

Parts of these results are included in the following paper:

Van Niekerk, M., Freeman, M., Paweska, J. T., Howell, P. G., Guthrie, A. J., Potgieter, A. C., Van Staden, V. and Huismans, H. (2003). Variation in the NS3 gene and protein in South African isolates of bluetongue and equine encephalosis viruses. *Journal of General Virology* **84**(3), 581-590.

Parts of these results have been submitted to GenBank as nucleotide sequence entries and obtained the following accession numbers:

EEV isolate	Accession number
S1REF*	AY115864
S1REF	AY115865
S2REF	AY115871
S3REF	AY115867
S4REF	AY115868
S5REF	AY115869
S6REF	AY115866
S7REF	AY115870
S1FLD1	AY115878
S1FLD2	AY115876
S1FLD3	AY115875
S1FLD4	AY115877
S3FLD1	AY115874
S6FLD1	AY115872
S6FLD2	AY115873

APPENDIX A

IUB/IUPAC CODES, ONE- AND THREE-LETTER CODES FOR THE COMMON AMINO ACIDS, PREDOMINANT STATE OF AMINO ACID SIDE CHAINS AT pH 7 AND PLASMID MAPS OF THE TWO VECTORS USED

Table A1. IUB/IUPAC codes

Code ^a	Bases	Derivation	Complements
A, C, G, T	A, C, G, T		T, G, C, A
R	A or G	puRine	Y
Y	T or C	pYrimidine	R
W	A or T	Weak ^b	W
S	C or G	Strong ^b	S
M	A or C	aMino	K
K	G or T	Keto	M
B	C, G or T	not A	V
D	A, G or T	not C	H
H	A, C or T	not G	D
V	A, C or G	not T	B
N	A, G, C or T	aNy	N

^a These single-letter codes are used to represent mixed or ambiguous bases. They are derived from shared features of the bases (refer to the third column).

^b Strong and weak refer to the strength of the hydrogen bonds between these pairs.

Table A2. One- and three-letter codes for the common amino acids

Amino acid	Abbreviation		Amino acid	Abbreviation	
	3-letter	1-letter		3-letter	1-letter
Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamine	Gln	Q	Serine	Ser	S
Glutamic acid	Glu	E	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V

Table A3. The predominant state of amino acid side chains at pH 7 (adapted from Campbell, 1995)

One-letter code	Amino acid	Predominant state at pH 7
A	Alanine	non-polar side chain
C	Cysteine	polar, uncharged side chain
D	Aspartic Acid	acidic side chain
E	Glutamic Acid	acidic side chain
F	Phenylalanine	non-polar side chain
G	Glycine	polar, uncharged side chain
H	Histidine	basic side chain
I	Isoleucine	non-polar side chain
K	Lysine	basic side chain
L	Leucine	non-polar side chain
M	Methionine	non-polar side chain
N	Asparagine	polar, uncharged side chain
P	Proline	non-polar side chain
Q	Glutamine	polar, uncharged side chain
R	Arginine	basic side chain
S	Serine	polar, uncharged side chain
T	Threonine	polar, uncharged side chain
V	Valine	non-polar side chain
W	Tryptophan	non-polar side chain
Y	Tyrosine	polar, uncharged side chain

