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GENOME ANNOUNCEMENT

Genome Sequence of Herpes Simplex Virus 1 Strain KOS

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Herpes simplex virus type 1 (HSV-1) strain KOS has been extensively used in many studies to examine HSV-1 replication, gene expression, and pathogenesis. Notably, strain KOS is known to be less pathogenic than the first sequenced genome of HSV-1, strain 17. To understand the genotypic differences between KOS and other phenotypically distinct strains of HSV-1, we sequenced the viral genome of strain KOS. When comparing strain KOS to strain 17, there are at least 1,024 small nucleotide polymorphisms (SNPs) and 172 insertions/deletions (indels). The polymorphisms observed in the KOS genome will likely provide insights into the genes, their protein products, and the *cis* elements that regulate the biology of this HSV-1 strain.

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 (10) which contains ~80 genes. Infection by HSV-1 can result in cold, ocular, and genital sores and encephalitis (reviewed in reference 10). Several strains of HSV-1 have been isolated that vary in their virulence (4), and this variance is probably due to base substitutions leading to amino acid or *cis* regulatory changes (3, 7, 13). One HSV-1 strain, KOS, was originally isolated from a human labial lesion and is frequently used to investigate HSV-1 gene function and pathogenesis (11, 12). KOS is less virulent than other HSV-1 strains, such as Mckrae and 17 (4, 6, 9), and this interesting difference in virulence led us to sequence the genome of strain KOS.

KOS genomic DNA (passage 12) was isolated from infected Vero (African green monkey kidney) cells using standard protocols (2), and an unpaired 42-bp Illumina library was generated and run at Genome Technology Access Center, Washington University. Since viral DNA was isolated from Vero cells, potential contaminating host reads that matched the Rhesus macaque and/or human genomes were removed using Bowtie (5). The remaining reads (16,494,831) were assembled into contigs using the Velvet *de novo* assembler (14). The resulting contigs that were >100 bp were assembled against the reference HSV-1 strain 17 genome (GenBank accession number NC_001806) with SeqMan Pro (DNASTAR, Inc.). Because the HSV-1 genome includes two pairs of inverted repeat regions, TRL/IRL and IRS/TRS, contigs assembling into one of the repeat units were reverse complemented and also placed into the other repeat unit.

The final KOS genome is 152,011 bp and has 13 gaps, exclusively at variable number tandem repeat (VNTR) regions, totaling 1,582 bp in length. In the GenBank annotation, the sequence and length of each VNTR was copied from strain 17. Using Bowtie to align the filtered reads against the *de novo* assembly, we determined that the average per-bp sequence coverage for the KOS genome was 4,257×. Gene annotations were transferred from the strain 17 genome using Rapid Annotation Transfer Tool (RATT) (8).

To identify nucleotide variants between the genomes of strains KOS and 17, we aligned the genomes using fast statistical alignment (FSA) (1) and applied custom Perl and R scripts. KOS differs from strain 17 by 1,024 single nucleotide polymorphisms (SNPs), 320 of which are nonsynonymous changes in 65 of 77 HSV-1 open reading frames. In addition, we identified previously reported mutations in the US9 and US8A genes (7). The two genomes also differ by 172 insertion/deletion events (indels), most of which are insertions or deletions of single bases in noncoding regions; however, 26 indels are in-frame additions or removals of codons. Future analyses comparing the KOS genome to the genomes of other HSV-1 strains will allow us to identify the genetic attributes of KOS that contribute to its pathogenesis.

Nucleotide sequence accession number. The HSV-1 strain KOS genome sequence has been deposited in GenBank under accession number JQ673480.

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