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Whole Genome Sequencing of Monkeypox on a PromethION from a Metagenomic Sample

Introduction

The current monkeypox epidemic warrants genomic surveillance. To this end, we performed genomic sequencing of a local monkeypox sample on a PromethION. In part to obtain the genetic sequence, in part to prepare and train for future epidemics that may have an unknown causative agent. Nanopore sequencing allowed for high viral coverage, beta-testing of adaptive sampling in host depletion mode, tracking of sample composition while sequencing, strain determination and preliminary assessment of mutational pattern, making nanopore sequencing a highly versatile method to characterise a virus in real time without lab enrichment.

Methods

Skin lesion swab from local monkeypox case

Metagenomic library prep, Ligation Sequencing Kit SQK-LSK110

On a P24: 1 Flow cell (R9) sequenced for 72 hrs; flow cell was split

1/2 adaptive sampling – host depletion hg38 | 1/2 control sequencing **Depletion of Unwanted Host Sequence**

Quality Statistics
NanoComp/NanoPlot

Draft Consensus Sequence/Analysis of Mutation Pattern
wf-mpx (EPI2ME Labs) Mafft Snipit

Real-time Surveillance of Metagenomic Composition of the Sample
Centrifuge Pavian

Results

The Run

After 72 hrs, 83.04 M reads were generated with an N50 of 856 bp, which resulted in a yield of 72.31 GB.

The Host Depletion

Adaptive sampling to reject human host reads, and thus to passively enrich the wanted target, was carried out on 1/2 of the flow cell. The rationale was to test adaptive sampling on a PromethION type of flow cell and have a control side left. As can be seen in Figure 1, the unblock length that designates adaptively sampled reads (light blue) for a PromethION device is at around 800 bp, currently higher than it would be on a MinION-like device set-up. Figure 2 shows the indirect effect sampling had on the monkeypox fraction of reads. 123,649 reads mapped to the monkeypox reference when adaptive host depletion was on, whereas just 66,394 reads mapped on the control side.

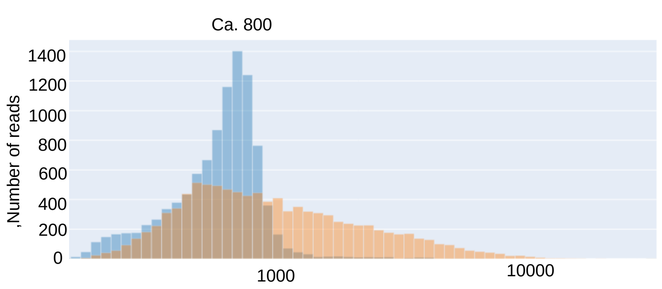

Figure 1: *Histogramm of log transformed read length. H1: all reads sequenced on the flow cell half where adaptive sampling was on. H2: control side exhibiting undisturbed read length distribution*

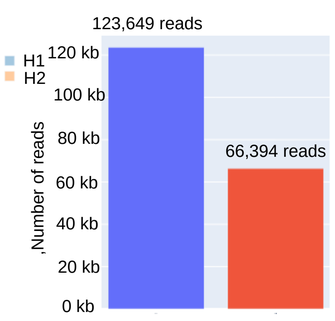

Figure 2: *Number of all re-basecalled reads mapping to the monkeypox reference, grouped and coloured after flow cell side. Dark blue: all reads that were sequenced on the adaptive sampling side of the flow cell, dark red: all reads mapping on the control side*

The Metagenomic Composition

Throughout the run the metagenomic composition was queried using Centrifuge to estimate species composition. In fact, after <10 min of sequencing time monkeypox could be reliably detected in the sample. The sankey plot below shows the full metagenomic composition after 72 hrs and re-basecalling. Reads mapping to human are omitted from the plot.

