Sequence analysis of mosquito-borne virus from imported cases

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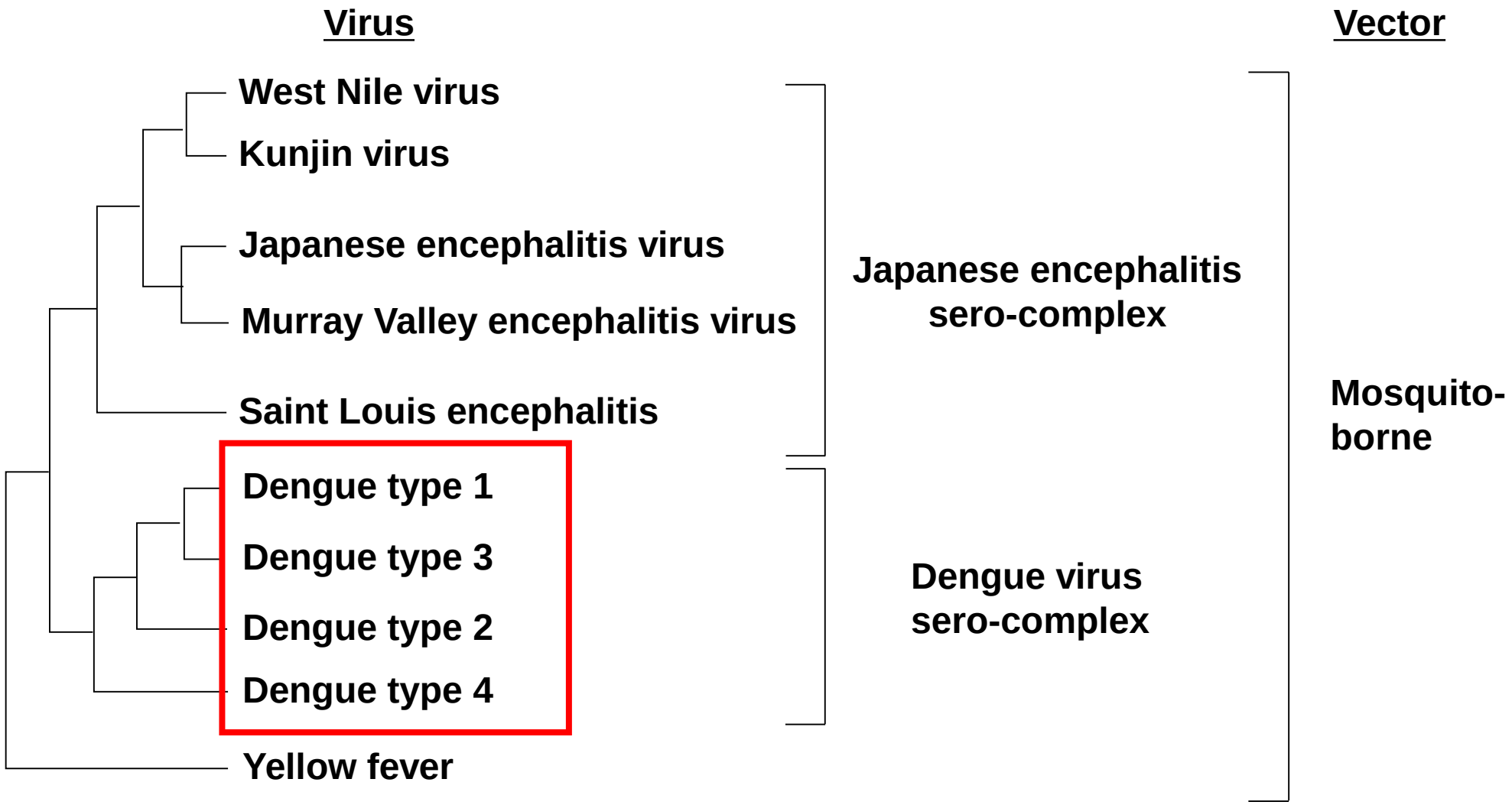
Mosquito-borne flavivirus



（Fields Virology）

National Epidemiological Surveillance for mosquito borne infectious Diseases in Japan

- **Case based surveillance**

  Category IV; Notifiable infectious disease by The Infectious Diseases Control Law