Diagram Courtesy of

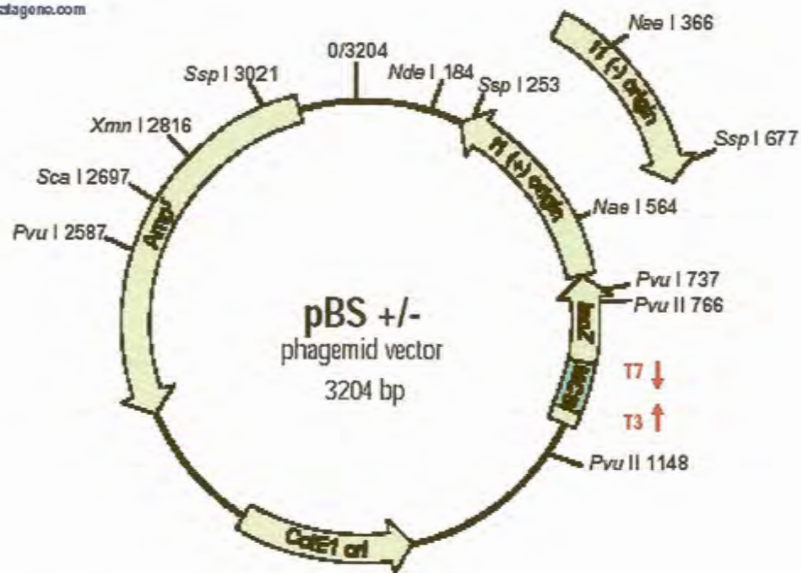


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Appendix A:
Maps and Restriction Endonuclease Sites for
pFastBac™ Expression Vectors

