An enhanced workflow for the Neo Comprehensive™ solid tumor genome profiling assay: comparison for Tecan Qubit vs KAPA library quantification

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Background

In next-generation sequencing (NGS), accurate quantification of library concentration is critical for achieving optimal sequencing results and maximizing data quality. This study compares fluorescence-based high sensitivity and quantitative PCR (qPCR)-based methods for Neo comprehensive genome profiling solid tumor library quantification, focusing on their efficacy in enhancing workflow efficiency for large NGS panels. We evaluated both methods across multiple NGS sequence runs to assess their data quality and final variants concordance.

Methods

Total one hundred and sixty (160) Neo Comprehensive solid tumor genome profiling final sequence libraries were selected after quantification by KAPA method. Those individual libraries were quantified by using Tecan Qubit method, molar concentration was converted from ng/ul. The new pooled libraries were sequenced on Illumina NovaSeq 6000 across three runs. Sequence data were processed through DRAGEN pipeline oncology software and output from DRAGEN were then passed through VAaAST (an internal variant annotation tool) for variant concordance comparison.

Overview of NGS Workflow

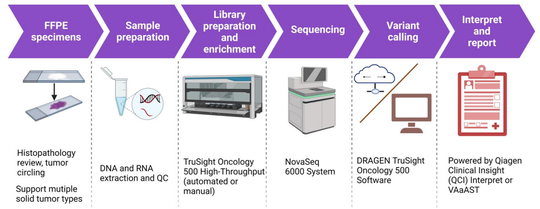

Fig 1A: Neo Comp Solid tumor NGS workflow

Parameter	TSO500 HT tissue
Sample types	FFPE tissue
Sample recommendation	> 20% tumor content
Chemistry	Hybrid-capture
Sequencing system	NovaSeq 6000
Panel size	1.94 Mb DNA, 358 kb RNA
Min. nucleic acid input	60 ng DNA, 80 ng RNA
Sample throughput	48 sample (or higher) per run
Deliverables	FASTQs, BAMs, QC report, VCFs, Fusions, MSI and TMB scores, and clinical report
Target TAT	~ 10 days real-time testing 2-3 weeks for batched testing

Fig 1B: Neo Comp Solid Tumor parameters

Results

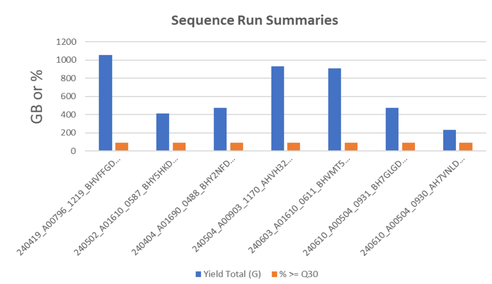

Fig 2: Sequence Batch Level QC.

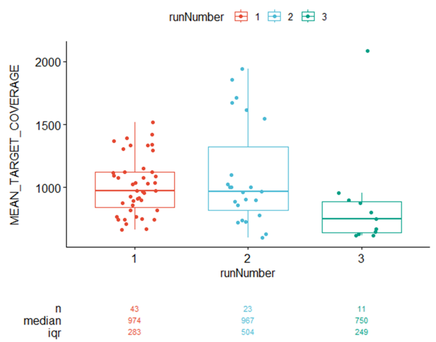

Fig 3: Neo Comp Solid Tumors DNA libraries metrics comparison. All samples reached > 500 mean target coverage.

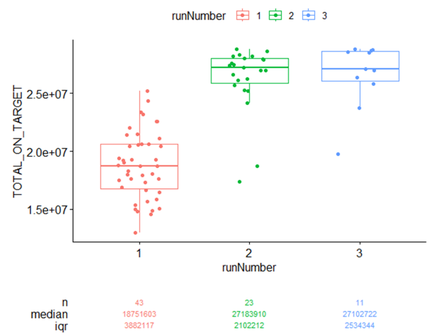

Fig 4: Neo Comp Solid Tumors RNA libraries metrics comparison. All samples reached > 5 million on target reads.

