

Draft Genome Sequence of *Tokyovirus*, a Member of the Family *Marseilleviridae* Isolated from the Arakawa River of Tokyo, Japan

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Members of the *Marseilleviridae* family are large DNA viruses with icosahedral particles that infect *Acanthamoeba* cells. This report presents a new *Marseilleviridae* family member discovered in a water/soil sample from a river in Tokyo, named *Tokyovirus*, with genome size of 370 to 380 kb.

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The *Marseilleviridae* family is a group of giant viruses with smaller particles of 200-nm diameter and with a genome size of 300 to 400 kb (1–9). This article reports the draft genome of a new virus belonging to the family *Marseilleviridae*, isolated from the coast of the Arakawa River, located in Tokyo, Japan. It was named *Tokyovirus* according to the rules of nomenclature for *Marseilleviridae*.

Water/soil samples were collected from a bank of the Arakawa River. After mud was removed by filtration (filter paper 43; Whatman PLC), samples were further filtered (0.8- μ m pore size, Millex-AA; Millipore). Filtered samples were concentrated by polyethylene glycol precipitation overnight at 4°C, followed by centrifugation at $1,500 \times g$ for 30 min at 4°C (10). After the supernatant was removed, the pellet was resuspended in 4 ml of peptone yeast extract-glucose (PYG) broth and then filtered again (Millex-AA; Millipore). Then, 4 ml of fresh PYG and a 1-ml suspension of amoeba cells were added to this viral solution, which was divided and cultured on 56 wells in a 96-well culture plate at 26°C. After 10 days, I found that amoeba cells in only 1 well out of 56 wells had delayed proliferation and had been almost round. The supernatant of the culture in this single well was inoculated to fresh amoeba cells of 3 wells in a 96-well culture plate. Almost all cells were rounded. The supernatant was inoculated to fresh amoeba cells in a 25 cm² culture flask. After 2 days, rounded amoeba cells were harvested. Then, the supernatant was stored at 4°C as an isolated virus solution. After virus cloning (11), genomic DNA of *Tokyovirus* (1.1 μ g) was prepared from PYG culture media including viral particles using NucleoSpin Tissue (Macherey-Nagel GmbH and Co. KG) according to the manufacturer's protocol. A DNA library for sequencing was prepared using the TruSeq Nano DNA LT library prep kit (Illumina, Inc.), and sequencing was performed on a HiSeq 2500 platform (Illumina, Inc.). Edena software was used to assemble 1,000,000 reads into 68 contigs with an average length of 5,481 nucleotides (nt) and a maximum contig length of 360,777 nt. The total length of the 68 contigs was 372,707 nt. Prediction of the coding region of the *Tokyovirus* genome was conducted using CRITICA version 1.05b and Glimmer 2 version 2.10. Prediction of tRNA was conducted

using tRNAScan-SE version 1.23, according to the manufacturer's protocols. The prediction of gene function was conducted using NCBI BLASTp in the NCBI NR and NCBI COG databases.

Genome analysis showed that *Tokyovirus* has a 370- to 380-kb genome. The total length of the 68 contigs was 372,707 nt, which is approximately equal to the genome sizes of other *Marseilleviridae* members (1, 2, 4, 7). This draft sequence is predicted to have 487 coding sequences (CDSs) and 2 tRNA genes (one of them is a pseudogene). Genome comparison revealed that *Tokyovirus* closely resembles *Marseillevirus* and *Melbournevirus*. *Tokyovirus* has 47 CDSs, which are predicted to be highly similar (>90% identical) with *Marseillevirus* homologs.

Nucleotide sequence accession number. The draft genomic sequence of *Tokyovirus* has been deposited in DDBJ/ENA/GenBank under the accession number [AP017398](https://www.ncbi.nlm.nih.gov/nuccore/AP017398).

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