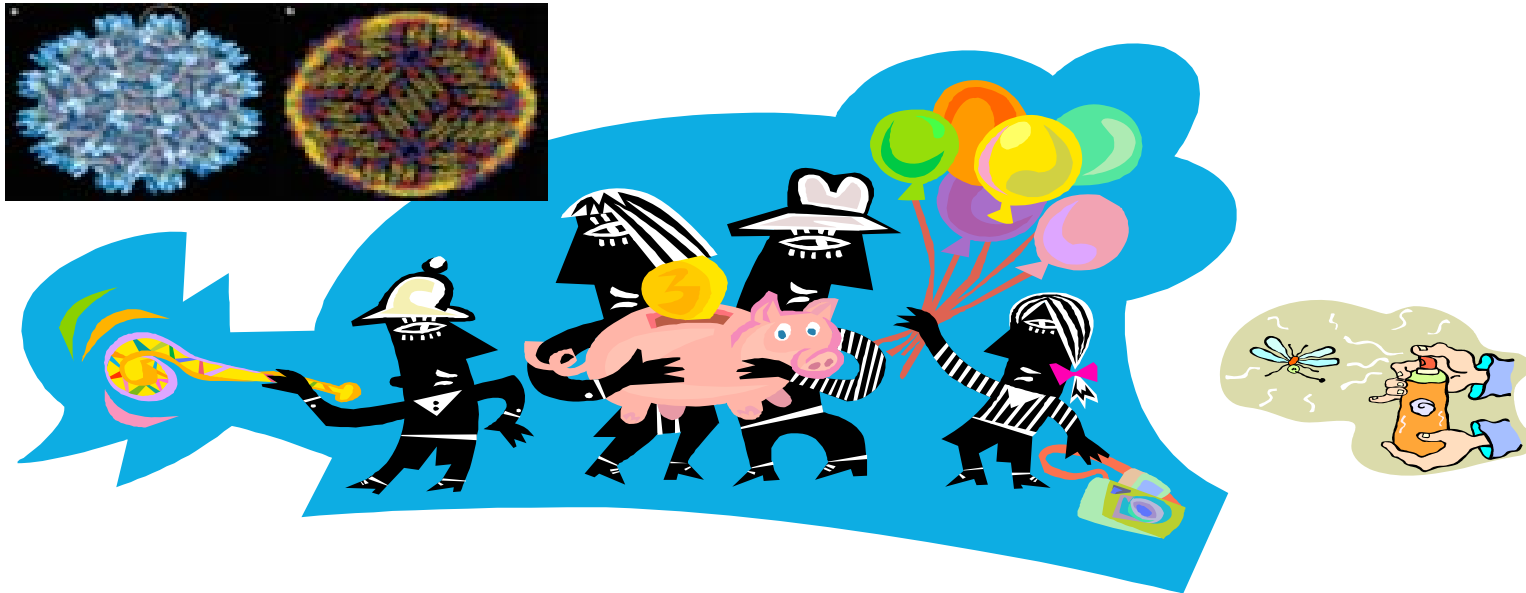


RECOMBINATION IN THE EVOLUTION OF *JAPANESE ENCEPHALITIS VIRUS*



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INTRODUCTION

- *Japanese encephalitis virus (JEV)*
- a single-stranded positive-sense ribonucleic acid (RNA)
- genus *Flavivirus*
- family *Flaviviridae*
- icosahedral and lipid envelope
- total size 50 nanometres (nm)

RNA LENGTH 11 KILOBASES (kb)

- One open reading frame
- 5'-C-preM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'
- 5' and 3' untranslated regions (UTR) are approximately 95 and 582 bases in long, respectively
- Three structural proteins
 - Capsid (C)
 - Premembrane (PrM)
 - Envelope (E)
- Genotype I-IV and a single member of putative 5th

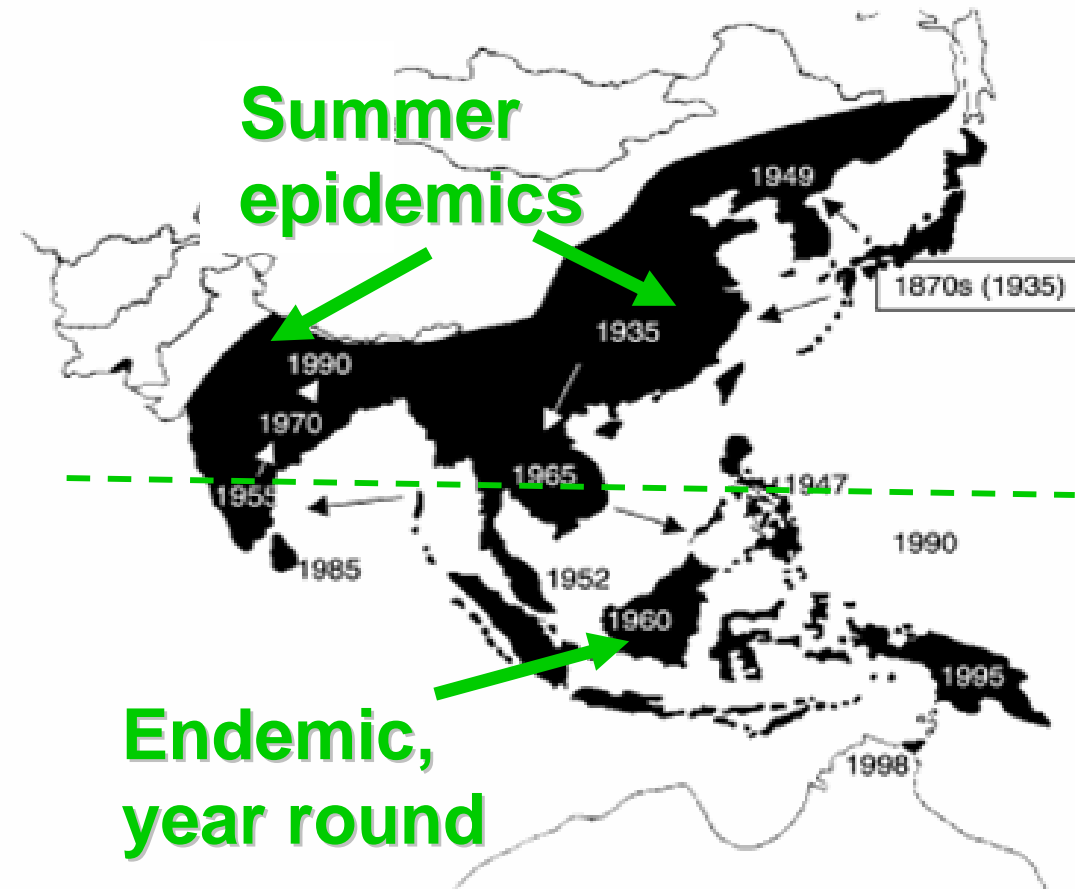
Japanese encephalitis - clinical epidemiology

- 35-50,000 cases/year
- 30% mortality



- sequelae in 50% of survivors
 - polio-like flaccid paralysis

Solomon, et al. Lancet 1998



Solomon T, Winter PM. Arch Virol Suppl. 2004

FIRST JEV ISOLATED

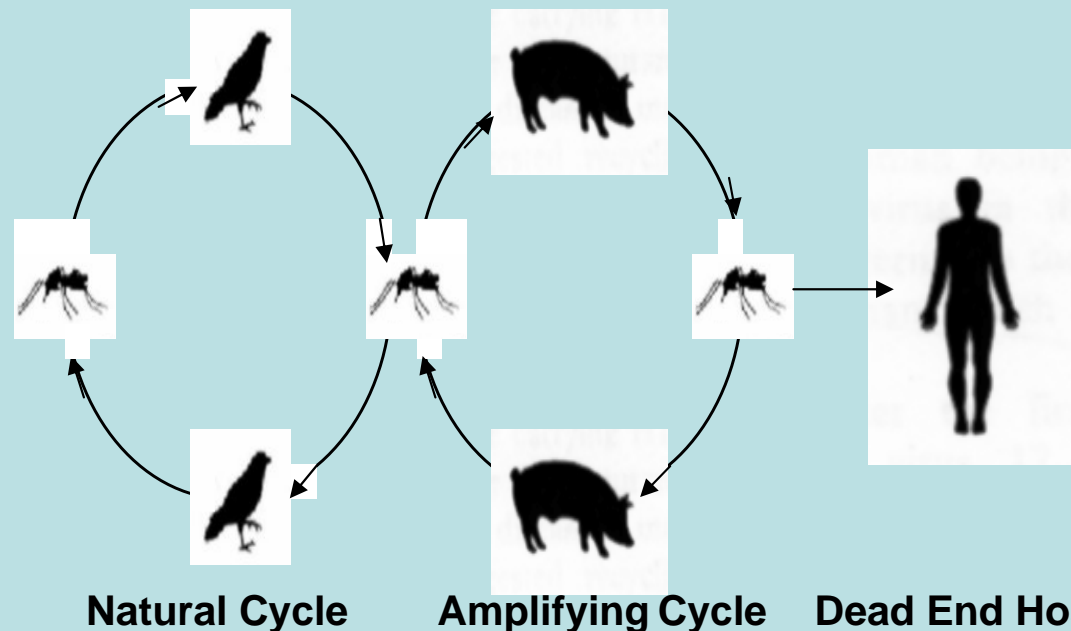
- JEV (prototype strain Nakayama) isolated from **human brain** in Japan in 1935

