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GENOME SEQUENCES

Complete Genome Sequence of Herpes Simplex Virus 1 Strain MacIntyre

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ABSTRACT Herpes simplex virus type 1 (HSV-1) strain MacIntyre has a severe defect in the anterograde spread after replication in the nucleus. To better understand and identify the genetic determinants that lead to the unique phenotypes of the MacIntyre strain, we sequenced its genome with PacBio single-molecule real-time sequencing technology and resolved the complete sequence.

Herpes simplex virus type 1 (HSV-1) is a member of the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family and has a linear double-stranded DNA genome (1), which contains ~80 genes. HSV-1 strain MacIntyre originated from the brain of a patient with fatal encephalitis (2–4). It egresses and infects only neurons that are presynaptic to the infected neuron (5–13).

We obtained the MacIntyre strain from Advanced Biotechnologies (Columbia, MD). The virus was propagated in Vero cells (ATCC, Gaithersburg, MD), and the viral stock was titrated using a plaque-forming assay (14). The genomic DNA was isolated by using a PureLink viral DNA minikit (Invitrogen, Carlsbad, CA) following the manufacturer's protocols. Viral DNA shearing was carried out using an M220 sonicator (Covaris, Woburn, MA). Size selection and subsequent cleanup were performed using a combination of BluePippin (Sage Science, Beverly, MA) with the red miniTUBE (Covaris) and AMPure PB beads (PacBio, Menlo Park, CA), respectively. After shearing, bands ~5 kb long were used for library construction using the SMRTbell template prep kit v1.0 (PacBio) with two alterations, an A-tailing step as well as overhang adapter ligation, as opposed to blunt adapter ligation. The constructed libraries were then sequenced using PacBio long-read sequencing on a Sequel system using v3.0 chemistry with a 6 pM loading concentration and a 10-h runtime.

A total of 4,230,138 subreads (mean length, 2,344 bp; maximum length, 22,799 bp; total number of bases, 9,917,428,361) from the 5′ end of the genome to the 3′ end were assembled into two contigs 126,677 and 25,074 bases long by using the PacBio *de novo* assembler Hierarchical Genome Assembly Process v4.0 (HGAP4) (15) (default settings were used, except that the seed length cutoff was set to 15 kbp, and the minimum required alignment length was set to 500 bp). We aligned the two contigs against the genome sequence of HSV-1 reference strain 17 (GenBank accession number JN555585) (16) and found a gap of ~600 bp between them in the repeat long (RL) region of strain 17. The two contigs were ordered and assembled into a draft sequence; the gap was filled by aligning all the reads to the draft sequence, and consensus was called by using the genome resequencing pipeline of SMRT Analysis v6.0 with default settings (17). The average depth per base pair over the final resolved genome sequence is 61,410 reads, a coverage that ensures high-quality consensus base calling (>99.99% accuracy) (15). The genome termini were determined by a short sequence, *a*, which is repeated at both ends of the genome and at the L-S junction (18). The *a* sequence (473

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bp) uncovered by this new assembly has unique features in its DR2 region compared to those of other HSV-1 strains that have been reported (19).

The finished length of the complete genome is 152,476 bases with a GC content of 68.21%. It resolves ~5.8 kbp of undetermined regions in the existing partial genome sequence (GenBank accession number KM222720) (20), with gaps closed in the genes *LAT*, *RL1*, *RL2*, *RS1*, *UL36*, *US1*, *US2*, *US9*, and *US12*. It also confirmed that three genes, *UL46*, *US7*, and *US9*, contain new stop-codon positions (previously reported under GenBank accession number KM222720) due to homopolymer-based frameshifts or single nucleotide variations. These variations may contribute to the unique phenotype of MacIntyre, but further experiments need to be conducted to associate observed differences with phenotype effects.

The whole-genome nucleotide similarity between the new genome and HSV-1 strain 17 is 97.48% based on BLASR (v2.0.0) alignment (21) with default parameters. The annotations for genes and coding sequences were transferred from HSV-1 strain 17 to the MacIntyre strain based on sequence homology (22, 23).

Data availability. This genome sequence was deposited at GenBank under the accession number MN136523, with SRA accession number SRR9719185.