Flavivirus

- ✓ ZIKAV,
- ✓ DENV,
- ✓ WNV,
- ✓ JEV

Alphavirus

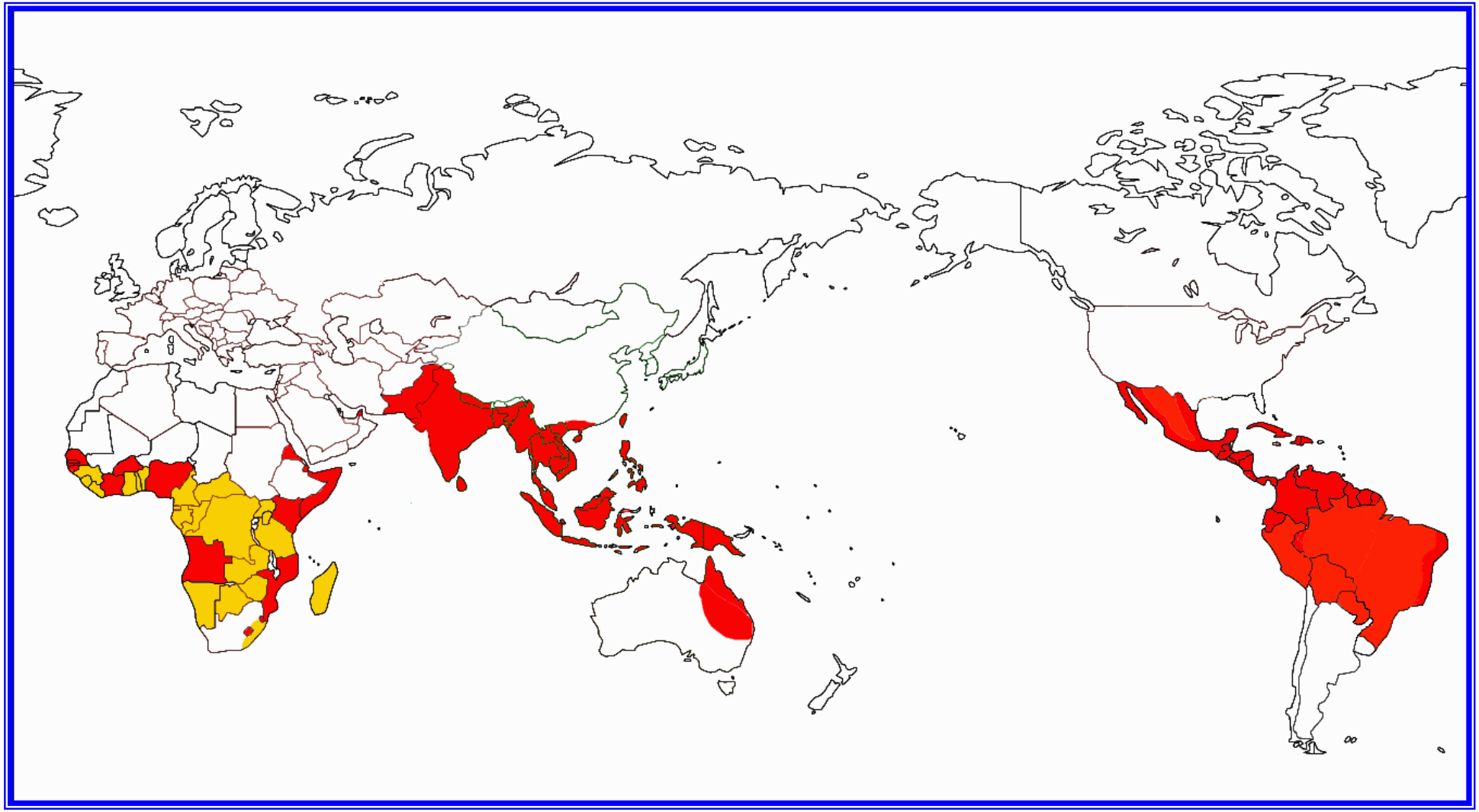
- ✓ CHIKV,
- ✓ VEEV,
- ✓ WEEV,
- ✓ EEEV

Laboratory Test for mosquito-borne diseases

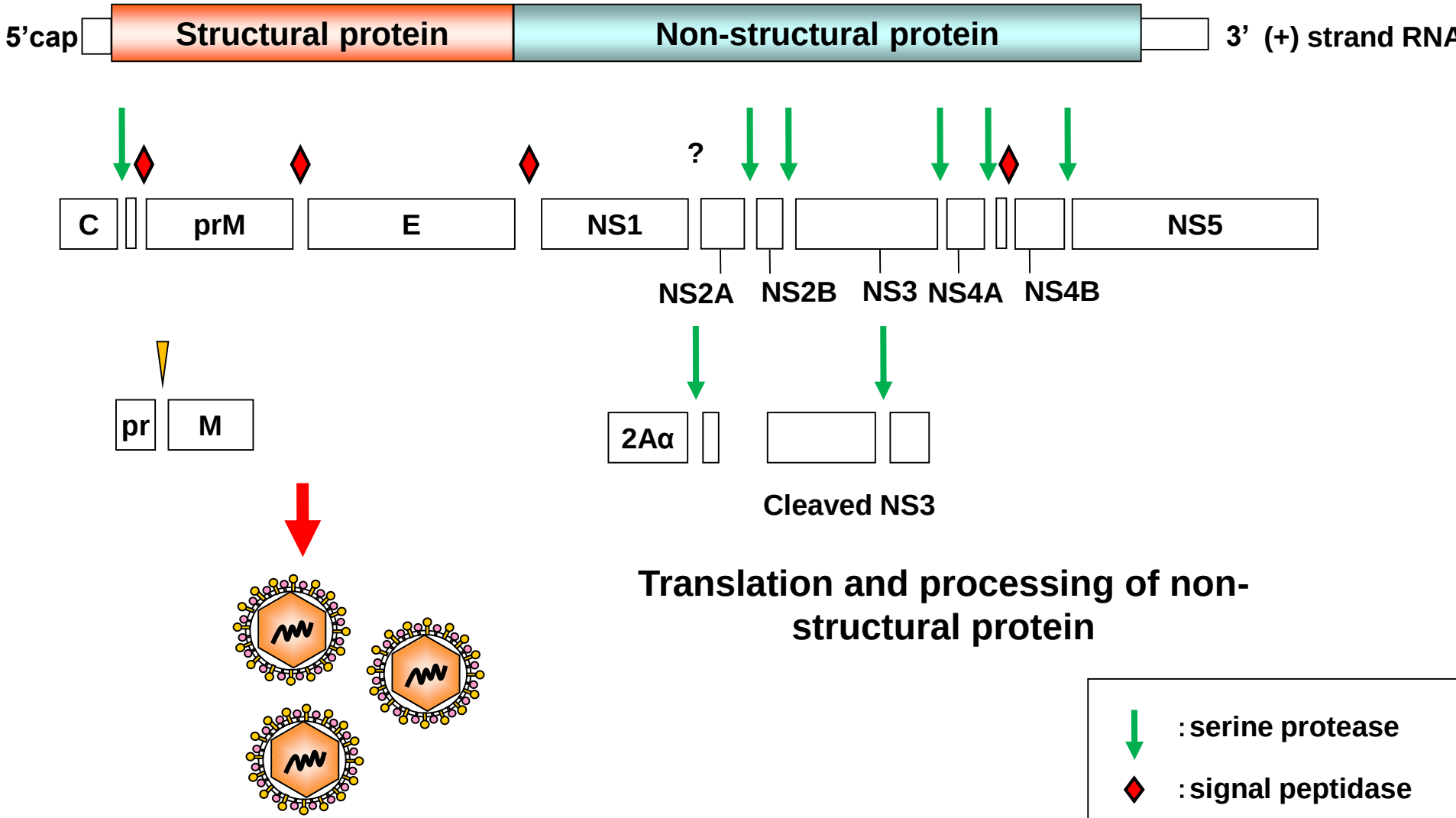
- **Speicemen: blood, serum, urine**
  - **Viral test**
    - **Detection of viral nucleic acid (RT-PCR, Real time RT-PCR, RT-Lamp）**
    - **Virus antigen detection (Dengue NS1 ELISA)**
    - **Virus isolation (cell culture; Vero and C6/36)**
  - **Serum test**
    - **IgM capture-ELISA**
    - **IgG ELISA**
    - **Neutralizing antibody test**

