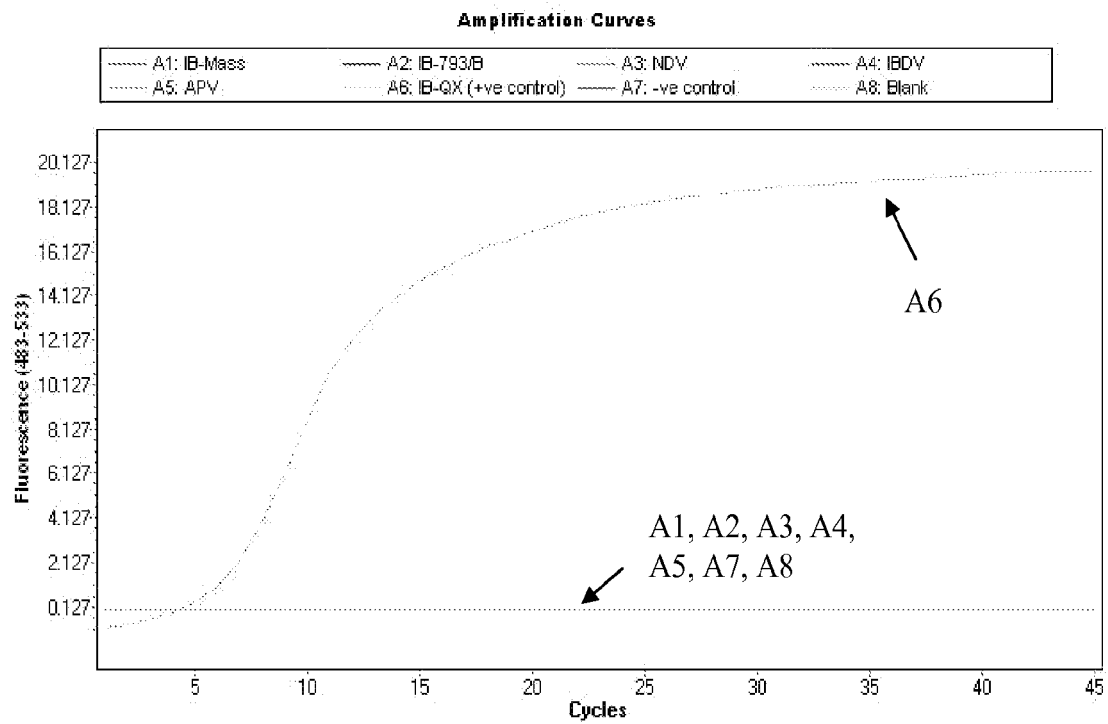


Figure 4

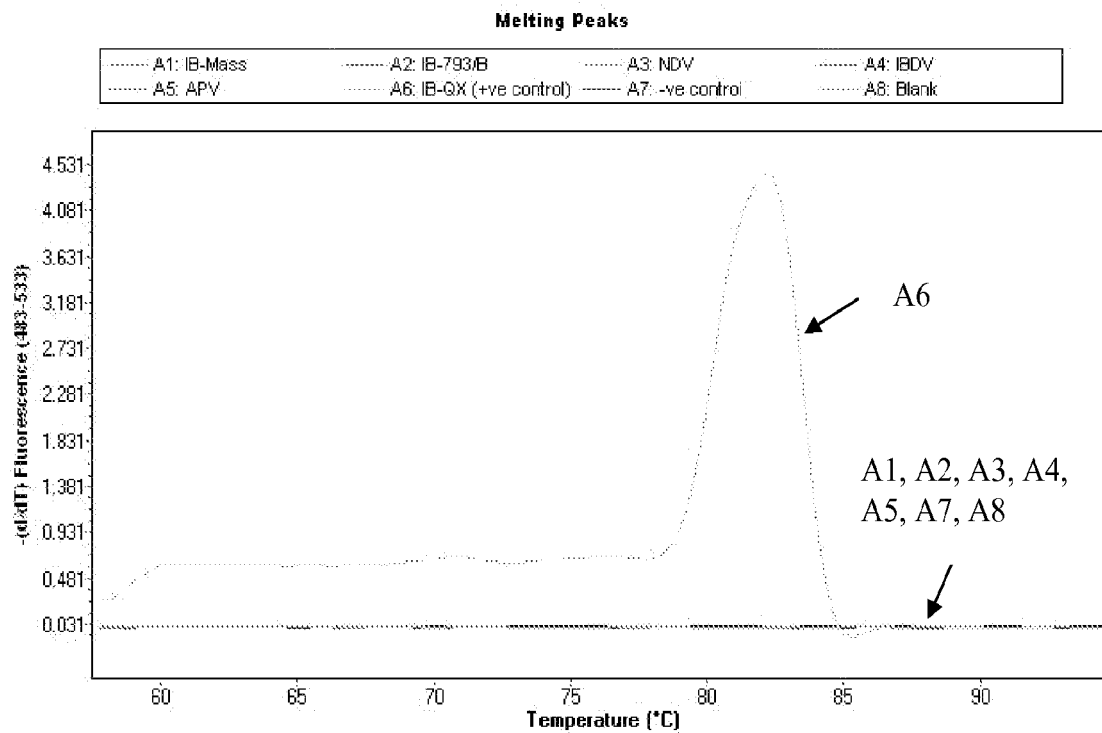
Melting peak (T_m) - IB-QX (Specificity)

Figure 5

SEQ ID NO:7 (S1 sequence of IB-QX strain)

1 GTTCTGCACAAGGGTGCAGTGTGGTGTTATTAAGGATGTTTATAATCAAAGTGTGGCTTCCATAGCTATGACAGCACCT 80
 81 CTTCAGGGTATGGCTTGGTCTAAGGCACAATTCTGTAGTGCACACTGTAACCTTTTCTGAAATTACAGTTTTTGTACACACA 160
 161TTGTATAGTAGTGGTAGTG 180

BLAST results

Sequences producing significant alignments:						
Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EU914939.1	Infectious bronchitis virus isolate UK/AV2150/07 spike glycoprotein S	333	333	100%	2e-88	100%
FJ807928.1	Infectious bronchitis virus isolate K1255/03 S1 glycoprotein (S1) ge	327	327	100%	1e-86	99%
FJ807927.1	Infectious bronchitis virus isolate K1019/03 S1 glycoprotein (S1) ge	327	327	100%	1e-86	99%
HM486955.1	Infectious bronchitis virus isolate K426/08 S1 glycoprotein gene, par	322	322	100%	5e-85	99%
EF621389.1	Infectious bronchitis virus isolate Kr/D75/05 S1 glycoprotein gene, p	322	322	100%	5e-85	99%
EF275415.1	Infectious bronchitis virus isolate NL/1-1449K/04 spike glycoprotein f	322	322	100%	5e-85	99%
JN622553.1	Infectious bronchitis virus isolate 082066/10 spike glycoprotein (S) c	316	316	100%	2e-83	98%
JN622562.1	Infectious bronchitis virus isolate 062561/09 spike glycoprotein (S) c	316	316	100%	2e-83	98%
JN622563.1	Infectious bronchitis virus isolate 062545/09 spike glycoprotein (S) c	316	316	100%	2e-83	98%
JN622564.1	Infectious bronchitis virus isolate 09622/10 spike glycoprotein (S) ge	316	316	100%	2e-83	98%
JN622565.1	Infectious bronchitis virus isolate 09621/10 spike glycoprotein (S) ge	316	316	100%	2e-83	98%
JN622566.1	Infectious bronchitis virus isolate 09623/10 spike glycoprotein (S) ge	316	316	100%	2e-83	98%
JF806088.1	Infectious bronchitis virus isolate K307/10 S1 glycoprotein gene, par	316	316	100%	2e-83	98%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
EU914939.1	Infectious bronchitis virus isolate UK/AV2150/07 spike glycoprotein S1 gene, partial cds	333	333	100%	2e-88	100%
FJ807928.1	Infectious bronchitis virus isolate K1255/03 S1 glycoprotein (S1) gene, partial cds	327	327	100%	1e-86	99%
FJ807927.1	Infectious bronchitis virus isolate K1019/03 S1 glycoprotein (S1) gene, partial cds	327	327	100%	1e-86	99%
HM486955.1	Infectious bronchitis virus isolate K426/08 S1 glycoprotein gene, partial cds	322	322	100%	5e-85	99%
EF621389.1	Infectious bronchitis virus isolate Kr/D75/05 S1 glycoprotein, partial gene, partial cds	322	322	100%	5e-85	99%

Figure 6

Comparison of the previously reported nepropathogenic IB (MH5365/95)
with sequences in Genbank

