High Resolution Whole-Genome Sequencing of Human Metapneumovirus Using Oxford Nanopore Technology

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Introduction:

Human metapneumovirus (hMPV), a member of the family Paramyxoviridae, is one of the leading causes of acute respiratory illness, especially in young children, the elderly and immunocompromised patients. Based on nucleotide sequence and antigenic variations, hMPV viruses consist of two main lineages, A and B, which are further divided into four sublineages, A1, A2, B1 and B2. We have developed high-throughput amplicon-based whole-genome sequencing (WGS) assays for accurate and rapid characterization of hMPV genomes using Oxford Nanopore technology. The hMPV WGS assays can facilitate large-scale viral genomic surveillance studies and drug resistance mutations studies regarding new antiviral treatments.

Materials & Methods:

The sensitivity of the hMPV whole-genome assay was assessed on virus stock dilutions of six hMPV strains, A1 (n=2), A2 (n=1), B1 (n=2), B2 (n=1) and hMPV positive nasopharyngeal (NP) swabs (n=16).

The hMPV whole-genome amplification approach and assay workflow (wet lab and dry lab) are presented in Figure 1.

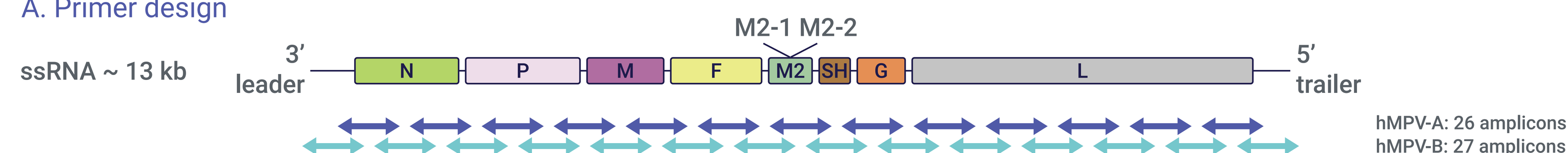
A. hMPV-A and hMPV-B whole-genome amplification (WGA) was performed with subgroup specific primers using a 2-primer-pool multiplex PCR-tiling approach, covering the full-length viral genome in respectively 26 and 27 overlapping amplicons of approximately 800 bp.

B. Viral RNA extraction was performed with the MagNA Pure 96 instrument and whole-genome amplification was performed with the respective hMPV subgroup specific primer pool set after DNase pre-treatment and cDNA synthesis using random hexamers. Successful amplification was confirmed by gel electrophoresis and the combined sample pools were sequenced with V14 kit on the MinION Mk1C instrument.

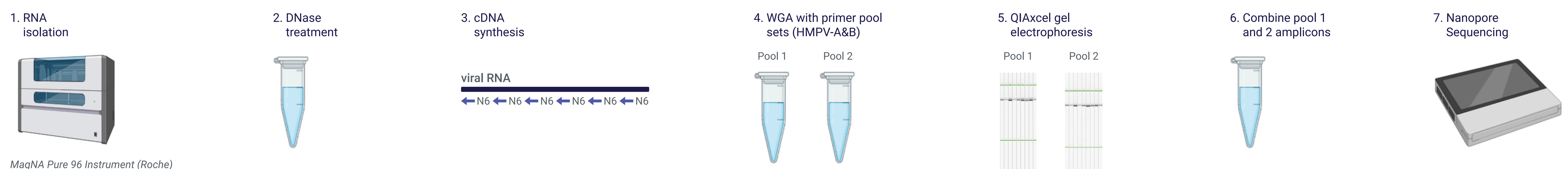
C. Data analysis was performed using Cerba Research's NL in-house developed bio-informatics pipeline, including subtyping of NP swab strains.

