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Shotgun metagenomics as a tool for the rapid diagnosis and genotyping of dengue virus

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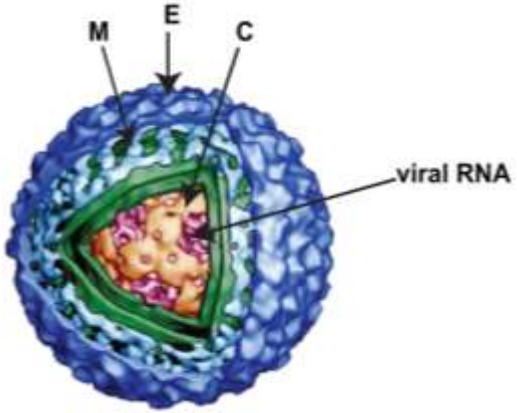
Transparency Declaration
Nothing to declare

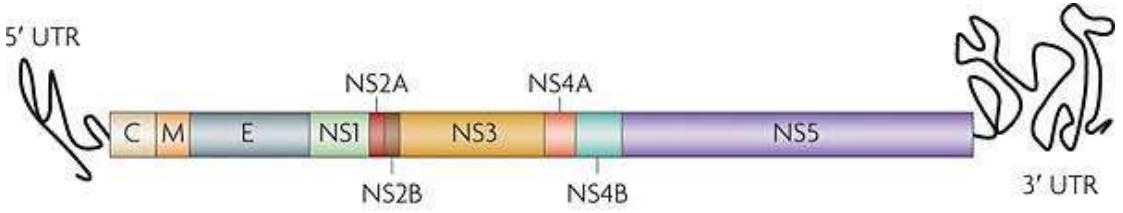
Dengue virus

- Flaviviridae
- ssRNA (+)
- 4 antigenically closely related serotypes (RNA virus)

DENV-1
DENV-2
DENV-3
DENV-4

- Dengue Genome: ≈11kb





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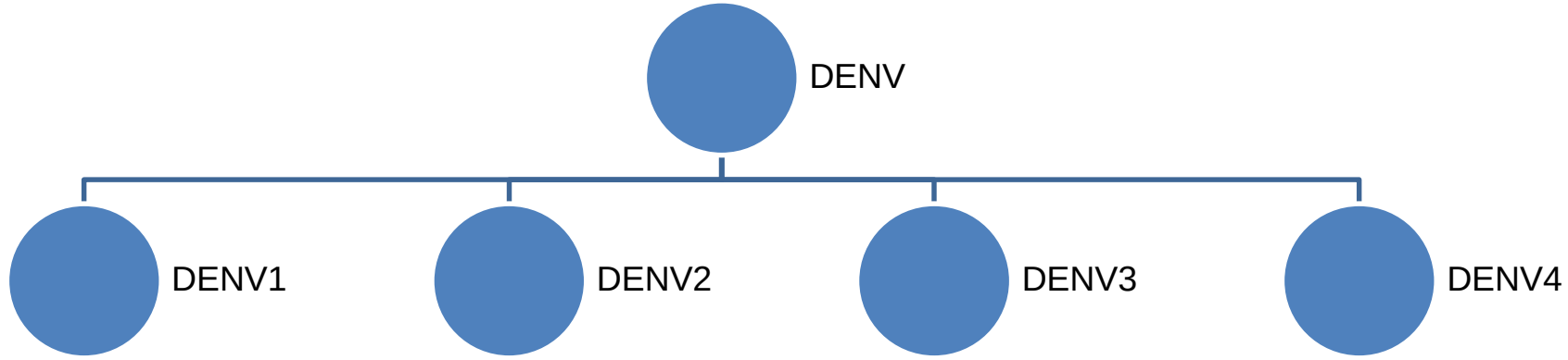

Genotypes: 5
(I-V)

Genotypes: 6
(Asian/American, Asian I, Asian II, Cosmopolitan, American, Sylvatic)

Genotypes: 5
(I-V)

Genotypes: 4
(I-III, Sylvatic)