Sequences producing significant alignments:						
Accession	Description	Max score	Total score	Query coverage	E value	Max ident
EH18783.1	Infectious bronchitis virus isolate MH5365/95 nonfunctional S1 glyco	2976	2976	100%	0.0	100%
GQ503613.1	Infectious bronchitis virus isolate THA50151 S1 glycoprotein (S1) ge	2326	2326	98%	0.0	93%
GQ503612.1	Infectious bronchitis virus isolate THA40151 S1 glycoprotein (S1) ge	2326	2326	98%	0.0	93%
GQ503610.1	Infectious bronchitis virus isolate THA20151 S1 glycoprotein (S1) ge	2326	2326	98%	0.0	93%
GQ503617.1	Infectious bronchitis virus isolate THA90151 S1 glycoprotein (S1) ge	2326	2320	98%	0.0	93%
GQ503614.1	Infectious bronchitis virus isolate THA60151 S1 glycoprotein (S1) ge	2320	2320	98%	0.0	93%
AF193423.1	Avian infectious bronchitis virus S1 spike protein (S1) gene, partial c	2320	1936	100%	0.0	88%
AY043312.1	Avian infectious bronchitis virus isolate A2 S1 protein (S1) gene, par	1936	1925	100%	0.0	88%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
GQ503613.1	Infectious bronchitis virus isolate THA50151 S1 glycoprotein (S1) gene	2976	2326	98%	0.0	93%
GQ503612.1	Infectious bronchitis virus isolate THA40151 S1 glycoprotein (S1) gene	2326	2326	98%	0.0	93%
GQ503610.1	Infectious bronchitis virus isolate THA20151 S1 glycoprotein (S1) gene	2326	2326	98%	0.0	93%
GQ503617.1	Infectious bronchitis virus isolate THA90151 S1 glycoprotein (S1) gene	2326	2320	98%	0.0	93%
GQ503614.1	Infectious bronchitis virus isolate THA60151 S1 glycoprotein (S1) gene	2320	2320	98%	0.0	93%
AF193423.1	Avian infectious bronchitis virus S1 spike protein (S1) gene, partial gene	2320	1936	100%	0.0	88%
AY043312.1	Avian infectious bronchitis virus isolate A2 S1 protein (S1) gene	1936	1925	100%	0.0	88%

Figure 7

Seq->	MH5365	V9/04	THA20151	THA40151	THA50151	THA60151	THA90151	AY043312	AF193423
MH5365	100	80.5	92.8	92.8	92.8	92.8	92.8	88.3	88.7
V9/04		100	80.6	80.6	80.6	80.7	80.7	78.3	78.7
THA20151			100	99.8	99.8	99.9	99.9	89	89.1
THA40151				100	99.7	99.8	99.8	88.9	89.1
THA50151					100	99.8	99.8	89	89.3
THA60151						100	100	89	89.1
THA90151							100	89	89.1
AY043312								100	98.1
AF193423									100

