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Performance Comparison of Illumina® MiSeq™ and MiSeq™ i100 Systems using Invivoscribe's LymphoTrack® Assays

INTRODUCTION

Next-generation sequencing (NGS) is revolutionizing how gene rearrangement information can be applied to hematological malignancies by overcoming the limitations of traditional PCR-based methods, especially in low tumor burden samples or those requiring complex interpretation. NGS offers sensitivity and specificity, enabling precise distinction between clonal and polyclonal gene rearrangements in both B-cell (Ig)[1] and T-cell receptor (TCR)[2]. In addition to clonality detection, the high resolution of NGS provides the DNA sequences for each gene rearrangement in a sample, with potential clinical applications including risk stratification, therapeutic optimization and measurable residual disease (MRD) detection to identify early relapse, monitor disease evolution, and provide endpoint surrogacy in drug trials. Some clinical guidelines are beginning to recommend MRD testing for several lymphoid cancers, including non-Hodgkin lymphoma (NHL), multiple myeloma (MM), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL).

Over the past decade, Invivoscribe has been at the forefront of NGS-based detection of clonal gene rearrangements and residual disease monitoring and offers LymphoTrack Assays for use with the MiSeq, commercially available, research use only (RUO) assay kits for the identification of Ig and TCR-gene rearrangement sequences to aid in the detection and monitoring of hematological malignancies. Improvements such as increased read depths, streamlined data generation and enhanced sequencing accuracy have generated demand to update existing NGS assays to take advantage of these new features and efficiencies. The newest addition to Illumina's portfolio is the Illumina MiSeq i100 series, which offer several advantages over the original MiSeq, including:

- Up to 4x faster sequencing enables same-day results
- Room temperature storage eliminates reagent thawing
- Simplified sequencing run setup in 3 steps and approximately 20 minutes

Here we present a performance comparison between the Illumina MiSeq and MiSeq i100 system using identical libraries generated by the full suite of B- and T-cell LymphoTrack Assays.

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LymphoTrack NGS Assay Overview

Invivoscribe's LymphoTrack Assays for MiSeq represent a significant improvement over existing fragment analysis assays due to their ability to accurately identify gene rearrangement sequences, the associated sequence frequencies, and associated V- and J-gene families. Therefore, these assays have three important and complementary uses:

1. Identifies clonal gene rearrangement sequences;
2. Generates detailed sequence information that can be used to determine somatic hypermutation status (with *IGH* and *IGHV* Leader); and
3. Facilitates measurable residual disease (MRD) assessment.

LymphoTrack Assays target immunoglobulin heavy chain *(IGH)* V_H-J_H (*IGHV* Leader as well as *IGH* FR1, FR2 and FR3), immunoglobulin light chain *(IGK),* T-cell receptor beta *(TRB)* and T-cell receptor gamma *(TRG)* gene rearrangements. These assays are internationally distributed and include assay controls and up to 24 uniquely indexed master mixes containing primers designed with Illumina adapters. This primer design streamlines the assay workflow, using one-step PCR and allowing target- and sample-level multiplexing by allowing a maximum of 24 different samples and any combination of targets to be batched for sequencing on a single flow cell. The supplemental LymphoTrack Software – MiSeq analyzes the resulting FASTQ files, providing a PDF sample report and tabulated output files for sample interpretation and data visualization.

NGS INSTRUMENT OVERVIEW

Illumina MiSeq and MiSeq i100 Systems

The Illumina MiSeq i100 Plus and MiSeq i100 systems are the newest benchtop sequencers designed to replace the Illumina MiSeq. Faster sequencing time and ease-of-use make the MiSeq i100 systems a cost-effective solution for small to medium-sized laboratories. Moreover, these new systems utilize the XLEAP-SBS chemistry, improving run time, accuracy and shipping stability. Table 1 describes the 2 x 300 bp paired-end read length run metrics and compares specifications of the Illumina MiSeq and MiSeq i100 systems.

