
## Supporting Information

## Supplementary material

**This appendix was part of the submitted manuscript and has been peer reviewed.**
**It is posted as supplied by the authors.**

Appendix to: Stewart A, Schlebusch S, Vlack S, et al. First case of mpox diagnosed in Queensland, Australia: clinical and molecular aspects. *Med J Aust* 2023; doi: 10.5694/mja2.51842.

**Methods**

Nucleic acids were extracted using the Qiagen EZ1 Virus Mini kit 2.0.

Libraries of the partial MPXV genes targeting the OPG105 (in-house, unpublished data) and OPG185 (ROPP et al 1995) were constructed from the generated amplicon using the Oxford Nanopore Rapid sequencing kit. The libraries were sequenced on the Oxford Nanopore Gridion-X5 with up to 1–2 Gbases generated depending on the sample. The libraries were sequenced on the Illumina NextSeq550 which generated between 30-50 million total paired reads (2x 150 nt).

Metagenomic sequencing libraries were constructed from extracted DNA using the Nextera XT DNA library kit with barcoded indices. The libraries were sequenced on the Illumina NextSeq550 using the NextSeq 500/550 Mid Output kit v2.5 which generated between 30-50 million total paired reads (2x 150 nt).

Low quality reads were removed, and adapters were trimmed using Trimmomatic (v0.39). Reads were then mapped to the Human Reference genome GRCh38 using minimap2 (v2.17-r941). Reads-pairs that had at least one read in the pair that mapped along more than 75% of their length at an identity of 90% or greater were removed. The remaining reads were then mapped to the MPXV genome (NC_063383.1). Consensus calling was done with Pilon (v1.24).

Following reference genome mapping, the consensus sequence(s) was then compared to other globally available sequences in the GenBank database using blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A multiple sequence alignment was generated using MAFFT v7.453 [Misawa K. et al 2002], incorporating the consensus sequence and sequences retrieved from GenBank which represented the most closely related sequence(s) and representative sequences from past and present MPXV circulating clades. The multiple sequence alignment was then trimmed to remove one of the inverted terminal repeat (ITR) sequences flanking the genome (ie. the 5'-terminal ITR, nucleotides 1-6422) leaving the 3'-terminal ITR (Happi, C. et al 2022) and

repeat regions. A number of repetitive regions were also masked out (https://github.com/nextstrain/monkeypox/blob/master/config/mask_overview.bed). Genetic variation analysis was conducted using snp-dists (https://github.com/tseemann/snp-dists) and a ML tree was generated using the Jukes-Cantor nucleotide substitution model and IQ-TREE v1.6.12 (Nguyen L. et al 2015). Tree visualization and plots were produced using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The genome reported passed QC throughout the method process described above.

## References


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