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USING NEXT-GENERATION SEQUENCING TO DETECT CLONAL *TRG* AND *TRB* GENE REARRANGEMENTS

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Background

T-cell malignancies arise from transformation and clonal expansion of a single cell. During T-cell development, the T cell receptor gamma (*TRG)* locus rearranges prior to the T cell receptor beta (*TRB)* locus. Combined, *TRG* and *TRB* can identify the vast majority of T-cell rearrangements. Historically, these clonal rearrangements are often identified by capillary electrophoresis (CE) methods which provide size distribution information, but not the sequence needed for tracking residual disease during the course of treatment. Recently, next-generation sequencing (NGS)-based approaches for immune receptor genes have been developed to improve the sensitivity of clonal detection and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We have developed and validated LymphoTrack® *TRG* & *TRB* clonality assays for the Illumina® MiSeq® platform.

Material and Methods

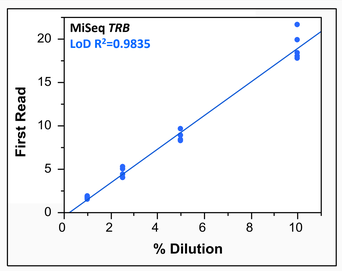
- Schematic Illustration of the *TRB* gene

•The workflow for the LymphoTrack® *TRG & TRB* Assays – MiSeq®

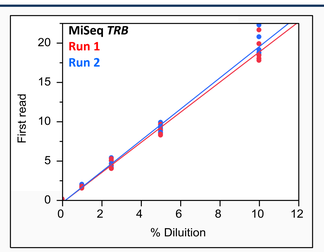
- The LymphoTrack® *TRG* and *TRB* assays for the MiSeq® were manufactured under cGMP standards and QC tested under a QSR-compliant regulatory system prior to use.
- Limit of detection (LoD), linearity, precision and reproducibility (P/R) were tested using clonal control DNA diluted in wild-type polyclonal (tonsil) DNA.
- 12 cell line DNA samples were tested with the *TRB* assay.
- DNA from 49 FFPE samples were extracted using common extraction methods by collaborators. All samples were tested with the *TRG* and *TRB* assays.
- Libraries were prepared with amplicons generated by PCR using proprietary multiplex master mixes with the consensus primers targeting all *TRG* and *TRB* V, (D) and J exon families, synthesized with MiSeq specific adapters and 24 index sequences optimized for NGS.
- Libraries were either sequenced for *TRB* and *TRG* individually or for *TRB* + *TRG* combined.
- LymphoTrack® Software – MiSeq® analyzed FASTQ data from the MiSeq.
- All statistical analyses were performed in JMP.

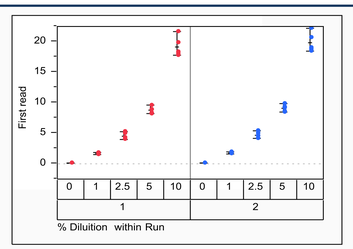
Results: LOD, LOB and Linearity

Clonal Control Dilutions (%)	N	*TRB* MiSeq				
		Size (bp)	Mean % Reads	CV%	Upper 95%	Lower 95%
10%	5	198	19.12	8.5	17.67	20.57
5%	5	198	8.78	6.3	8.29	9.27
2.5%	5	198	4.5	12.5	4.00	5.00
1%	5	198	1.7	8.2	1.57	1.83
Tonsil	3	197	0.1	30.6	0.07	0.13