Genotype III

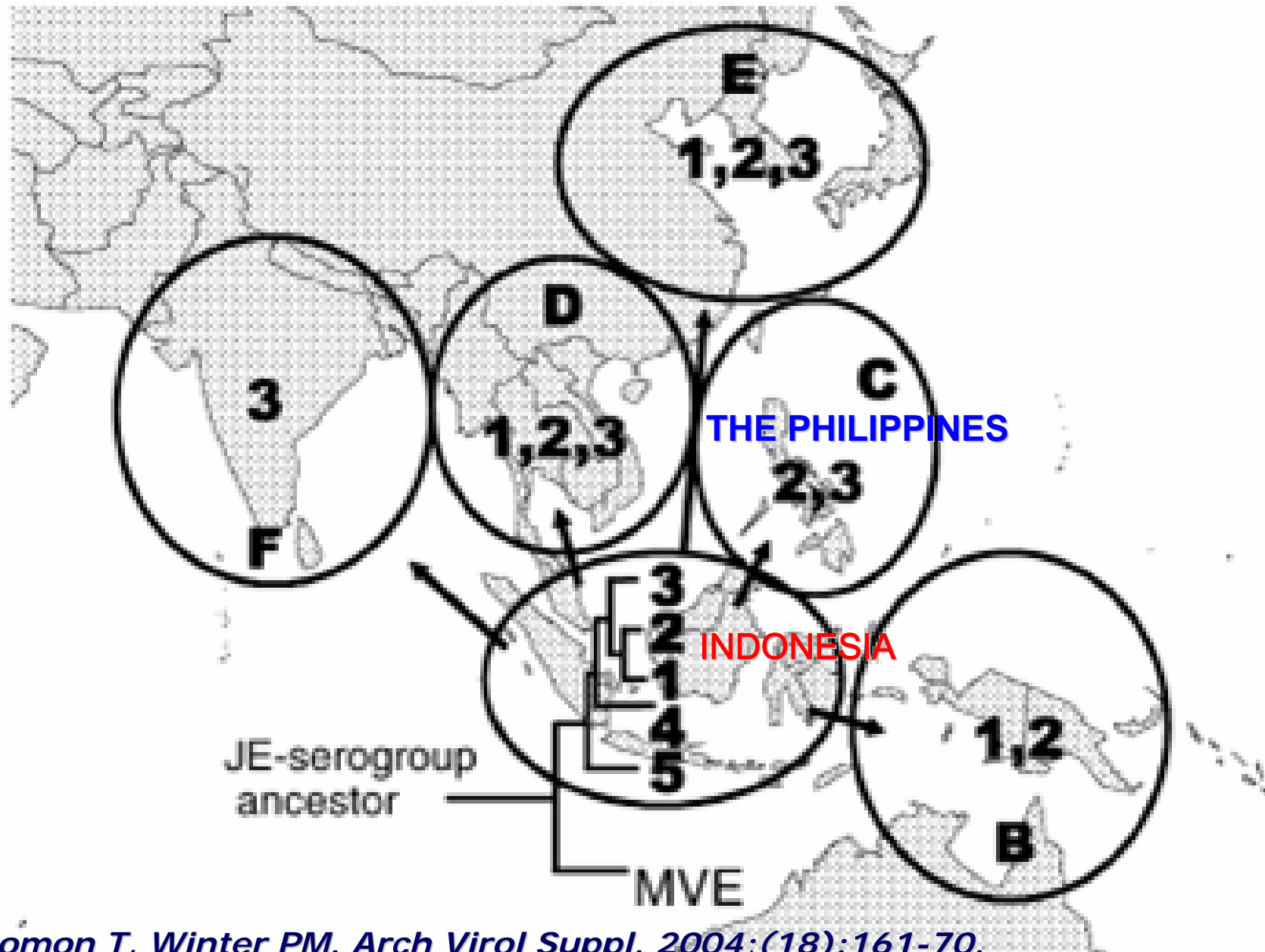
- JEV (prototype strain JaGAr 01) isolated from **Culex mosquitoes** in Japan in 1959

Genotype III

- Arbovirus



Showing the Postulated Origin of JEV from a JE-Serogroup Ancestor in the Indonesia–Malaysia Region



Solomon T, Winter PM. *Arch Virol Suppl.* 2004;(18):161-70.

OBJECTIVES

- **LOOK FOR EXISTENCE OF NEW GENOTYPES AMONG 6 FILIPINO ISOLATES AND 21 INDONESIAN ISOLATES BETWEEN 1978-1987**
- **APPLY PHYLOGENETIC ANALYSIS TO DETECT RECOMBINATION BETWEEN CIRCULATING GENOTYPES**

WATERIALS AND METHODS

6 Filipino and 21 Indonesian JEV Strains isolated during 1978-1987

No	Isolate #	Specimen	Date Collected	Source	Cell line*	Original	Cell line**
1	PHI/PIG/84/NM/002	<i>Sus domesticus</i>	1984	Luzon Island, the Philippines	AP61-1	Dr Curtis Hayes ¹	C6/36
2	PHI/PIG/84/NM/003	<i>Sus domesticus</i>	1984	Luzon Island, the Philippines	Vero-1	Dr Curtis Hayes ¹	Vero C1008
3	PHI/PIG/84/NM/004	<i>Sus domesticus</i>	1984	Luzon Island, the Philippines	Vero-1	Dr Curtis Hayes ¹	Vero C1008
4	PHI/PIG/84/NM/005	<i>Sus domesticus</i>	1984	Luzon Island, the Philippines	AP61-2	Dr Curtis Hayes ¹	C6/36
5	PHI/PIG/85/NM/007	<i>Sus domesticus</i>	1985	Luzon Island, the Philippines	Vero-1	Dr Curtis Hayes ¹	Vero C1008
6	PHI/PIG/86/NM/011	<i>Sus domesticus</i>	1986	Luzon Island, the Philippines	AP61-1	Dr Curtis Hayes ¹	C6/36
7	JKT451	<i>Cx. tritaeniorhynchus</i>	May 16, 1978	Lombok	NA	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
8	JKT654	<i>Cx. tritaeniorhynchus</i>	October 26, 1978	Kapuk, West Jakarta	C6/36-1, Vero-1, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
9	JKT811	<i>Cx. tritaeniorhynchus</i>	January 8, 1979	Kapuk, West Jakarta	BHK21-2, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
10	JKT220507	NA	Jan-79	Indonesia	Tox-1, Vero-1	Dr Robert Tesh ³	C6/36
11	JKT1110	<i>Cx. tritaeniorhynchus</i>	February 5, 1979	Kapuk, West Jakarta	Vero-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
12	JKT2254	<i>An. annularis</i>	March 24, 1979	Lombok	Vero-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
13	JKT1729	<i>Cx. tritaeniorhynchus</i>	April 2, 1979	Kapuk, West Jakarta	Vero-2, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
14	JKT1754	<i>Cx. tritaeniorhynchus</i>	April 16, 1979	Kapuk, West Jakarta	Vero-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
15	JKT2212	<i>Cx. tritaeniorhynchus</i>	October 15, 1979	Kapuk, West Jakarta	Vero-2, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
16	JKT2303	<i>Cx. tritaeniorhynchus</i>	November 13, 1979	Kapuk, West Jakarta	Vero-2, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
17	JKT2329	<i>Cx. tritaeniorhynchus</i>	November 13, 1979	Kapuk, West Jakarta	BHK21-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
18	JKT2352	<i>Cx. tritaeniorhynchus</i>	November 27, 1979	Kapuk, West Jakarta	Vero-2, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
19	JKT2362	<i>Cx. tritaeniorhynchus</i>	November 27, 1979	Kapuk, West Jakarta	BHK21-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
20	JKT2380	<i>Cx. vishnui</i>	November 27, 1979	Kapuk, West Jakarta	Vero-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
21	JKT4312	<i>Cx. gelidus</i>	December 11, 1979	Kapuk, West Jakarta	NA	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
22	JKT4331	<i>Cx. tritaeniorhynchus</i>	December 11, 1979	Kapuk, West Jakarta	Vero-1, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
23	JKT4332	<i>Cx. tritaeniorhynchus</i>	December 11, 1979	Kapuk, West Jakarta	Vero-3, SMB-1	Ratna Tan/ Dr Patrick J. Blair ²	C6/36
24	JKT7180	<i>Cx. tritaeniorhynchus</i>	7/26-27/1981	Indonesia	Vero-1	Dr Robert Tesh ³	C6/36
25	JKT27-085	Mosquitoes	Jan-87	Indonesia	C6/36-2	Dr Robert Tesh ³	C6/36
26	JKT27-087	Mosquitoes	Jan-87	Indonesia (Java Central)	C6/36-2	Dr Robert Tesh ³	C6/36
27	PRS222682	NA	NA	Indonesia	MOSQ?, C6/36-1	Dr Robert Tesh ³	C6/36

*Cell Line from Original, **Cell line in our Laboratory

¹Hayes CG, Viral Diseases Department, Naval Medical Research Center, Silver Spring, USA

²Patrick J Blair, Viral Diseases Program, U.S. NAMRU-2 Jakarta, Kompleks P2MPLP/LITBANGKES, JL.Percetakan Negara No. 29, Jakarta Pusat, Indonesia

