TT45

# Detection of Clonal *TRG* and *TRB* Gene Rearrangements Using Next-Generation Sequencing

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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 T cell receptor beta (*TRB)* locus. Combined, *TRG* and *TRB* assays can identify the vast majority of T-cell rearrangements in T-cell as well as some B-cell malignancies (e.g., B-ALL). Historically, clonal rearrangements have been identified using capillary electrophoresis (CE) methods, which provide size distribution information, but not the sequence needed for tracking minimal residual disease (MRD) throughout the course of treatment. Recently, we have developed next-generation sequencing (NGS) assays for immune receptor genes that improve both the sensitivity of clonality detection and identify the specific V(D)J DNA sequences required to track clones in follow-up MRD testing. Here we present data from the LymphoTrack® *TRG* and *TRB* Clonality Assays developed for the Illumina® MiSeq® platform and compare results with the IdentiClone® CE-based assays.

## Material and Methods

- Schematic Illustration of the *TRG* gene


- Schematic Illustration of the *TRB* gene

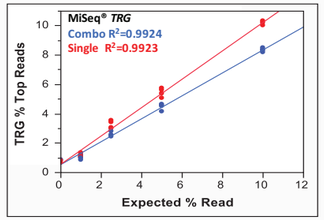

- Workflow for the LymphoTrack® *TRG & TRB* Assays - MiSeq®

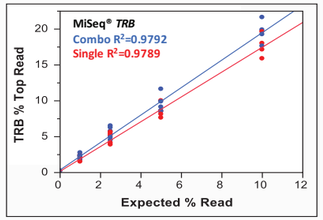

- The LymphoTrack® *TRG* and *TRB* Assays for the MiSeq® were cGMP manufactured, QC tested, and analyzed using LymphoTrack® bioinformatics software developed under full design control consistent with our ISO13485-compliant regulatory system.
- Limit of detection (LoD), linearity, precision and reproducibility (P/R) were tested using clonal control DNA diluted in wild-type polyclonal (tonsil) DNA for *TRB* assay.
- DNA from 60 formalin-fixed paraffin-embedded (FFPE) samples were extracted using common extraction methods by collaborators. All samples were tested with *TRG* and *TRB* Assays .
- Contrived cell line was used for MRD detection at $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ sensitivity with an internal control spiked in to reactions at approximately 1000 cell equivalence.
- 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 fa``milies, synthesized with MiSeq® specific adapters and 24 index sequences optimized for the NGS platform.
- Libraries were either sequenced for *TRB* and *TRG* individually or for *TRB* + *TRG* combined.
- LymphoTrack® Software - MiSeq® was used to analyze FASTQ data generated from the MiSeq®.
- When comparing testing results, only samples that met the specimen and data acceptance criteria for both methods were evaluated
- All statistical analyses were performed in JMP®.

## Conclusion

- The LymphoTrack® MiSeq® *TRB* assay demonstrated excellent linearity ($R^2$>0.90), sensitivity (as low as 2.5%), and reproducibility (<20%CV) testing serial dilutions of contrived cell line DNA.
- Concordance between the LymphoTrack® *TRB* - MiSeq® and IdentiClone® *TRB* assays was 86.4%.
- Concordance between the LymphoTrack® *TRG* - MiSeq® and IdentiClone® *TRG* assays was 94.1%
- Concordance between the combined *TRB+TRG* LymphoTrack® MiSeq® and IdentiClone® assays was 84.1%
- Excellent linearity ($R^2$>0.90) for contrived cell line, reproducibility (<20% CV) for control cell line, and clonal detection for MRD at $10^{-5}$ sensitivity was demonstrated for LymphoTrack® MiSeq® *TRG* and *TRB* assays.

