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Using Nanopore Technology to Sequence a Pool of Novel Mycobacteriophage Genomes and Analysis of these Sequences.

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Nanopore long-read sequencing is an inexpensive sequencing technology that allows more aspects of the sequencing process to be brought into the classroom. We sequenced the genomes of 12 novel mycobacteriophages isolated at the University of Colorado Boulder. We generated libraries from unfragmented DNA using the barcoded native ligation sequencing kit and sequenced them on a minION flowcell (R9.4.1). We sequenced 20-50k reads per sample with a median read length of 10kb. We conducted basecalling and barcode demultiplexing using Guppy (GPU-enabled super accuracy model, 98.3% modal raw read accuracy). We then assembled each genome using Flye, achieving >1000x coverage and assembled genomes ranged from 45-69 kb. To validate the accuracy of the final assemblies, we sequenced DNA from Cluster O phage Blessica, which was previously sequenced with both Illumina technology (University of Pittsburgh). Our nanopore sequence had about 300-fold coverage and had 99.987% accuracy as compared to the Illumina sequence. The differences between the sequences were mostly found in repetitive sequences. Dot plot analysis of the other 8 sequences showed that 6 were in cluster B1, 1 in Cluster A3 and 1 in Cluster P1. We performed further preliminary analyses of these sequences using DNA Master and BLAST and found them to be similar to other members of their respective clusters.