Figure 8
HRM analysis to compare IB-QX, IBnC90, Mass and 793/B

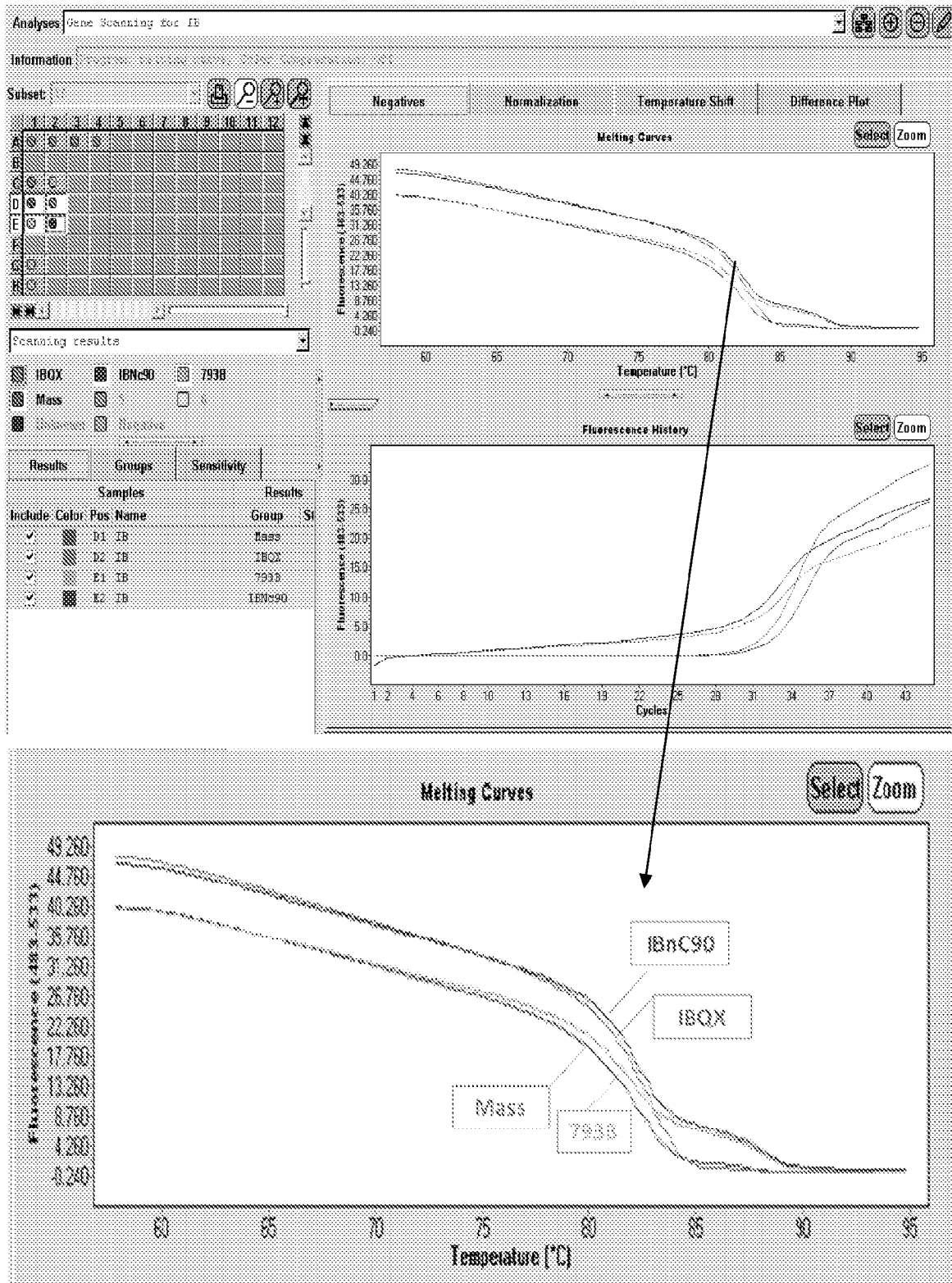


Figure 9
HRM analysis to compare/detect IB-QX and IBnC90

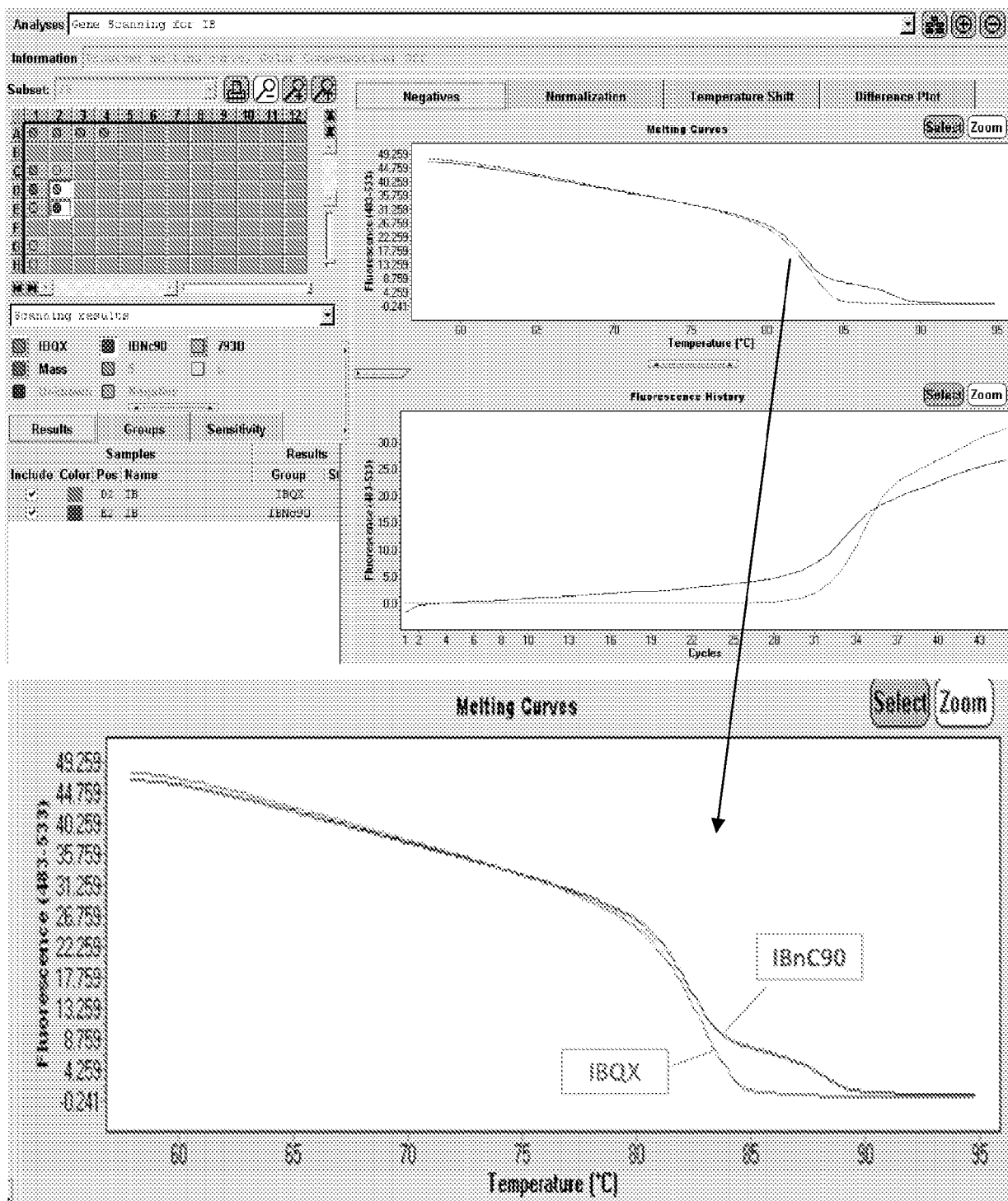


Figure 10

Sequence ID: LBK (wild-type) (SEQ ID NO:10)

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1      TAGCAAGACTGGGAATAGGTACATACCTGGAGAGGGCCACACCCTGCAAGGAACACACTATGAAGAACTGGTGCTAGCAA      80
81     GAAAGCAGGTCAACAACTTTCAAGGGACAGACAGGTATAATCTAGGTCCAATACTCAATATGGTGCTAAGGAGGCTGAGA      160
161    GICATGATGATGACCTTGATTGGGAGAGCGGTATGAGCGTGGTTAACCCGCGAICTGGACCCGCTATTAGGACTCTATTG      240
241    TAGATAACACTATTTATTTTATTATTIAGATATTACTATTTGTTTATTATITATTTATTGAATGAGTAAGAAGTGGT      320
321    ACAAACACTCCTCGTGTTACCACTACACTCATTTTAAACAGCACTT      367

```

BLAST**Sequences producing significant alignments:**

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident	Links
D49532.1	Hog cholera virus (strain ALD) complete sequence, encoding a polyprotein	538	538	100%	1e-149	93%	
AF326963.1	Classical swine fever virus strain Eystrup, complete genome	529	529	100%	7e-147	93%	
HM237795.1	Classical swine fever virus strain CSFV/1.1/dp/CSF0382/XXXX/Koslov	521	521	100%	1e-144	92%	
DQ127910.1	Classical swine fever virus strain SWH, complete genome	520	520	100%	4e-144	92%	
EU497410.1	Classical swine fever virus strain J1(06), complete genome	516	516	100%	5e-143	92%	

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
D49532.1	Hog cholera virus (strain ALD) complete sequence, encoding a polyprotein	538	538	100%	1e-149	93%
AF326963.1	Classical swine fever virus strain Eystrup, complete genome	529	529	100%	7e-147	93%
HM237795.1	Classical swine fever virus strain CSFV/1.1/dp/CSF0382/XXXX/Koslov	521	521	100%	1e-144	92%
DQ127910.1	Classical swine fever virus strain SWH, complete genome	520	520	100%	4e-144	92%
EU497410.1	Classical swine fever virus strain J1(06), complete genome	516	516	100%	5e-143	92%

Figure 11

Sequence of VRI (wild-type) (SEQ ID NO:11)

```

1      TAGCAAGACTGGGAACAGGTACACCCCGGGGAAGGCCACACCCCTGCAAGGAACACACTATGAAGAACTGGTGTAGCAA      80
81     GAAAGCAGGTCAACAACCTTTCAAGGGACAGACAGGTATAATCTAGGTCCAATAGTCAATATGGTGCTAAGGAGGCTGAGA      160
161    GTCATGATGATGACCTTGATTGGGAGAGGGGTATGAGCGTGGTTAACC CGCAICTGGACCCGCTATTAGGACTCTATTG      240
241    TAGATAACACTATTTATTTTCTATTATTIAGATATTACTATTTGTTTATTIATTTATTTATTGAATGAGTAAGAACTGGT      320
321    ACAAACACCTCGTGTACCACACTACACTCATTTTAAACAGCAGCTT      367

```

BLAST**Sequences producing significant alignments:**

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident
D49532.1	Hog cholera virus (strain ALD) complete sequence, encoding a polyprotein	510	510	100%	2e-141	92%
AF326963.1	Classical swine fever virus strain Eystrup, complete genome	501	501	100%	1e-138	91%
U0127910.1	Classical swine fever virus strain SWH, complete genome	497	497	100%	2e-137	91%
HM237795.1	Classical swine fever virus strain CSFV/1.1/dp/CSF0382/XXXX/Koslov	494	494	100%	2e-136	91%
EU497410.1	Classical swine fever virus strain J1(06), complete genome	494	494	100%	2e-136	91%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
D49532.1	Hog cholera virus (strain ALD) complete sequence, encoding a polyprotein	510	510	100%	2e-141	92%
AF326963.1	Classical swine fever virus strain Eystrup, complete genome	501	501	100%	1e-138	91%
HM237795.1	Classical swine fever virus strain CSFV/1.1/dp/CSF0382/XXXX/Koslov	497	497	100%	2e-137	91%
DQ127910.1	Classical swine fever virus strain SWH, complete genome	494	494	100%	2e-136	91%
EU497410.1	Classical swine fever virus strain J1(06), complete genome	494	494	100%	2e-136	91%

Figure 12

Sequence of Pestiffa (SEQ ID NO:12)

```

1      GTACAGGGGGGGGAAGGGAGACATACCCGGGGGGGAAACAGCTTGCACGGAAGACATTATGAAGAACTGGTGTGGCA      80
81     AGAAAAACAGATCAACAACCTTCAAGGGACAGACAGGTACAACCTAGGCCCAATAGTCAACATGGTGTAAAGGAGGCTGAG      160
161    AGTCATGATGATGACGCTGATAGGGAGAGGGGCATGACGCGGGTAACCCGGGATCTGAACCCGCCAGTAGGACCCCTATT      240
241    GIAGATAACACTAATTTTTTTTTTTTTTTTTTTTTTTTTTTAGACATTATTATTATTTTTTTTTTTTTTTTTTAAAAAA      320
321    AAAAAAATTTTTTTAACTACCTCAAGTACCACTACACTCACTTTTATAGGGGGGGG      379

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BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
Z46258.1	Hog cholera virus (Classical swine fever virus) "Chinese" strain (C-str	503	503	92%	4e-139	93%
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	496	496	92%	7e-137	92%
AY805221.1	Classical swine fever virus strain CC/HVRI, complete genome	490	490	92%	3e-135	92%
AY663656.1	Classical swine fever virus, complete genome	490	490	92%	3e-135	92%
AY382481.1	Classical swine fever virus, complete genome	490	490	92%	3e-135	92%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
Z46258.1	Hog cholera virus (Classical swine fever virus) "Chinese" strain (C-strain	503	503	92%	4e-139	93%
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	496	496	92%	7e-137	92%
AY805221.1	Classical swine fever virus strain CC/HVRI, complete genome	490	490	92%	3e-135	92%
AY663656.1	Classical swine fever virus, complete genome	490	490	92%	3e-135	92%
AY382481.1	Classical swine fever virus, complete genome	490	490	92%	3e-135	92%