Dengue virus

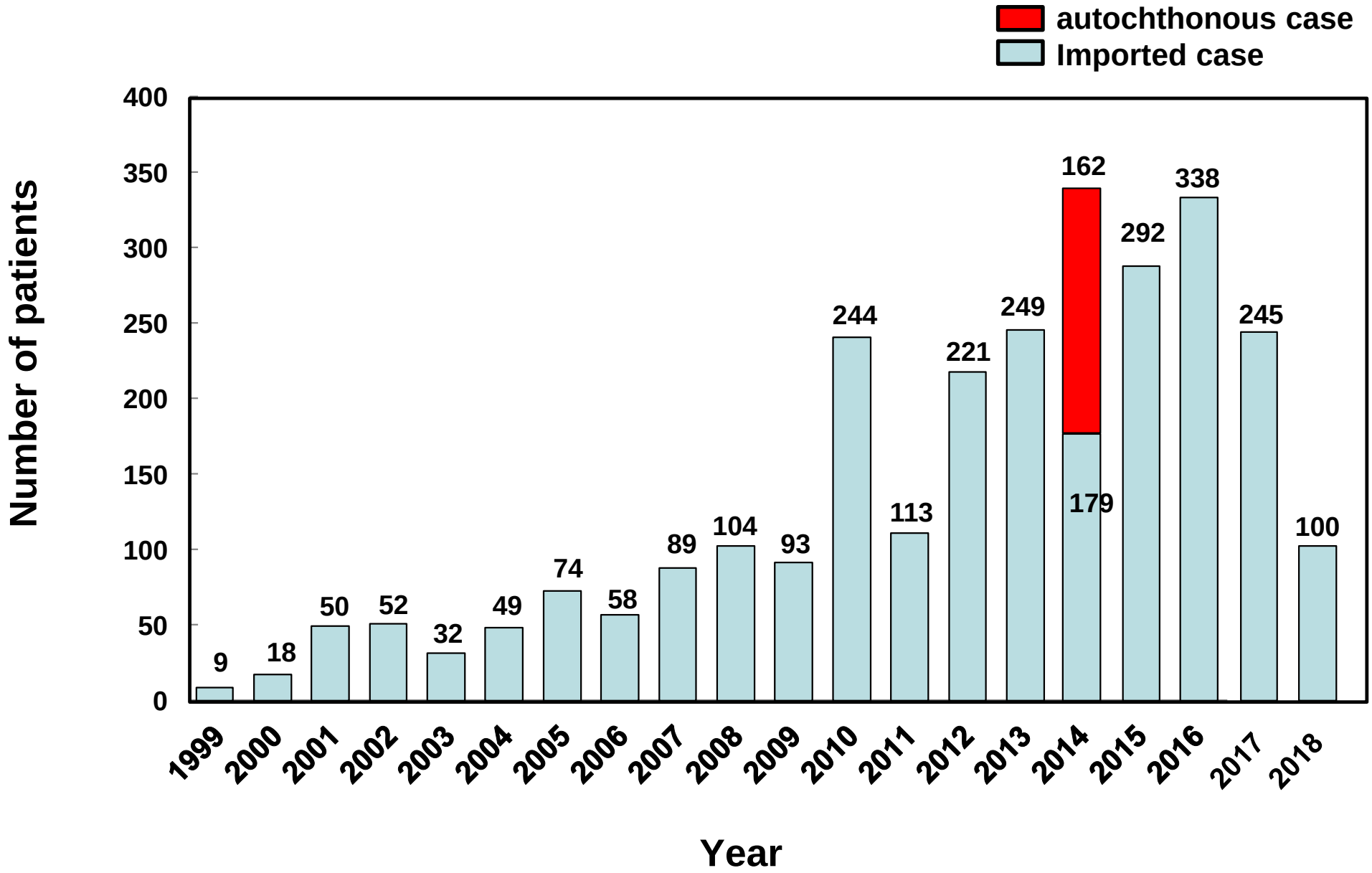
The distribution of dengue virus



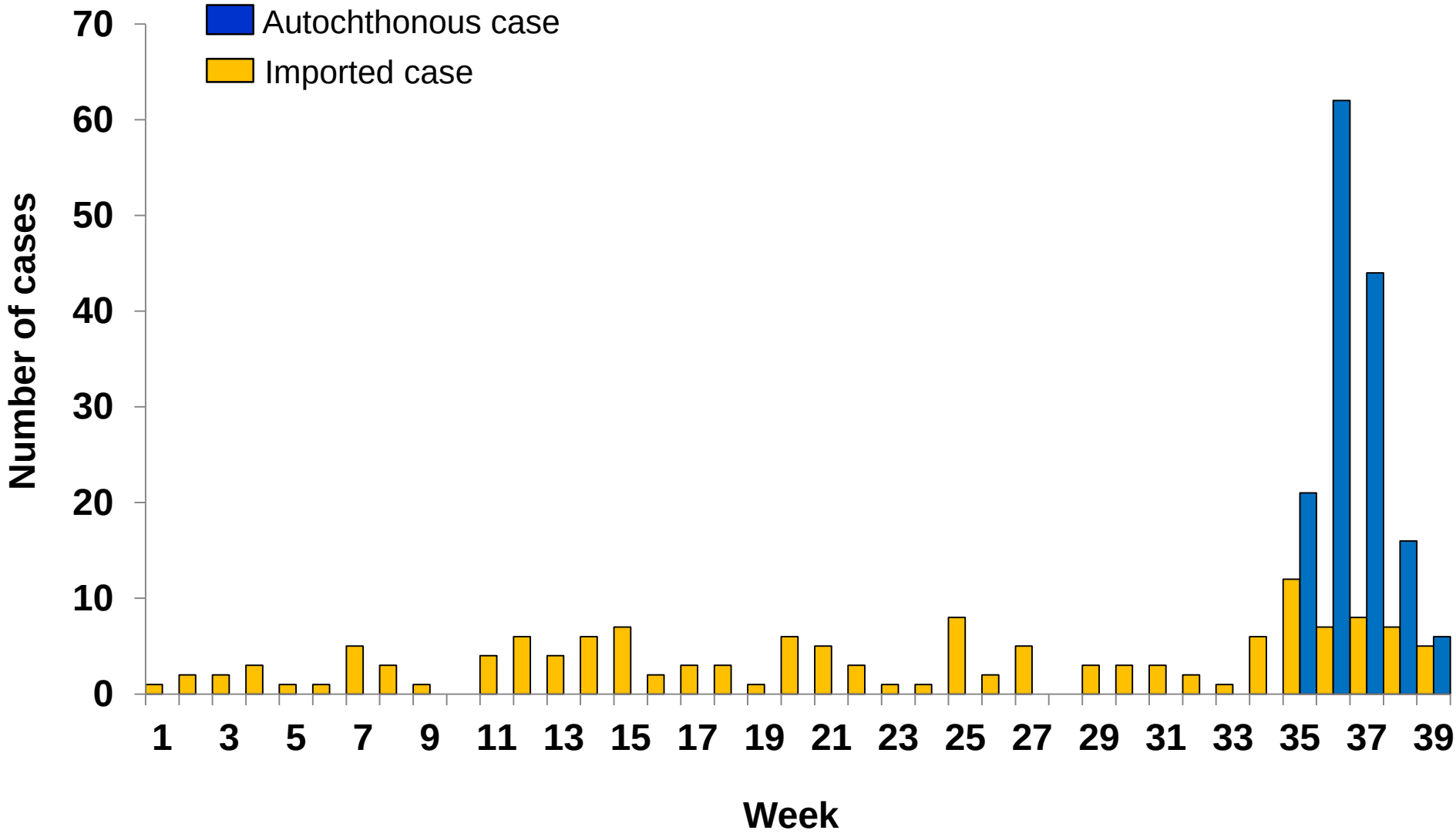