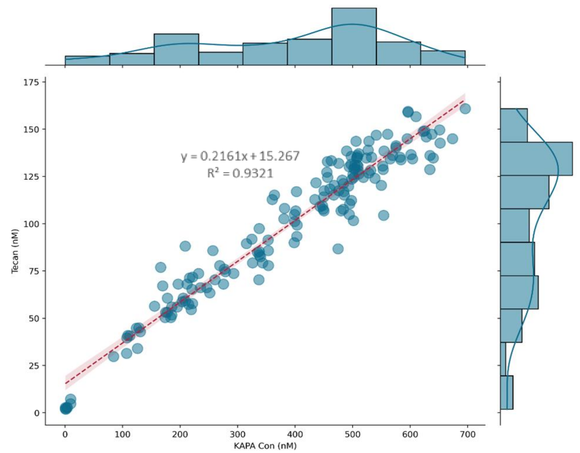

Fig 5: Tecan Qubit vs. KAPA library quant. Tecan quant and KAPA quant showed good correlation. The histogram distribution for KAPA and Tecan are quite similar.

SNV	
Number of concordant	78369
Number of totals	78686
Average concordant (Number of concordant/ Number of totals) *100	99.6%
InDel	
Number of concordant	2095
Number of totals	2151
Average concordant (Number of concordant/ Number of totals) *100	97.40%
Clinical Relevant SNV/InDel	
Number of concordant	938
Number of totals	961
Average concordant (Number of concordant/ Number of totals) *100	97.61%
RNA fusion/splice variant	
Concordant	54
Discordant	1
Totals	55
Concordant (concordant/totals)	98.20%

Fig 6a: Functional variant concordance analysis. variant analysis showed concordance rate for SNVs, Indels, clinical relevant small variants and fusion/splice are 99.6%, 97.40%, 97.61 and 98.20% respectively.

File Name	Gene Symbol	Cdot	Pdot	Read Depth	Allele Frequenc
AVTSO3011-CondC	PIK3C2B	c.4386_4394del	p.Val1464_Glu1466del	1246	0.0586
AVTSO3011-CondC	NOTCH3	c.4762A>C	p.Asn1588His	757	0.0687
AVTSO3042-CondB	FGFR3	c.2076dup	p.Ser693LeufsTer124	855	0.0515
AVTSO3042-CondC	FGFR3	c.2076dup	p.Ser693LeufsTer124	983	0.0519
AVTSO3042-CondD	CCND1	c.869T>A	p.Val290Glu	921	0.0586

Fig 6b: Variants missed in validation runs. Variants only found in original runs (discordant variants) were close to the 5% variant allele frequency (VAF).

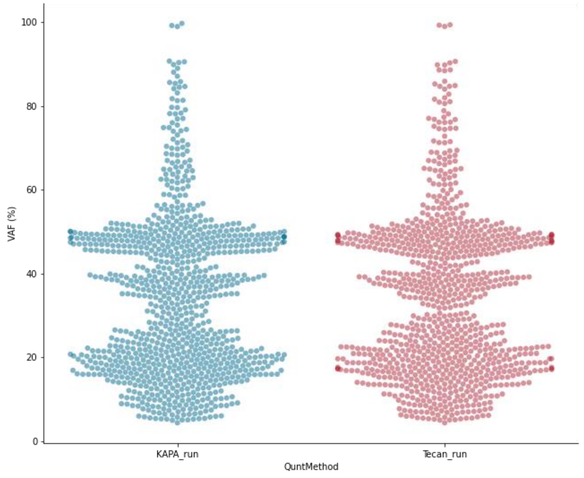

Fig 7: Concordant variant analysis between KAPA and Tecan runs. Swamp plot showed comparable VAF distribution between KAPA runs and Tecan runs.

Key findings

- A total of 160 samples were successfully re-sequenced across three sequence runs covering different NovaSeq 6000 flow cells.
- The library concentration between KAPA and Tecan showed highly correlation with R^2 is 0.93.
- DNA and RNA variant analysis showed concordance rate for SNVs, Indels, and fusion/splice are 99.6%, 97.4%, and 98.2% respectively.
- Tecan quantification improved NGS workflow efficiency by reduced operational time and less error prone.

Conclusions

- Tecan quantification result showed equivalent performance with operational benefits.
- These findings provided the rationale for loading the libraries at the recommended concentrations for the sequencer which resulted an improved workflow in the clinical implementation.

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