Figure 13

Sequence of MVP (SEQ ID NO: 13)

```

1      TAGTTAGTAGAAAGACGGGAAAAGGGTACACCCCGGGAGGGGCCACCCCTGCAGGGGAGACATTATGAAGAACTGGTG      80
81     TTGGCAAGAAAACAGATCAAAAACTTTCAAGGGACAGACAGGTACAATCTAGGCCCAATAGTCAACATGGTGTTAAGGAG      160
161    GCTGAGAGTCATATTATTGCCCTTATTGGGGAGGGGGTATGAGCGCGGGCAACCCGGGATCTGGACCCGCCAGTAGGAC      240
241    CCTATTGTAGATAACACTAACTTTTATTTATTTAGATATTATTACTTATTTATTTATTTATTTATTGAATGAGTAAGAA      320
321    CIGGCACAAACTACCTCAAGTTACCACACCTCCCTCTTTTACAGCCCTTTTAATGG      379

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BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident	Length
D49533.1	Hog cholera virus (strain GPE-) complete sequence, encoding a polyprotein	545	545	98%	7e-152	93%	379
U90951.1	Pestivirus type 2 strain Affort A19, complete genome	529	529	98%	7e-147	92%	1200
X87939.1	Classical swine fever virus, strain Affort/157 complete genome	529	529	98%	7e-147	92%	1200
U45460.1	Hog cholera virus strain Gientof polyprotein gene, putative NS5 prot	521	521	98%	1e-144	92%	1000
D49532.1	Hog cholera virus (strain A1D) complete sequence, encoding a polyprotein	518	518	98%	1e-143	92%	379

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
D49533.1	Hog cholera virus (strain GPE-) complete sequence, encoding a polyprotein	545	545	98%	7e-152	93%
U90951.1	Classical swine fever virus strain C-ZJ-2008, complete genome	529	529	98%	7e-147	92%
X87939.1	Classical swine fever virus strain CC/HVRI, complete genome	529	529	98%	7e-147	92%
U45460.1	Classical swine fever virus, complete genome	521	521	98%	1e-144	92%
D49532.1	Classical swine fever virus, complete genome	518	518	98%	1e-143	92%

Figure 14

Sequence of QYHC (SEQ ID NO: 14)

```

1      TCTAGCAAGACTGGGAATAGGTACATACCCGGAGAGGGTCACACCCTGCAAGGAAGACATTATGAAGAAGCTGGTGTGGC      80
81     AAGAAAACAGATCAACAACCTTCAAGGGACAGACAGGTACAACCTAGGCCCAATAGTCAACATGGTGTAAAGGAGGCTGA      160
161    GAGTCATGATGATGACGCTGATAGGGAGAGGGGCATGAGCGCGGGTAACCCGGGATCTGAACCCGCCAGTAGGACCCTAT      240
241    TCTAGATAACACTAATTTTCTTTTCTTTTCTTTTATTTATTTAGATATTATTATTTATTTATTTATTTATTTATTTATGAATGAG      320
321    TAAGAAGCTGGTATAAACACCTCAAGTCACCACACTACACTCATTTTAAACAGCACTTTA      379

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BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident	Links
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	678	678	99%	0.0	99%	
AY663656.1	Classical swine fever virus, complete genome	678	678	99%	0.0	99%	
AY382481.1	Classical swine fever virus, complete genome	678	678	99%	0.0	99%	
AF531433.1	Classical swine fever virus strain HCV, complete genome	678	678	99%	0.0	99%	
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	673	673	99%	0.0	99%	

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	678	678	99%	0.0	99%
AY663656.1	Classical swine fever virus, complete genome	678	678	99%	0.0	99%
AY382481.1	Classical swine fever virus, complete genome	678	678	99%	0.0	99%
AF531433.1	Classical swine fever virus strain HCV, complete genome	678	678	99%	0.0	99%
HM175885.1	Classical swine fever virus strain, C-ZJ-2008, complete genome	673	673	99%	0.0	99%

Figure 15

Sequence of ZBC (SEQ ID NO: 15)

```

1      TGTAGCAAGACTGGGAATAGGTACATCCCCGGGAAGGTCACAACATGCAAGGAAGACATTATGAAGAACTGGTGTGGC      80
81     AAGAAAACAGATCAACAACCTTCAAGGGACAGACAGGTACAACCTAGGCCCAATAGTCAACATGGTGTAAAGGAGGCTGA      160
161    GAGTCATGATGATGACGCTGATAGGGAGAGGGGCATGAGCGCGGGTAACCCGGGATCTGAACCCGCCAGTAGGACCCTAT      240
241    TGTAGATAACACTAATTTTCCTTTTCTTTTTATTATTATTAGATATTATTATTATTATTATTATTATTATTATTGAATGA      320
321    GTAAGAACTGGTATAAACACCTCAAGACCACACTACACTCATTTTAAACAGCACTTTAA      379

```

BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	649	649	99%	0.0	98%
AY663656.1	Classical swine fever virus, complete genome	649	649	99%	0.0	98%
AY382481.1	Classical swine fever virus, complete genome	649	649	99%	0.0	98%
AF531433.1	Classical swine fever virus strain HCLV, complete genome	649	649	99%	0.0	98%
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	645	645	99%	0.0	97%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	649	649	99%	0.0	98%
AY663656.1	Classical swine fever virus, complete genome	649	649	99%	0.0	98%
AY382481.1	Classical swine fever virus, complete genome	649	649	99%	0.0	98%
AF531433.1	Classical swine fever virus strain HCV, complete genome	649	649	99%	0.0	98%
HM175885.1	Classical swine fever virus strain, C-ZJ-2008, complete genome	645	645	99%	0.0	97%

Figure 16

Sequence of YSC (SEQ ID NO: 16)

```

1      GGGGGCCCCCGGAAAAGTTAAACCTGCACGAGACATTCAATGAAGAACTGGTGTTCGAAGAAAACAGATCAACAAC  80
81     TTCAAGGGACAGACAGGTACAACCTAGGCCCAATAGTCAACATGGTGTTAAGGAGGCTGAGAGTCATGATGATGACGCTG  160
161    ATAGGGAGAGGGGCATGAGCGCGGGTAACCCGGGATCTGAACCGCCAGTAGGACCCATTGTAGACAACACTAATCTTT  240
241    TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATATTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAAAGCAA  320
321    AAACTGGTATAAACCCCCCCCCCCCCCCCCCTTTTTTTTTTAAAAAACTTTAGC  379

```

BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E-value	Max ident
U45456.1	Hog cholera virus strain Riems polyprotein gene, putative NS5 protein	455	455	82%	1e-124	93%
AY259122.1	Classical swine fever virus strain Riems, complete genome	398	398	65%	2e-107	96%
Z46258.1	Hog cholera virus (Classical swine fever virus) "Chinese" strain (C-strain)	385	385	63%	2e-103	95%
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	383	383	63%	6e-103	95%
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	381	381	63%	2e-102	95%

Accession Number	Description	Max Score	Total Score	Query coverage	E-value	Max identity
U45456.1	Hog cholera virus strain Riems polyprotein gene, putative NS5 protein	455	455	82%	1e-124	93%
AY259122.1	Classical swine fever virus strain Riems, complete genome	398	398	65%	2e-107	96%
Z46258.1	Hog cholera virus (Classical swine fever virus) "Chinese" strain (C-strain)	385	385	63%	2e-103	95%
HM175885.1	Classical swine fever virus strain C-ZJ-2008, complete genome	383	383	63%	6e-103	95%
AY805221.1	Classical swine fever virus strain C/HVRI, complete genome	381	381	63%	2e-102	95%

