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## Ultrasensitive Capture of Human Herpes Simplex Virus Genomes Directly from Clinical Samples Reveals Extraordinarily Limited Evolution in Cell Culture

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**ABSTRACT** Herpes simplex viruses (HSVs) are difficult to sequence due to their large DNA genome, high GC content, and the presence of repeats. To date, most HSV genomes have been recovered from culture isolates, raising concern that these genomes may not accurately represent circulating clinical strains. We report the development and validation of a DNA oligonucleotide hybridization panel to recover nearly complete HSV genomes at abundances up to 50,000-fold lower than previously reported. Using copy number information on herpesvirus and host DNA background via quantitative PCR, we developed a protocol for pooling for cost-effective recovery of more than 50 HSV-1 or HSV-2 genomes per MiSeq run. We demonstrate the ability to recover  $\geq 99\%$  of the HSV genome at  $\geq 100\times$  coverage in 72 h at viral loads that allow whole-genome recovery from latently infected ganglia. We also report a new computational pipeline for rapid HSV genome assembly and annotation. Using the above tools and a series of 17 HSV-1-positive clinical swabs sent to our laboratory for viral isolation, we show limited evolution of HSV-1 during viral isolation in human fibroblast cells compared to the original clinical samples. Our data indicate that previous studies using low-passage-number clinical isolates of herpes simplex viruses are reflective of the viral sequences present in the lesion and thus can be used in phylogenetic analyses. We also detect superinfection within a single sample with unrelated HSV-1 strains recovered from separate oral lesions in an immunosuppressed patient during a 2.5-week period, illustrating the power of direct-from-specimen sequencing of HSV.

**IMPORTANCE** Herpes simplex viruses affect more than 4 billion people across the globe, constituting a large burden of disease. Understanding the global diversity of herpes simplex viruses is important for diagnostics and therapeutics as well as cure research and tracking transmission among humans. To date, most HSV genomics has been performed on culture isolates and DNA swabs with high quantities of virus. We describe the development of wet-lab and computational tools that enable the accurate sequencing of near-complete genomes of HSV-1 and HSV-2 directly from clinical specimens at abundances  $>50,000$ -fold lower than previously sequenced and at significantly reduced cost. We use these tools to profile circulating HSV-1 strains in the community and illustrate limited changes to the viral genome during the viral isolation process. These techniques enable cost-effective, rapid sequencing of HSV-1 and HSV-2 genomes that will help enable improved detection, surveillance, and control of this human pathogen.

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