



# Genome Sequences of West Nile Virus Reference Materials

Hanna Roth,<sup>a</sup> Julia Kreß,<sup>a</sup> Michael Chudy,<sup>a</sup> Johannes Blümel,<sup>a</sup> Jonas Schmidt-Chanasit,<sup>b</sup> Dániel Cadar,<sup>b</sup> Matthias Niedrig,<sup>c</sup> Emőke Ferenczi,<sup>d</sup> Maike Herrmann,<sup>a</sup>  Sally A. Baylis,<sup>a</sup> Csaba Miskey<sup>a</sup>

<sup>a</sup>Paul-Ehrlich-Institut, Langen, Germany

<sup>b</sup>Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

<sup>c</sup>Robert Koch Institute, Berlin, Germany

<sup>d</sup>National Center for Epidemiology, Budapest, Hungary

**ABSTRACT** We report the sequences of two West Nile virus (WNV) strains (lineages 1 and 2) developed by the Paul-Ehrlich-Institut as reference materials. The materials are calibrated against the 1<sup>st</sup> World Health Organization WNV RNA International Standard and are intended for use in nucleic acid technology assays supporting transfusion safety.

West Nile virus (WNV) is a *Flavivirus* (family *Flaviviridae*) transmitted by *Culex* mosquitoes and causing infections in birds, horses, and humans (1). First isolated from a Ugandan patient in 1937 (2), WNV subsequently spread within Africa, Asia, the Middle East, North America, and Europe (3). Typically, WNV infections are asymptomatic; however, some individuals develop West Nile fever and occasionally neuroinvasive disease (4). With the expansion of WNV in Europe, including Germany (5), transmission by blood transfusion is a concern, and implementation of nucleic acid amplification technique (NAT)-based donor screening is necessary once human cases become endemic and for travelers returning from affected areas (6). To support testing by transfusion services and NAT assay developers, reference materials were prepared by the Paul-Ehrlich-Institut for WNV lineages 1 (NY99; flamingo) and 2 (Héja; goshawk), reflecting circulating European clades (7, 8). The isolates were passaged once in Vero E6 cells and heat-inactivated as previously described (9); no infectivity was detected following heat inactivation. Heat-inactivated stocks were diluted in human plasma, dispensed into vials, and lyophilized; batches of reference material prepared from NY99 and Héja were designated 13299/19 and 13300/19, respectively. RNA was extracted using the ExiPrep Dx viral RNA kit (Bioneer Corp., Daejeon, Republic of Korea) (10). Libraries were prepared using a modified version of the “not not so random priming” method (11). Following cDNA synthesis, barcoded Illumina libraries were prepared by PCR amplification using NEBNext Ultra II master mix (New England Biolabs, Frankfurt, Germany); amplicons were recovered and sequenced using a MiSeq instrument with the paired-end (2 × 300-bp) setting as previously described (12).

Majority consensus sequences were generated from the processed and mapped reads based on the reference sequences (13); default parameters were applied unless otherwise stated. The sequencing statistics are shown in Table 1. Fastp v0.20.0 (14) was used for quality trimming and adapter removal. After quality control, the reads were mapped using BWA-MEM v0.7.12-r1039 (15). Host-derived sequences (*Chlorocebus sabeus*; GenBank accession number [GCA\\_000409795.2](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000409795.2)) were removed by specifying the minimum seed length (-k 31). Unmapped reads were extracted using SAMtools v1.7 (16) and bamtofastq v2.17.0 (17) and subsequently mapped to the WNV reference genomes submitted under GenBank accession numbers [AF196835.2](https://www.ncbi.nlm.nih.gov/nuccore/AF196835.2) (lineage 1) or [DQ116961.1](https://www.ncbi.nlm.nih.gov/nuccore/DQ116961.1) (lineage 2). Host-free alignments were deduplicated using MarkDuplicates in the Picard toolkit (<http://broadinstitute.github.io/picard>) and left-aligned using LeftAlignIndels in GATK v4.0 (18) Variant calling was performed using LoFreq v2.1.3 (19).

**Citation** Roth H, Kreß J, Chudy M, Blümel J, Schmidt-Chanasit J, Cadar D, Niedrig M, Ferenczi E, Herrmann M, Baylis SA, Miskey C. 2021. Genome sequences of West Nile virus reference materials. *Microbiol Resour Announc* 10:e00740-21. <https://doi.org/10.1128/MRA.00740-21>.

