H-24

Performance Comparison of Nine Different Assays Between Next Generation Sequencing (NGS) Platforms

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Introduction

Over the past decade, Invivoscribe® has developed 9 different assays on Illumina's MiSeq™ NGS platform which aid in the identification and monitoring of target-specific sequences associated with hematologic disease. With NGS technology and instrumentation advancements in read depth, data generation, and sequencing accuracy; there is a high demand to adapt existing assays to the higher-throughput NextSeq 1000™ NGS platform. Here we present a performance comparison between Illumina's MiSeq and NextSeq 1000 platforms using identical libraries generated by the Research Use Only (RUO) LymphoTrack® Assays, *FLT3* ITD MRD and *NPM1* MRD Assays.

Methods

Positive DNA from cell lines or clinical samples were contrived using polyclonal DNA as a background in order to create sensitivity panels for each assay. The evaluated assays included V-J rearrangement assays for immunoglobulin heavy chain *IGH* (IGHV Leader, FR1, FR2 and FR3), immunoglobulin light chain (*IGK*), T-cell receptor gamma (*TRG*), T-cell receptor beta (*TRB*), and assays for measurable residual disease (MRD) in internal tandem duplications (ITD) for fms-like tyrosine kinase 3 (*FLT3*) gene and mutations for nucleophosmin (*NPM1*) gene.

The workflow for the NGS assays is shown below:

* Clonality assays = 1 round
MRD assays = 2 rounds

Three pooled libraries were generated:

- Library A: Comprised of 7 V-J Clonality Rearrangement Assay Samples
- Library B: Comprised of *FLT3* ITD MRD Assay Samples
- Library C: Comprised of *NPM1* MRD Assay Samples

Library A was sequenced at 2x301 cycles on the MiSeq using v3 reagent kit and on the NextSeq 1000 using a P1 reagent kit. FASTQ files from both platforms were analyzed using Invivoscribe's RUO LymphoTrack MiSeq Software (v2.4.3). Top % reads from each of the target specific V-J rearrangements were compared between the NGS platforms.

Libraries B (*FLT3*) and C (*NPM1*) were sequenced separately each at 2x301 cycles on the MiSeq using v3 reagent kits and sequenced together in a combined library on the NextSeq 1000 using a P2 reagent kit. FASTQ files from both platforms were analyzed using Invivoscribe's RUO *FLT3*-ITD MRD Software (v1.2) and *NPM1* MRD Software (v1.1.1). Detected variant read frequencies (VRF) from each of the target specific mutations were compared between the NGS platforms.