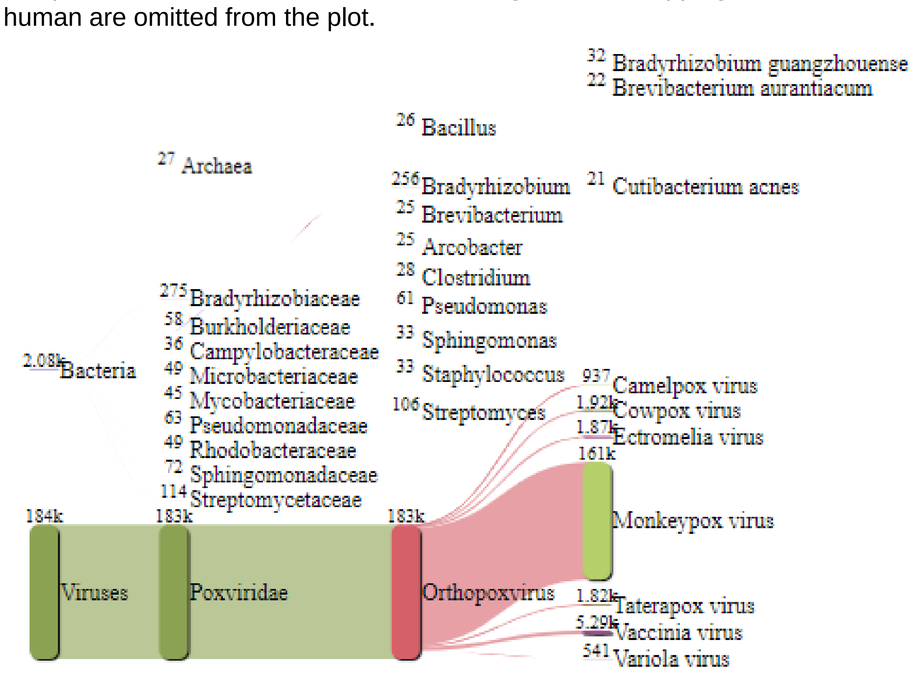

Figure 3: *Sankey plot of the metagenomic composition after the run time had ended and the reads had been re-basecalled*

The Preliminary Draft Sequence and Mutational Pattern

A preliminary draft consensus was made via the wf-mpx pipeline. In comparision to the reference (MT903344.1) characteristic basepair substitutions can be found, which are connected to the activity of the hosts APOBEC3 enzymes and have been observed for the sequences of the current outbreak.

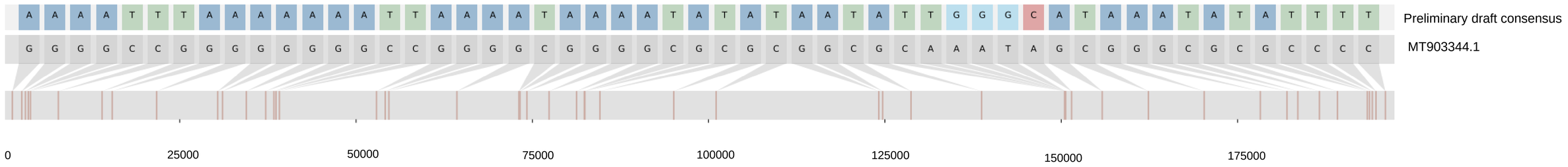

Figure 4: *Mutation Pattern. Highlighted are selected mutations that the preliminary draft sequence has in comparision to the reference over the full length of the monkeypox genome*

Conclusion

Nanopore sequencing has been deployed in outbreaks since the Zika virus as a lightweight and fast method to rapidly characterise a sequence. The current monkeypox outbreak is no exception. Taken together with the increased throughput of a PromethION, high coverage of lowly abundant samples is feasible and will likely add to the popularity of nanopore sequencing.

Literature

Isidro, Joana, et al. "Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus." *Nature medicine* 28.8 (2022): 1569-1572.
Payne, Alexander, et al. "Readfish enables targeted nanopore sequencing of gigabase-sized genomes." *Nature biotechnology* 39.4 (2021): 442-450.

https://virological.org/t/initial-observations-about-putative-apobec3-deaminase-editing-driving-short-term-evolution-of-mpxv-since-2017/830