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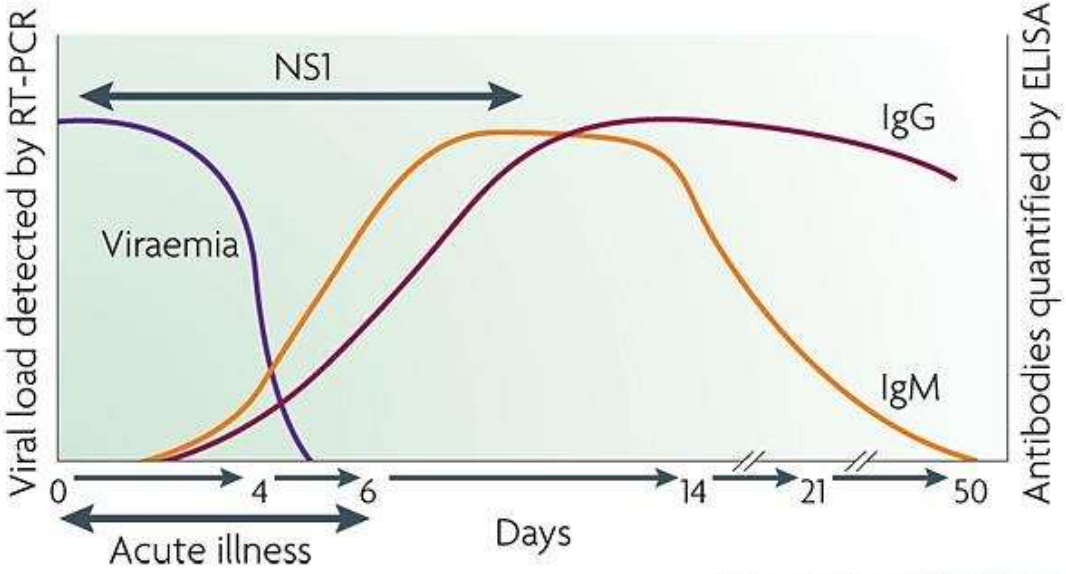
Dengue virus detection

Diagnosis of Dengue can be performed:

1. Acute febrile illness:
- RT-PCR, RT-*q*PCR
- Viral Culture
- NS1 (antigen test)

2. Convalescent phase
- Dengue IgM detection
- Dengue IgG detection



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Dengue virus genotyping

The majority of the genotyping and phylogeny studies are based on:

- Envelope gene: Fast but partial information
- Partial genome: Partial information
- Complete genome: Requires multiple primers and PCR amplification steps

Classic approach: Sanger sequencing

New methodologies are required to improve rapid detection and genotyping of DENV and other viruses directly from clinical material

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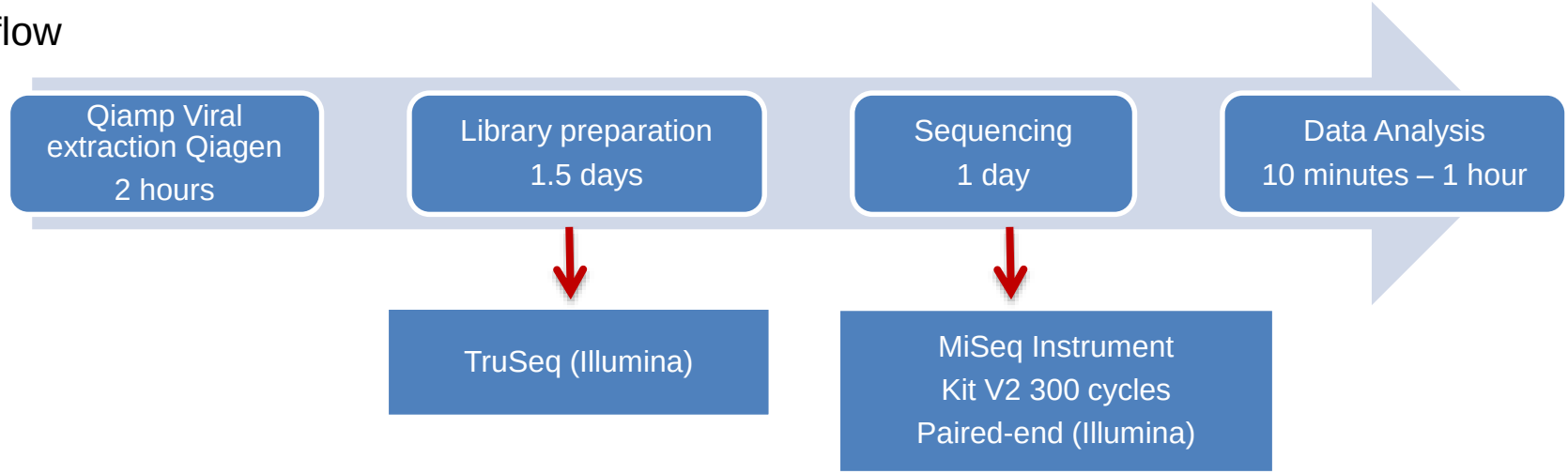
Shotgun metagenomics

