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A new IRMA module for analyzing whole-genome sequences from human metapneumovirus

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ABSTRACT The large amount of genetic diversity in human metapneumovirus makes reference-based alignments difficult. We created a new module for the Iterative Refinement Meta-Assembler (IRMA) that performs alignment and consensus sequence generation without requiring subtyping and can handle duplications in the glycoprotein. This module increases the feasibility of genomic surveillance.

KEYWORDS human metapneumovirus, HMPV, genome analysis, iterative refinement meta-assembler, consensus sequence, genomic surveillance

Human metapneumovirus (HMPV) causes a significant number of respiratory infections each year, especially in young children (1). HMPV is genetically diverse with two antigenically distinct lineages (A and B) that cocirculate (2, 3). These two lineages have each split into two sublineages (A1, A2, B1, and B2), and A2 has further split into A2.1 and A2.2 (4). Most of the genetic diversity among subtypes is in G, the glycoprotein (5, 6). G is also highly variable within subtypes, with strains containing either a 111 or 180 nucleotide duplication currently circulating within A2.2 (7, 8).

There has been limited whole-genome sequencing of HMPV, despite potential public health benefits of genomic surveillance. One of the barriers to whole-genome sequencing is efficiently analyzing the sequence data due to the large amount of genetic diversity. Current library preparation methods do not require subtyping (9–11). However, the genetic diversity of HMPV hinders the ability to use a single reference to accurately assemble genomes for all samples.

To address this problem, we have developed an HMPV IRMA module. IRMA was developed for assembling highly variable RNA viruses (12). IRMA is reference-based, but it iteratively gathers reads and edits the reference genome, minimizing the effects of distance from the initial reference. It also allows for a different reference genome for each subtype, making prior subtyping unnecessary. To create the reference, we downloaded all whole genomes available on GenBank (accessed Oct. 18, 2024, "Metapneumovirus hominis"). Sequences were aligned using MAFFT v7 (13), and IQ-TREE 2 (14) was used

TABLE 1 The number of genomes used to create the consensus reference sequences[a]

Lineage	Number of genomes
A1	15
A2.1	47
A2.2	140
A2.2 (111)	103
A2.2 (180)	6
B1	92
B2	118
Total	**521**

[a]Number in parentheses is the size of the G duplication.

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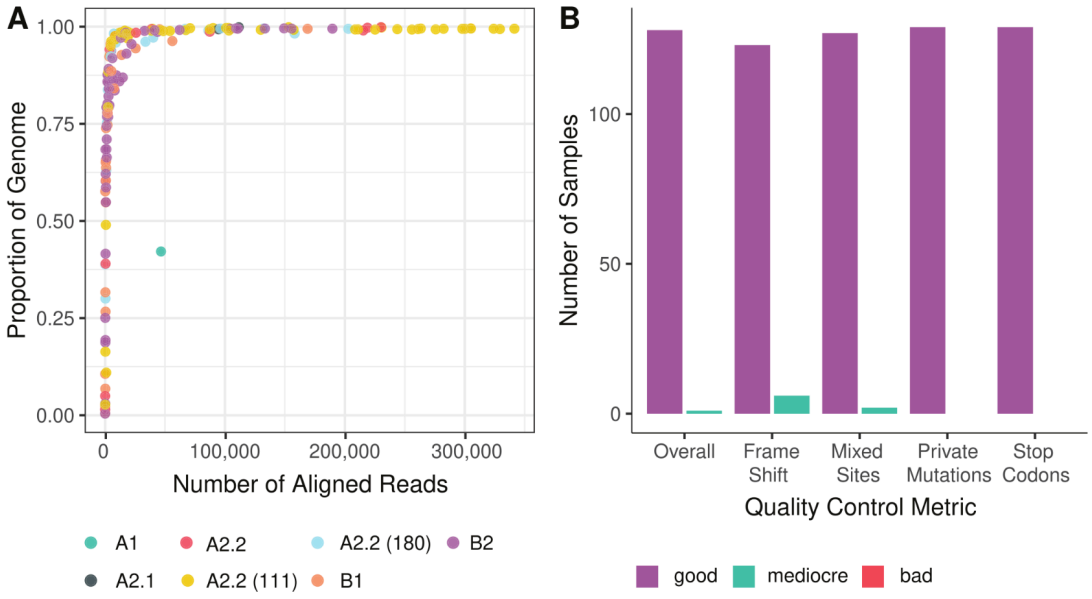

FIG 1 The IRMA module creates complete and high-quality HMPV consensus genomes. (A) Genome completeness. The number of reads aligned to the final reference versus the proportion of nucleotides present in the consensus genome. The color is the reference genome used for each sample. (B) Quality control metrics from Nextclade for samples with at least 75% coverage of the genome.

to create a phylogeny. We used previously typed samples and the phylogeny to assign samples to A1, A2, A2.1, A2.2, A2.2 +111 nt duplication, A2.2 +180 nt duplication, B1, or B2 (Table 1). For each sublineage, we created a plurality consensus sequence using EMBL consensus generator (15) and a hidden Markov model using IRMA.

To test the IRMA pipeline, we sequenced 181 samples from the Investigating Respiratory Viruses in the Acutely Ill (IVY) study (November 2024–April 2025) (16, 17) and from the Household Influenza Vaccine Effectiveness (HIVE) study (2011–2022) (18). Nasal swabs were sequenced using the Respiratory Virus Oligos Panel v2 on an Illumina NextSeq 2000 (2 × 300, P1 chemistry).

The consensus sequences generated by IRMA were complete or nearly complete genomes (Fig. 1A). A2.1, A2.2, B1, and B2 lineages were present (19). Lineages were consistent with previous qPCR subtyping (A or B) (20). We were able to detect the 111-nt (42 samples) and 180-nt (11 samples) insertions in a subset of A2.2 samples, showing that the IRMA module can handle samples with or without a duplication. No systematic issues were detected in the alignments (Fig. 1B). The IRMA module is suitable for Illumina and Nanopore sequencing. For Nanopore sequencing, the config file would need to be altered (see Flu module in IRMA for example). For Illumina sequencing, read lengths shorter than 300 bp compromise accurate detection of duplications.

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DATA AVAILABILITY

The module and consensus sequences are available at https://github.com/laurin-glab/HMPV_IRMA_module. To use, place the file inside the modules folder of IRMA and follow the instructions at https://wonder.cdc.gov/amd/flu/irma/index.html. Sequences are available at BioProject PRJNA1304962.

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