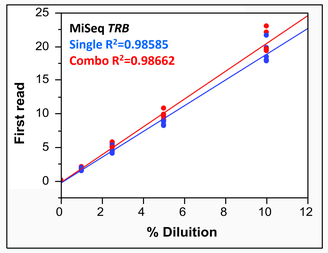


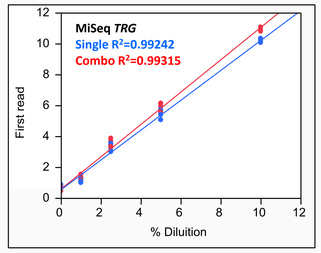
Results: Precision and Reproducibility





Results: *TRG* + *TRB* Combo Assay





Results: Top 5 Sequences % for Normal PB Samples

Sample	Rank	V-gene	J-gene	% total reads
PB 17.7	1	TRBD1	TRBJ1-1	0.35
	2	TRBD1	TRBJ1-6	0.26
	3	TRBD2	TRB_J2-7	0.23
	4	TRBV12-4	TRBJ1-1	0.21
	5	TRBV4-1	TRB_J2-7	0.21
PB 34.5	1	TRBV29-1	TRBJ1-1	0.25
	2	TRBD2	TRB_J2-1	0.18
	3	TRBD1	TRBJ1-1	0.18
	4	TRBD2	TRB_J2-7	0.15
	5	TRBV14	TRB_J2-2	0.11
PB 43.3	1	TRBV11-2	TRBJ1-1	1.08
	2	TRBD2	TRB_J2-7	0.36
	3	TRBD2	TRB_J2-1	0.29
	4	TRBD1	TRBJ1-2	0.27
	5	TRBV2	TRBJ1-5	0.26
PB 45.4	1	TRBD2	TRBJ1-2	0.23
	2	TRBV4-1	TRB_J2-2	0.19
	3	TRBV9	TRB_J2-7	0.14
	4	TRBV4-2	TRB_J2-2	0.13
	5	TRBV29-1	TRB_J2-7	0.13
PB 48.2	1	TRBV2	TRB_J2-5	0.86
	2	TRBV20-1	TRB_J2-7	0.52
	3	TRBV6-4	TRBJ1-4	0.51
	4	TRBV20-1	TRB_J2-7	0.32
	5	TRBV12-4	TRB_J2-7	0.25
PB 9.4	1	TRBD2	TRB_J2-5	0.85
	2	TRBD1	TRBJ1-5	0.49
	3	TRBV14	TRBJ1-1	0.45
	4	TRBV23-1	TRBJ1-1	0.35
	5	TRBD2	TRB_J2-5	0.31

Results: Cell Lines

Sample	Rank	V-gene	J-gene	Total Reads %
CCR-CEM	1	TRBV3-1	TRB_J2-3	95.29
CML-T1	1	TRBV19	TRB_J2-5	75.86
DND-41	1	TRBV18	TRBJ1-2	69.85
	2	TRBV6-3	TRB_J2-7	15.79
HPB-ALL	1	TRBV7-3	TRB_J2-5	52.75
	2	TRBV5-5	TRB_J2-5	24.42
LOUCY	1	TRBV20-1	TRB_J2-2	78.50
	2	TRBV5-6	TRB_J2-1	7.65
MOLT-3/4	1	TRBV20-1	TRB_J2-1	40.04
	2	TRBV10-3	TRB_J2-5	40.71
PEER	1	TRBV4-2	TRB_J2-3	91.04
PF-382	1	TRBV20-1	TRB_J2-1	48.08
	2	TRBV7-9	TRB_J2-7	18.85
RPMI-8402	1	TRBV15	TRBJ1-5	83.68
	2	TRBV19	TRB_J2-7	8.20
MOLT-13	1	TRBV10-1	TRBJ1-1	37.33
	2	TRBD2	TRB_J2-3	24.96
JURKAT	1	TRBV12-4	TRBJ1-2	76.17
	2	TRBD1	TRBJ1-3	17.33
HSB-2	1	TRBV5-1	TRBJ1-1	92.37

Results: Clinical Study

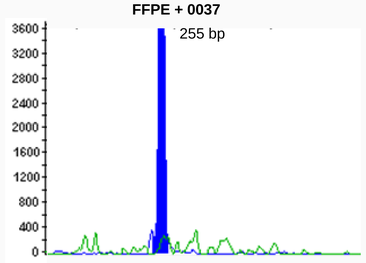
TRB MiSeq	
Clonal (%)	15/49 (31%)
Non-Clonal (%)	34/49 (69%)