Genome structure of dengue virus



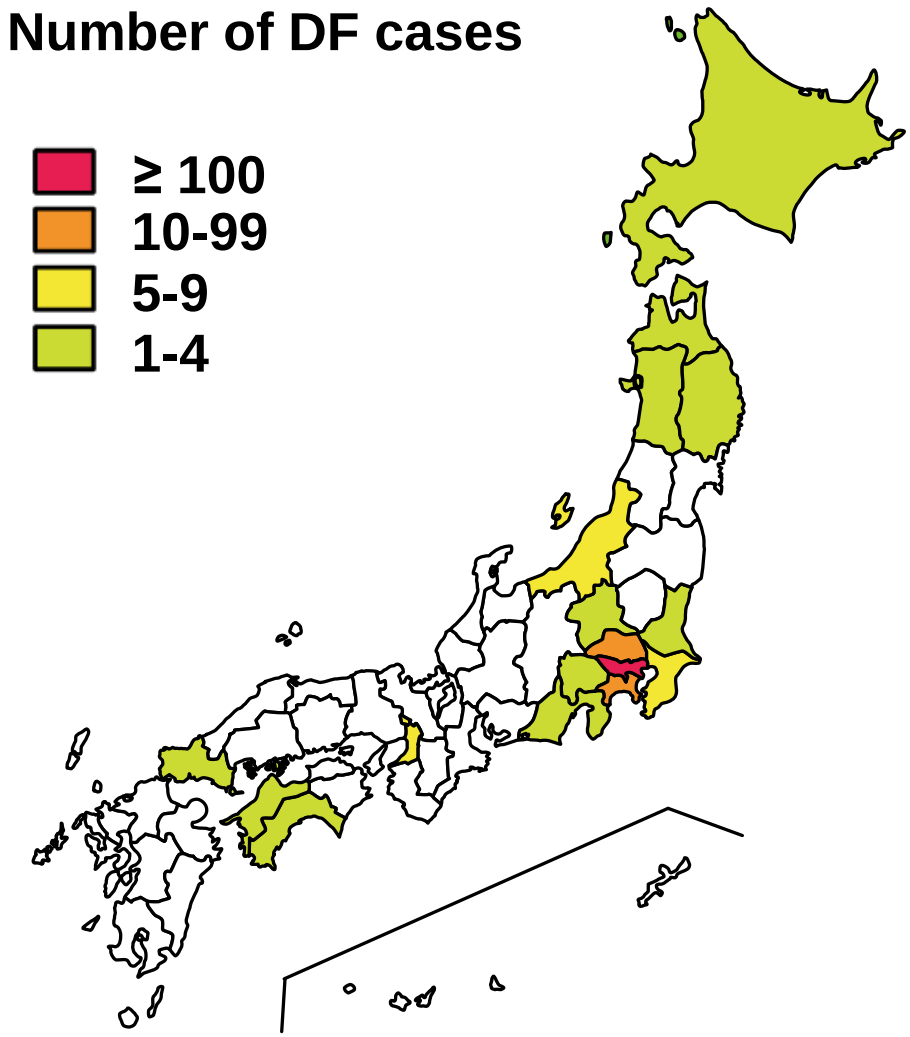
The dengue fever (DF) cases in Japan, 1999-August 2018