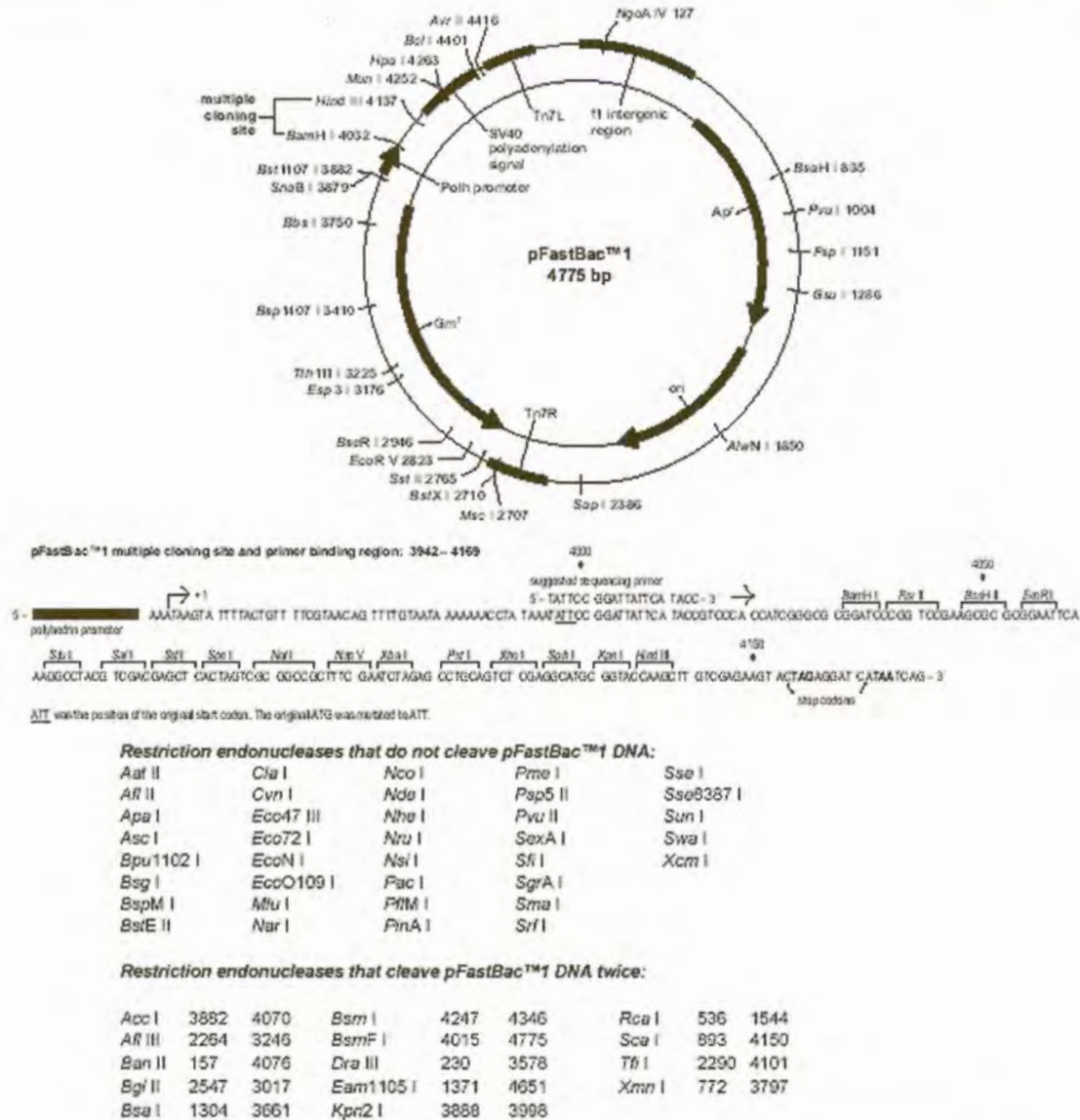


Figure 8. Map and restriction endonuclease sites for pFastBac™ 1. Restriction endonucleases that cleave pFastBac™ 1 once are shown on the outer circle. The nucleotide position refers to the 5' base of the recognition sequence.

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. Vector sequences, restriction information, and maps can be found in the Vector Data area of our web site, www.invitrogen.com. 31

APPENDIX B

LIST OF BIOINFORMATICS TOOLS AND RESOURCES USED IN THIS STUDY

Table B1. List of bioinformatics tools and resources used in this study, compiled and adapted from information contained in Alpey, 1997

Name	Description	Where available	Reference (where applicable)
*AnTheProt (ANalyse THE PROTeins) version 5.0	A suite of programs that allows the user to perform different analyses such as secondary structure prediction, protein profile analyses, detection of known protein domains or patterns, similarity searches, detection of potential cleavage site of a signal peptide and determination of titration curves using the same interface. (The main idea was to integrate into a single package most of the methods designed for protein sequence analysis).	http://pbil.ibcp.fr/ANTHEPROT	Deléage <i>et al.</i> , 1988; Deléage <i>et al.</i> , 1989; Geourjon <i>et al.</i> , 1991; Geourjon and Deléage, 1995
#BLAST (Basic Local Alignment Search Tool) (BLASTP version 2.2.1.)	A program for comparing an unknown sequence against a sequence database. Uses an approach based on matching short sequence fragments, and a powerful statistical model to find the best local alignments between the unknown sequence and the database. Will only match continuous sequence – an alignment with insertions and deletions will be displayed as a number of separate fragment matches.	http://www.ncbi.nlm.nih.gov/BLAST/	Altschul <i>et al.</i> , 1990; Altschul <i>et al.</i> , 1997
#BLASTP + BEAUTY search	A combination search consisting of a BLAST search (BLASTP 2.0.4 Feb 24 1998) of the National Center for Biotechnology Information's NR Protein Database and BEAUTY post-processing provided by the Human Genome Sequencing Center, Baylor College of Medicine.		BEAUTY: Worley <i>et al.</i> , 1995 BLASTP: Altschul <i>et al.</i> , 1997

Name	Description	Where available	Reference (where applicable)
*CBS (Center for Biological Sequence Analysis)	A prediction server to analyse nucleotide and amino acid sequences, contains links to SignalP, TargetP and TMHMM.	http://www.cbs.dtu.dk/services/	
%CDD (Conserved Domain Database) (version 1.54) at NCBI	A database of conserved domains present in protein sequences. Contains domains derived from two collections namely Smart and Pfam.	http://www.ncbi.nlm.nih.gov/Structure/cdd	
ClustalX1.81	A program used to perform multiple sequence alignments of nucleotide and amino acid sequences. Details of algorithms, implementation and useful tips on usage of Clustal programs can be found in the mentioned references.	ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/ or ftp.embl-heidelberg.de or ftp.ebi.ac.uk or http://www-igbmc.u-strasbg.fr/BioInfo/	Higgins and Sharp, 1988; Higgins and Sharp, 1989; Higgins <i>et al.</i> , 1996; Thompson <i>et al.</i> , 1997; Jeanmougin <i>et al.</i> , 1998
COILS version 1999_2.2	A program to predict regions of coiled-coil arrangements of α -helices in proteins. It compares a sequence to a database of known parallel two-stranded coiled-coils and derives a similarity score. By comparing this score to the distribution of scores in globular and coiled-coil proteins, the program then calculates the probability that the sequence will adopt a coiled-coil conformation.	http://www.ch.embnet.org/cgi-bin/COILS	Lupas <i>et al.</i> , 1991; Lupas, 1996
%DART (Domain Architecture Retrieval Tool)	A program at NCBI used to search for proteins with similar domain architectures than your query protein.		