Figure 1: Workflow

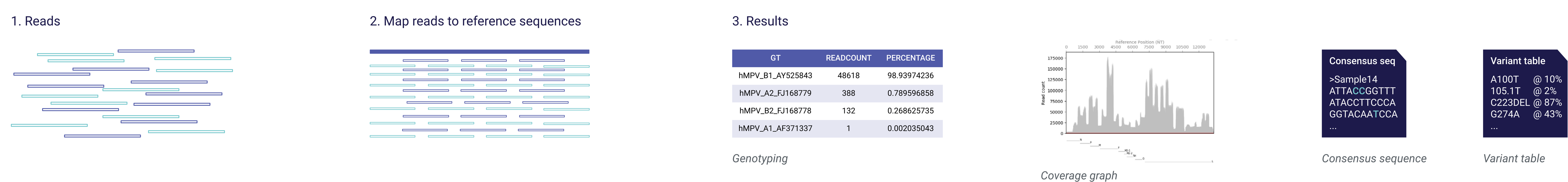
A. Primer design



B. Workflow wet lab



C. Workflow dry lab

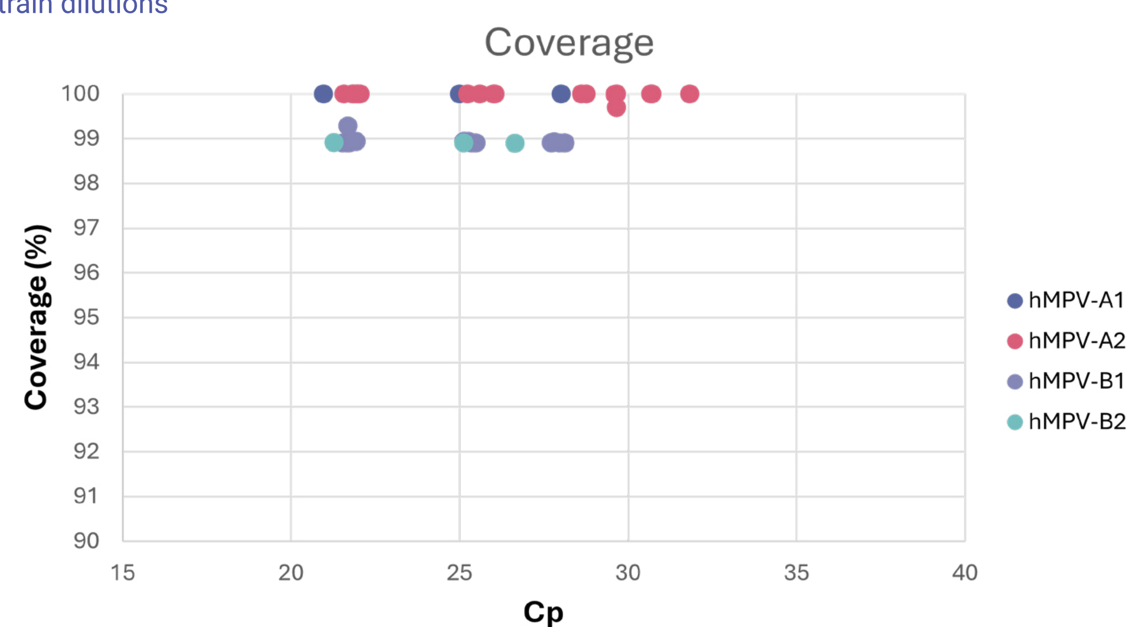


Results:

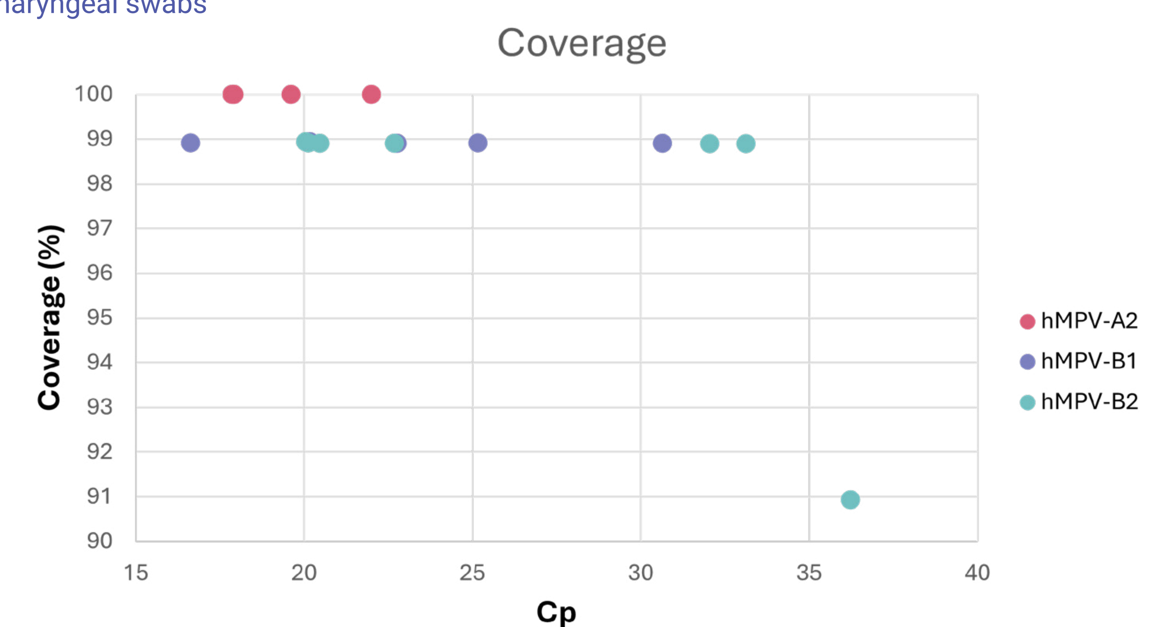
1. The NP swab strains belonged to hMPV A2 (n=4), B1 (n=5) and B2 (n=7) sub-lineages.
2. For **hMPV strain dilutions** with viral RNA concentrations ranging from 3.9 log10 copies/mL (Cp 31.8) to 7.0 log10 copies/mL (Cp 21.0), 99% to 100% of the whole-genome was sequenced (figure 2A) with a mean read depth per sample ranging from 3942 to 97310 reads (figure 2C and 2D).
3. For **NP swabs** with viral RNA concentrations ranging from 2.7 log10 copies/mL (Cp 36.2) to 8.3 log 10 copies/mL (Cp 16.6), between 91% to 100% of the whole-genome was sequenced (figure 2B) with a mean read depth per sample ranging from 724 to 50010 reads (figure 2C and 2D). For hMPV B2 virus with a lower viral RNA concentration of 2.7 log10 copies/mL (Cp 36.2) we achieved 91% coverage of the full genome (figure 2B).
4. Full-length **F-gene** sequence was obtained from all hMPV strain dilutions and 15 (94%) NP swabs. Similarly, for hMPV B2 virus with a lower viral RNA concentration of 2.7 log10 copies/mL (Cp 36.2) a partial sequence of the F-gene was obtained.
5. Full-length **G-gene** sequence was obtained from all hMPV strain dilutions and NP swabs.

Figure 2: The hMPV WGS coverage for virus stock dilutions (A) and nasopharyngeal swab samples (B). The violin plots summarize the read depth distribution per nucleotide position of all hMPV-A (C) and hMPV-B (D) samples.