Differentiation of wild-type vs. vaccine-type of CSFV

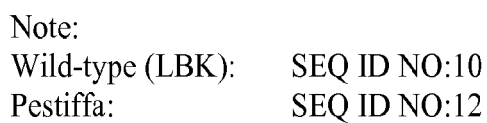


Figure 18

HRM assay to differentiate different vaccine-type CSFV

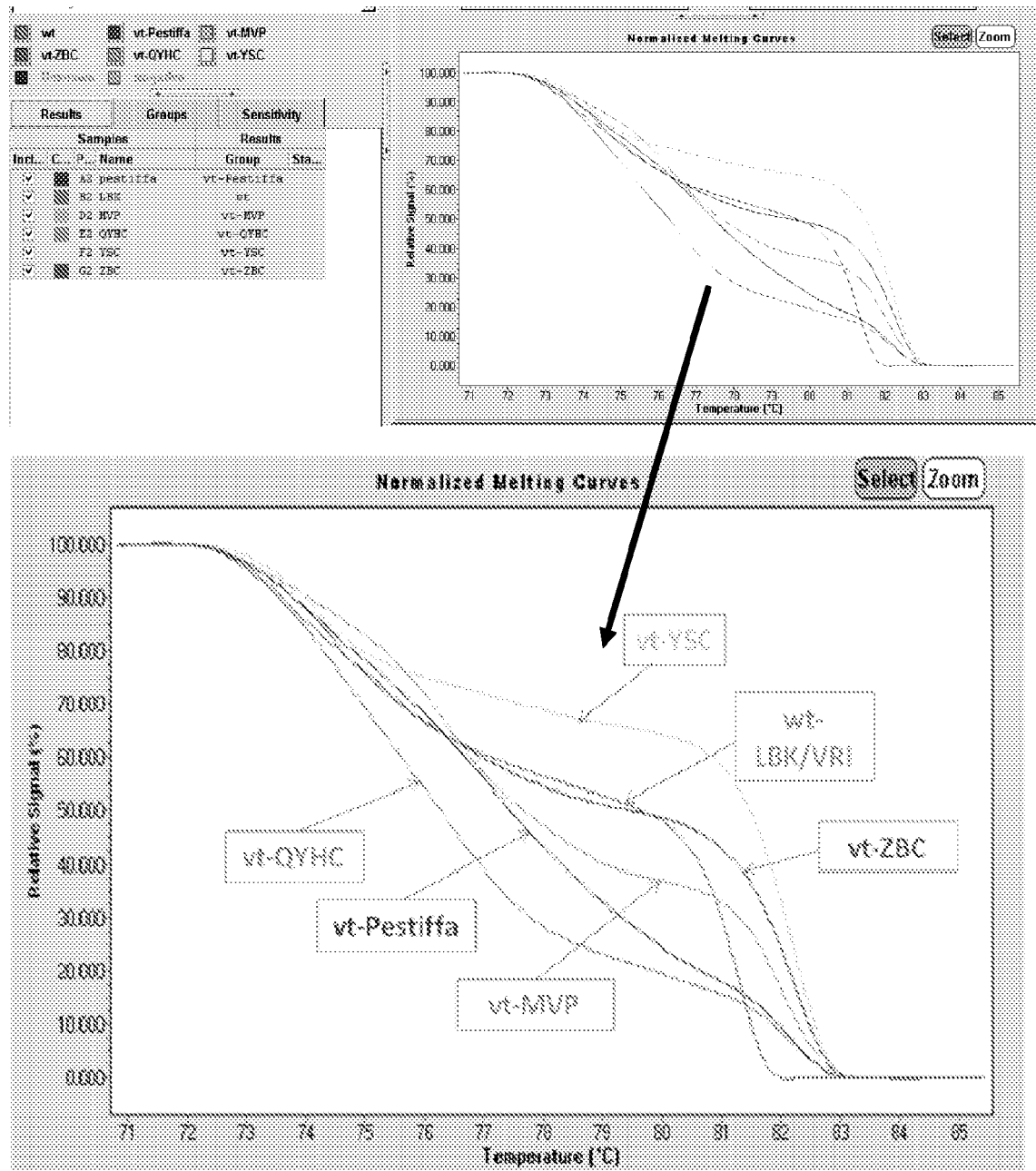
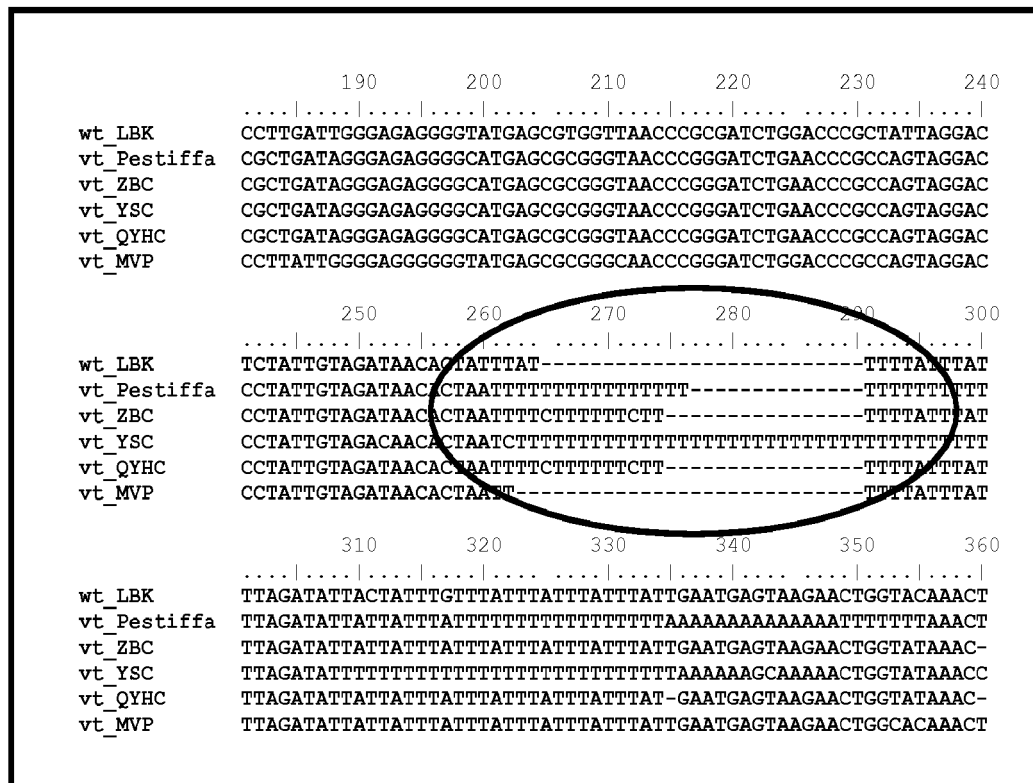


Figure 19

A portion of the nucleotide sequence alignment of the wild-type (LBK) and the vaccine-types (Pestiffa, ZBC, YSC, QYHC and MVP)