**Editor** Jelle Matthijnssens, KU Leuven

**Copyright** © 2021 Roth et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Julia Kreß, [Julia.Kress@pei.de](mailto:Julia.Kress@pei.de), or Sally A. Baylis, [Sally.Baylis@pei.de](mailto:Sally.Baylis@pei.de).

**Received** 3 August 2021

**Accepted** 15 September 2021

**Published** 28 October 2021

**TABLE 1** West Nile Virus NY99 and Héja sequencing statistics

Parameter <sup>a</sup>	Data for isolate:	
	NY99	Héja
GenBank accession no.	<a href="#">MZ605381</a>	<a href="#">MZ605382</a>
BioProject accession no.	<a href="#">PRJNA759393</a>	<a href="#">PRJNA759393</a>
WNV lineage	1	2
PEI reference material code no. <sup>b</sup>	13299/19	13300/19
Length (bp)	11,025	11,028
Potency	6.25 log <sub>10</sub> IU/ml	5.88 log <sub>10</sub> IU/ml
No. of reads		
Raw reads	2,624,302	3,084,270
After QC	2,530,266	2,979,944
After removal of host sequences	461,663	447,665
Total length of reads (bp)	94,234,259	89,678,647
Avg read length (bp)	204	200
No. of mapped reads	350,568	351,140
Proportion mapped		
% of raw reads	13.4	11.4
% of QC reads	13.9	11.8
% of reads after removal of host sequences	75.9	78.4
Mean depth of coverage (×)	6,522	6,497

<sup>a</sup>QC, quality control.<sup>b</sup>PEI, Paul-Ehrlich-Institut.

The sequence determined for isolate NY99 was 11,025 bp long, with seven nucleotide changes (all synonymous) compared to the prototype ([AF196835.2](#)). The Héja isolate, 11,028 bp long, is closely related to viruses isolated from goshawks in Central Europe, confirming its position within the Central European lineage 2 clade. Héja showed 27 nucleotide differences to [DQ116961.1](#) (>99% identity), resulting in 6 amino acid changes (3 nonsynonymous). The Héja virus has not always been adequately detected in external quality assessment programs (8); therefore, knowledge of the sequence is important for improving assays to ensure detection of similar viruses going forward.

Both reference materials are calibrated against the World Health Organization International Standard for WNV for NAT-based assays (20) and are considered “secondary standards” (21).

**Data availability.** The sequences of strains NY99 and Héja reported here have been deposited in GenBank under the accession numbers [MZ605381](#) and [MZ605382](#), respectively. The sequencing read data have been deposited in the NCBI SRA under accession number [PRJNA759393](#). The reference materials are available from the Paul-Ehrlich-Institut ([www.pei.de](#)).

## ACKNOWLEDGMENT

The WNV isolates NY99 and Héja were kindly provided by the Bernhard Nocht Institute for Tropical Medicine.

## REFERENCES

- Habarugira G, Suen WW, Hobson-Peters J, Hall RA, Bielefeldt-Ohmann H. 2020. West Nile virus: an update on pathobiology, epidemiology, diagnostics, control and “One Health” implications. *Pathogens* 9:589. <https://doi.org/10.3390/pathogens9070589>.
- Smithburn KC, Hughes TP, Burke AW, Paul JH. 1940. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med Hyg* s1-20: 471–492. <https://doi.org/10.4269/ajtmh.1940.s1-20.471>.
- Chancey C, Grinev A, Volkova E, Rios M. 2015. The global ecology and epidemiology of West Nile virus. *Biomed Res Int* 2015:376230. <https://doi.org/10.1155/2015/376230>.
- Bai F, Thompson EA, Vig PJS, Leis AA. 2019. Current understanding of West Nile virus clinical manifestations, immune responses, neuroinvasion, and immunotherapeutic implications. *Pathogens* 8:193. <https://doi.org/10.3390/pathogens8040193>.
- Ziegler U, Santos PD, Groschup MH, Hattendorf C, Eiden M, Höper D, Eisermann P, Keller M, Michel F, Klopffleisch R, Müller K, Werner D, Kampen H, Beer M, Frank C, Lachmann R, Tews BA, Wylezich C, Rinder M, Lachmann L, Grünewald T, Szentiks CA, Sieg M, Schmidt-Chanasit J, Cadar D, Lühken R. 2020. West Nile Virus epidemic in Germany triggered by epizootic emergence, 2019. *Viruses* 12:448. <https://doi.org/10.3390/v12040448>.
- Domanović D, Gossner CM, Lieshout-Krikke R, Mayr W, Baroti-Toth K, Dobrota AM, Escoval MA, Henseler O, Jungbauer C, Liumbruno G, Oyonarte S, Politis C, Sandić I, Vidović MS, Young JJ, Ushiro-Lumb I, Nowotny N. 2019. West Nile and Usutu virus infections and challenges to blood safety