DF cases in Japan, wk 1-39, 2014

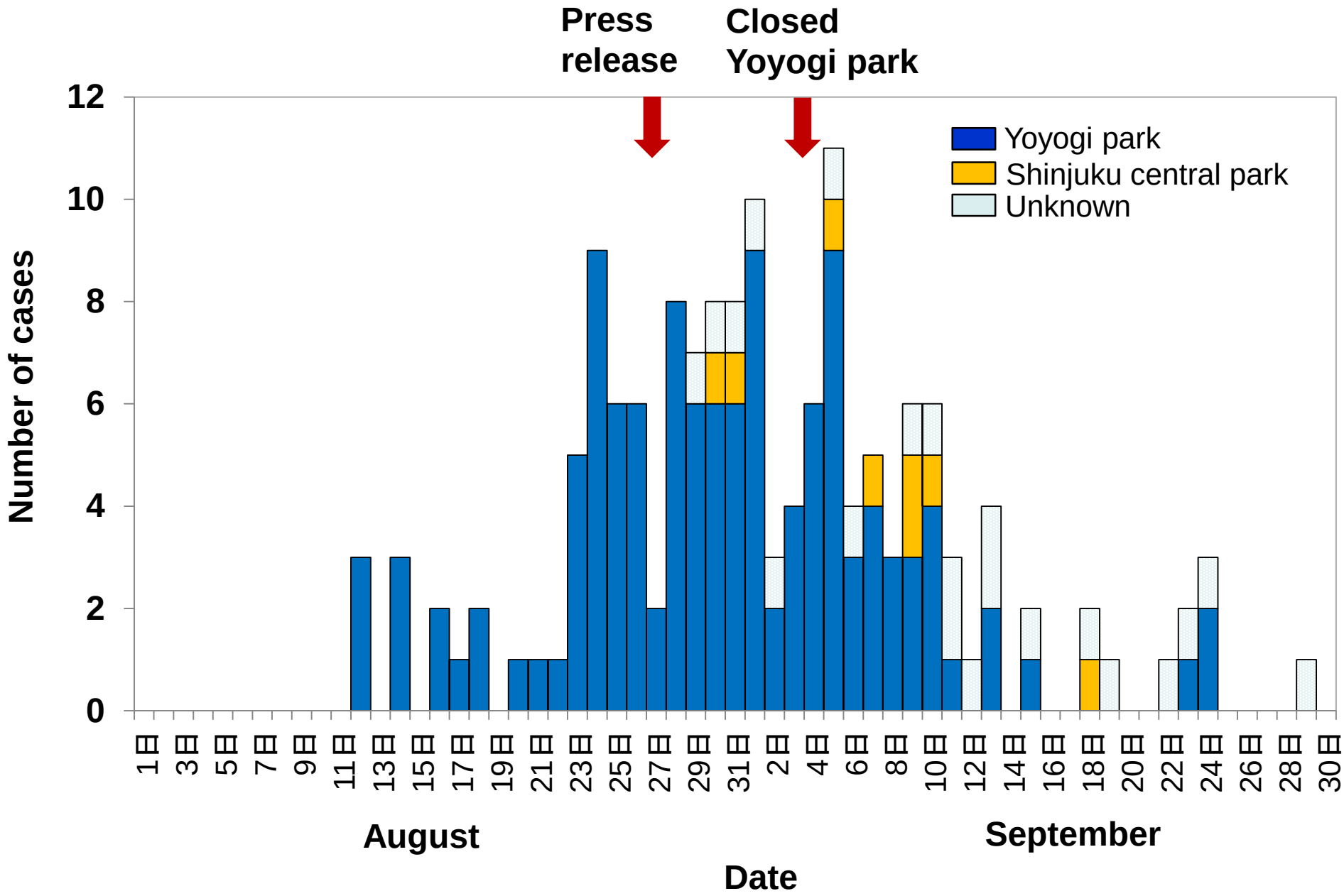


Distribution of autochthonous DF cases in Japan, 2014

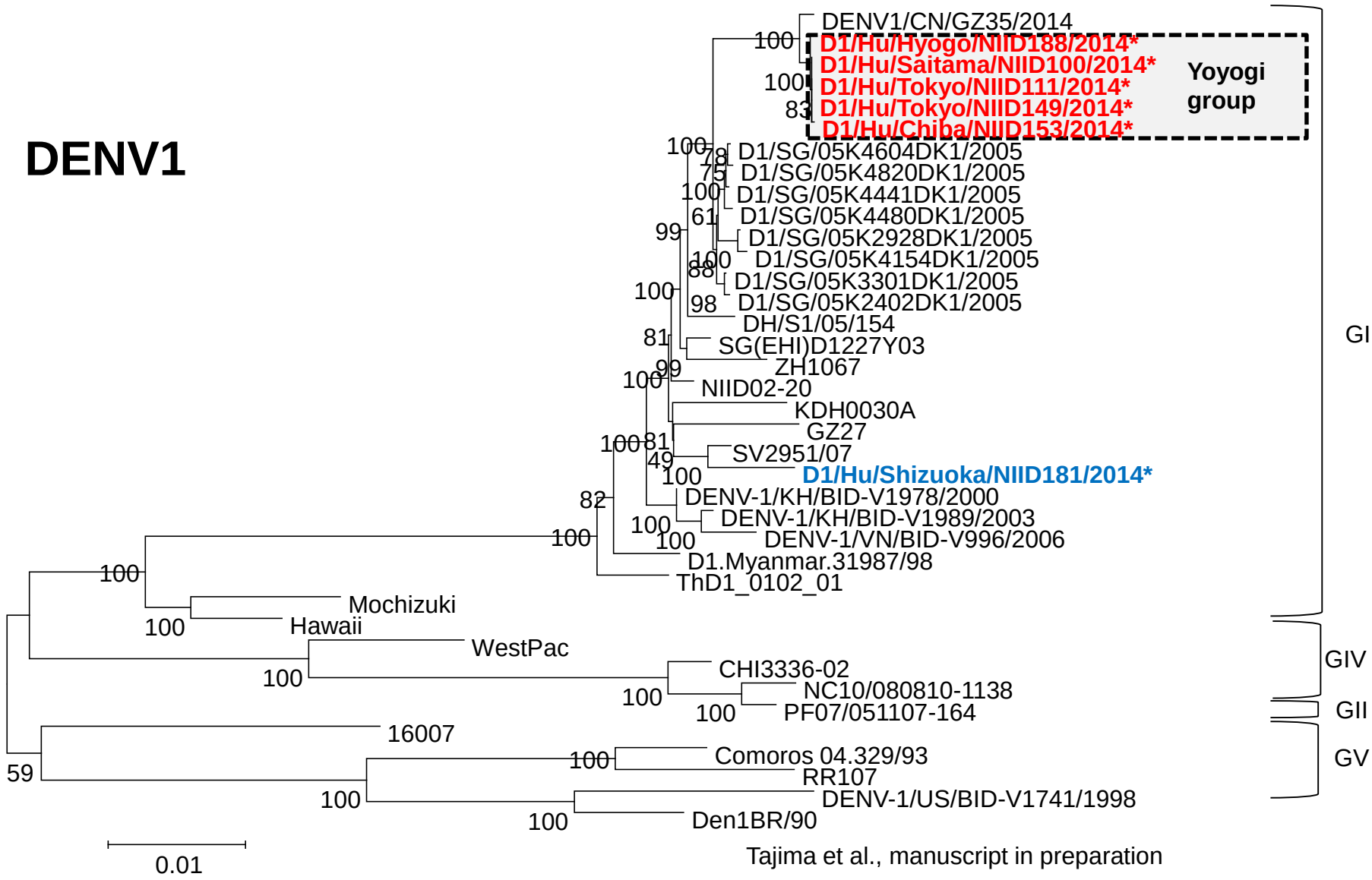


Yoyogi Park, Tokyo

Date of onset of the autochthonous dengue cases in Japan in 2014



Phylogenic analysis of whole genome DENV isolates from the autochthonous DF cases in Japan in 2014



Tajima et al., manuscript in preparation

Specific Aim

To understand DENV epidemic and distribution, we constructed genomic database of mosquito-borne viruses together with epidemiological data by using GenEpid-J System based on imported DEN cases in Japan