LBK: SEQ ID NO:10
 Pestiffa: SEQ ID NO:12
 ZBC: SEQ ID NO:15
 YSC: SEQ ID NO:16
 QYHC: SEQ ID NO:14
 MVP: SEQ ID NO:13

Figure 20

HRM assay of NDV vaccine strains

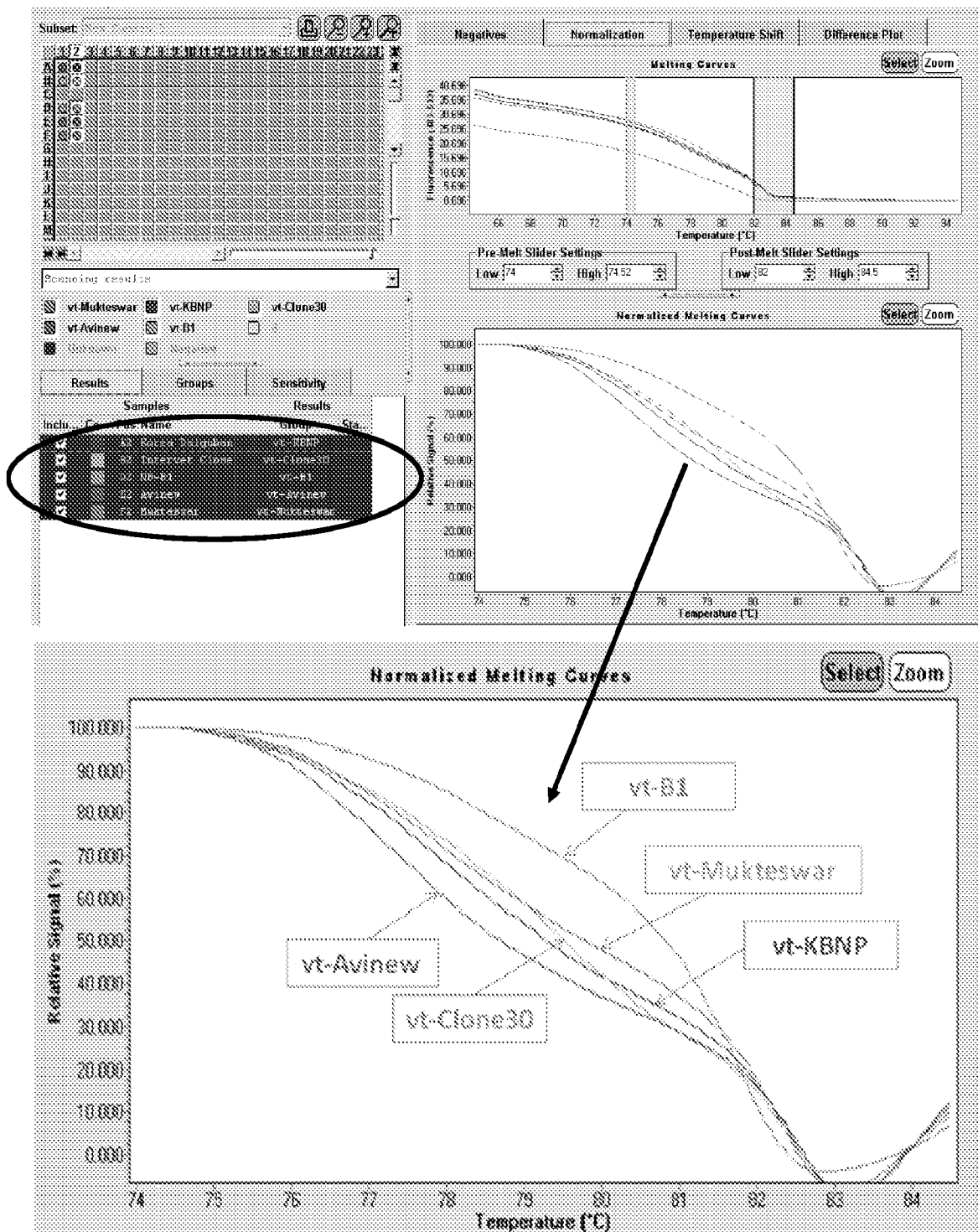


Figure 21A

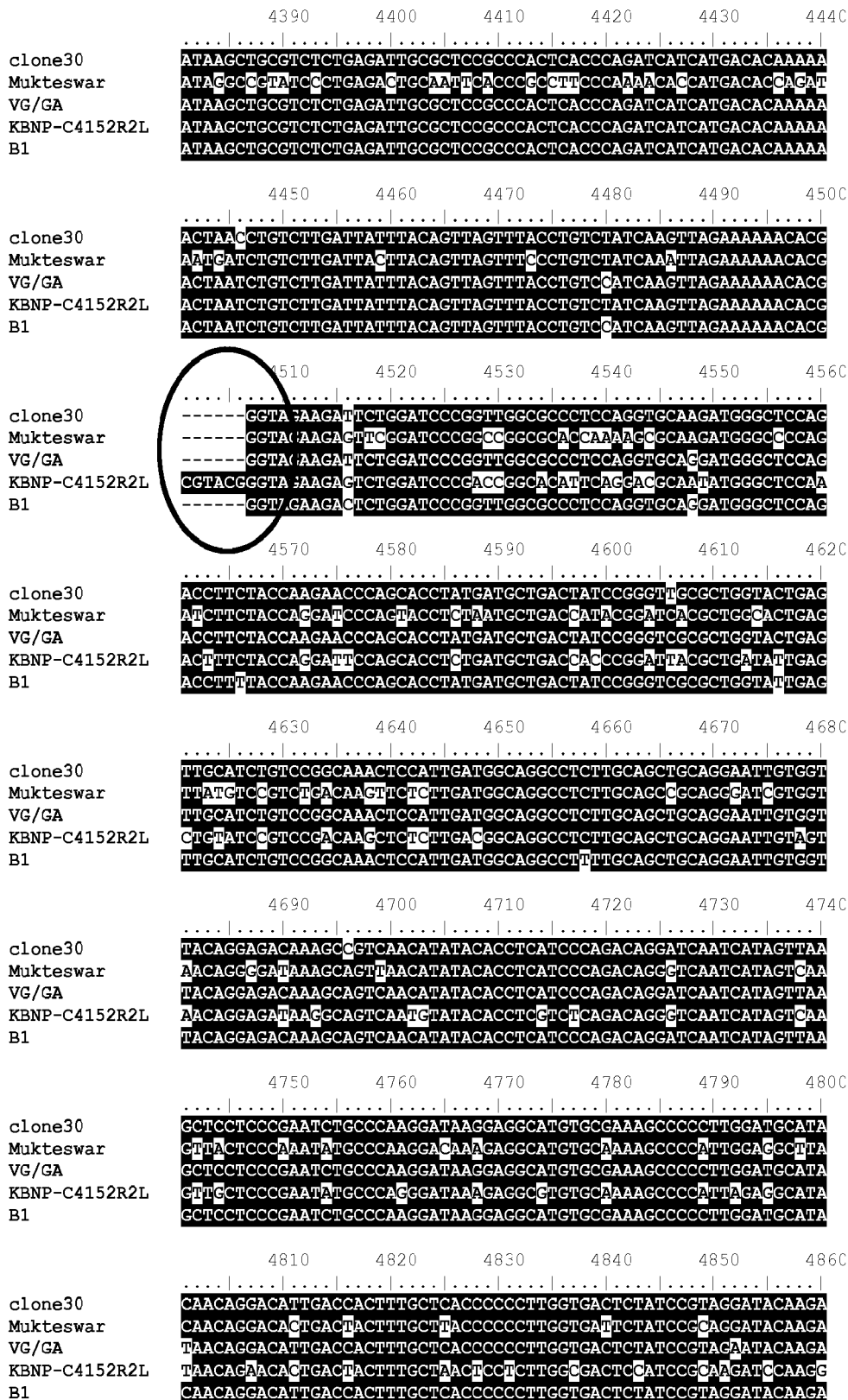


Figure 21B

4870 4880 4890 4900 4910 4920

clone30 GTCTGTGACTACATCTGGAGGGGGGAGACAGGGCGCCTTATAGGCGCCATTATTGGCGG

Mukteswar GTCTGTGACTACATCGGAGGAAAGGAGACAGAGACGCTTTATAGGTGCCATTATTGGCAG

VG/GA GTCTGTGACTACATCTGGAGGGGGGAGACAGGGCGCCTTATAGGCGCCATTATTGGCGG

KBNP-C4152R2L GTCTGTGTCCACCTCTGGAGGAGGACAGACAGCAGCCTGATAGGTGCTGTTATTGGCAG

B1 GTCTGTGACTACATCTGGAGGGGGGAGACAGGGCGCCTTATAGGCGCCATTATTGGCGG

clone30: SEQ ID NO:19; Mukteswar: SEQ ID NO:20; B1: SEQ ID NO:21

VG/GA: SEQ ID NO:22' KBNP-C4152R2L: SEQ ID NO:23;