Name	Description	Where available	Reference (where applicable)
* [#] DAS	A server to predict transmembrane segments.	http://www.biokemi.su.se/~server/DAS/	Cserzo <i>et al.</i> , 1997
*@EASY	A program that simultaneously searches a number of databases (BLOCKS, Pfam, PRINTS-BLOCKS, PRINTS, PROFILES, PROSITE) using BLASTP and FASTA3.	http://proline.sbc.man.ac.uk/cgi-bin/dbbrowser/easy	
*ExpASy (Expert Protein Analysis System)	A molecular biology server dedicated to the analysis of protein sequences and structures. Includes links to databases, tools and software packages, education and services, documentation, lists of molecular biology resources, major molecular biology servers, mirror sites, miscellaneous, announcements and new features, and local links. Proteomics tools listed include tools for protein identification and characterization, DNA translation to protein, similarity searches, pattern and profile searches, post-translational modification prediction, primary structure analysis, secondary structure prediction, tertiary structure prediction, transmembrane regions detection, and sequence alignment.	http://www.expasy.ch/	
#FASTA	A program for comparing an unknown sequence against a sequence database. The program makes approximations to try and concentrate only on alignments that are likely to be significant. Sometimes misses weak but significant scores. Attempts to find global alignments.	http://www.ebi.ac.uk/htbin/fasta.py?request	Lipman and Pearson, 1985
#GenBank	A DNA database based at the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine, located on the National Institutes for Health (NIH) campus in the US.	www.ncbi.nlm.nih.gov/web/search/index.html	

Name	Description	Where available	Reference (where applicable)
#GeneFIND	A gene family identification system which aims at high-throughput full-scale gene family identification by taking advantages of the strengths of various search methods using multi-level filters, and incorporating ProClass family information.	http://pir.georgetown.edu/gf-cgi/genefind.pl	Wu <i>et al.</i> , 1996; Wu <i>et al.</i> , 1998
#MaxHom version 1.99.04	A multiple sequence alignment program which finds similar sequences in a database.		Sander and Schneider, 1991
*MEGA (Molecular Evolutionary Genetic Analysis) version 2.1	A program that carries out parsimony, distance matrix and likelihood methods for molecular data. It can also be used calculate basic sequence statistics.	MEGA2 is available as Windows95/98/NT/2000 executables, and MEGA is available as a DOS executable, at http://www.megasoftware.net . The MEGA manual is also available on-line in HTML at http://evolgen.biol.metrou.ac.jp/MEGA/manual/default.html .	Kumar <i>et al.</i> , 2001
*META PredictProtein	A service for sequence analysis and prediction. It includes database searches such as prediction-based threading, also incorporating purely sequence-based database searches (FRSVR) and hidden Markov model method (SAM-T98) for finding remote homologs of protein sequences. It also includes predictions of presence and location of signal peptide cleavage sites in amino acid sequences from different organisms (SignalP), location and orientation of transmembrane helices (TMHMM, TOPPRED), and location of transmembrane helices (DAS).	http://www.embl-heidelberg.de/predictprotein	

