

Strain Kaplan of Pseudorabies Virus Genome Sequenced by PacBio Single-Molecule Real-Time Sequencing Technology

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Pseudorabies virus (PRV) is a neurotropic herpesvirus that causes Aujeszky's disease in pigs. PRV strains are widely used as transsynaptic tracers for mapping neural circuits. We present here the complete and fully annotated genome sequence of strain Kaplan of PRV, determined by Pacific Biosciences RSII long-read sequencing technology.

Received 3 June 2014 Accepted 26 June 2014 Published 17 July 2014

Citation Tombác D, Sharon D, Oláh P, Csabai Z, Snyder M, Boldogkői Z. 2014. Strain Kaplan of pseudorabies virus genome sequenced by PacBio single-molecule real-time sequencing technology. *Genome Announc*. 2(4):e00628-14. doi:10.1128/genomeA.00628-14.

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Pseudorabies virus (PRV), also known as Aujeszky's disease virus or suid herpesvirus 1, a member of the *Alphaherpesvirinae* subfamily, causes significant abortion and morbidity in pigs, the natural host of the virus (1). PRV is a useful model organism for studies of the pathogenesis of herpesviruses. The genetically modified strains are powerful tracers for mapping neuronal circuits (2–6), are tools in gene and cancer therapy (7), and serve as viral vectors for gene delivery into mammalian neurons (3, 4) and cardiomyocytes (8); PRVs have also been employed as live vaccines against Aujeszky's disease (9–11). Further, attenuated vaccine strains of PRV are valuable models for novel vaccine development against varicella-zoster virus (VZV) and herpes simplex virus 1 and 2 (HSV-1 and HSV-2, respectively) (12).

The currently available genome sequences of PRV contain several discrepancies, mainly in intergenic repetitive regions (GenBank accession no. JF797218.1), and the totally annotated version of genome sequence is a composite of six different PRV strains (GenBank accession no. NC_006151.1). We have sequenced the PRV Kaplan genome with Pacific Biosciences single-molecule long-read sequencing technology (Pacific Biosciences, Menlo Park, CA, USA) in order to upgrade the draft sequences, reconstruct the GC-rich and repetitive regions of the genome, and extract epigenetic information. The availability of the completely annotated genome and the single-base resolution methylation map of strain Kaplan will aid in understanding the control of viral gene expression at different levels. Investigations of the PRV genome and gene functions are expected to result in the development of effective vaccines and direct practical applications in gene, cancer, and antiviral therapies.

Sequencing of purified virion DNA was carried out on the Pacific Biosciences RSII sequencer. SMRTbell template libraries were prepared from the DNA, as previously described (13, 14), using standard protocols for 6-kb and 20-kb library preparation. Sequencing was performed in five single-molecule real-time (SMRT) cells with P5 DNA polymerase and C3 chemistry (P5-C3) yielding a total of 78,111 reads and an extremely high coverage (1,200×) throughout the genome.

The sequencing reads were processed and mapped to the respective reference sequences with the BLASR mapper (<https://github.com/PacificBiosciences/blasr>) and the Pacific Biosciences SMRT Analysis pipeline (<https://github.com/PacificBiosciences/SMRT-Analysis/wiki/SMRT-Pipe-Reference-Guide-v2.0>) using the standard mapping protocol.

The protein-coding genes were predicted by GATU (15). Manual annotation was used to identify other genomic features. Annotation of a previously unknown noncoding RNA (named Close to OriL [CTO]), a newly discovered splice site of the early protein 0 gene, and new isoforms of 11 protein-coding genes are based on RNAseq data (our unpublished data). MicroRNA (miRNA) annotation was based on the precursor miRNAs found in strains NIA-3 and Ea.

The complete genome of strain Kaplan of PRV is characterized as a double-stranded linear DNA composed of 143,423 bp, with an average G+C content of 73.59%. PRV contains 70 protein-coding genes (11 genes have different isoforms), two latency-associated transcripts, and a long noncoding RNA, and its genome predicts 16 miRNAs.

Nucleotide sequence accession number. The complete genome of strain Kaplan of pseudorabies virus was assigned DDBJ/EMBL/GenBank accession no. [KJ717942](https://www.ncbi.nlm.nih.gov/nuccore/KJ717942).

ACKNOWLEDGMENTS

This project was supported by the Swiss-Hungarian Cooperation Programme grant (SH/7/2/8) to Z.B. This research was also supported by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program" to D.T.