Sequence analysis of DENV genome by Next Generation Sequencer (NGS)

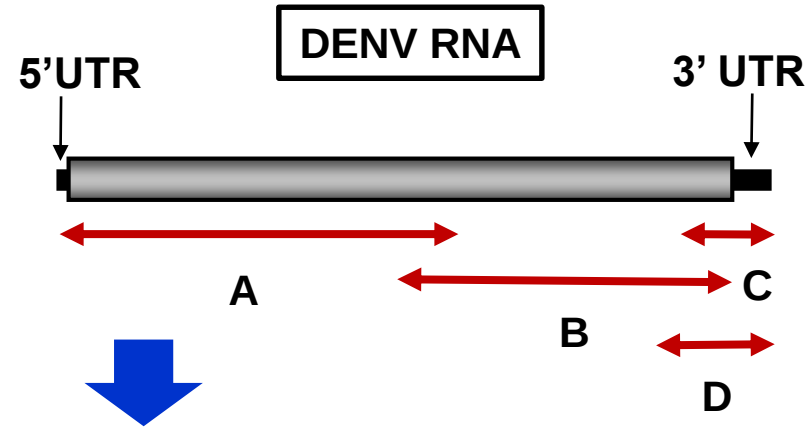
Extract Viral genome from patient serum

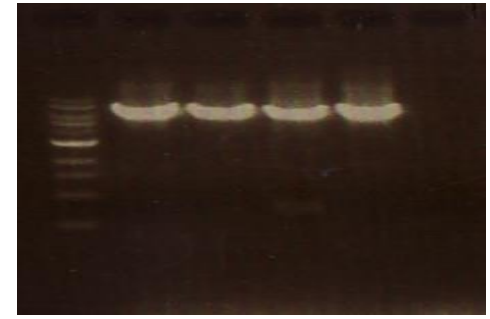
Synsesis cDNA and amplify target region by RT-PCR

Prepare library for NGS

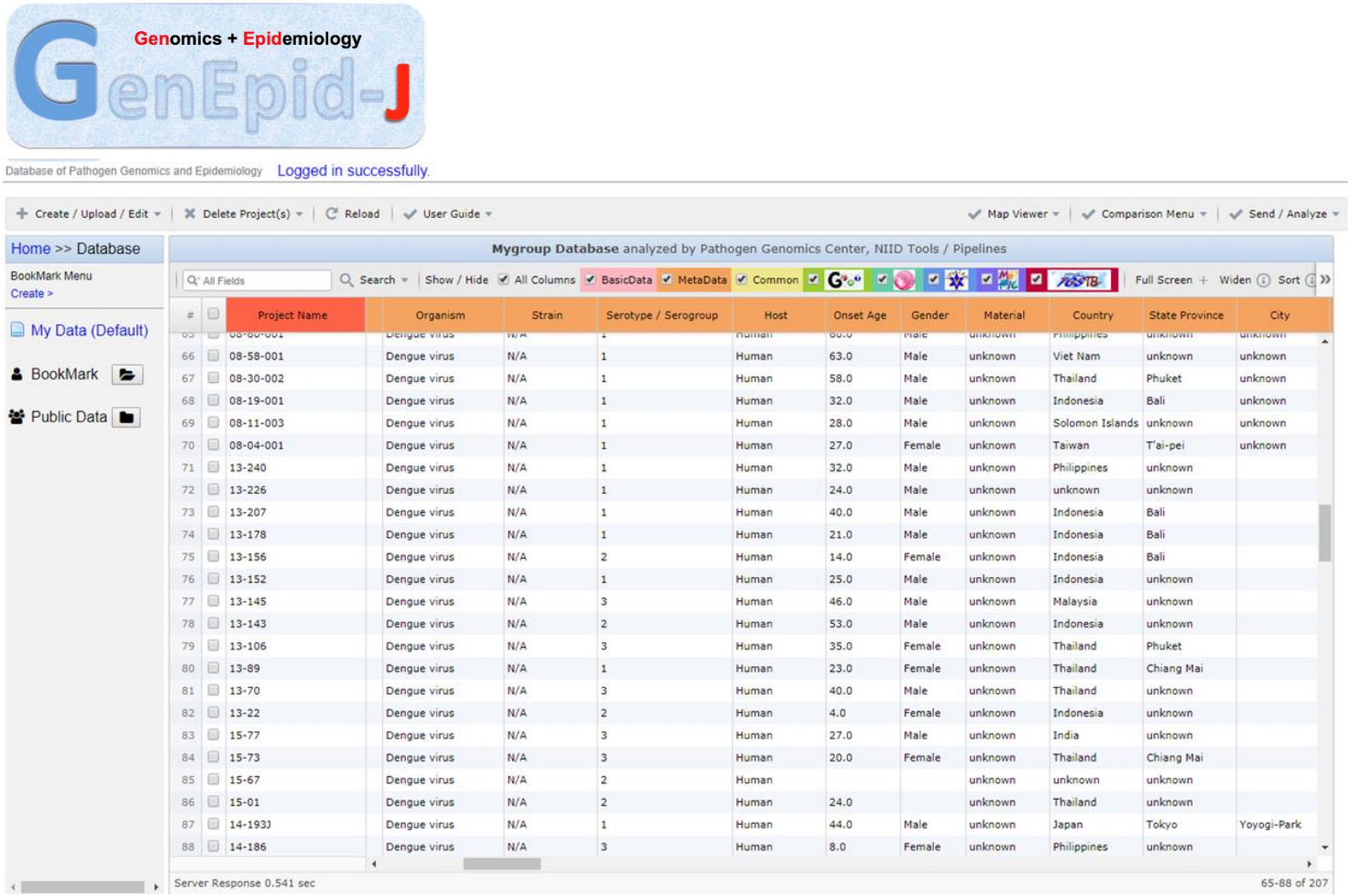
Emulsion PCR

Sequence analysis by NGS





Registration of DENV genomic data to GenEpid-J obtained by NGS and Sanger sequencer