Name	Description	Where available	Reference (where applicable)
*PHD (Profile fed neural network systems from Heidelberg) version 1.96	A suite of programs consisting of three different programs that predicts one-dimensional structure from multiple sequence alignments. The three programs are PHDsec that predicts secondary structure, PHDacc that predicts solvent accessibility and PHDhtm for prediction of helical transmembrane regions.		Rost, 1996a
§PHDhtm version 1.96	A program that predicts the location and topology of transmembrane helices from multiple sequence alignments.	www.emblheidelberg.de/Services/sander/predictprotein/predictprotein.html	Rost <i>et al.</i> , 1996
PHDsec	A program that predicts the secondary structure of a protein from multiple sequence alignments.	http://www.embl-heidelberg.de/predictprotein/predictprotein.html	Rost and Sander, 1993; Rost and Sander, 1994; Rost <i>et al.</i> , 1994
#PIR (Protein Information Resource)	A community resource that provides protein databases and analysis tools to support research on molecular evolution, functional genomics and computational biology. Includes PIR-PSD (PIR-International Protein Sequence Database) which is a protein sequence database in which entries are annotated and classified into family groups. This database is used in a comparison of an amino acid sequence to the other amino acid sequences in the database using a BLAST search.	http://pir.georgetown.edu/ or http://pir.georgetown.edu/cgi-bin/pirwww/blast.pl	Barker <i>et al.</i> , 2001
*PredictProtein version 1.99.08	A service for sequence analysis and structure prediction. It includes database searches such as PROSITE to detect functional motifs, ProDom to detect protein domains, MaxHom to generate multiple sequence alignments and Topits for fold recognition by prediction-based threading. It also includes predictions of	http://maple.bioc.columbia.edu/pp/ or http://www.embl-heidelberg.de/predictprotein	Rost, 1996b

Name	Description	Where available	Reference (where applicable)
	secondary structure (PHDsec, PROFsec), residue solvent accessibility (PHDacc, PROFacc), transmembrane helix location and topology (PHDhtm, PHDtopology), coiled-coil regions (COILS), and cysteine bonds (CYSPRED).		
@PRINTS	A profile database to identify motifs in a protein sequence.	http://www.biochem.ucl.ac.uk/bsm/dbbrowser/PRINTS/PRINTS.html	Attwood <i>et al.</i> , 1994
%ProDom release 2001.2 Aug 2001	A profile database to identify domains in a protein sequence.	http://protein.toulouse.inra.fr/prodom/prodom.html or http://www.toulouse.inra.fr/prodom.html	Sonnhammer and Kahn, 1994; Corpet <i>et al.</i> , 1998; Corpet <i>et al.</i> , 2000
@PROSITE (release 16.51 of 25 Nov 2001)	A database that consists of biologically significant patterns and profiles formulated in such a way that with appropriate computational tools it can help to determine to which known family of protein (if any) a new sequence belongs, or which known domain(s) it contains.	http://www.expasy.ch/sprot/prosite.html	Bairoch <i>et al.</i> , 1997; Hofmann <i>et al.</i> , 1999
*ProtParam tool	A tool that allows the computation of various physical and chemical parameters for a given protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).	http://ca.expasy.org/cgi-bin/protparam	

Name	Description	Where available	Reference (where applicable)
PSORT (Prediction of Protein Localization Sites) version 6.4	A program that analyses prokaryotic or eukaryotic sequences and searches for protein sorting signals. The program reports back a probability for the protein being localized to different compartments within the cell.	http://psort.nibb.ac.jp/form.html	
#SAM-T99	A program consisting of three functional sections covering alignment, database search results and secondary structure prediction. The database that it searches is the PDB database.	http://www.cse.ucsc.edu/~farmer/target99-query	
*SAPS (Statistical Analysis of Protein Sequences) version of April 11, 1996	A program that uses statistical criteria to evaluate a wide range of protein sequence properties.	http://www.isrec.isb-sib.ch/cgi-bin/SAPS	Brendel <i>et al.</i> , 1992
SignalP version 1.1	A program that predicts the presence and location of leader sequences and cleavage sites within both prokaryotes and eukaryotes.	http://www.cbs.dtu.dk/services/SignalP	Nielson <i>et al.</i> , 1997
SSpro version 2.0	A server for protein secondary structure prediction based on an ensemble of 11 bi-directional recurrent neural networks.	http://promoter.ics.uci.edu/BRNN-PRED/	Baldi <i>et al.</i> , 1999
SSpro8 version 2.0	An experimental extension to SSpro using the full DSSP 8 classes instead of three classes to classify the secondary structure of a protein.	http://promoter.ics.uci.edu/BRNN-PRED/	Baldi <i>et al.</i> , 1999