³Dr Robert Tesh, UTMB

PHILIPPINES

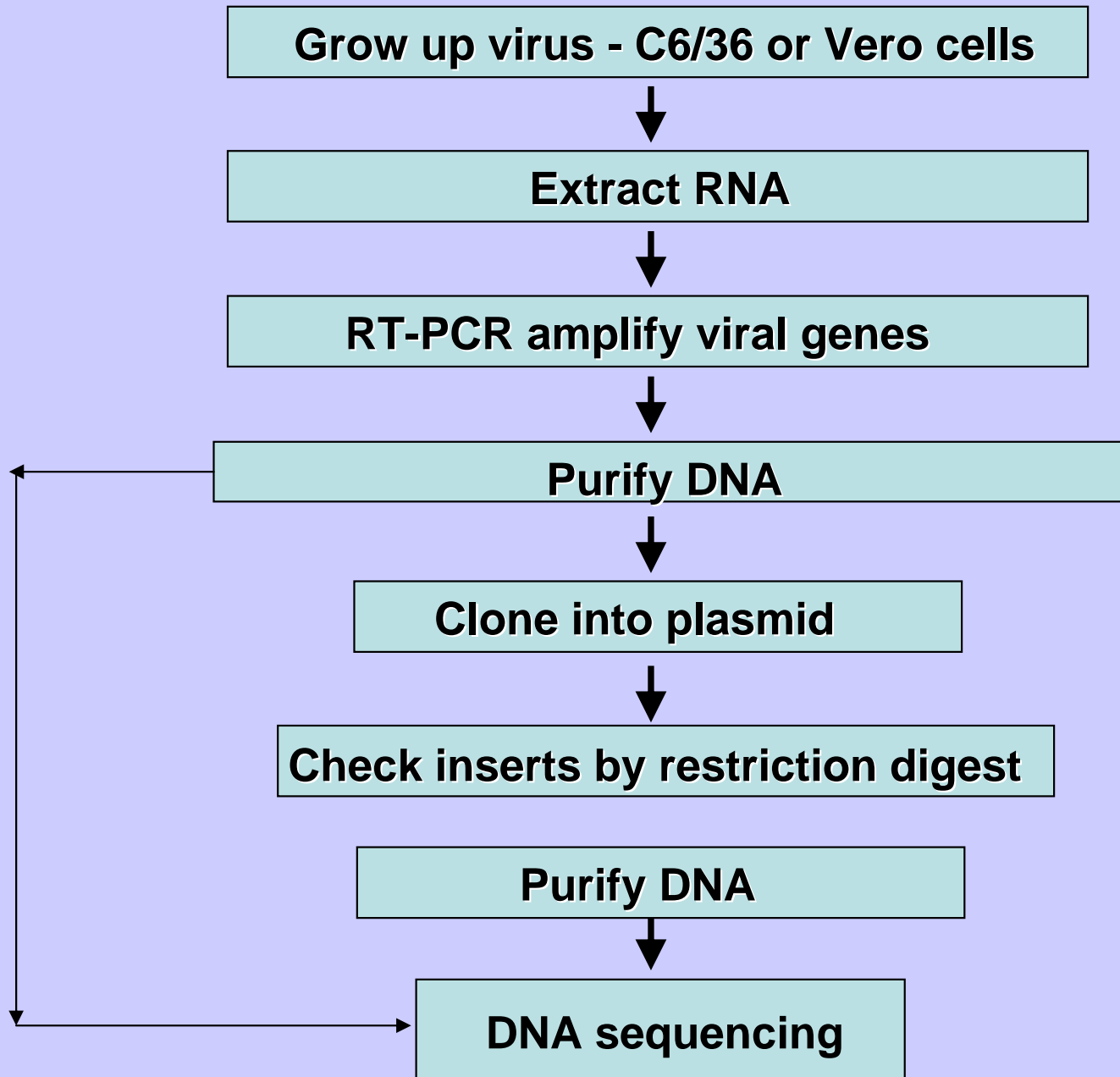
Regions and Provinces



Indonesia



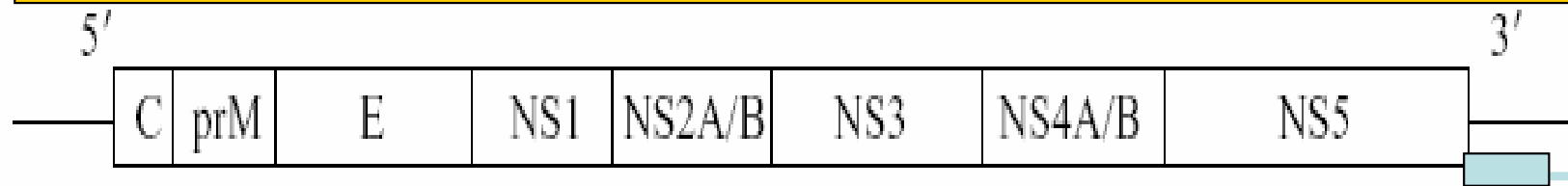
Lab Methods



JEV Nucleotide and Base Sequences of JEV Sense Primer and Anti-Sense Primer(GENOSYS)

VD8 : G G G T C T C C T C T A A C C T C T A G

EMF : T G G A T G A C G A C G G A A G A C A T G



879s : G C T T T C C T G G C G G C G G T A C T T G G

1720a : G T G G C G T G C G C C T C T T C A A A

1577s : G T T T T A C G T C A T G A C C G T G G G G G

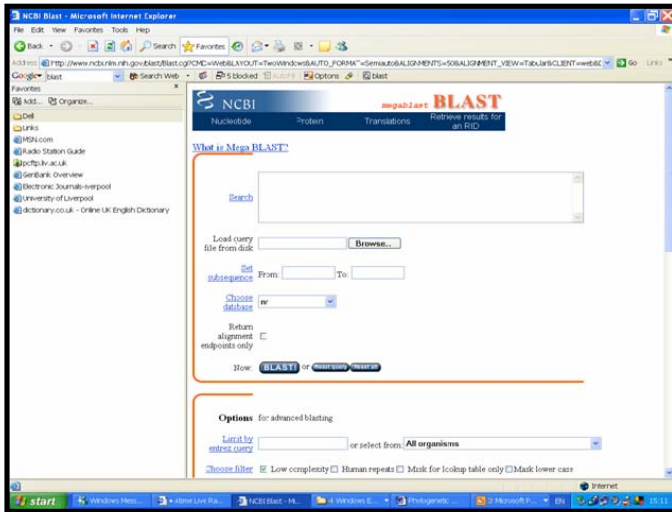
2518a : C C A C A C C T C A T C T C T T T T C T T G

10201s: C A T A C G T G G G A A A G C G T G A G G A C A
T C T G G

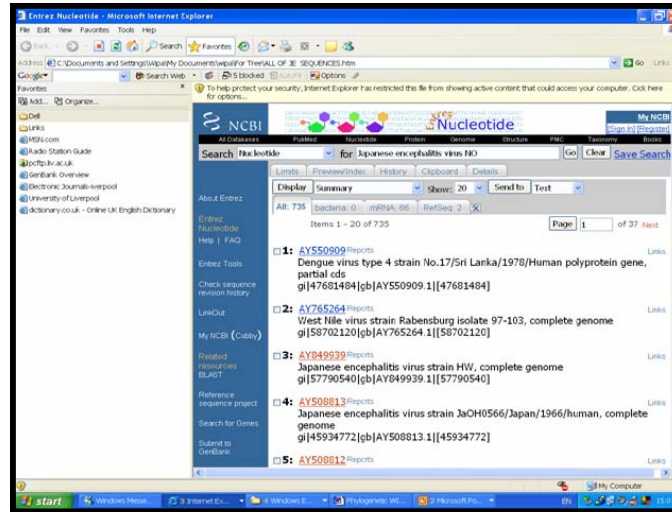
10944a: C C A C C A G C T A C A T G T T T C G G C G C T C

PHYLOGENETIC METHODS

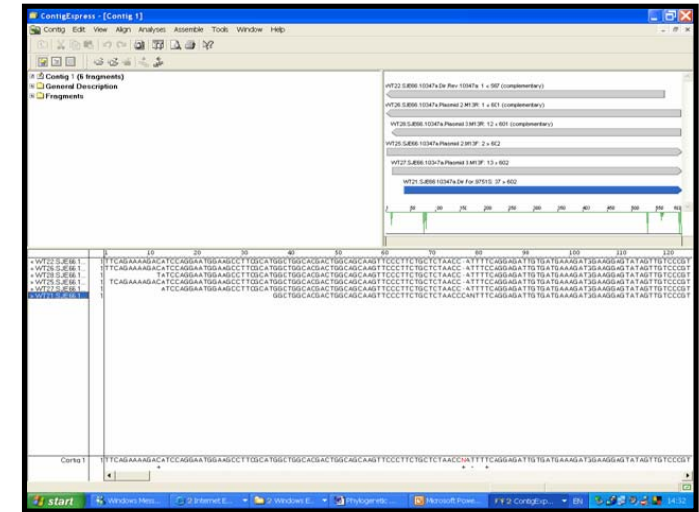
1. DNA sequence of interest... **BLAST** Search



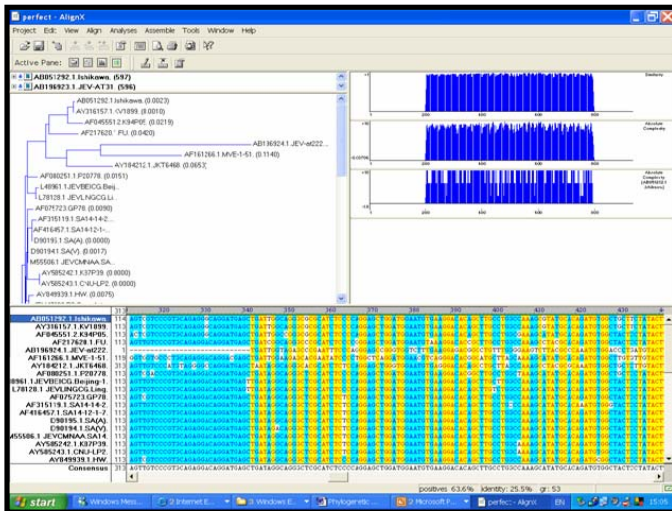
2. Other related sequences **NCBI** Search



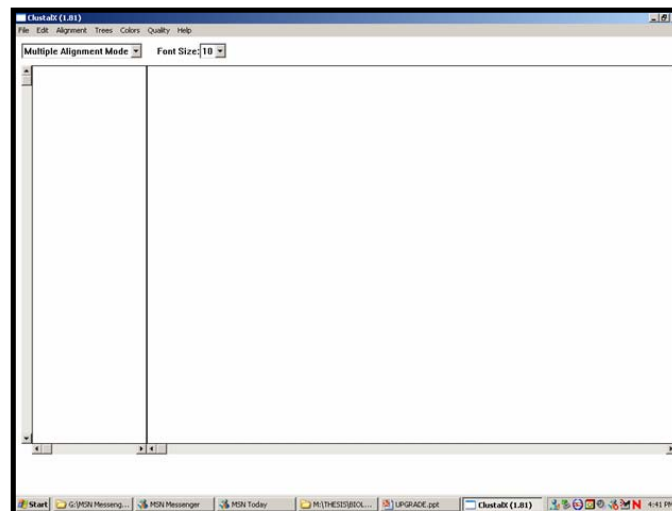
3. Align by **Invitrogen-ContigExpress®** Module