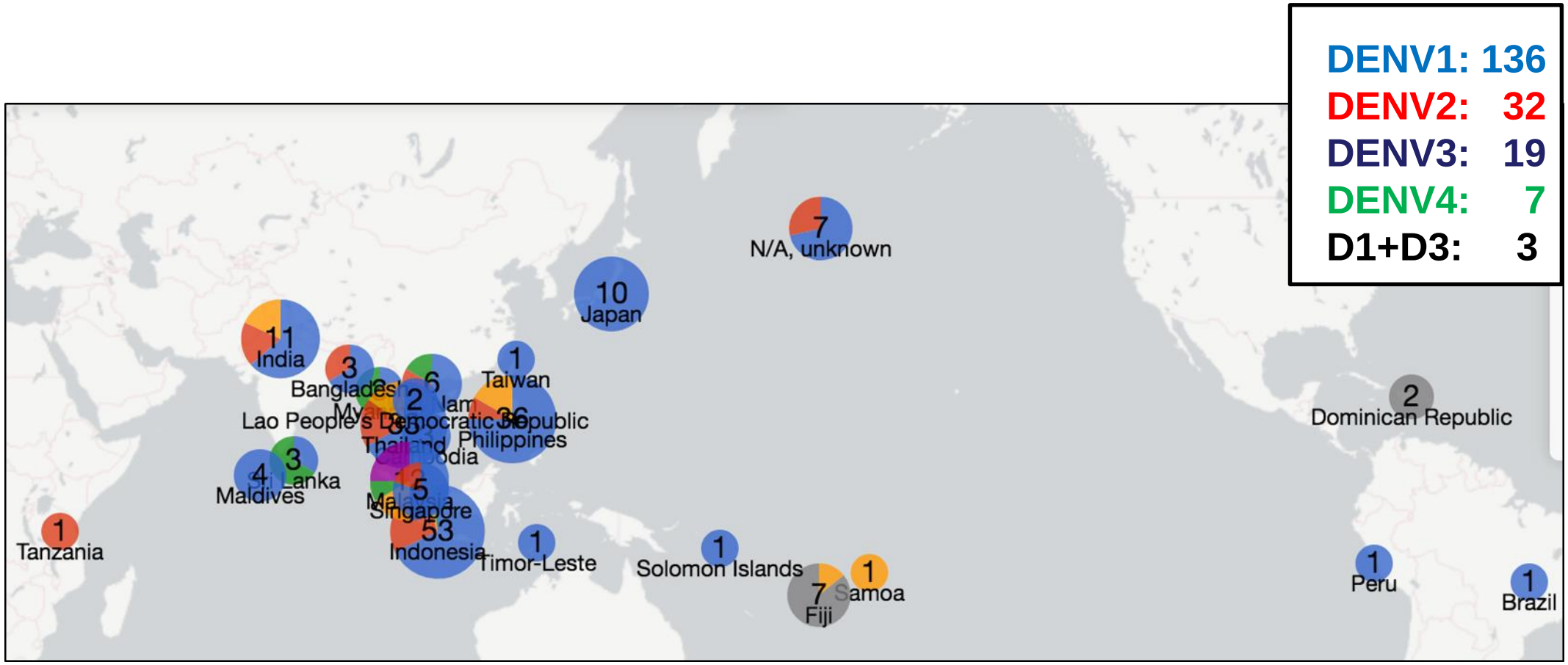


#	Project Name	Organism	Strain	Serotype / Serogroup	Host	Onset Age	Gender	Material	Country	State Province	City
66	08-58-001	Dengue virus	N/A	1	Human	63.0	Male	unknown	Viet Nam	unknown	unknown
67	08-30-002	Dengue virus	N/A	1	Human	58.0	Male	unknown	Thailand	Phuket	unknown
68	08-19-001	Dengue virus	N/A	1	Human	32.0	Male	unknown	Indonesia	Bali	unknown
69	08-11-003	Dengue virus	N/A	1	Human	28.0	Male	unknown	Solomon Islands	unknown	unknown
70	08-04-001	Dengue virus	N/A	1	Human	27.0	Female	unknown	Taiwan	T'ai-pei	unknown
71	13-240	Dengue virus	N/A	1	Human	32.0	Male	unknown	Philippines	unknown	
72	13-226	Dengue virus	N/A	1	Human	24.0	Male	unknown	unknown	unknown	
73	13-207	Dengue virus	N/A	1	Human	40.0	Male	unknown	Indonesia	Bali	
74	13-178	Dengue virus	N/A	1	Human	21.0	Male	unknown	Indonesia	Bali	
75	13-156	Dengue virus	N/A	2	Human	14.0	Female	unknown	Indonesia	Bali	
76	13-152	Dengue virus	N/A	1	Human	25.0	Male	unknown	Indonesia	unknown	
77	13-145	Dengue virus	N/A	3	Human	46.0	Male	unknown	Malaysia	unknown	
78	13-143	Dengue virus	N/A	2	Human	53.0	Male	unknown	Indonesia	unknown	
79	13-106	Dengue virus	N/A	3	Human	35.0	Female	unknown	Thailand	Phuket	
80	13-89	Dengue virus	N/A	1	Human	23.0	Female	unknown	Thailand	Chiang Mai	
81	13-70	Dengue virus	N/A	3	Human	40.0	Male	unknown	Thailand	unknown	
82	13-22	Dengue virus	N/A	2	Human	4.0	Female	unknown	Indonesia	unknown	
83	15-77	Dengue virus	N/A	3	Human	27.0	Male	unknown	India	unknown	
84	15-73	Dengue virus	N/A	3	Human	20.0	Female	unknown	Thailand	Chiang Mai	
85	15-67	Dengue virus	N/A	2	Human			unknown	unknown	unknown	
86	15-01	Dengue virus	N/A	2	Human	24.0		unknown	Thailand	unknown	
87	14-193J	Dengue virus	N/A	1	Human	44.0	Male	unknown	Japan	Tokyo	Yoyogi-Park
88	14-186	Dengue virus	N/A	3	Human	8.0	Female	unknown	Philippines	unknown	