Name	Description	Where available	Reference (where applicable)
#SWISS-PROT Release 39.0 (5/00)	A protein database consisting of properly checked and annotated translations of the EMBL database. It distinguishes itself from other protein sequence databases by three distinct criteria namely annotations, minimal redundancy and integration with other databases.	http://www.expasy.ch/sprot/sprot/ or http://www.ebi.ac.uk/swissprot/	Bairoch and Apweiler, 2000
TargetP (version 1.0)	A program that predicts the subcellular localization of proteins, specifically whether the protein is localized within the mitochondria, the chloroplast, is part of the secretory pathway or other which does not include the previously mentioned locations.	http://www.cbs.dtu.dk/services/	Emanuelsson <i>et al.</i> , 2000
\$TMbase Release 25	A database of naturally occurring TM helices based on SWISS-PROT release 25.		Hofmann and Stoffel, 1993
\$TMHMM version 2.0.	A program to predict the location and topology of transmembrane helices.	http://www.cbs.dtu.dk/services/TMHMM-1.0/ or http://www.cbs.dtu.dk/	Sonnhammer <i>et al.</i> , 1998
\$TMPRED	A program that predicts the location and topology of transmembrane helices.	http://www.ch.embnet.org/software/TMPRED_form.html or http://www.ch.embnet.org/cgi-bin/TMPRED_form_parser	
\$TopPred2	A program to predict the location and orientation of transmembrane helices.	http://bioweb.pasteur.fr/seqanal/tmp/toppred or http://www.biokemi.su.se/~server/toppred2/	Von Heijne, 1992; Claros and Von Heijne, 1994; Cserzo <i>et al.</i> , 1997

Molecular Biology Servers, services or suites of programs which are available on the internet that can serve as a platform from which links are available to most of the regularly used bioinformatics tools or software on the world wide web are indicated with an asterisk (*), tools used in similarity searches are indicated by #, tools used to identify transmembrane regions or helices are indicated by \$, tools used to identify protein domains or profiles are indicated by %, tools used to identify protein motifs are indicated by @ and tools used for secondary structure prediction are indicated by &.

APPENDIX C

 LIST OF NUCLEOTIDE AND AMINO ACID SEQUENCES OBTAINED FROM GENBANK USED IN THIS STUDY FOR
 PHYLOGENETIC ANALYSES

Table C1. Relevant information regarding all orbivirus nucleotide and amino acid sequences obtained from GenBank used in this study