Table 1: Instrument Specifications - Illumina MiSeq and MiSeq i100

Metric (per Flow Cell)	MiSeq (Reagent Kit v3)	MiSeq i100 (25M Flow Cell)
PF Reads	44M - 50M reads	50M reads
Run Time	~56 hours	~15 hours
Output	13.2Gb - 15Gb	15Gb
Quality Score	> 70%	≥ 85%
Reagent Type	V3 Standard-SBS	XLEAP-SBS
Reagent Storage	Cartridge: Frozen / Flow Cell: Refrigerated	Cartridge + Flow Cell: Room Temperature

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MATERIALS AND METHODS

LymphoTrack 7-Target Library Construction

Contrived samples were prepared from DNA isolated from clonal (positive) and polyclonal (negative) cell lines, in which the clonal DNA was diluted in polyclonal DNA to create panels containing:

- *IGH* FR1 at 0.5%, 1.0%, 2.5%, 5.0% and 10%
- *IGH* FR2, *IGH* FR3, *IGHV* Leader, *TRB* and *TRG* at 1.0%, 2.5%, 5.0% and 10%
- *IGK* at 2.5%, 5.0%, 10.0% and 20.0%

Varying clonal dilutions were used for each target in order to obtain Percent (%) Reads values within the same range for comparison; these dilutions were previously characterized.

Testing was conducted using the LymphoTrack Assay for the anticipated clonal target, following the associated instructions for use (IFU) procedures. Briefly, target PCR was performed using 50 ng of contrived sample DNA with the LymphoTrack Assay kit for the anticipated clonal target. The amplified targets were purified, quantified, and normalized; finally, equimolar quantities of each target were pooled to create the final 7-target library. This 7-target library was then sequenced on the Illumina MiSeq and MiSeq i100 instruments. An illustration representing the LymphoTrack Assay workflow is shown in Figure 1.

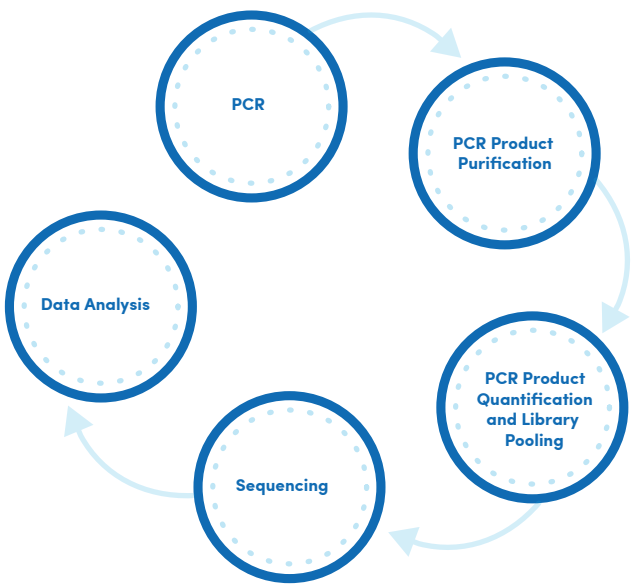

Figure 1: Depiction of LymphoTrack Assay – MiSeq workflow.

LymphoTrack 7-Target Library Sequencing

Aliquots of the 7-target library were sequenced on Illumina's MiSeq and MiSeq i100. The MiSeq sequencing run used the 600 cycle MiSeq v3 reagent kit[A] with paired-end sequencing at a read length of 2 x 300 bp and 12 pM of the 7-target library. The loading concentration of the 7-target library for the MiSeq i100 was calculated using the Illumina loading concentration guidelines; thus, 78 pM of the 7-target library was sequenced with the MiSeq i100 using the 600 cycle MiSeq i100 25M reagent kit[B] with paired-end sequencing at a read length of 2 x 300 bp.

Table 2: Illumina Materials

Catalog #	Description
[A]MS-102-3003	MiSeq Reagent Kit v3 (600-cycle)
[B]20115696	MiSeq i100 25M Reagent Kit (600-cycle)

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Data Analysis using LymphoTrack Software - MiSeq

Sequencing results were analyzed using the LymphoTrack Software – MiSeq version 3.0.0 using the default analysis parameters (which included 500 unique sequences, applied a 2 bp maximum mismatch allowance and referenced the IMGT/V-QUEST database to align sample sequences). The validity of each sequencing run was assessed by verifying results generated by the positive and negative controls for all 7 targets; likewise, sample validity was established by applying a minimum requirement of 20,000 reads. Performance of the MiSeq versus MiSeq i100 was evaluated comparing:

1. the metrics generated by each system:
   - Q30 run quality score;
   - number of pass-filter (PF) reads generated by LymphoTrack Assays; AND
2. LymphoTrack Software analysis results:
   - total number of reads generated per sample per target;
   - classification of the most prevalent / clonal sequence detected—V- and J-gene family assignment; and
   - the Percent (%) Reads value of the Rank 1 sequence (i.e., the most prevalent / clonal sequence).