We studied five samples from five dengue symptomatic patients (acute phase <4 days)

- RT-qPCR positive for Dengue 2, collected 2015 (non epidemic year)

Workflow



- Library validation — Qubit (Quantity and purity)
- Cluster generation — Determine fragment length

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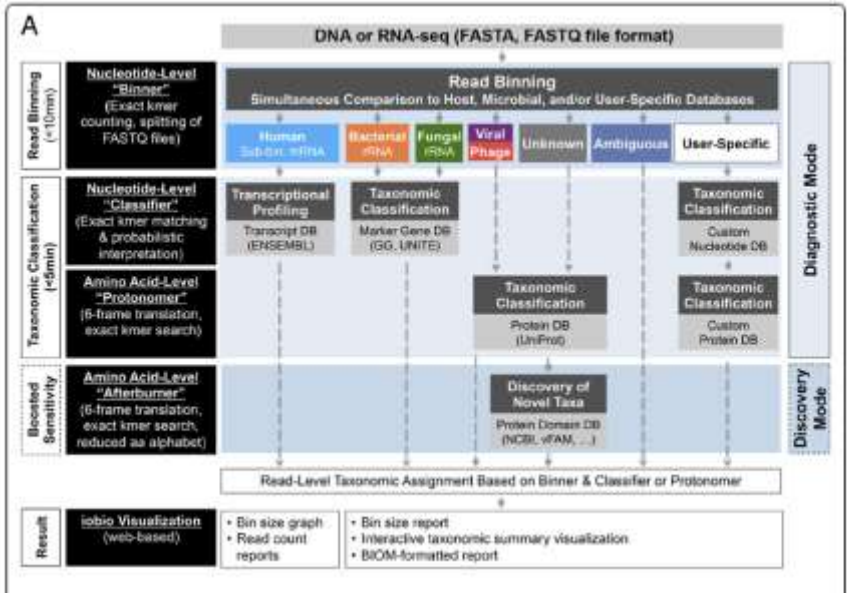
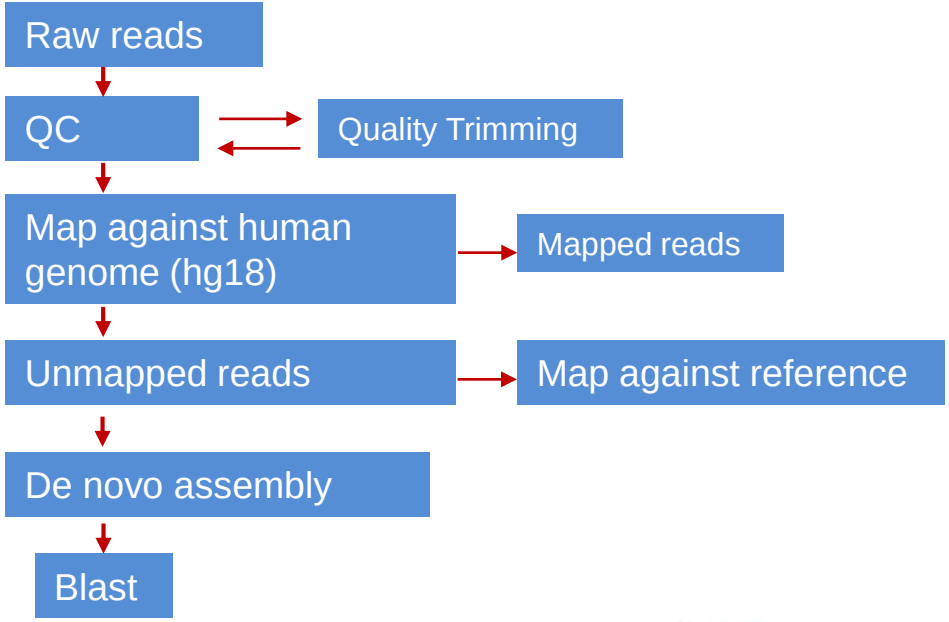
Bioinformatics analysis