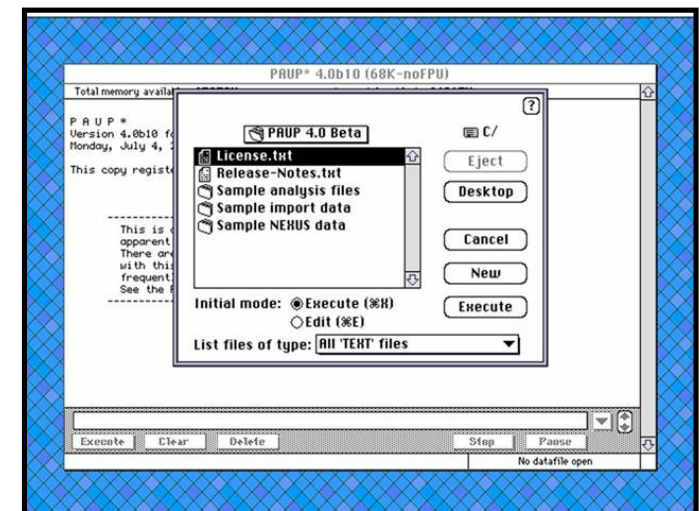
4. **NTI Advance™ 9.0**



5. **ClustalX** programme



6. **PAUP** package



RECOMB. METHODS

Looked at our new strains plus all on GenBank:

1. Sliding window analysis - neighbour-joining trees
2. RAT (recombination analysis tool)
<http://cbr.jic.ac.uk/dicks/software/RAT/index.html> to generate diversity plots
3. LARD (Likelihood Analysis of Recombination in DNA) Version 2.2 Copyright, 1999 Andrew Rambaut to find breakpoints or crossing over points

RESULTS

VIRAL CULTURE & RT-PCR

Day 0

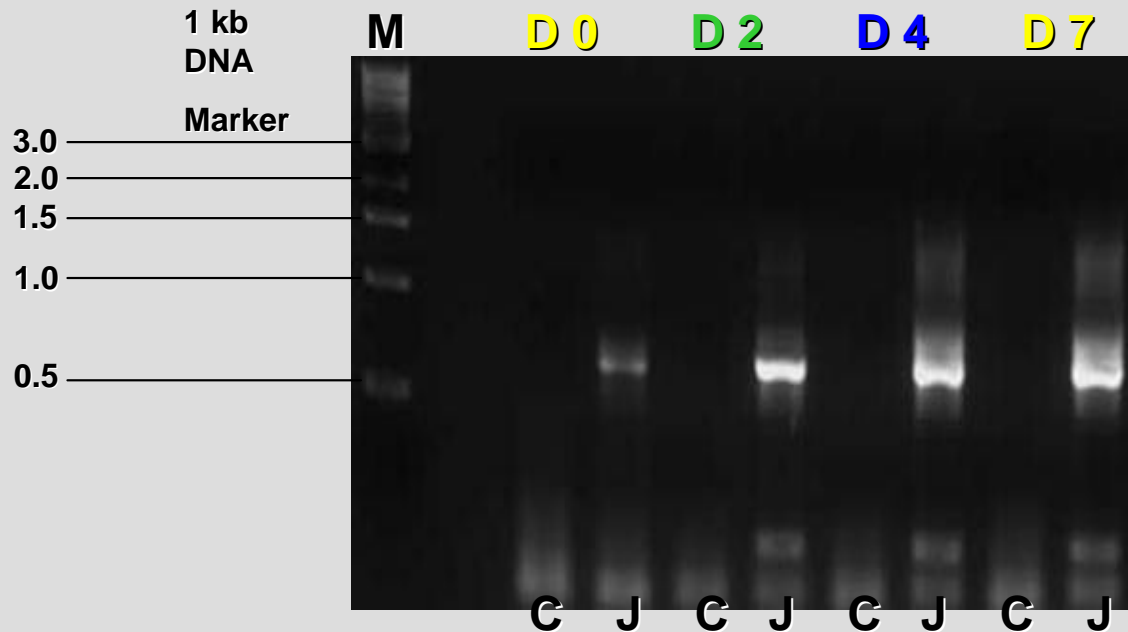
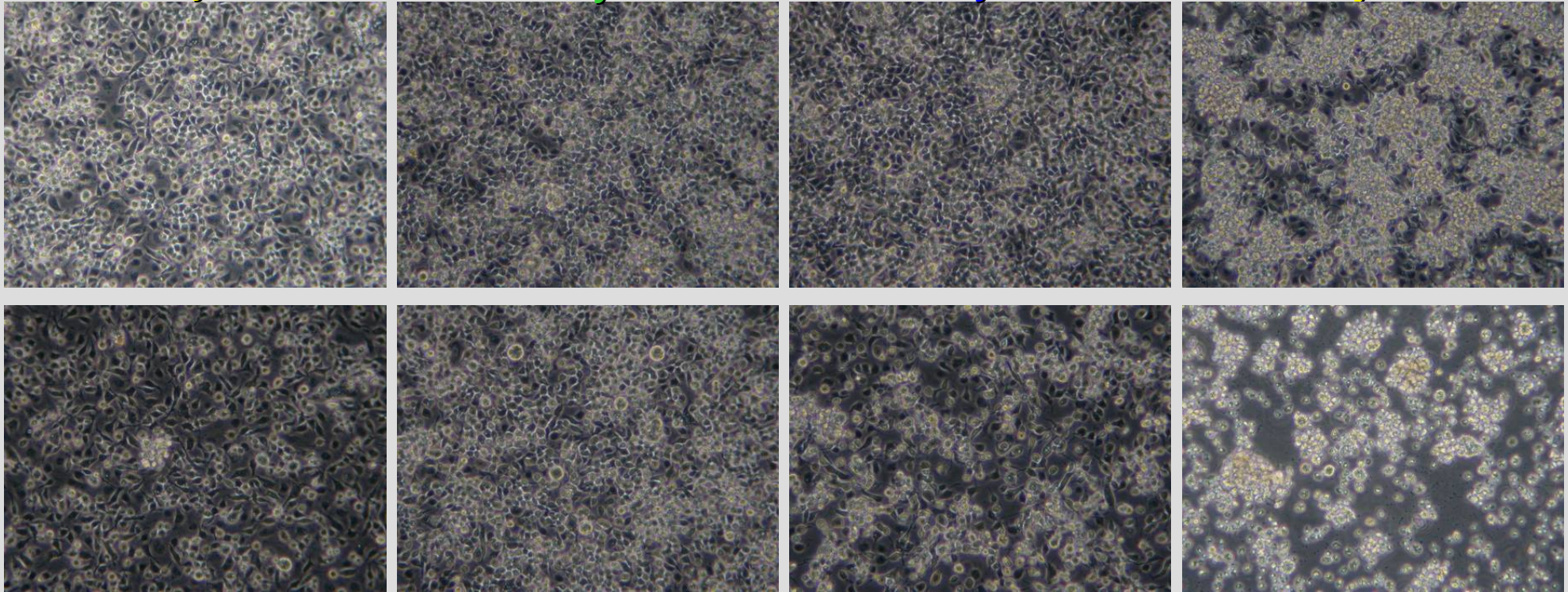
Day 2

Day 4

Day 7

FORZANO

UNIVERSITY OF LIVERPOOL



**V
D
8
&
E
M
F**

RT-PCR

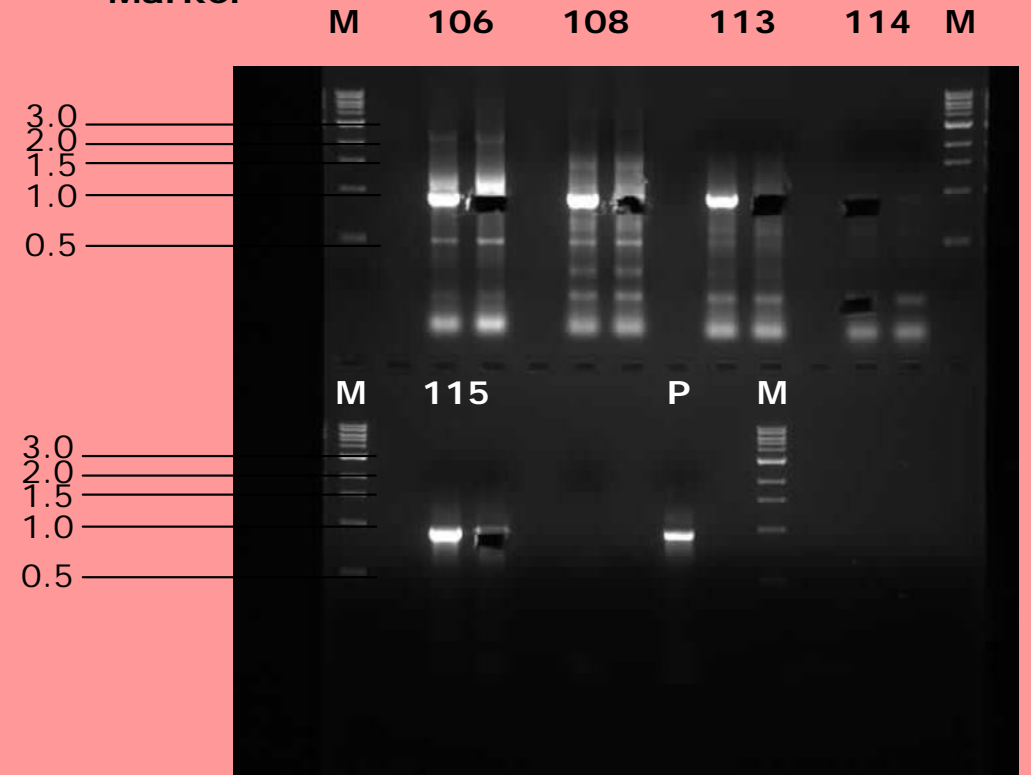
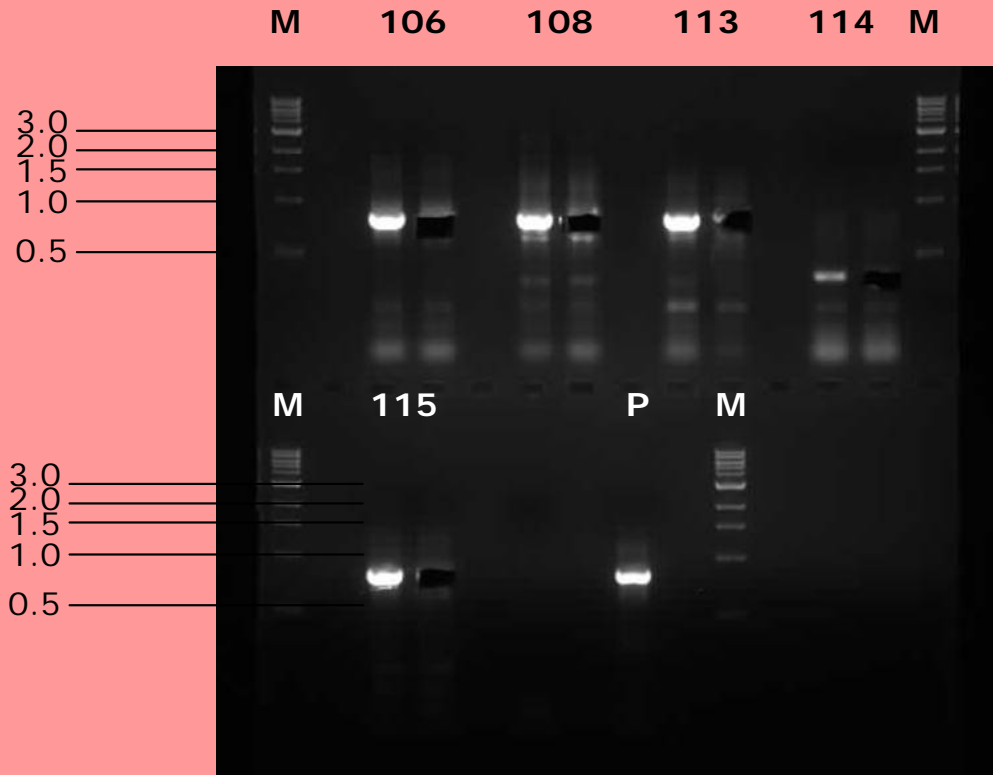
ENVELOPE OF SJE106-115