Figure 22

	10	20	30	40	50	60	70																																																															
Clone30	M	G	S	R	P	S	T	K	N	P	A	M	M	L	T	I	R	V	A	L	V	L	S	C	I	P	A	N	S	I	D	G	R	P	L	A	A	G	I	V	V	T	G	D	K	A	V	N	I	Y	T	S	S	Q	T	G	S	I	I	V	K	L	L	P	N	L	P			
Mukteswar	M	G	P	R	S	T	K	N	P	A	M	M	L	T	I	R	V	A	L	V	L	S	C	I	P	A	N	S	I	D	G	R	P	L	A	A	G	I	V	V	T	G	D	K	A	V	N	I	Y	T	S	S	Q	T	G	S	I	I	V	K	L	L	P	N	L	P				
Avinew	M	G	S	R	P	S	T	K	N	P	A	M	M	L	T	I	R	V	A	L	V	L	S	C	I	P	A	N	S	I	D	G	R	P	L	A	A	G	I	V	V	T	G	D	K	A	V	N	I	Y	T	S	S	Q	T	G	S	I	I	V	K	L	L	P	N	L	P			
KBNP	M	G	S	R	P	S	T	K	N	P	A	M	M	L	T	I	R	V	A	L	V	L	S	C	I	P	A	N	S	I	D	G	R	P	L	A	A	G	I	V	V	T	G	D	K	A	V	N	I	Y	T	S	S	Q	T	G	S	I	I	V	K	L	L	P	N	L	P			
B1	M	G	S	R	P	S	T	K	N	P	A	M	M	L	T	I	R	V	A	L	V	L	S	C	I	P	A	N	S	I	D	G	R	P	L	A	A	G	I	V	V	T	G	D	K	A	V	N	I	Y	T	S	S	Q	T	G	S	I	I	V	K	L	L	P	N	L	P			
	80	90	100	110	120	130	140																																																															
Clone30	K	D	K	E	A	C	A	K	A	P	L	D	A	Y	N	R	T	L	T	T	L	T	P	L	G	S	I	R	R	I	Q	E	S	V	T	T	S	G	G	R	Q	R	L	I	G	A	I	G	G	V	A	L	G	V	A	A	Q	I	T	A	A	A	A							
Mukteswar	K	D	K	E	A	C	A	K	A	P	L	D	A	Y	N	R	T	L	T	T	L	T	P	L	G	S	I	R	R	I	Q	E	S	V	T	T	S	G	G	R	Q	R	L	I	G	A	I	G	G	V	A	L	G	V	A	A	Q	I	T	A	A	A	A							
Avinew	K	D	K	E	A	C	A	K	A	P	L	D	A	Y	N	R	T	L	T	T	L	T	P	L	G	S	I	R	R	I	Q	E	S	V	T	T	S	G	G	R	Q	R	L	I	G	A	I	G	G	V	A	L	G	V	A	A	Q	I	T	A	A	A	A							
KBNP	K	D	K	E	A	C	A	K	A	P	L	D	A	Y	N	R	T	L	T	T	L	T	P	L	G	S	I	R	R	I	Q	E	S	V	T	T	S	G	G	R	Q	R	L	I	G	A	I	G	G	V	A	L	G	V	A	A	Q	I	T	A	A	A	A							
B1	K	D	K	E	A	C	A	K	A	P	L	D	A	Y	N	R	T	L	T	T	L	T	P	L	G	S	I	R	R	I	Q	E	S	V	T	T	S	G	G	R	Q	R	L	I	G	A	I	G	G	V	A	L	G	V	A	A	Q	I	T	A	A	A	A							
	150	160	170	180	190	200	210																																																															
Clone30	L	I	Q	A	K	Q	N	A	N	I	L	R	L	K	E	S	I	A	A	T	N	E	A	V	H	E	V	T	D	G	L	S	Q	L	A	V	A	V	G	K	M	Q	Q	F	V	N	D	Q	F	N	K	T	A	Q	E	L	D	C	I	K	I	A	Q	Q	V	G	V	E	L	
Mukteswar	L	I	Q	A	K	Q	N	A	N	I	L	R	L	K	E	S	I	A	A	T	N	E	A	V	H	E	V	T	D	G	L	S	Q	L	A	V	A	V	G	K	M	Q	Q	F	V	N	D	Q	F	N	K	T	A	Q	E	L	D	C	I	K	I	A	Q	Q	V	G	V	E	L	
Avinew	L	I	Q	A	K	Q	N	A	N	I	L	R	L	K	E	S	I	A	A	T	N	E	A	V	H	E	V	T	D	G	L	S	Q	L	A	V	A	V	G	K	M	Q	Q	F	V	N	D	Q	F	N	K	T	A	Q	E	L	D	C	I	K	I	A	Q	Q	V	G	V	E	L	
KBNP	L	I	Q	A	K	Q	N	A	N	I	L	R	L	K	E	S	I	A	A	T	N	E	A	V	H	E	V	T	D	G	L	S	Q	L	A	V	A	V	G	K	M	Q	Q	F	V	N	D	Q	F	N	K	T	A	Q	E	L	D	C	I	K	I	A	Q	Q	V	G	V	E	L	
B1	L	I	Q	A	K	Q	N	A	N	I	L	R	L	K	E	S	I	A	A	T	N	E	A	V	H	E	V	T	D	G	L	S	Q	L	A	V	A	V	G	K	M	Q	Q	F	V	N	D	Q	F	N	K	T	A	Q	E	L	D	C	I	K	I	A	Q	Q	V	G	V	E	L	
	220	230	240	250	260	270	280																																																															
Clone30	N	L	Y	L	T	E	L	T	T	V	F	G	P	Q	I	T	S	P	A	L	N	K	L	T	I	Q	A	L	N	L	A	G	C	N	M	D	Y	L	L	T	K	L	G	I	G	N	Q	L	S	S	L	I	G	S	G	L	I	T	G	N	P	I	L	Y	D	S	Q	T		
Mukteswar	N	L	Y	L	T	E	L	T	T	V	F	G	P	Q	I	T	S	P	A	L	N	K	L	T	I	Q	A	L	N	L	A	G	C	N	M	D	Y	L	L	T	K	L	G	I	G	N	Q	L	S	S	L	I	G	S	G	L	I	T	G	N	P	I	L	Y	D	S	Q	T		
Avinew	N	L	Y	L	T	E	L	T	T	V	F	G	P	Q	I	T	S	P	A	L	N	K	L	T	I	Q	A	L	N	L	A	G	C	N	M	D	Y	L	L	T	K	L	G	I	G	N	Q	L	S	S	L	I	G	S	G	L	I	T	G	N	P	I	L	Y	D	S	Q	T		
KBNP	N	L	Y	L	T	E	L	T	T	V	F	G	P	Q	I	T	S	P	A	L	N	K	L	T	I	Q	A	L	N	L	A	G	C	N	M	D	Y	L	L	T	K	L	G	I	G	N	Q	L	S	S	L	I	G	S	G	L	I	T	G	N	P	I	L	Y	D	S	Q	T		
B1	N	L	Y	L	T	E	L	T	T	V	F	G	P	Q	I	T	S	P	A	L	N	K	L	T	I	Q	A	L	N	L	A	G	C	N	M	D	Y	L	L	T	K	L	G	I	G	N	Q	L	S	S	L	I	G	S	G	L	I	T	G	N	P	I	L	Y	D	S	Q	T		
	290	300	310	320	330	340	350																																																															
Clone30	Q	L	L	G	I	Q	V	T	L	P	S	V	G	N	L	N	M	R	A	T	Y	L	E	T	L	S	V	T	T	K	G	F	A	S	A	L	V	P	K	V	V	T	Q	V	G	S	V	I	E	E	L	D	T	S	Y	C	I	E	T	D	L	D	L	Y	C	T	R	I		
Mukteswar	Q	L	L	G	I	Q	V	T	L	P	S	V	G	N	L	N	M	R	A	T	Y	L	E	T	L	S	V	T	T	K	G	F	A	S	A	L	V	P	K	V	V	T	Q	V	G	S	V	I	E	E	L	D	T	S	Y	C	I	E	T	D	L	D	L	Y	C	T	R	I		
Avinew	Q	L	L	G	I	Q	V	T	L	P	S	V	G	N	L	N	M	R	A	T	Y	L	E	T	L	S	V	T	T	K	G	F	A	S	A	L	V	P	K	V	V	T	Q	V	G	S	V	I	E	E	L	D	T	S	Y	C	I	E	T	D	L	D	L	Y	C	T	R	I		
KBNP	Q	L	L	G	I	Q	V	T	L	P	S	V	G	N	L	N	M	R	A	T	Y	L	E	T	L	S	V	T	T	K	G	F	A	S	A	L	V	P	K	V	V	T	Q	V	G	S	V	I	E	E	L	D	T	S	Y	C	I	E	T	D	L	D	L	Y	C	T	R	I		
B1	Q	L	L	G	I	Q	V	T	L	P	S	V	G	N	L	N	M	R	A	T	Y	L	E	T	L	S	V	T	T	K	G	F	A	S	A	L	V	P	K	V	V	T	Q	V	G	S	V	I	E	E	L	D	T	S	Y	C	I	E	T	D	L	D	L	Y	C	T	R	I		
	360	370	380	390	400	410	420																																																															
Clone30	V	T	F	P	M	S	P	G	I	Y	S	C	L	S	G	N	T	S	A	C	M	Y	S	K	T	E	G	A	L	T	T	P	Y	M	T	I	K	G	S	V	I	A	N	C	K	M	T	T	C	R	C	V	N	P	P	G	I	I	S	Q	N	Y	G	E	A	V	S	L	I	D
Mukteswar	V	T	F	P	M	S	P	G	I	Y	S	C	L	S	G	N	T	S	A	C	M	Y	S	K	T	E	G	A	L	T	T	P	Y	M	T	I	K	G	S	V	I	A	N	C	K	M	T	T	C	R	C	V	N	P	P	G	I	I	S	Q	N	Y	G	E	A	V	S	L	I	D
Avinew	V	T	F	P	M	S	P	G	I	Y	S	C	L	S	G	N	T	S	A	C	M	Y	S	K	T	E	G	A	L	T	T	P	Y	M	T	I	K	G	S	V	I	A	N	C	K	M	T	T	C	R	C	V	N	P	P	G	I	I	S	Q	N	Y	G	E	A	V	S	L	I	D
KBNP	V	T	F	P	M	S	P	G	I	Y	S	C	L	S	G	N	T	S	A	C	M	Y	S	K	T	E	G	A	L	T	T	P	Y	M	T	I	K	G	S	V	I	A	N	C	K	M	T	T	C	R	C	V	N	P	P	G	I	I	S	Q	N	Y	G	E	A	V	S	L	I	D
B1	V	T	F	P	M	S	P	G	I	Y	S	C	L	S	G	N	T	S	A	C	M	Y	S	K	T	E	G	A	L	T	T	P	Y	M	T	I	K	G	S	V	I	A	N	C	K	M	T	T	C	R	C	V	N	P	P	G	I	I	S	Q	N	Y	G	E	A	V	S	L	I	D
	430	440	450	460	470	480	490																																																															
Clone30	K	Q	S	C	N	V	L	S	L	G	G	I	T	L	R	L	S	G	E	F	D	V	T	Y	Q	K	N	I	S	I	Q	D	S	Q	V	I	T	G	N	L	D	I	S	T	E	L	G	N	V	N	S	I	S	A	L	N	K	L	E	S	N	R	K	L	D	K				
Mukteswar	K	Q	S	C	N	V	L	S	L	G	G	I	T	L	R	L	S	G	E	F	D	V	T	Y	Q	K	N	I	S	I	Q	D	S	Q	V	I	T	G	N	L	D	I	S	T	E	L	G	N	V	N	S	I	S	A	L	N	K	L	E	S	N	R	K	L	D	K				
Avinew	K	Q	S	C	N	V	L	S	L	G	G	I	T	L	R	L	S	G	E	F	D	V	T	Y	Q	K	N	I	S	I	Q	D	S	Q	V	I	T	G	N	L	D	I	S	T	E	L	G	N	V	N	S	I	S	A	L	N	K	L	E	S	N	R	K	L	D	K				
KBNP	K	Q	S	C	N	V	L	S	L																																																													