REFERENCES

1. Aujeszky A. 1902. A contagious disease, not readily distinguishable from rabies, with unknown origin. *Veterinarius*. 12:387–396. (In Hungarian.)
2. Card JP, Kobiler O, Ludmir EB, Desai V, Sved AF, Enquist LW. 2011. A dual infection pseudorabies virus conditional reporter approach to identify projections to collateralized neurons in complex neural circuits. *PLoS One* 6:e21141. <http://dx.doi.org/10.1371/journal.pone.0021141>.
3. Granstedt AE, Kuhn B, Wang SS, Enquist LW. 2010. Calcium imaging

- of neuronal circuits in vivo using a circuit-tracing pseudorabies virus. *Cold Spring Harb. Protoc.* 2010:pdb.prot5410. <http://dx.doi.org/10.1101/pdb.prot5410>.
4. Boldogkői Z, Balint K, Awatramani GB, Balya D, Buskamp V, Viney TJ, Lagali PS, Duebel J, Pásti E, Tombácz D, Tóth JS, Takács IF, Scherf BG, Roska B. 2009. Genetically timed, activity-sensor and rainbow trans-synaptic viral tools. *Nat. Methods* 6:127–130. <http://dx.doi.org/10.1038/nmeth.1292>.
 5. Song CK, Enquist LW, Bartness TJ. 2005. New developments in tracing neural circuits with herpesviruses. *Virus Res.* 111:235–249. <http://dx.doi.org/10.1016/j.virusres.2005.04.012>.
 6. Yang M, Card JP, Tirabassi RS, Miselis RR, Enquist LW. 1999. Retrograde, transneuronal spread of pseudorabies virus in defined neuronal circuitry of the rat brain is facilitated by gE mutations that reduce virulence. *J. Virol.* 73:4350–4359.
 7. Boldogkői Z, Nógrádi A. 2003. Gene and cancer therapy—pseudorabies virus: a novel research and therapeutic tool? *Curr. Gene Ther.* 3:155–182. <http://dx.doi.org/10.2174/1566523034578393>.
 8. Prorok J, Kovács PP, Kristóf AA, Nagy N, Tombácz D, Tóth JS, Ördög B, Jost N, Virág L, Papp JG, Varró A, Tóth A, Boldogkői Z. 2009. Herpesvirus-mediated delivery of a genetically encoded fluorescent Ca(2+) sensor to canine cardiomyocytes. *J. Biomed. Biotechnol.* 2009:361795. <http://dx.doi.org/10.1155/2009/361795>.
 9. Klingbeil K, Lange E, Teifke JP, Mettenleiter TC, Fuchs W. 2014. Immunization of pigs with an attenuated pseudorabies virus recombinant expressing the hemagglutinin of pandemic swine origin H1N1 influenza A virus. *J. Gen. Virol.* 95:948–959. <http://dx.doi.org/10.1099/vir.0.059253-0>.
 10. Maresch C, Lange E, Teifke JP, Fuchs W, Klupp B, Müller T, Mettenleiter TC, Vahlenkamp TW. 2012. Oral immunization of wild boar and domestic pigs with attenuated live vaccine protects against pseudorabies virus infection. *Vet. Microbiol.* 161:20–25. <http://dx.doi.org/10.1016/j.vetmic.2012.07.002>.
 11. Zhu L, Yi Y, Xu Z, Cheng L, Tang S, Guo W. 2011. Growth, physico-chemical properties, and morphogenesis of Chinese wild-type PRV Fa and its gene-deleted mutant strain PRV SA215. *Viol. J.* 8:272. <http://dx.doi.org/10.1186/1743-422X-8-272>.
 12. Szpara ML, Tafuri YR, Parsons L, Shamim SR, Verstrepen KJ, Legendre M, Enquist LW. 2011. A wide extent of inter-strain diversity in virulent and vaccine strains of alphaherpesviruses. *PLoS Pathog.* 7:e1002282. <http://dx.doi.org/10.1371/journal.ppat.1002282>.
 13. Travers KJ, Chin CS, Rank DR, Eid JS, Turner SW. 2010. A flexible and efficient template format for circular consensus sequencing and SNP detection. *Nucleic Acids Res.* 38:e159. <http://dx.doi.org/10.1093/nar/gkp817>.
 14. Clark TA, Murray IA, Morgan RD, Kislyuk AO, Spittle KE, Boitano M, Fomenkov A, Roberts RJ, Korlach J. 2012. Characterization of DNA methyltransferase specificities using single-molecule, real-time DNA sequencing. *Nucleic Acids Res.* 40:e29. <http://dx.doi.org/10.1093/nar/gkr1146>.
 15. Tcherepanov V, Ehlers A, Upton C. 2006. Genome annotation transfer utility (GATU): rapid annotation of viral genomes using a closely related reference genome. *BMC Genomics* 7:150. <http://dx.doi.org/10.1186/1471-2164-7-150>.