Genomic Surveillance of SARS-CoV-2: A Comparison of Four Different Methods

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Objective

The purpose of this study was to evaluate four SARS-CoV-2 next generation whole genome sequencing methods as tools for genomic surveillance, comparing genome coverage, ambiguities, depth of coverage, concordance of base calling and Pangolineage assignment between the methods.

Methodology

- 86 archived SARS-CoV-2-positive samples were selected for comparative testing on Illumina, Ion Torrent S5 XL, and the Clear Dx™ system (GridIon x5).
- A subset of 40 samples were also run on the Genexus instrument.
- ARTIC Illumina sequencing was performed using a modified version of the ARTIC V4[1] amplicon panel and sequenced on a HiSeq instrument.
- Ion Torrent sequencing was performed with the Ion AmpliSeq™ Insight Panel on an Ion S5 XL or using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay GX on the Genexus instrument.
- Clear Dx™ sequencing was performed on a Clear Dx™ instrument containing a GridION by Oxford Nanopore Technologies, using a minION v9.4 flow cell with ARTIC v3 primers from Clear Labs.

Analysis

- Consensus sequences were generated with a 20x depth of coverage threshold and > 50% agreement for base calling.
- Sequences with <90% genome coverage and/or >10% ambiguous or missing bases were excluded from the downstream analysis (Figure 1).

Genome Coverage

Depth of Coverage

Gaps and Ambiguities

Pangolin Lineage Assignment

Technologies

Clear Dx™	S5 XL and Genexus	Illumina
		
Automated Library Prep	Automated Library Prep	Manual Library Prep (Can be automated)
ARTIC primers	AmpliSeq Insight primers	ARTIC primers
NanoPore Sequencing	Ion Torrent Sequencing	Illumina Sequencing

Images sourced from Technical Note: Clear Dx SARS-CoV-2 WGS V3.0, thermofisher.com, illumina.com

Results

- Pangolin assigned a lineage in 89.5% (77/86), 90.7% (78/86), 96.5% (83/86), and 100% (40/40) of the samples sequenced using the Clear Dx™, Illumina, S5 XL, and Genexus instruments, respectively (Table 1).
- There were no disagreements in lineage assignments across the methods.
- Samples sequenced using the Genexus instrument maintained the highest depth of coverage across Ct values (Figure 2).
- Samples sequenced on the Clear Dx™ and Genexus instruments had the highest percentage per site ambiguous bases in spike region of the genome (Figure 3).

Pangolin Lineage Assignment

Ct range	Clear Dx™	Illumina	S5 XL	Genexus
20-25	28/29 (97%)	29/29 (100%)	28/29 (97%)	14/14 (100%)
25-30	27/30 (90%)	30/30 (100%)	30/30 (100%)	14/14 (100%)
30+	22/27 (81%)	19/27 (70%)	25/27 (93%)	12/12 (100%)

Table 1: The number of consensus sequences that Pangolin COVID-19 Lineage Assigner was able to assign a lineage for each method, stratified by Ct range.

Figure 1a

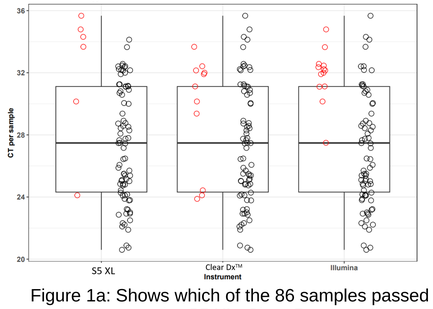

Figure 1b

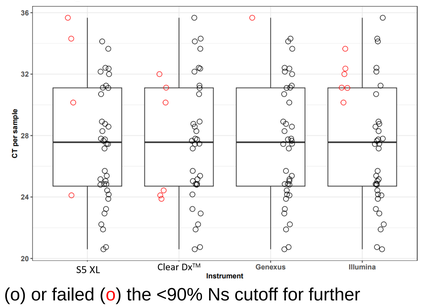

Figure 1a: Shows which of the 86 samples passed (o) or failed (o) the <90% Ns cutoff for further analysis across the S5 XL, Clear Dx™ and Illumina instruments.
Figure 1b: Shows the same for the subset of 40 samples that were also sequenced on the Genexus instrument.

Data

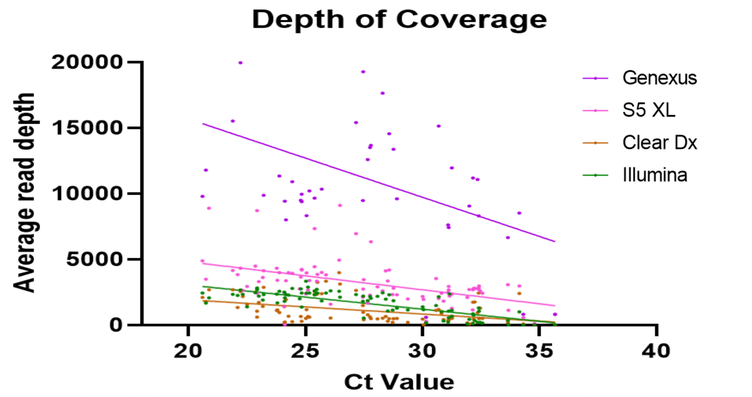

Figure 2: The average depth of coverage per base by Ct values of samples.

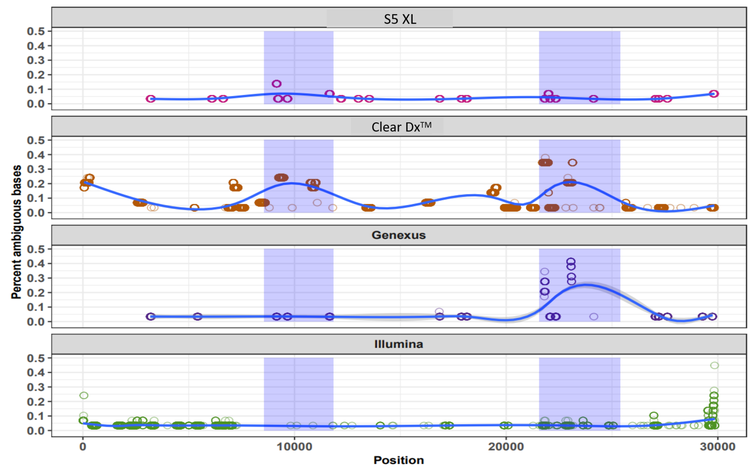

Figure 3: Percent ambiguous bases per site. Nsp4-nsp6 and Spike regions of genome are highlighted.

Conclusion

- The Illumina HiSeq has the highest throughput potential, while Clear Dx™, S5 XL and Genexus provides automated library prep and faster turn-around times.
- In samples with the highest Ct values, Clear Dx™ and Illumina generated lower coverage.
- There were no disagreements in lineage assignment between the methods; however, both S5 XL and Genexus were able to assign a lineage to more samples with Ct values greater than 30.
- Ambiguous base calls were observed at a higher frequency for Clear Dx™ and Genexus in the spike region.

References

- [1] Plitnick J, et al. "Whole-genome Sequencing of Sars-CoV-2: Assessment of the Ion Torrent Ampliseq Panel and Comparison with the Illumina MiSeq ARTIC Protocol." *Journal of Clinical Microbiology* 59.12 (2021): e00649-21.