1720a

2518a

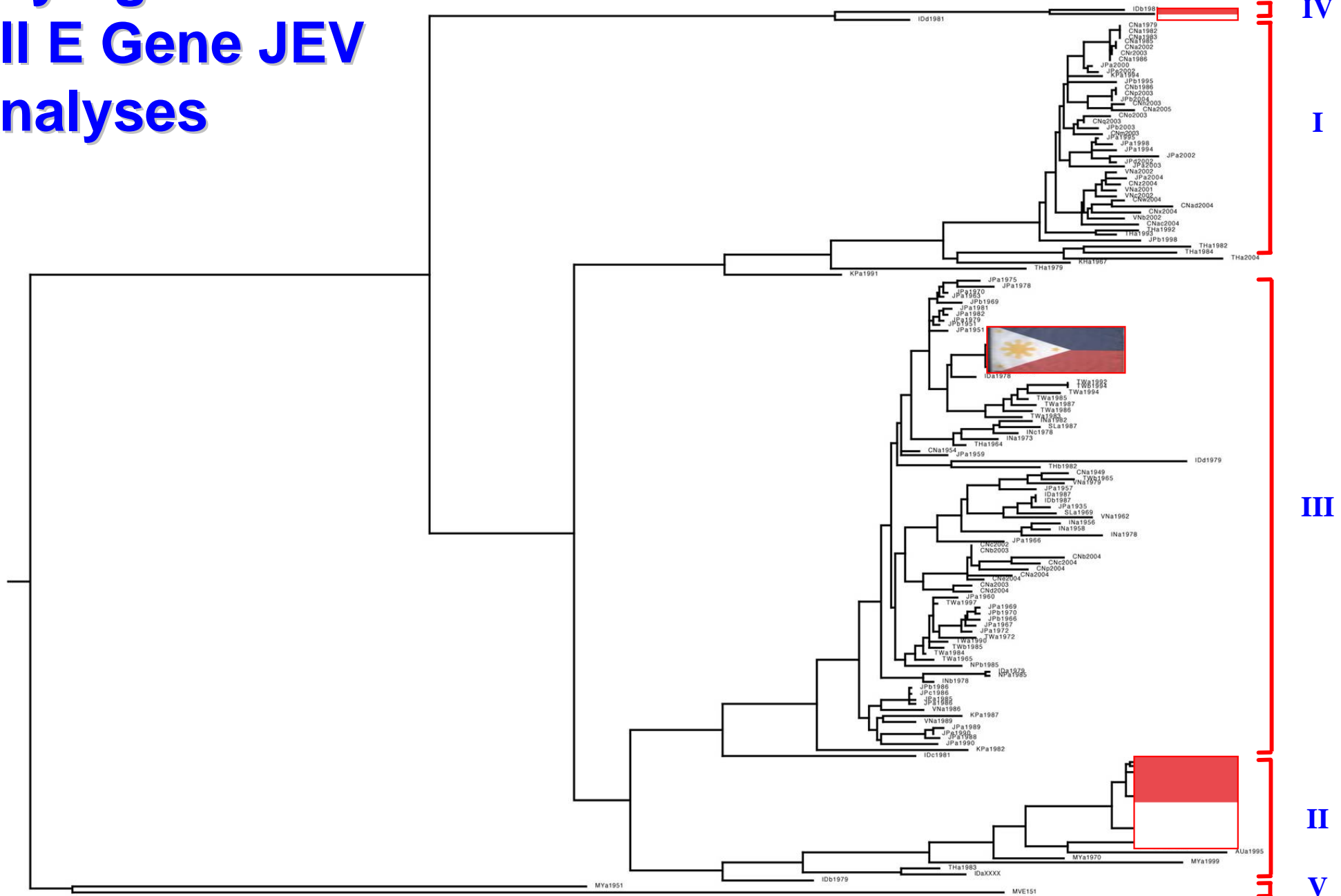
1 kb
DNA
Marker

1 kb
DNA
Marker

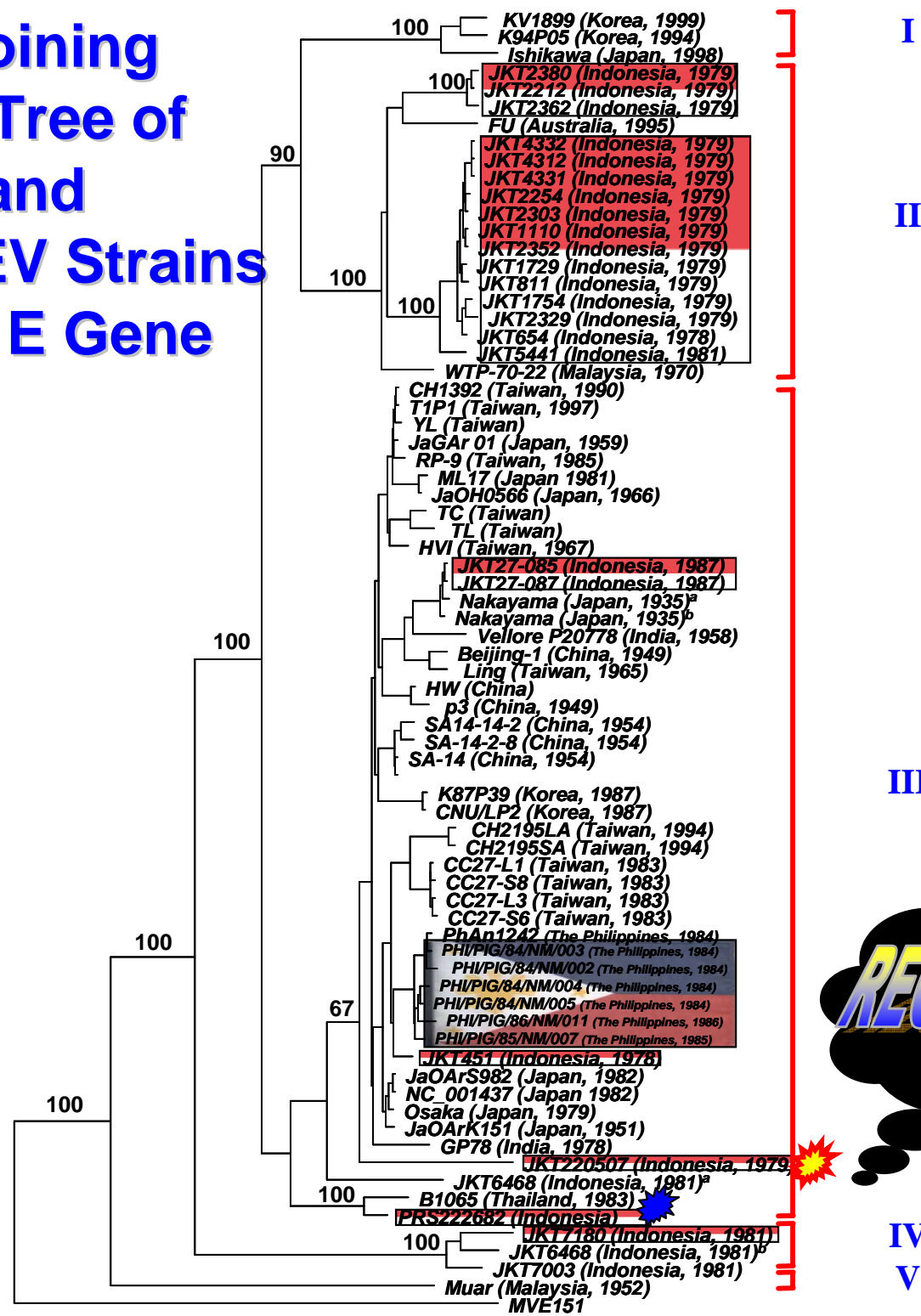


M: MARKER, P: POSITIVE CONTROL

Neighbour-Joining Phylogenetic Tree of All E Gene JEV Analyses

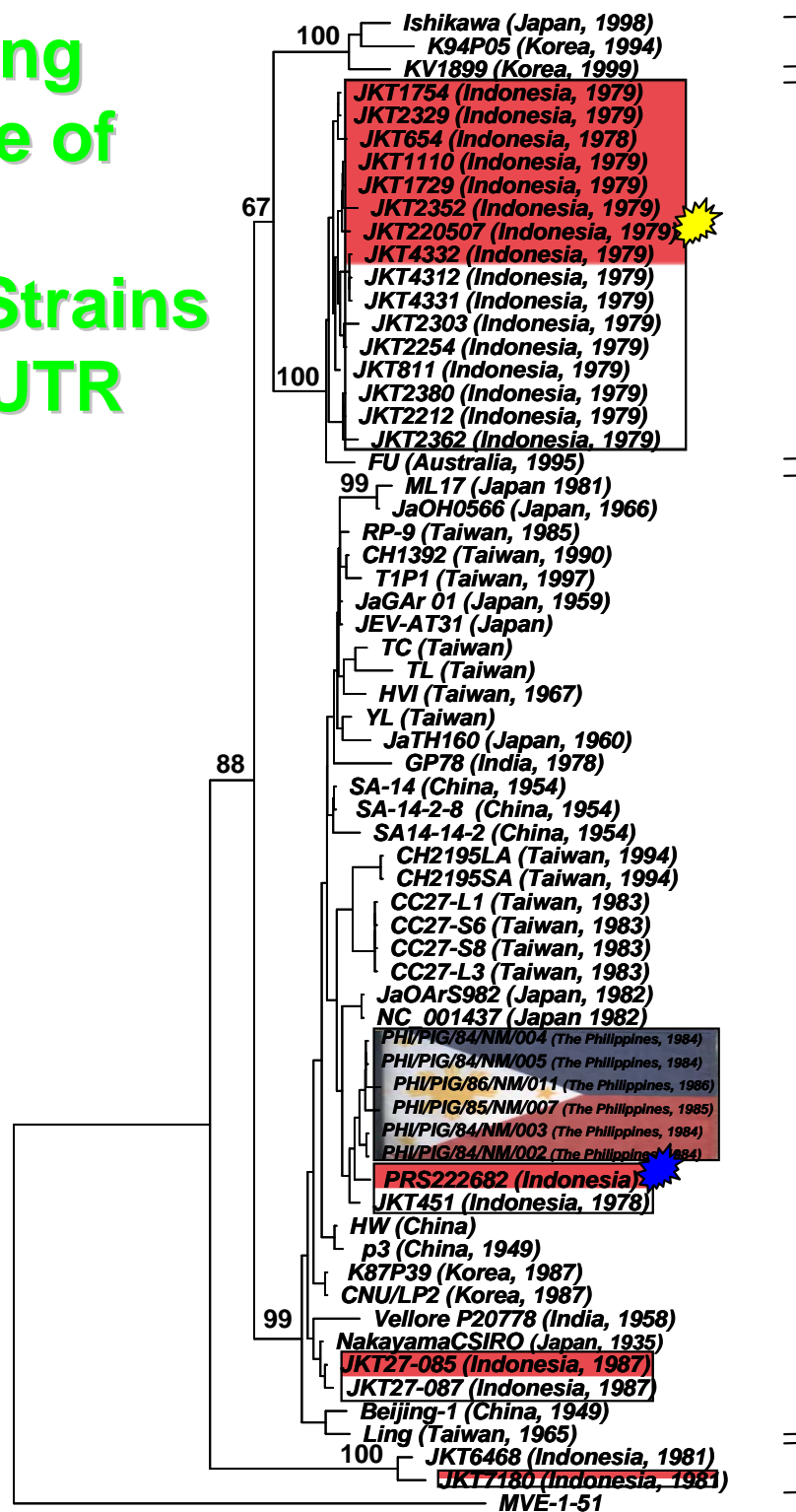


Neighbour-Joining Phylogenetic Tree of 6 Filipino and 21 Indonesian JEV Strains Determined by E Gene



- 0.002 Substitutions/site

Neighbour-Joining Phylogenetic Tree of 6 Filipino and 21 Indonesian JEV Strains Determined by 3'UTR