Registered Information

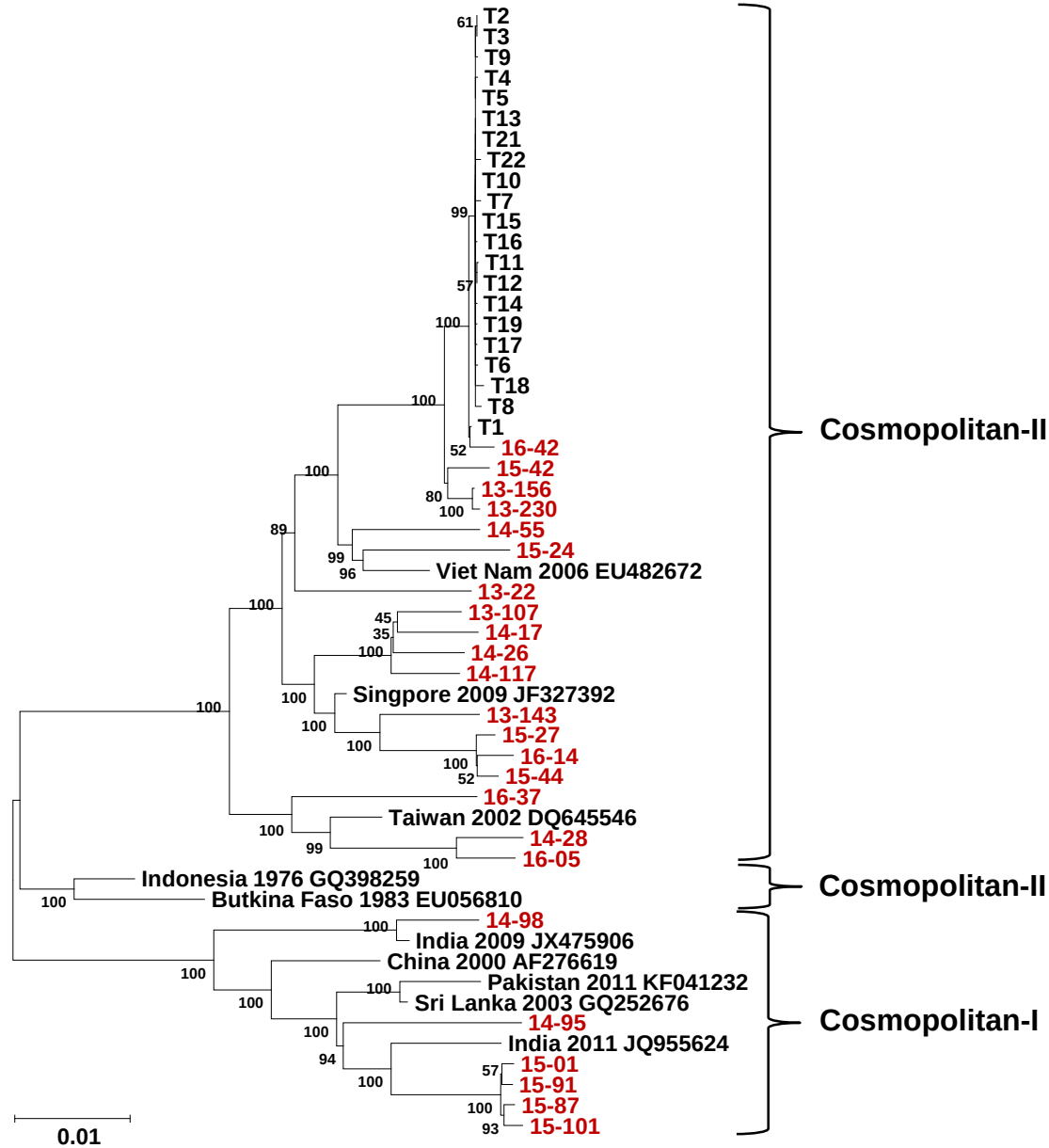
- Age
- Sex
- Date of onset
- year
- Date of sample collection
- Whole sequence result
- DEN virus (sero) type
- Ct value of TaqMan PCR
- ex) country and region

Global Map of DENV by patient data, DENV type, and year with GenEpid-J

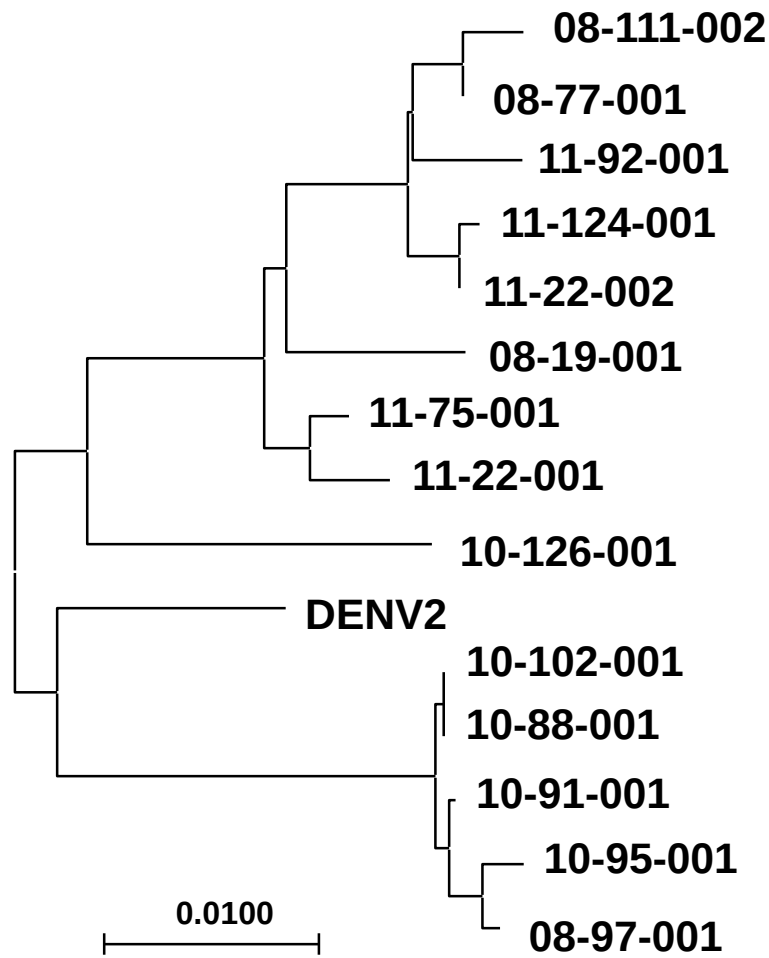


The 197 out of 207 Imported dengue cases were analyzed by NGS

Phylogenic analysis of DENV2 genome obtained by NGS



Phylogenic analysis of imported DENV2 strain from 2008 to 2011



Summary

- We analyzed 197 out of 207 imported DENV strains by NGS, and constructed DENV genomic database by using GenEpid-J System
- Our analysis revealed that 24 out of 32 imported DENV2 strains were classified DENV2 cosmopolitan genotype, and 14 of DENV2 cosmopolitan genotype were imported to Japan during 2008 to 2011
- GenEpid-J is useful tool for analysis of DENV infection

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