RESULTS

Illumina MiSeq and MiSeq i100 performance comparison using 7- LymphoTrack targets

Both sequencing runs met the minimum Q30 score requirement for the respective instrument (MiSeq: 77.02%, >70%, and MiSeq i100: 96.89%, ≥ 85%). Both sequencing systems produced similar levels of output; the 7-target library generated 34 million pass filter (PF) reads (20.7 Gb output) on the MiSeq i100, compared to the 32 million PF reads generated on the MiSeq (19.6 Gb output). However, the MiSeq i100 run time (14 hours) was over 3-times faster than that of the MiSeq (53 hours), demonstrating its enhanced efficiency without impacting the quality or amount of sequencing data.

Table 3: Comparison of run metrics using LymphoTrack 7-target library

Metric (per Flow Cell)	MiSeq (Reagent Kit v3)	MiSeq i100 (25M Flow Cell)
Loading Concentration	12 pM	78 pM
Reads Pass Filter	32 M	34 M
Run Time	53 hours	14 hours
Output	19.6 Gb	20.7 Gb
Quality Score	77.02%	96.89%

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To compare the amount of sequencing information produced by each platform, the validity of sequencing results for each sample was evaluated by verifying the total reads generated met the minimum requirement (> 20,000). Both the MiSeq and MiSeq i100 generated more than 20,000 reads for all samples and all LymphoTrack targets; Figure 2 illustrates a comparison of Total Reads values generated by each target for the MiSeq and MiSeq i100 systems.

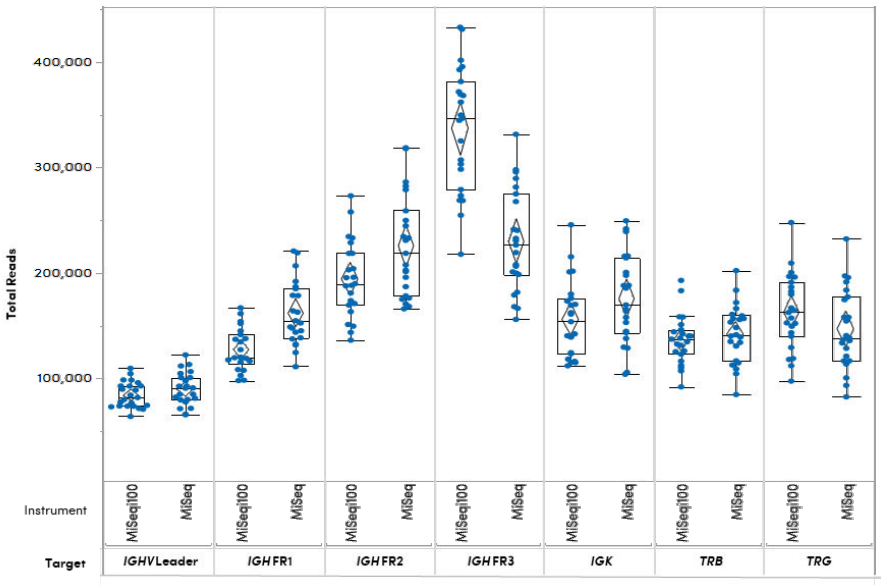


Figure 2: Total Reads Values, MiSeq versus MiSeq i100.