Serogroup and serotype	Accession number	Origin of virus and year of isolation	Number of basepairs	Reference
AHSV-1	U02711	South Africa (UN)	764 bp	De Sá <i>et al.</i> , 1994
*AHSV-2	U59279	South Africa (UN)	764 bp	Sailleau <i>et al.</i> , 1997
AHSV-3	AJ007303	South Africa (UN)	758 bp	Martin <i>et al.</i> , 1998
*AHSV-4	AJ007305	South Africa (UN)	758 bp	Martin <i>et al.</i> , 1998
AHSV-5	AJ007309	South Africa (UN)	758 bp	Martin <i>et al.</i> , 1998
AHSV-6	U60189	South Africa (UN)	755 bp	Sailleau <i>et al.</i> , 1997
*AHSV-7	AJ007306	South Africa (UN)	758 bp	Martin <i>et al.</i> , 1998
AHSV-8	AJ007307	South Africa (UN)	764 bp	Martin <i>et al.</i> , 1998
AHSV-9	AJ007308	South Africa (UN)	756 bp	Martin <i>et al.</i> , 1998
*Broadhaven NS3	M83197	Scotland, St. Abb's Head (UN)	702 bp	Moss <i>et al.</i> , 1992
BTV-1	AF135223	China, Shizong (1986)	785 bp	Bonneau <i>et al.</i> , 1999
*BTV-2	AF135224	Jinghong, China (1996)	785 bp	Bonneau <i>et al.</i> , 1999
BTV-3	AF135225	Ershang, China (1996)	785 bp	Bonneau <i>et al.</i> , 1999
BTV-4	AF135226	Dehong, China (1996)	785 bp	Bonneau <i>et al.</i> , 1999
BTV-10	AF044372	United States, California (1953)	822 bp	Pierce <i>et al.</i> , 1998
BTV-11	AF044373	United States, Texas (1962)	822 bp	Pierce <i>et al.</i> , 1998
*BTV-12	AF135227	China, Jinghong (1996)	785 bp	Bonneau <i>et al.</i> , 1999
BTV-13	AF044374	United States, Idaho (1967)	822 bp	Pierce <i>et al.</i> , 1998
BTV-15	AF135228	China, Ershang (1996)	785 bp	Bonneau <i>et al.</i> , 1999

Serogroup and serotype	Accession number	Origin of virus and year of isolation	Number of basepairs	Reference
BTV-16	AF135229	China, Wuxi (1988)	785 bp	Bonneau <i>et al.</i> , 1999
BTV-17	AF044707	United States, California, Kern (1981)	822 bp	Pierce <i>et al.</i> , 1998
*Chuzan NS3	AB018091	Japan (1985)	728 bp	Yamakawa <i>et al.</i> , 1999
EEV-1 (S1FLD1)	AY1158Y8	South Africa, W. Cape, Stellenbosch (1999)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-1 (S1FLD2)	AY115876	South Africa, W. Cape, Stellenbosch (1999)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-1 (S1FLD3)	AY115875	South Africa, W. Cape, Stellenbosch (1999)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-1 (S1FLD4)	AY115877	South Africa, W. Cape, Stellenbosch (1999)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-1 (S1REF*)	AY115864	UN	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-1 (S1REF)	AY115865	South Africa, N. Cape, Colesberg (1976).	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-2 (S2REF)	AY115871	South Africa, N. Cape, Kimberley (1967).	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-3 (S3FLD1)	AY115874	South Africa, Gauteng, Kempton Park (2000)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-3 (S3REF)	AY115867	South Africa, Limpopo, Naboomspruit (1971)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-4 (S4REF)	AY115868	South Africa, Gauteng, Onderstepoort (1974)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-5 (S5REF)	AY115869	South Africa, Gauteng, Johannesburg (1974)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-6 (S6FLD1)	AY115872	South Africa, Limpopo, Hoedspruit (2000)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-6 (S6FLD2)	AY115873	South Africa, Gauteng, Roodeplaat (2000)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-6 (S6REF)	AY115866	South Africa, N. West, Potchefstroom (1991)	759 bp	Van Niekerk <i>et al.</i> , 2003
EEV-7 (S7REF)	AY115870	South Africa, KwaZulu-Natal, St. Lucia (2000)	759 bp	Van Niekerk <i>et al.</i> , 2003
*EHDV-1	L29023	United States, New Jersey (UN)	809 bp	Jensen and Wilson, 1995
*EHDV-2	L29022	United States, South Carolina (UN)	809 bp	Jensen <i>et al.</i> , 1994

REF denotes reference strain; FLD denotes field strain; (UN) denotes year of virus isolation unknown; UN denotes information unknown; N. Cape denotes Northern Cape province, S.A.; W. Cape denotes Western Cape province, S.A.; N. West denotes North West province, S.A.; Limpopo denotes the province previously known as the Northern Province, S.A. and the asterisk (*) indicates selected sequences for use in the second nucleotide or amino acid dataset

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