Optimizing Read Depth With Real-World Sequencing Data in a Clinical Setting

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Introduction

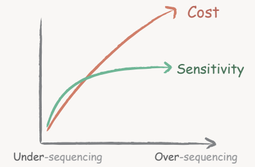

Fig 1. The sequencing cost and NGS assay sensitivity increase at different pace as the sequencing depth increases.

- Higher sequencing depths increase the sensitivity of an NGS assay.
- Once a sufficient depth is reached, additional sequencing provides limited improvement in assay sensitivity.
- It is essential to use real-world sequencing data to find the optimal sequencing depth.

Methods

Table 1. Comparison of Illumina NovaSeq 6000 Flow Cells.

Flow Cells (300 cycles)	Price	Run Time	Paired-end Reads
S2	~$10,000	36 hr	6.6 – 8.2 B
S1	~$5,500	25 hr	2.6 – 3.2 B
SP	~$3,100	25 hr	1.3 – 1.6 B

- Three flow cells S2, S1 and SP were used to sequence the same clinical batch of 93 patient samples on NeoGenomics' amplicon-based solid tumor NGS assay, covering over 300 genes.
- Concordance between the flow cells were assessed, including QC, SNV, InDel, microsatellite instability (MSI) and tumor mutational burden (TMB).

Results

QC Comparison of Samples Sequenced on Different Flow Cells

- Sample Total Read on the SP was ~22% that on the S2;
- Consensus Read on the SP was ~ 25 - 70% that on the S2;
- Average Coverage on the SP was ~ 25 - 70% that on the S2;
- %Target>=100cov and Uniformity barely changed except in a few failed samples.

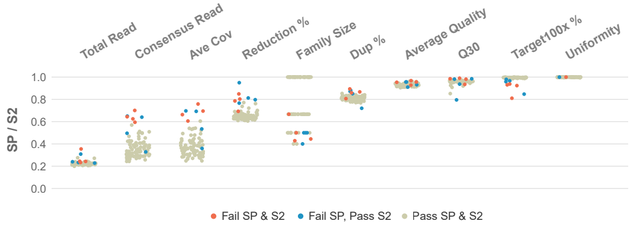

Fig 2. QC metrics comparison of the same batch of 93 clinical samples sequenced on SP and S2 flow cells. y-axis, ratio of SP/S2 of all the QC metrics. Each dot denotes a sample. Red, samples failed on both flow cells; blue, samples failed on SP, but passed on S2; Beige, samples passed on both flow cells.

Increased Failure Rate on Smaller Flow Cells

- When switching from S2 flow cell to S1 or SP, failure rate of new samples increases from 8.1% to 10.6% and 15.2%, respectively.
- Failure rate of repeated samples only increased slightly when switched to smaller flow cell.

Table 2. Predicted sample failure rate if switching from S2 to S1 and SP flow cells, using the QC metrics of clinical samples sequenced on S2 flow cell in June and July 2022. Prediction is based on sample's QC metrics of %Target 100x on S2: SP<99.3%; S1<99.0%.

	S2	S1	SP
New Samples	8.1%	10.6%	15.2%
Repeated Samples	62.8%	67.4%	73.4%
Re-extraction Samples	65.0%	69.4%	75.4%

Coverage of Targets Difficult to Amplify

- Certain targets which are difficult to amplify. e.g. *TERT* Promoter, tend to get less coverage. This challenge becomes more pronounced when using smaller flow cells.
- To keep the same sensitvity, the criteria for *TERT* variant calling may need to be adjusted.

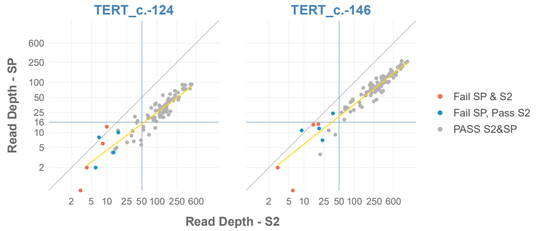

Fig 3. Comparison of coverage of two TERT promoter hotspots, c.-124 and c.-146 on SP (y-axis) and S2 (x-axis) flow cell. Each dot denotes one of the 93 clinical samples in the batch.

MSI and TMB callings on Different Flow Cells

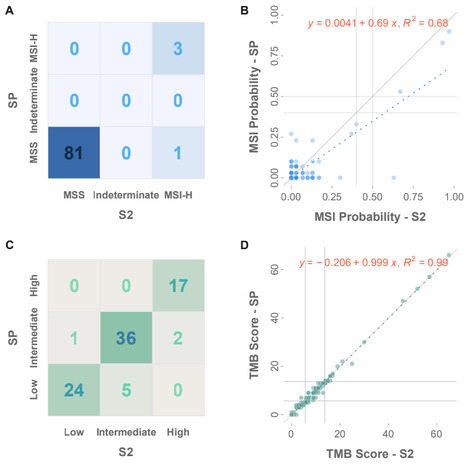

Fig 4. Comparison of MSI (A&B) and TMB (C&D) calling between SP and S2 flow cells on the same clinical batch. QC failed samples were removed from analysis.

Summary

Switching from S2 to SP flow cell

Pros

High concordance between SP and S2:

- SNV & Indel: 96.3%,
- MSI: 98.8%,
- TMB: 90.6%

Saves:

- $1.7M (from $2.5M to $0.8M) per year on flow cells,
- 11 hours TAT (from 36hr to 25 hr) per batch on sequencing,
- 80% of the data storage cost with reduced reads,
- Computation power and time on running pipeline with reduced reads.

Cons

- Sample failure rate increases from 8.1% to 15.2,
- Certian targets, which are difficult to amplify, tend to get even lower coverage.

Conclusion

- In this feasibility study, despite the huge difference of sequencing depth between the flow cells, no significant differences in term of sensitivity for SNV or InDel detection, TMB or MSI calling were observed.
- This study indicates that a SP flow cell is sufficient to sequence a batch of 93 patient samples for this NGS assay.