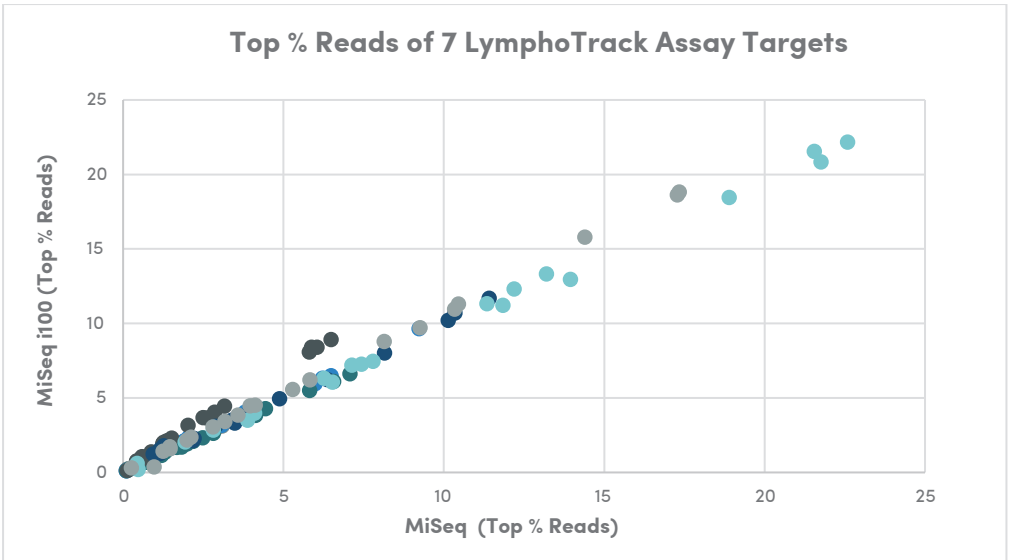
Both the Illumina MiSeq and MiSeq i100 exhibit a similar distribution of Total Reads across the seven targets. The increase in total reads observed for the *IGH* FR3 target in the MiSeq i100 could be explained by increased representation on the patterned MiSeq i100 flow cell; the MiSeq employs a traditional non-patterned flow cell.

Sequence data was analyzed by the LymphoTrack Software – MiSeq (v3.0.0) and evaluation of the V- and J-gene of the most prevalent sequence detected by each platform were compared. All 7 LymphoTrack targets demonstrated concordant results between both sequencing systems. Table 4 describes the known clonal V- and J-gene expected and detected in each of the seven assay targets.

Table 4: Comparison of the Top Read V- and J-genes generated by the 7-LymphoTrack targets between MiSeq and MiSeq i100

LymphoTrack Assay	Clonal Positive Cell Line	Expected Clonal V-Gene	Detected Clonal V-gene		Expected Clonal J-Gene	Detected Clonal J-gene	
			Illumina MiSeq i100	Illumina MiSeq		Illumina MiSeq i100	Illumina MiSeq
IGHV Leader	IVS-0013	IGHV1-46_03	IGHV1-46_03	IGHV1-46_03	IGHJ4_02	IGHJ4_02	IGHJ4_02
IGH FR1			IGHV1-46_03	IGHV1-46_03		IGHJ4_02	IGHJ4_02
IGH FR2			IGHV1-46_03	IGHV1-46_03		IGHJ4_02	IGHJ4_02
IGH FR3			IGHV1-46_03	IGHV1-46_03		IGHJ4_02	IGHJ4_02
IGK	IVS-0030	IGKV3D-20_01	IGKV3D-20_01	IGKV3D-20_01	IGKDEL	IGKDEL	IGKDEL
TRB	IVS-0061	TRBV12-4_01	TRBV12-4_01	TRBV12-4_01	TRBJ1-2_01	TRBJ1-2_01	TRBJ1-2_01
TRG	IVS-0009	TRGV8_01	TRGV8_01	TRGV8_01	TRGJ1_02/ TRGJ2_01	TRGJ1_02/ TRGJ2_01	TRGJ1_02/ TRGJ2_01

The Top Percent (%) Reads values for both Illumina MiSeq and Illumina MiSeq i100 were plotted against one another to compare performance of the assay between both sequencing systems, see Figure 3. Correlation (R^2 value) of the best fit lines was > 0.99 for all seven targets (B-cell and T-cell), indicating strong agreement between Top % Reads results from both the MiSeq and MiSeq i100.



Target	*IGH* FR1	*IGH* FR2	*IGH* FR3	*IGHV* Leader	*IGK*	*TRB*	*TRG*
Dilution Range	0.5% – 10%	1% - 10%	1% - 10%	1% - 10%	2.5% - 20%	1% - 10%	1% - 10%
Correlation (R^2)	0.999	0.998	0.999	0.999	0.997	0.998	0.999

Figure 3: Correlation plot demonstrating Top % Reads generated by MiSeq and MiSeq i100 using 7 LymphoTrack targets

CONCLUSION

In summary, the assessment of NGS metrics generated from a 7-target library prepared with Invivoscribe's RUO LymphoTrack Assays demonstrated that both the MiSeq and the MiSeq i100 produce similar outputs in terms of reads per run (50M reads), with the MiSeq i100 taking less than half the sequencing time of the original MiSeq, allowing for a faster time to result. Comparable results were obtained by sequencing with the MiSeq Standard SBS and MiSeq i100 XLEAP-SBS chemistries, including downstream analyses generated by LymphoTrack Software – MiSeq, which included >20,000 reads per sample in all 7 targets, identical V- and J- gene family identification, and no significant differences between Top % Reads values (R^2 > 0.99) of the Rank 1 sequence for all targets.