		TRB Identiclone	
		Clonal	Non-Clonal
TRB MiSeq	Clonal	8	0
	Non-Clonal	1	11

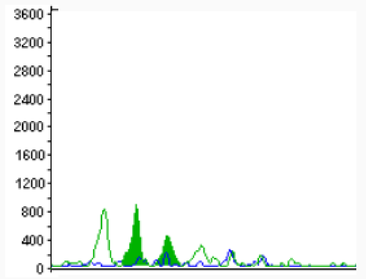
TRB MiSeq vs. Identiclone	
Concordance (%)	95
Sensitivity (%)	89
Specificity (%)	100
PPV (%)	100
NPV (%)	92

CE

FFPE + 0037

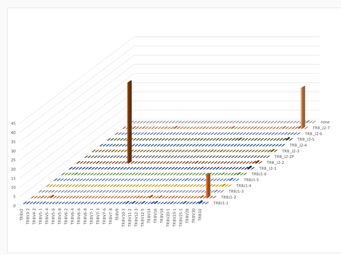


FFPE - 0020



TRB MiSeq

FFPE + 0037



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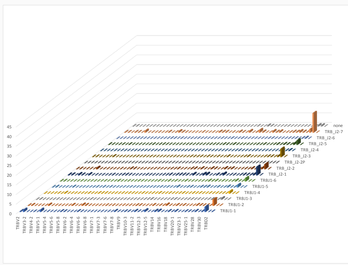

Sample	V-gene	J-gene	*TRB* Total Reads %	*TRB* MiSeq	*TRG* MiSeq	*TRB* Identiclone (CE)
Sample_0020	TRBD2	TRB_J2-2	0.69	Non-clonal	Non-clonal	Non-clonal
Sample_0021	TRBV11-2	TRBJ1-2	0.50	Non-clonal	Non-clonal	Non-clonal
Sample_0022	TRBV14	TRBJ1-2	0.43	Non-clonal	Non-clonal	Non-clonal
Sample_0024	TRBV12-4	TRB_J2-5	39.96	clonal	clonal	clonal
Sample_0025	TRBD1	TRBJ1-5	22.22	clonal	clonal	clonal
Sample_0026	TRBD2	TRB_J2-2	0.53	Non-clonal	Non-clonal	Non-clonal
Sample_0027	TRBD2	TRB_J2-2	2.46	Non-clonal	Non-clonal	Non-clonal
Sample_0028	TRBV14	TRBJ1-1	0.84	Non-clonal	Non-clonal	Non-clonal
Sample_0029	TRBV12-4	TRBJ1-2	0.89	Non-clonal	Non-clonal	Non-clonal
Sample_0030	TRBV29-1	TRBJ1-2	1.18	Non-clonal	Non-clonal	Non-clonal
Sample_0031	TRBD2	TRB_J2-2	0.68	Non-clonal	Non-clonal	Non-clonal
Sample_0032	TRBD1	TRB_J2-7	37.96	clonal	clonal	clonal
Sample_0033	TRBV28	TRB_J2-7	33.65	clonal	clonal	clonal
Sample_0034	TRBV18	TRBJ1-6	5.10	Non-clonal	Non-clonal	clonal
Sample_0035	TRBV12-4	TRBJ1-2	0.92	Non-clonal	Non-clonal	Non-clonal
Sample_0036	TRBD1	TRBJ1-4	22.96	clonal	clonal	clonal
Sample_0037	TRBV6-4	TRB_J2-2	37.47	clonal	clonal	clonal
Sample_0038	TRBV14	TRBJ1-1	4.85	Non-clonal	Non-clonal	Non-clonal
Sample_0039	TRBD1	TRBJ1-1	28.61	clonal	clonal	clonal
Sample_0040	TRBV4-3	TRBJ1-2	15.69	clonal	clonal	clonal

Conclusions

- The LymphoTrack® *TRB* Assay – MiSeq® was able to consistently detect all known *TRB* clonal rearrangements from cell line DNA.
- Excellent linearity (R^2>0.90), sensitivity of clonality (2.5%), and reproducibility (<20% CV) were demonstrated with serial dilutions of contrived cell line DNA.
- Concordance between the LymphoTrack® *TRB* and CE assays was 95% and between the LymphoTrack® *TRG* and *TRB* assays was 94%.