- in the European Union. *Emerg Infect Dis* 25:1050–1057. <https://doi.org/10.3201/eid2506.181755>.
7. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286:2333–2337. <https://doi.org/10.1126/science.286.5448.2333>.
  8. Linke S, Mackay WG, Scott C, Wallace P, Niedrig M. 2011. Second external quality assessment of the molecular diagnostic of West Nile virus: are there improvements towards the detection of WNV? *J Clin Virol* 52: 257–260. <https://doi.org/10.1016/j.jcv.2011.08.010>.
  9. Baylis SA, Hanschmann K-MO, Schnierle BS, Trösemeier J-H, Blümel J, Zika Virus Collaborative Study Group. 2017. Harmonization of nucleic acid testing for Zika virus: development of the 1st World Health Organization International Standard. *Transfusion* 57:748–761. <https://doi.org/10.1111/trf.14026>.
  10. Roth H, Schneider L, Eberle R, Lausen J, Modlich U, Blümel J, Baylis SA. 2020. Zika virus infection studies with CD34<sup>+</sup> hematopoietic and megakaryocyte-erythroid progenitors, red blood cells and platelets. *Transfusion* 60:561–574. <https://doi.org/10.1111/trf.15692>.
  11. Levin JZ, Yassour M, Adiconis X, Nusbaum C, Thompson DA, Friedman N, Gnirke A, Regev A. 2010. Comprehensive comparative analysis of strand-specific RNA sequencing methods. *Nat Methods* 7:709–715. <https://doi.org/10.1038/nmeth.1491>.
  12. Brown RJP, Tegtmeyer B, Sheldon J, Khera T, Anggakusuma, Todt D, Vieyres G, Weller R, Joecks S, Zhang Y, Sake S, Bankwitz D, Welsch K, Ginkel C, Engelmann M, Gerold G, Steinmann E, Yuan Q, Ott M, Vondran FWR, Krey T, Ströh LJ, Miskey C, Ivics Z, Herder V, Baumgärtner W, Lauber C, Seifert M, Tarr AW, McClure CP, Randall G, Baktash Y, Ploss A, Thi VLD, Michailidis E, Saeed M, Verhoye L, Meuleman P, Goedecke N, Wirth D, Rice CM, Pietschmann T. 2020. Liver-expressed Cd302 and Cr11 limit hepatitis C virus cross-species transmission to mice. *Sci Adv* 6:eabd3233. <https://doi.org/10.1126/sciadv.abd3233>.
  13. Hörner C, Schürmann C, Auste A, Ebenig A, Muraleedharan S, Dinnon KH, III, Scholz T, Herrmann M, Schnierle BS, Baric RS, Mühlebach MD. 2020. A highly immunogenic and effective measles virus-based Th1-biased COVID-19 vaccine. *Proc Natl Acad Sci U S A* 117:32657–32666. <https://doi.org/10.1073/pnas.2014468117>.
  14. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ pre-processor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
  15. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
  16. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
  17. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841–842. <https://doi.org/10.1093/bioinformatics/btq033>.
  18. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20:1297–1303. <https://doi.org/10.1101/gr.107524.110>.
  19. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, Khor CC, Petric R, Hibberd ML, Nagarajan N. 2012. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. *Nucleic Acids Res* 40:11189–11201. <https://doi.org/10.1093/nar/gks918>.
  20. Kempster SL, Minhas R, Hockley JG, Morris CL. 2020. A collaborative study to evaluate the proposed 1st WHO International Standard for West Nile Virus (WNV) RNA for Nucleic Acid Amplification Techniques (NAT). Report WHO/BS/2020.2397. World Health Organization, Geneva, Switzerland.
  21. Baylis SA, Wallace P, McCulloch E, Niesters HGM, Nübling CM. 2019. Standardization of nucleic acid tests: the approach of the World Health Organization. *J Clin Microbiol* 57:e01056-18.