Thus, samples tested with LymphoTrack Assays can be sequenced on the MiSeq i100 to identify gene rearrangements and assess clonality. Table 5 lists the recommended sequencing length for different target combinations of LymphoTrack Assays.

Table 5: Read Length compatibility of LymphoTrack Targets

Target Combination(s)	MiSeq Read Length	MiSeq i100 Read Length
Leader plus any combination of LymphoTrack targets	2 x 300 bp	2 x 300 bp
IGH FR1, *IGH* FR2, *IGH* FR3, *IGK*, *TRB*, *TRG*	2 x 250 bp	2 x 250 bp
IGH FR3, *TRG*	2 x 150 bp	2 x 150 bp

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ABOUT INVIVOSCRIBE

Invivoscribe is a global, vertically integrated biotechnology company dedicated to Improving Lives with Precision Diagnostics®. For thirty years, Invivoscribe has enhanced the quality of healthcare worldwide by providing high-quality, standardized reagents, tests, and bioinformatics tools to advance the field of precision medicine. Invivoscribe has a successful track record developing and commercializing companion diagnostics through pharmaceutical partnerships as well as providing expertise in both regulatory compliance and laboratory services. Providing distributable reagents and kits as well as clinical services through our international clinical lab subsidiaries (LabPMM), Invivoscribe is an ideal partner from proof of concept through commercialization.

LymphoTrack Assays and Software for use with MiSeq

Catalog #	Product Description	Quantity
71210059	LymphoTrack *IGHV* Leader Somatic Hypermutation Assay Kit A – MiSeq	8 indices – 5 reactions each
71210069	LymphoTrack *IGHV* Leader Somatic Hypermutation Assay Panel – MiSeq	24 indices – 5 reactions each
71210009	LymphoTrack IGH FR1 Assay Kit A – MiSeq	8 indices – 5 reactions each
71210039	LymphoTrack *IGH* FR1 Assay Panel – MiSeq	24 indices – 5 reactions each
71210149	LymphoTrack *IGH* FR1 Assay Panel B – MiSeq	24 indices – 5 reactions each
71210089	LymphoTrack *IGH* FR2 Assay Kit A – MiSeq	8 indices – 5 reactions each
71210099	LymphoTrack *IGH* FR2 Assay Panel – MiSeq	24 indices – 5 reactions each
71210109	LymphoTrack *IGH* FR3 Assay Kit A – MiSeq	8 indices – 5 reactions each
71210119	LymphoTrack *IGH* FR3 Assay Panel – MiSeq	24 indices – 5 reactions each
71210129	LymphoTrack *IGH* FR1/2/3 Assay Kit A – MiSeq	8 indices per FR region– 5 reactions each
71210139	LymphoTrack *IGH* FR1/2/3 Assay Panel – MiSeq	24 indices per FR region – 5 reactions each
71220009	LymphoTrack *IGK* Assay Kit A – MiSeq	8 indices – 5 sequencing reactions each
71220019	LymphoTrack *IGK* Assay Panel – MiSeq	24 indices – 5 sequencing reactions each
72250009	LymphoTrack *TRB* Assay Kit A – MiSeq	8 indices – 5 reactions each
72250019	LymphoTrack *TRB* Assay Panel – MiSeq	24 indices – 5 reactions each
72270019	LymphoTrack *TRG* Assay Kit A – MiSeq	8 indices – 5 reactions each
72270009	LymphoTrack *TRG* Assay Panel – MiSeq	24 indices – 5 reactions each
S100010	LymphoTrack Software - MiSeq	1 software package

To learn more about how you can use Invivoscribe's assays on Illumina's MiSeq i100 system, **please email inquiry@invivoscribe.com**. To learn more about Invivoscribe and LabPMM, **visit us online at invivoscribe.com** or **call us at +1.858.224.6600.**

References 1. Ho et al. J Mol Diag. 2021 May;23(7): 805–815. | 2. Arcila et al. J Mol Diag. 2019 March;21(2): 330–342.