1. Taxonomer
(Robert Schlaberg, Utah University)



2. CLC Genomics Workbench



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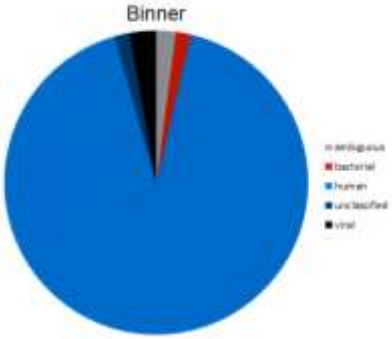
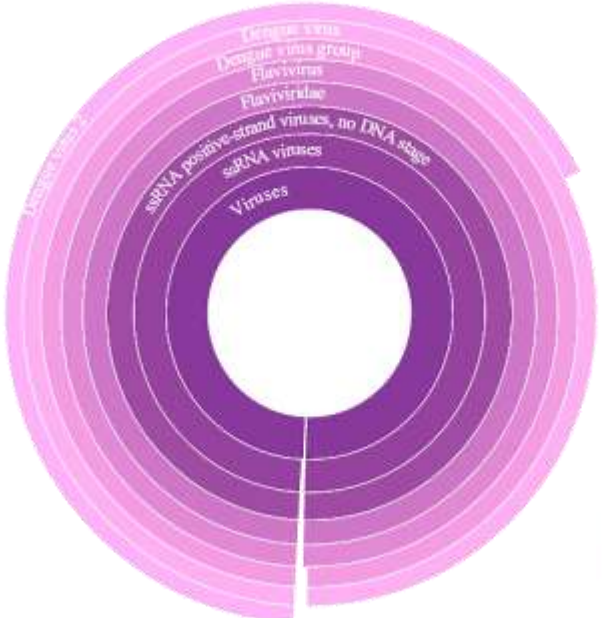
Taxonomer

Classified reads

	human	viral	bacterial	ambiguous	unclassified
Sample1	1,799,273 (91.9%)	55,286 (2.8%)	32,116 (1.6%)	40,762 (2.1%)	31,664 (1.6%)
Sample2	1,061,246 (83.0%)	11,865 (0.9%)	29,354 (2.3%)	37,695 (3.0%)	137,676 (10.8%)
Sample3	1,059,851 (75.6%)	144,024 (10.3%)	31,810 (2.3%)	51,882 (3.7%)	113,368 (8.1%)
Sample4	1,448,970 (79.6%)	83,284 (4.6%)	46,983 (2.6%)	76,781 (4.2%)	163,584 (9.0%)
Sample5	1,046,940 (74.5%)	28,552 (2.0%)	42,867 (3.1%)	79,974 (5.7%)	206,749 (14.7%)

Dengue 2 (DENV2) was identified in all samples





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CLC workbench results

Mapping against human genome

Sample	Total number of reads	Mapped reads against hg18	Unmapped reads against hg18	Dengue-2 mapped reads	Average coverage	Longest contig
1	3,918,662	3,671,259 (93.7 %)	247,403 (6.3 %)	110,770 (2.83%)	1,480.6	10,694
2	2,556,120	2,186,315 (85.5 %)	369,805 (14.5 %)	13,517 (0.53%)	176.5	10,712
3	2,802,530	2,220,772 (79.2 %)	581,758 (20.8 %)	288,694 (10.30%)	3,747.2	10,736
4	3,640,058	3,038,591 (83.5 %)	601,467 (16.5 %)	165,422 (4.52%)	2,146.9	10,711
5	2,810,772	2,251,377 (80.1 %)	559,395 (19.9 %)	53,517 (1.90%)	704.6	10,619