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REFERENCES

1. Whitley RJ, Kimberlin DW, Roizman B. 1998. Herpes simplex viruses. Clin Infect Dis 26:541–553. quiz 554–555. https://doi.org/10.1086/514600.
2. Sosa-Martinez J, Lennette EH. 1955. Studies on a complement fixation test for herpes simplex. J Bacteriol 70:205–215.
3. Pauls FP, Dowdle WR. 1967. A serologic study of herpesvirus hominis strains by microneutralization tests. J Immunol 98:941–947.
4. Dowdle WR, Nahmias AJ, Harwell RW, Pauls FP. 1967. Association of antigenic type of *herpesvirus hominis* with site of viral recovery. J Immunol 99:974–980.
5. Cook ML, Stevens JG. 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. Infect Immun 7:272–288.
6. Pepose JS, Foos RY, Stevens JG. 1986. Herpes simplex virus immediate-early protein ICP4 in murine models of latency. Graefes Arch Clin Exp Ophthalmol 224:341–345. https://doi.org/10.1007/BF02150027.
7. McFarland DJ, Hotchin J. 1987. Contrasting patterns of virus spread and neuropathology following microinjection of herpes simplex virus into the hippocampus or cerebellum of mice. J Neurol Sci 79:255–265. https://doi.org/10.1016/0022-510x(87)90233-4.
8. McLean JH, Shipley MT, Bernstein DI. 1989. Golgi-like, transneuronal retrograde labelling with CNS injections of herpes simplex virus type 1. Brain Res Bull 22:867–881. https://doi.org/10.1016/0361-9230(89)90032-4.
9. Krinke GJ, Dietrich FM. 1990. Transneuronal spread of intraperitoneally administered herpes simplex virus type 1 from the abdomen via the vagus nerve to the brains of mice. J Comp Pathol 103:301–306. https://doi.org/10.1016/S0021-9975(08)80050-3.
10. Garner JA, LaVail JH. 1999. Differential anterograde transport of HSV type 1 viral strains in the murine optic pathway. J Neurovirol 5:140–150. https://doi.org/10.3109/13550289909021996.
11. LaVail JH, Topp KS, Giblin PA, Garner JA. 1997. Factors that contribute to the transneuronal spread of herpes simplex virus. J Neurosci Res 49:485–496. https://doi.org/10.1002/(SICI)1097-4547(19970815)49:4<485::AID-JNR9>3.0.CO;2-4.
12. Zemanick MC, Strick PL, Dix RD. 1991. Direction of transneuronal transport of herpes simplex virus 1 in the primate motor system is strain-dependent. Proc Natl Acad Sci U S A 88:8048–8051. https://doi.org/10.1073/pnas.88.18.8048.
13. Hoover JE, Strick PL. 1993. Multiple output channels in the basal ganglia. Science 259:819–821. https://doi.org/10.1126/science.7679223.
14. Harper DR. 2000. Determination of viral infectivity: HSV, VZV, and CMV, p 119–127. *In* Kinchington D, Schinazi RF (ed), Antiviral methods and protocols. Humana Press, Totowa, NJ. https://doi.org/10.1385/1-59259-245-7:119.
15. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
16. Davison AJ. 2011. Evolution of sexually transmitted and sexually transmissible human herpesviruses. Ann N Y Acad Sci 1230:E37–E49. https://doi.org/10.1111/j.1749-6632.2011.06358.x.
17. Morse AM, Calabro KR, Fear JM, Bloom DC, McIntyre LM. 2017. Reliable detection of herpes simplex virus sequence variation by high-throughput resequencing. Viruses 9:E226. https://doi.org/10.3390/v9080226.
18. Umene K. 1993. Herpes-simplex virus type-1 variant a-sequence generated by recombination and breakage of the a-sequence in defined regions, including the one involved in recombination. J Virol 67:5685–5691.
19. Umene K, Oohashi S, Yoshida M, Fukumaki Y. 2008. Diversity of the a sequence of herpes simplex virus type 1 developed during evolution. J Gen Virol 89:841–852. https://doi.org/10.1099/vir.0.83467-0.
20. Szpara ML, Tafuri YR, Parsons L, Shreve JT, Engel EA, Enquist LW. 2014. Genome sequence of the anterograde-spread-defective herpes simplex

virus 1 strain MacIntyre. Genome Announc 2:e01161-14. https://doi.org/10.1128/genomeA.01161-14.
21. Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. BMC Bioinformatics 13:238. https://doi.org/10.1186/1471-2105-13-238.
22. McGeoch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, McNab D, Perry LJ, Scott JE, Taylor P. 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J Gen Virol 69:1531–1574. https://doi.org/10.1099/0022-1317-69-7-1531.
23. McGeoch DJ, Dolan A, Donald S, Brauer DH. 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res 14:1727–1745. https://doi.org/10.1093/nar/14.4.1727.