Recommendations for analysis of the xGen[™] Respiratory Virus Amplicon Panel

Analysis workflow

The following flowchart may serve as a general guide for analyzing sequence data derived from the xGen Respiratory Virus Amplicon Panel. The full panel was designed to detect six common respiratory viruses (see **Genomes used as targets in panel design**). This workflows includes the major steps and publicly available tools for the genomic characterization of a sample, regardless of its complexity.



* In cases where a sample contains more than one targeted virus, partitioning reads to their respective genomes of origin may be helpful in further characterizing the target sample. One approach is to use the alignment information, i.e., to assign reads to one of the six genomes with which they have the highest alignment scores using the "optional" custom steps. For more information contact us at www.idtdna.com/ContactUs.

† Review Genome assembly approaches below.

Genome assembly approaches

In addition to an alignment-based approach to assess preliminary panel performance, additional information can be obtained through assembly-based approaches. The following are open-source tools, that have not been tested by IDT.

De novo assemblers:

Megahit (https://github.com/voutcn/megahit)

Virus_assembly (https://github.com/jsede/virus_assembly)

Reference-based assemblers:

IRMA (https://wonder.cdc.gov/amd/flu/irma/)

Reference genomes used as targets in panel design:

The maximum percent aligned for these genomes should correspond to values in Table 1.

- 1. RSV A: OP890336
- 2. RSV B: OP965707
- 3. Influenza A H1N1 (A/California/7/2009) (8 segments: NC_026438.1, NC_026435.1, NC_026437.1, NC_026433.1, NC_026436.1, NC_026434.1, NC_026431.1, NC_026432.1)
- 4. Influenza A H3N2 (A/New York/392/2004) (8 segments: NC_007373.1, NC_007372.1, NC_007371.1, NC_007366.1, NC_007369.1, NC_007368.1, NC_007367.1, NC_007370.1)
- 5. Influenza B (B/Malaysia/2506/2004) (8 segments: CY040455.1, CY040456.1, CY040454.1, CY040449.1, CY040452.1, CY040451.1, CY040450.1, CY040453.1)
- 6. SARS-CoV-2 (Wuhan-1): MN908947.3

Table 1. xGen Respiratory Virus Amplicon Panel NGS metrics.

	RSV A	RSV B	Influenza A H1N1	Influenza A H3N2	Influenza B	SARS-CoV-2
% mapping	86%	93.60%	99.20%	99.40%	99.50%	98.50%
% on-target (base)	97.20%	96.70%	96.50%	96.30%	96.60%	97.20%
% base uniformity	67.40%	70.10%	93.20%	93.10%	77.80%	92.90%
x % genome >10X coverage	94.50%	98.90%	100%	100%	100%	100%

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