hg18: human genome

- Near full-length genome sequences in all samples

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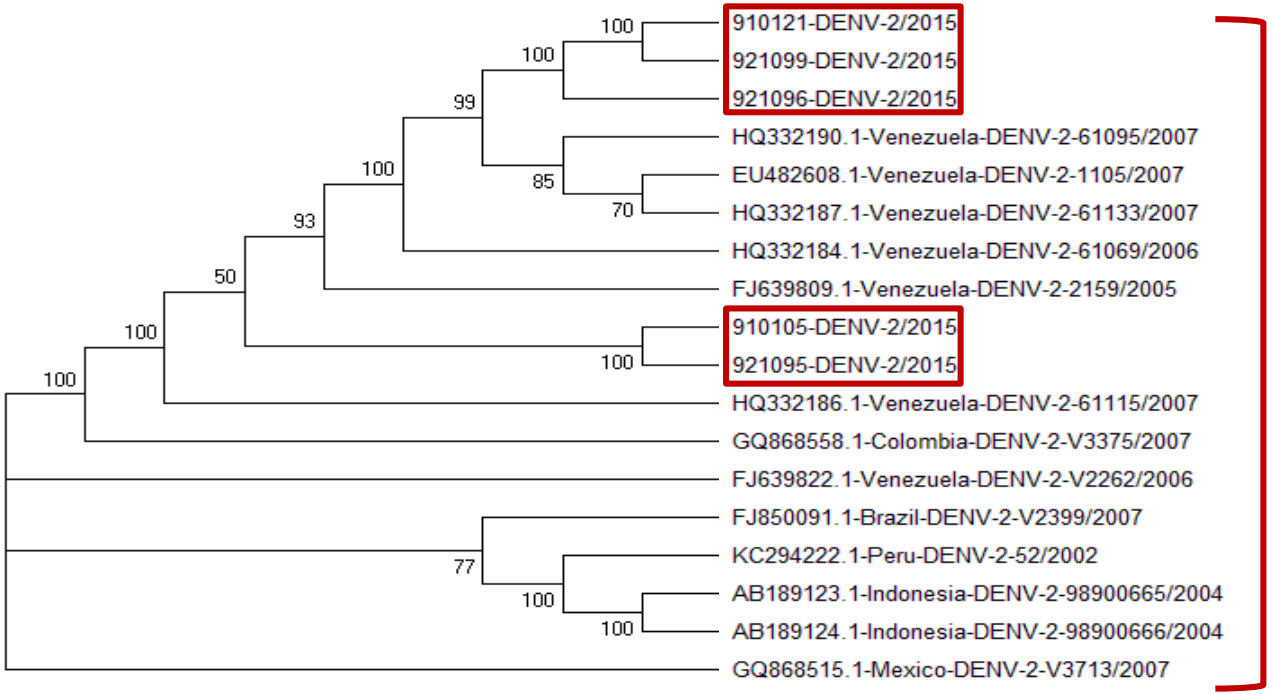
Phylogenetic analysis



American/Asian Genotype

Sub-cluster B

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. Software MEGA 7.

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Shotgun Metagenomics

- Improves the time frame to detect and genotyping of dengue virus
- Superior resolution of DENV strains given by phylogenetic analysis of complete genome
- Allows detection of genetic variants within the sample
- Important in countries where dengue-like viruses co-circulate

What to improve?

- The proportion of human DNA should be as low as possible (human DNA depletion)
- Increase the coverage per sample
- Decrease the costs per sample (by improving the aforementioned items)

What's next?

- Incorporate the remaining serotypes DENV1, DENV3 and DENV4.
- Apply the methodology for Zika and Chikungunya.

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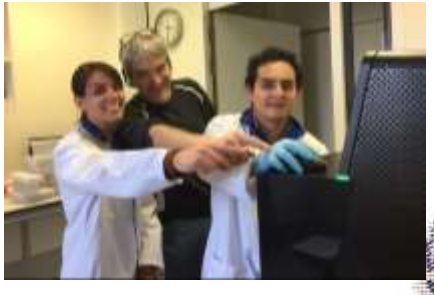

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