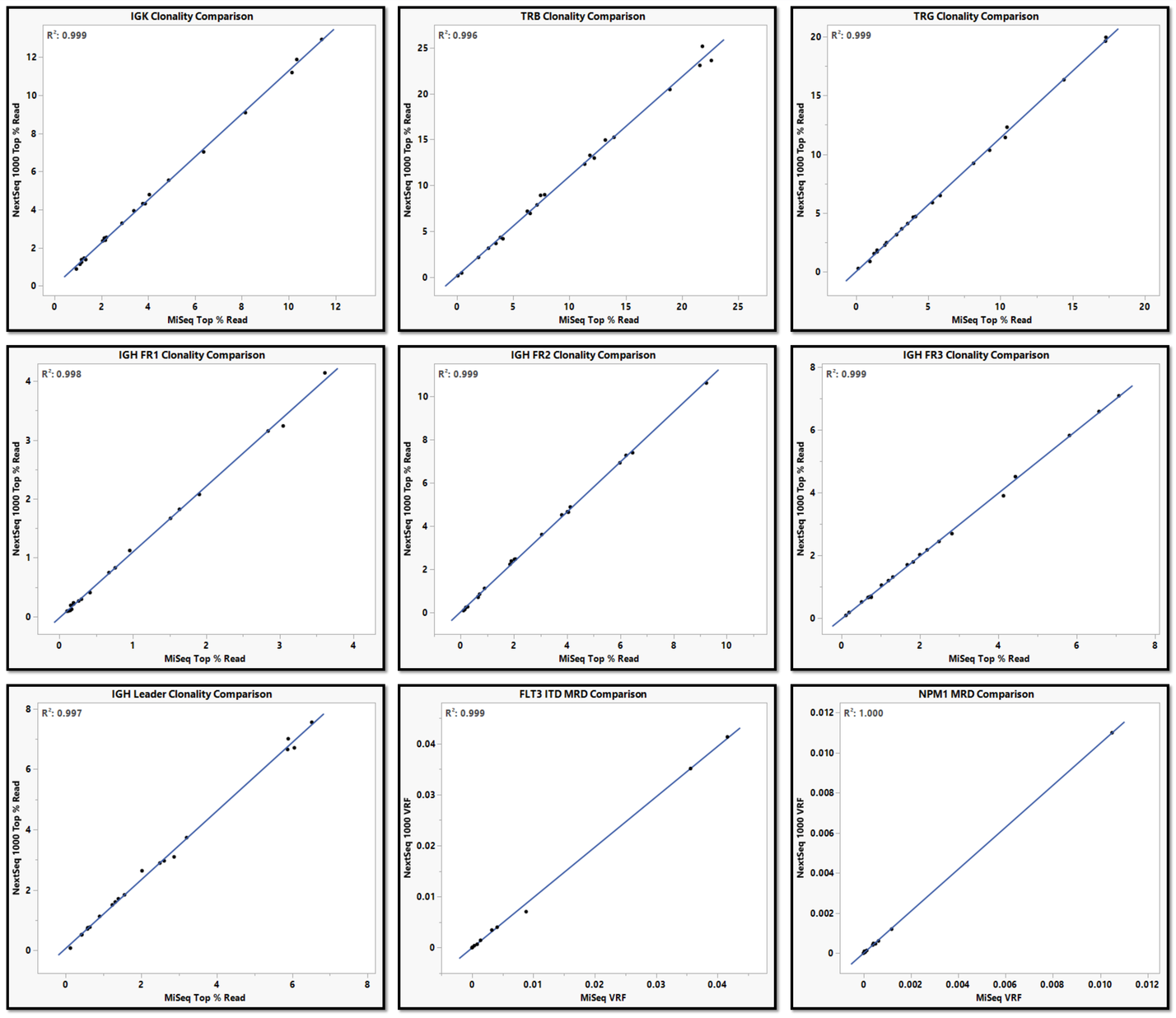
Conclusions

Excellent correlation ($R^2 > 0.99$) was observed between NGS platforms. This demonstrates that all of Invivoscribe's RUO LymphoTrack MiSeq assays and AML NGS MRD assays are compatible with the NextSeq 1000. It also shows that libraries may be pooled and sequenced together on the NextSeq 1000 for greater cost efficiency. Additionally, the increased read depth of the NextSeq 1000 platform enabled the detection of 3 dilution levels missed by the MiSeq. This provides evidence of enhanced sensitivity, which is important when assessing residual disease.

Results: Instrument Correlation and Data Generation Metrics

Library	Assay	Instrument Correlation (R^2)	Pass Filter Reads	
			MiSeq	NextSeq 1000
Library A	*IGHV* Leader	0.997	32 million	161 million
	IGH FR1	0.998		
	IGH FR2	0.999		
	IGH FR3	0.999		
	IGK	0.999		
	TRG	0.999		
	TRB	0.996		
Library B	*FLT3* ITD MRD	0.999	19 million	413 million
Library C	*NPM1* MRD	1.000	14 million	

Results: Instrument Correlation Results



Results: MRD Detection Capability by Instrument

FLT3 ITD MRD Detection Results			
ITD Insertion Length	VRF Target	MiSeq (VRF)	NextSeq 1000 (VRF)
Medium Insert: 42 bp	5.00E-02	3.56E-02	3.51E-02
	5.00E-03	3.23E-03	3.46E-03
	5.00E-04	4.11E-04	3.96E-04
	5.00E-05	3.28E-05	3.88E-05
	1.00E-06	Not Detected	Not Detected
Long Insert: 172 bp	5.00E-02	1.39E-03	1.45E-03
	5.00E-03	9.04E-05	1.11E-04
	5.00E-04	1.93E-05	1.18E-05
	5.00E-05	Not Detected	1.34E-06
	1.00E-06	Not Detected	Not Detected

NPM1 MRD Detection Results			
NPM1 Mutation Type	VRF Target	MiSeq (VRF)	NextSeq 1000 (VRF)
Type: A	1.00E-05	1.30E-05	9.55E-06
	1.00E-06	Not Detected	Not Detected
Type: B	5.00E-05	1.18E-05	8.74E-06
	1.00E-05	Not Detected	2.27E-06
Type: D	5.00E-05	5.03E-05	4.59E-05
	1.00E-05	5.57E-06	1.03E-05
Type: Other	5.00E-05	3.86E-05	9.68E-05
	1.00E-05	Not Detected	1.62E-06

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