I

II

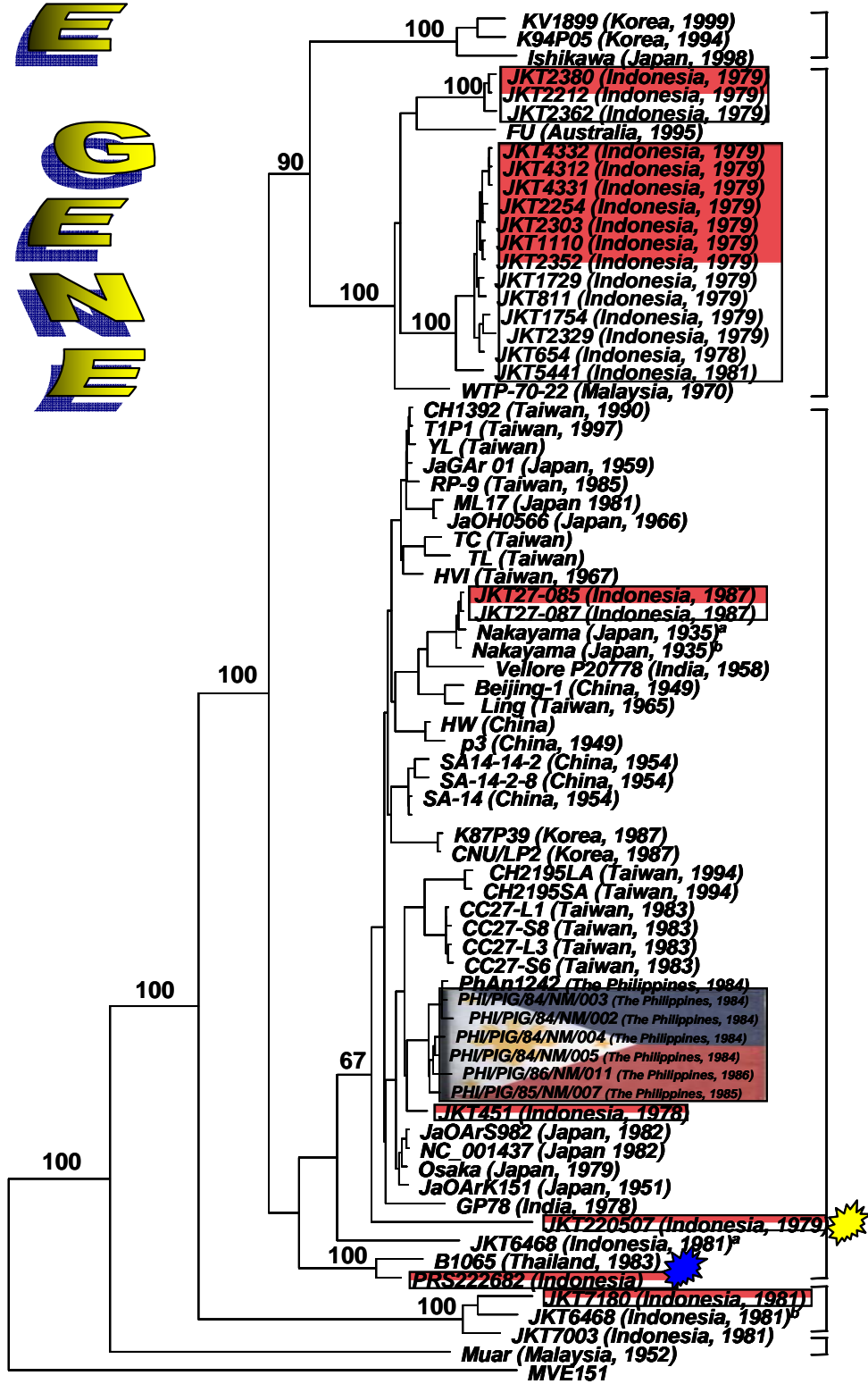
III

IV





E N Z Y M E



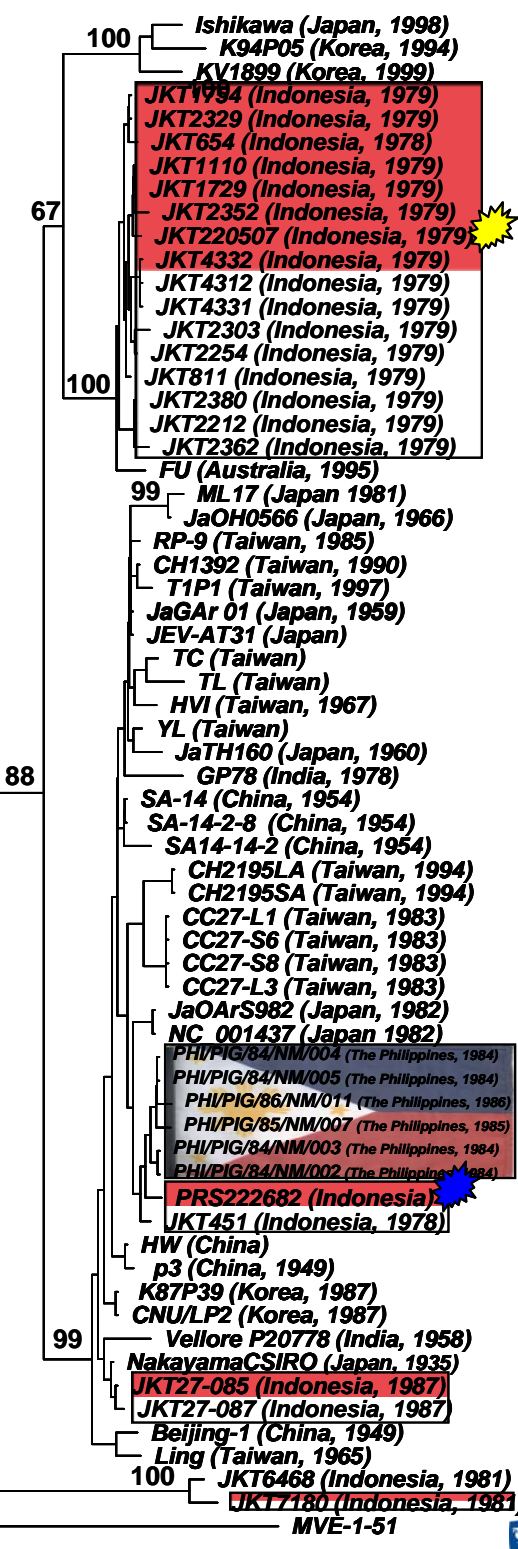
- 0.002 Substitutions/site

I
II
III
IV
V

J
K
T
2
2
0
5
0
7

P
R
S
2
2
2
6
8
2

- 0.002 Substitutions/site

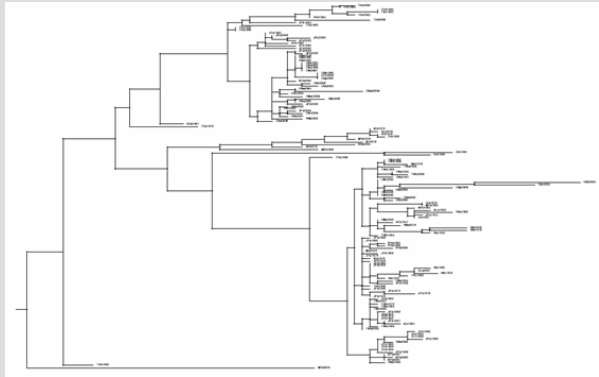


3
U
T
R

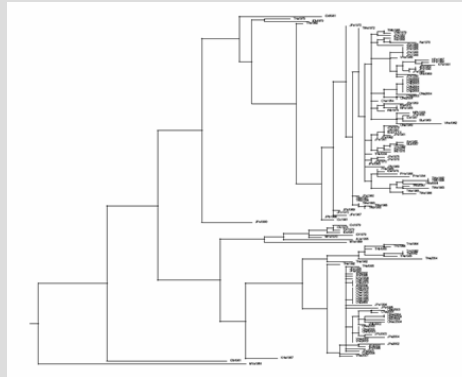
III

IV

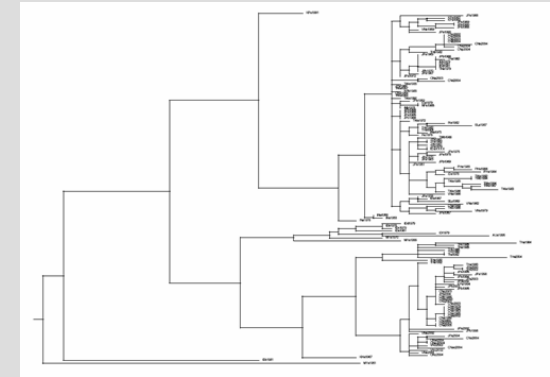
9 NJ Trees of Envelope Gene



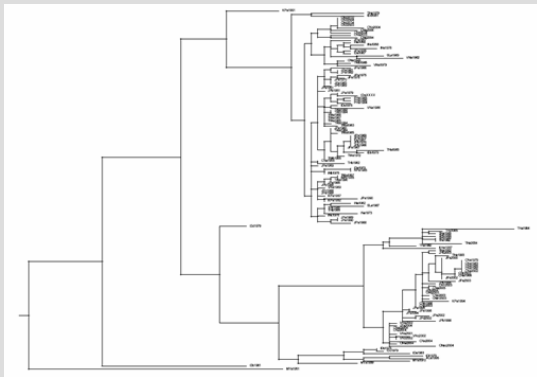
1–300 bp



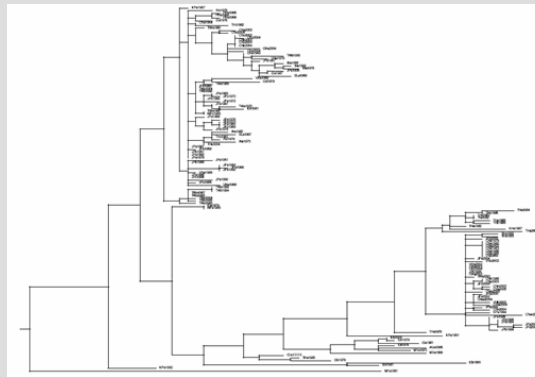
150–450 bp



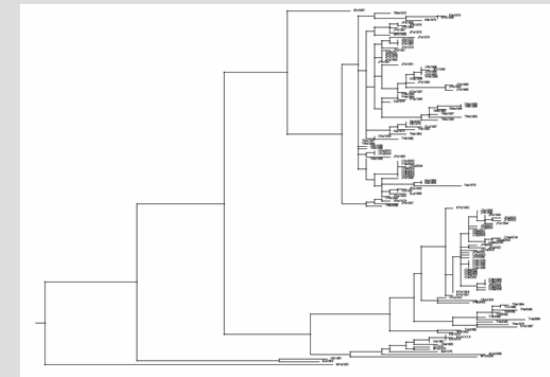
300–600 bp



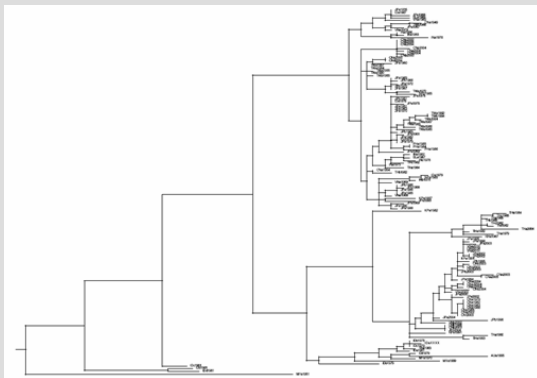
450–750 bp



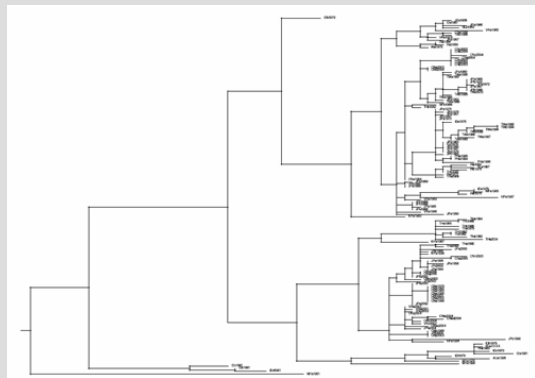
600–900 bp



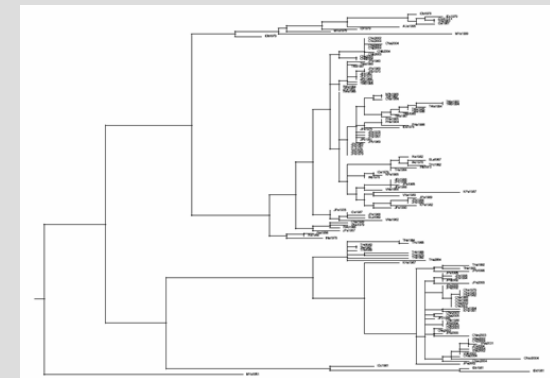
750–1050 bp



900–1200 bp



1050–1350 bp



1200–1500 bp

Sub-Genotypes of 9 Trees of All E Gene JEV Analyses

TipDate Code	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6	Tree 7	Tree 8	Tree 9
JP_a_1935	3	3	3	3	3	3	3	3	3
CN_a_1949	3	3	3	3	3	3	3	3	3
JP_a_1951	3	3	3	3	3	3	3	3	3
TH_a_1979	1	3	3	3	1	1	1	1	1
ID_b_1979	3	3	3	3	?	4	3	3	2/3
ID_a_1979	3	3	3	3	3	3	3	3	3
JP_a_1979	3	3	3	3	3	3	3	3	3
VN_a_1979	3	3	3	3	3	3	3	3	3
ID_a_1981	2	2	2	2	2	2	2	2	2
JP_a_1981	3	3	3	3	3	3	3	3	3
ID_b_1981	4	4	4	4	?	4	4	4	4
ID_c_1981	3	3	3	3	3	3	4	4	(4)
ID_d_1981	4	(3)	3	3	?	4	4	4	4