Figure 23

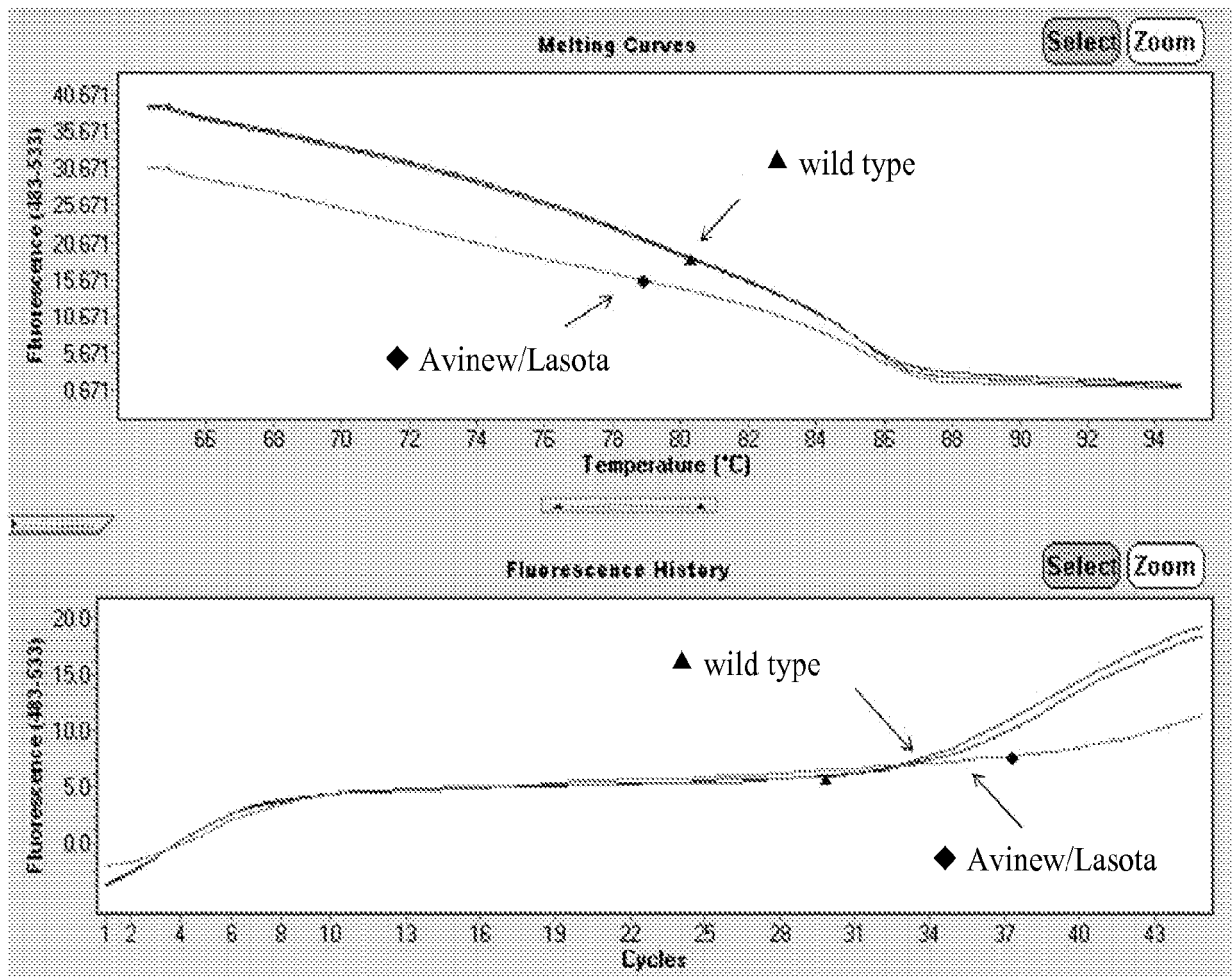
Fig. 24 A Sequence identity matrix of the whole genome of the vaccine-type NDV

Seq->	clone30	Mukteswar	Avinew	KBNP-C4152R2L	B1
Clone30		87.6	99	95.5	98.9
Mukteswar			87.7	86.9	87.6
Avinew				94.9	99.5
KBNP-C4152R2L					94.8
B1					

Fig. 24B Sequence identity matrix of the amino acid residues for the fusion protein gene of the vaccine-type NDV

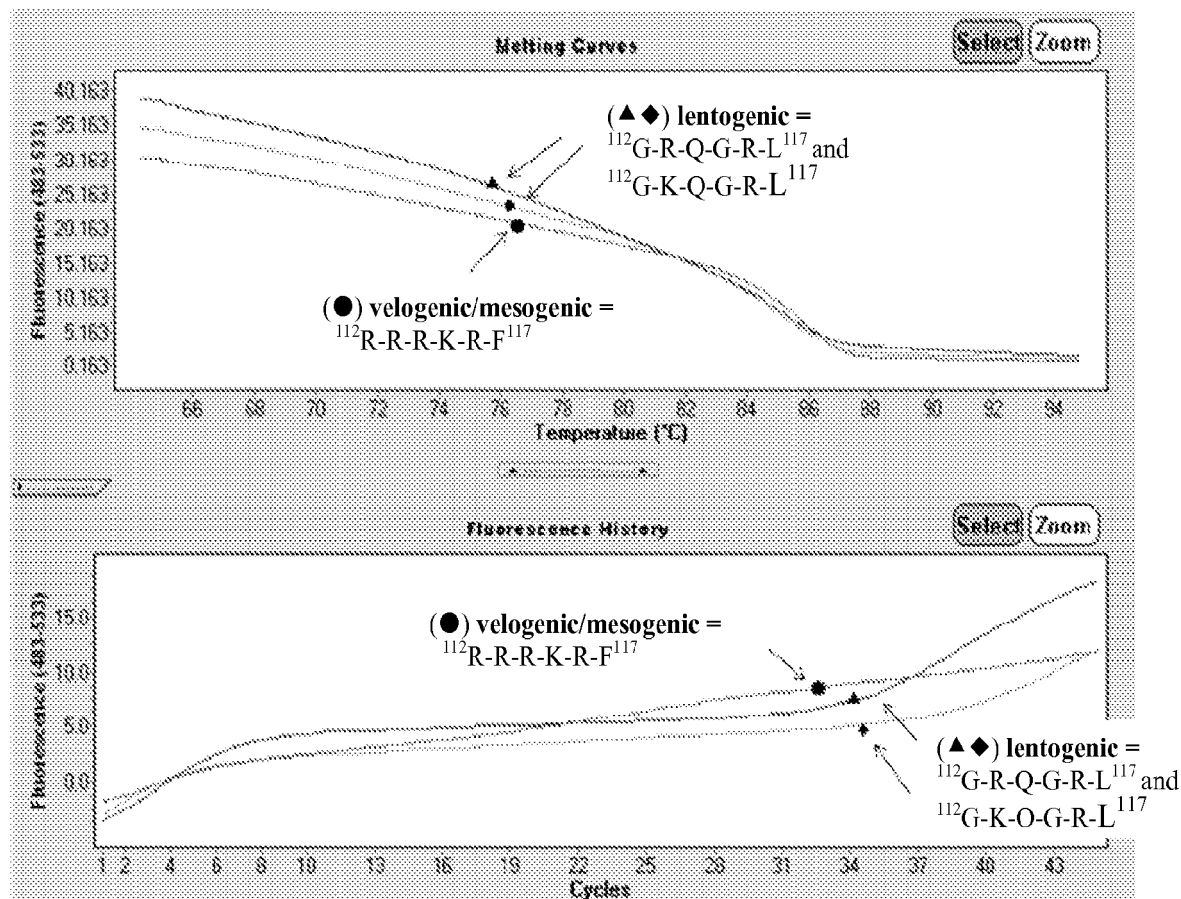
Seq->	Clone 30	Mukteswar	Avinew	KBNP-C4152R2L	B1
Clone 30		89.80	99.00	89.10	99.20
Mukteswar			88.90	90.00	89.00
Avinew				88.60	99.00
KBNP-C4152R2L					88.70
B1					

Figure 24



Samples were compared with Merial Vaccine (Avineu/Lasota). The wild-type (▲) and vaccine type (◆) is distinguished by the distinct melting curves.

Figure 25



Samples were compared with known vvNDV (velogenic) obtained from PDC. The velogenic/mesogenic (●) and lentogenic (▲◆) is distinguished by the distinct melting curves.