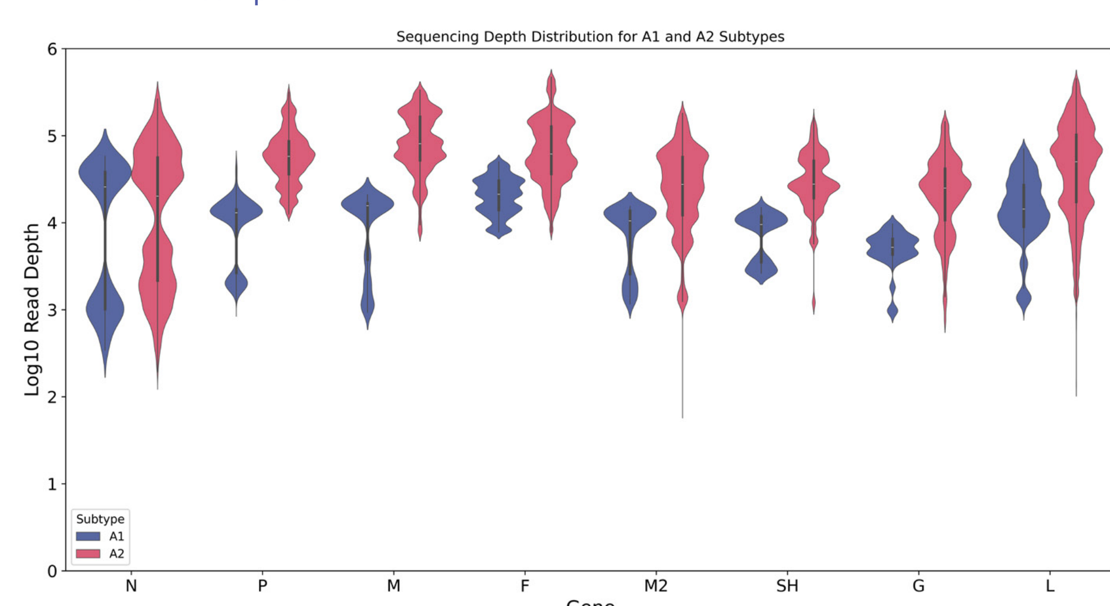
A. Coverage hMPV strain dilutions



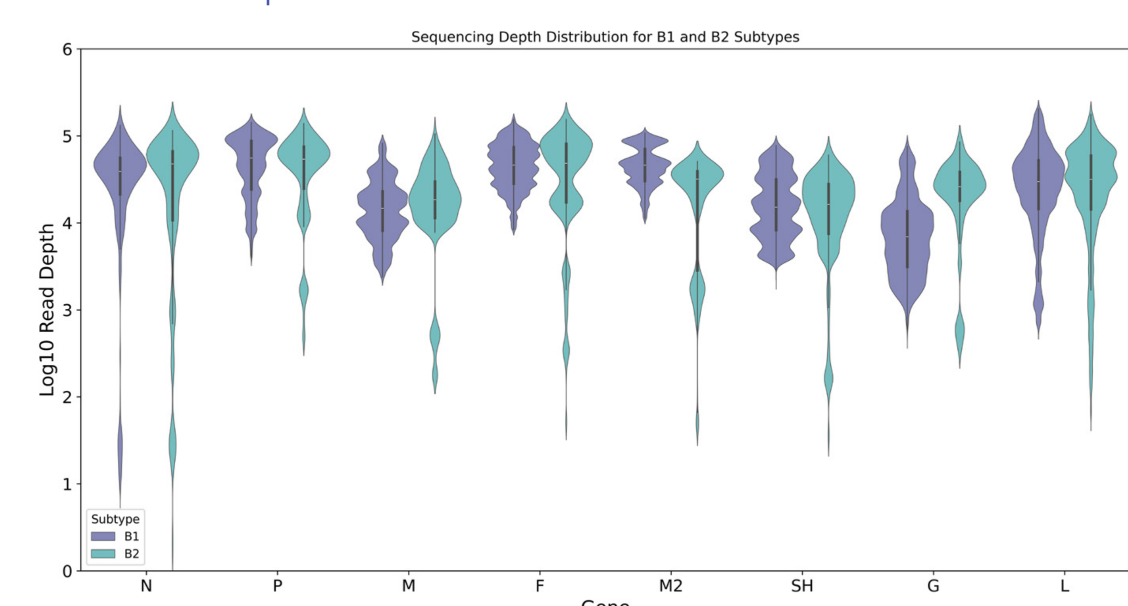
B. Coverage Nasopharyngeal swabs



C. Sequencing depth hMPV A1 & A2 samples



D. Sequencing depth hMPV B1 & B2 samples



Conclusion:

- All The hMPV amplicon-based WGS assays demonstrate robust performance and high sensitivity.
- This assay can be applied directly on respiratory samples for large-scale viral genomic surveillance studies and investigating drug resistance mutations of new antiviral therapies.

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Authors are all Cerba Research NL employees with no conflict of interest.