TH_a_1982	1	1	1	1	1	1	1	1	1
CN_a_1982	1	1	1	1	1	1	1	1	1
IN_a_1982	3	3	3	3	3	3	3	3	3
JP_a_1982	3	3	3	3	3	3	3	3	3
CN_a_1983	1	1	1	1	1	1	1	1	1
TH_a_1983	3	3	3	3	?	2	2	2	2
TW_a_1983	3	3	3	3	3	3	3	3	3
TH_a_1984	1	1	1	1	1	1	1	1	1
TW_a_1984	3	3	3	3	3	3	3	3	3
CN_p_2004	3	3	3	3	3	3	3	3	3
CN_a_2005	1	1	1	1	1	1	1	1	1
ID_d_1979	2	2	2	2	3	3	3	3	3
ID_a_1987	3	3	3	3	3	3	3	3	3
ID_c_1979	2	2	2	2	2	2	2	2	2
ID_a_1978	3	3	3	3	3	3	3	3	3
ID_i_1979	2	2	2	2	2	2	2	2	2
ID_b_1978	2	2	2	2	2	2	2	2	2

2372

JKT1749

JKT6468

JKT9092

B1065

JKT220507

Recombinant detected by BLAST Search

Strain	Primer	Sequencing Code No.	Results from Nucleotide-nucleotide BLAST (blastn)
PRS222682	879s	WTA59279_33_879s_B08.ab1	M18370.1 Japanese encephalitis virus (strain JaOArS982), complete genome 1528
PRS222682	1720a	WTA59279_34_1720a_C08.ab1	M18370.1 Japanese encephalitis virus (strain JaOArS982), complete genome 1497
PRS222682	M13F	WT475-M13F	M18370.1 Japanese encephalitis virus (strain JaOArS982), complete genome 1515
PRS222682	M13R	WT476-M13R	U70414.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1459
PRS222682	M13F	WT477-M13F	M18370.1 Japanese encephalitis virus (strain JaOArS982), complete genome 1497
PRS222682	M13R	WT478-M13R	U70414.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1439

G III



Strain	Primer	Sequencing Code No.	Results from Nucleotide-nucleotide BLAST (blastn)
PRS222682	1577s	BWT435-1577s	U70406.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1505
PRS222682	2518a	BWT436-2518a	U70406.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1445
PRS222682	M13F	WT563-M13F	U70406.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1041
PRS222682	M13R	WT564-M13R	U70388.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1249
PRS222682	M13F	WT565-M13F	U70406.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1162
PRS222682	M13R	WT566-M13R	U70388.1 Japanese encephalitis virus envelope protein mRNA, partial cds 1257

G II

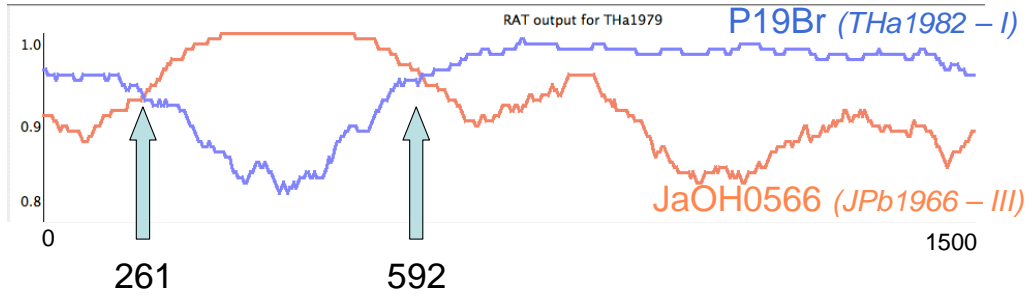


G II / III



Genetic Diversity Plot for 2372

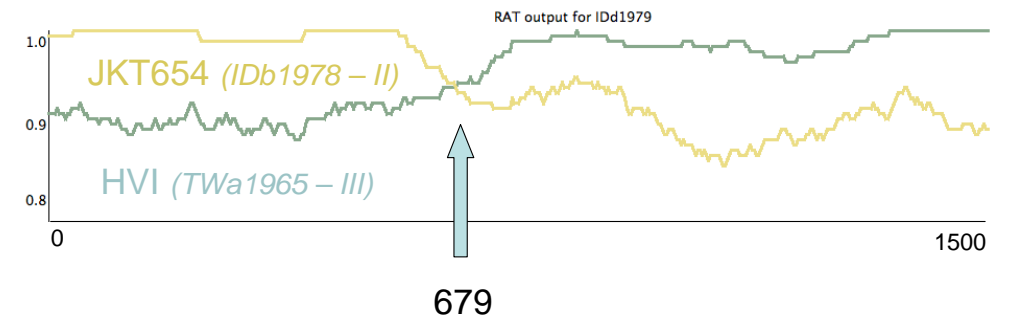
(Thailand, 1979: **G/I**)



LR = 73

Genetic Diversity Plot for JKT220507

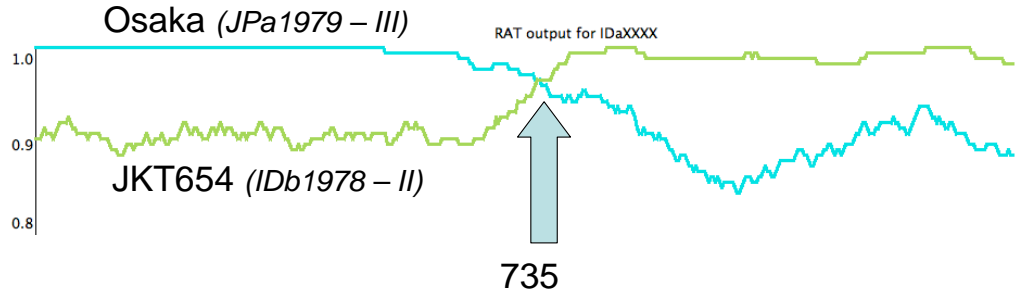
(Indonesia, 1979: **G/II**)



LR = 89

Diversity Plot for PRS222682

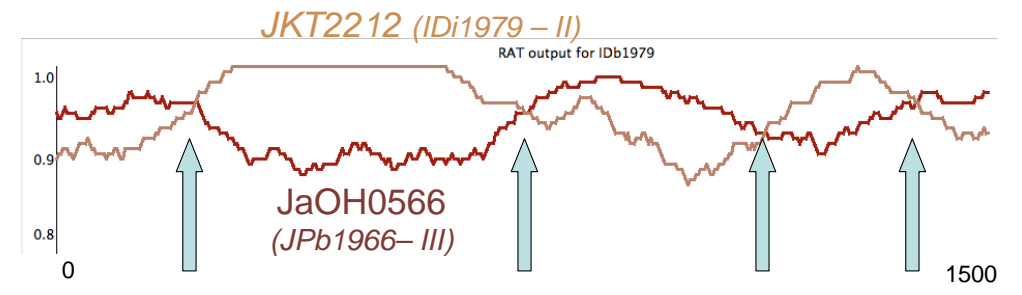
(Indonesia : **G/III**)



LR=105









Genetic Diversity Plot for JKT1749

(Indonesia, 1979: **G/IV**)



LR = xx

Recombinant Strains, Their Closest 'Parental' Sequences

JEV Strain	Genotype	Isolate	Region of E Gene	Parents (Isolate)	Sub-Genotype
JKT1749	IV	Indonesia, 1979	??	JaOH0566 (Japan, 1966)	
			??	JKT2212 (Indonesia, 1979)	II
			??	JaOH0566 (Japan, 1966)	
			??	JKT2212 (Indonesia, 1979)	II
JKT220507	II	Indonesia, 1979	1-679	JKT654 (Indonesia, 1978)	II
			680-1500	HVI (Taiwan, 1965)	
2372	I	Thailand, 1979	1-261	P19Br (Thailand, 1982)	I
			262-592	JaOH0566 (Japan, 1966)	
			593-1500	P19Br (Thailand, 1982)	I
JKT6468	IV	Indonesia, 1981	1-1009	JaOH0566 (Japan, 1966)	
			1010-1500	JKT7003 (Indonesia, 1981)	IV
JKT9092	IV	Indonesia, 1981	1-265	JKT7003 (Indonesia, 1981)	IV
			266-723	JaOH0566 (Japan, 1966)	
			724-1500	JKT7003 (Indonesia, 1981)	IV
B1065	II or III	Thailand, 1983	1-728	JaOH0566 (Japan, 1966)	
			729-1500	JKT5441 (Indonesia, 1981)	II
PRS222682	III	Indonesia	1-735	Osaka (Japan, 1979)	
			736-1500	JKT654 (Indonesia, 1978)	II

SUMMARY & CONCLUSIONS

6 Filipino strains belonged to genotype III

20 Indonesian isolates were categorised into three genotypes

Genotype II (15)

Genotype III (4)

Genotype IV (1)

1 isolate (JKT220507) was categorised as genotype III by E gene and II by 3'UTR, suggesting that recombination had occurred.

Seven further isolates, from Indonesia and Thailand, were identified as recombinants.

All involve genotype III.

Why?

Most widely dispersed?

FURTHER WORK

The role of recombination in JEV evolution and spread of JEV merits further investigation.

ACKNOWLEDGEMENTS



The Royal Thai Government Scholarship, THAILAND



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Viral Brain Infections Group Website

<http://www.liv.ac.uk/neuroscience>

The Completed Nucleotide Sequences of Japanese encephalitis virus

GenBank Accession No.	Name	Year	Source	Gene Type	Place
AB051292.1	Ishikawa	1998	Mosquito, Swine	I	Japan
AF045551.2	K94P05	1994	Mosquito	I	Korea
AY316157.1	KV1899	1999	Swine	I	Korea
AF217620.1	FU	1995	Human	II	Australia
AF014161.1	RP-9	1985	Mosquito	III	Taiwan
AF069076.1	JaGAr 01	1959	Mosquito	III	Japan
AF075723.1	GP78	1978	Human	III	India
AF080251.1	Vellore P20778	1958	Human	III	India
AF098735.1	HVI	1967	Human, Mosquito	III	Taiwan
AF098736.1	TC	IU*	Mosquito	III	Taiwan
AF098737.1	TL	IU*	Mosquito	III	Taiwan
AF221499.1	CH2195LA	1994	Mosquito	III	Taiwan
AF221500.1	CH2195SA	1994	Mosquito	III	Taiwan
AF254452.1	CH1392	1990	Mosquito	III	Taiwan
AF254453.1	T1P1	1997	Mosquito	III	Taiwan
AF315119.1	SA14-14-2	1954	SA-14 derivative	III	China
AF486638.1	YL	IU*	Swine Vaccine	III	Taiwan
AY303795.1	CC27-L1	1983	CC27 derivative	III	Taiwan
AY303796.1	CC27-L3	1983	CC27 derivative	III	Taiwan
AY303797.1	CC27-S6	1983	CC27 derivative	III	Taiwan
AY303798.1	CC27-S8	1983	CC27 derivative	III	Taiwan
AY508813.1	JaOH0566	1966	Human	III	Japan
AY585242.1	K87P39	1987	Mosquito	III	Korea
AY849939.1	HW	IU*	IU*	III	China
L48961.1	Beijing-1	1949	Human	III	China
L78128.1	Ling	1965	Human, Mosquito	III	Taiwan
M18370.1	JaOArS982	1982	Mosquito	III	Japan
M55506.1	SA-14	1954	Mosquito	III	China
U47032.1	p3	1949	Mosquito	III	China
NC_001437.1	JaOArS982	1982	Mosquito	III	Japan
U15763.1	SA-14 derivative	1954	SA-14 derivative	III	China
AY508812.1	ML17	1981	Swine vaccine strain	IU*	Japan
AY585243.1	CNU/LP2	1987	K87P39 derivative	IU*	Korea
AY184212.1	JKT6468	1981	Mosquito	IV	Indonesia
Muar	Muar	1952	Human	V	Malaysia

*Information Unavailable

**AF161266.1 *Murray Valley encephalitis virus* strain MVE-1-51 Used for Outgroup of *Japanese encephalitis virus*