## Results: *TRG* /*TRB* LoD, LoB and Linearity



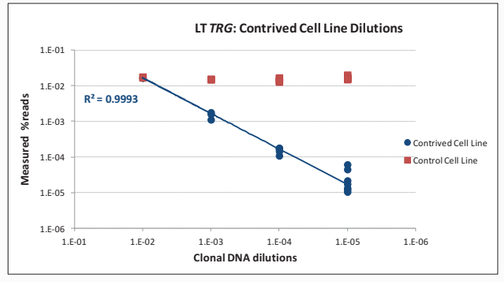




## Results: *TRG*/*TRB* Precision and Reproducibility

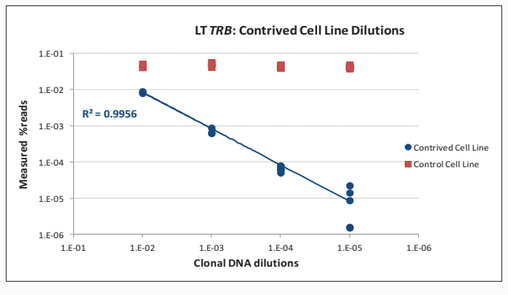
| Clonal Control Dilutions (%) | *TRG* MiSeq® Size (bp) | *TRG* MiSeq® Mean % Reads | *TRG* MiSeq® CV% | *TRB* MiSeq® Size (bp) | *TRB* MiSeq® Mean % Reads | *TRB* MiSeq® CV% |
|---|---|---|---|---|---|---|
| 10 | 147 | 8.66 | 10.4 | 198 | 17.63 | 16.7 |
| 5 | 147 | 4.59 | 12.0 | 198 | 9.35 | 18.4 |
| 2.5 | 147 | 2.39 | 11.7 | 198 | 5.25 | 19.6 |
| 1 | 147 | 0.94 | 13.8 | 198 | 2.04 | 20.0 |
| 0 | varies | 0.62 | 40.3 | varies | 0.17 | 46.8 |

## Results: *TRG/TRB* MRD Detection

LymphoTrack® *TRG* Assay-MiSeq®

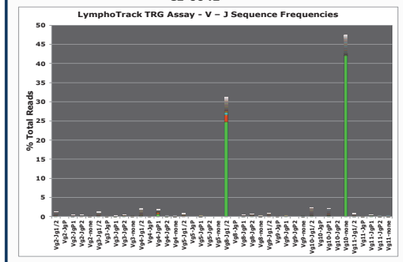



LymphoTrack® *TRB* Assay-MiSeq®



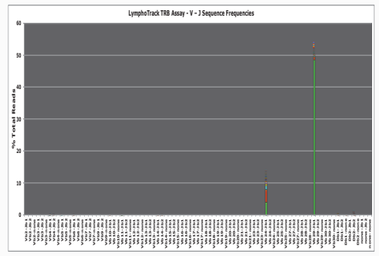

## Results: *TRG/TRB* Positive Clinical Sample

CE-0041




*TRG* Positive (Clonal)

CE-0041




*TRB* Positive (Clonal)

## Results: Clinical Study between *TRB* MiSeq® and IdentiClone® Assays

n=44

| *TRB* MiSeq® | *TRB* Identiclone: Clonal | *TRB* Identiclone: Non-Clonal |
|---|---|---|
| Clonal | 18 | 0 |
| Non-Clonal | 6 | 20 |

| *TRB* MiSeq® vs. *TRB* IdentiClone® (Tube A/B/C) | |
|---|---|
| Concordance (%) | 86.4 |
| Sensitivity (%) | 75.0 |
| Specificity (%) | 100.0 |
| PPV (%) | 100.0 |
| NPV (%) | 76.9 |

## Results: Clinical Study Comparing *TRG* MiSeq® and IdentiClone® Assays

n=51

| *TRG* MiSeq® | *TRG* Identiclone *2.0*: Clonal | *TRG* Identiclone *2.0*: Non-Clonal |
|---|---|---|
| Clonal | 14 | 1 |
| Non-Clonal | 2 | 34 |

| *TRG* MiSeq® vs. *TRG* IdentiClone® 2.0 | |
|---|---|
| Concordance (%) | 94.1 |
| Sensitivity (%) | 87.5 |
| Specificity (%) | 97.1 |
| PPV (%) | 93.3 |
| NPV (%) | 94.4 |

## Results: Clinical Study for LymphoTrack® *TRG* + *TRB* Assays - MiSeq®

| | *TRB* MiSeq® | *TRG* MiSeq® | *TRB + TRG* MiSeq® |
|---|---|---|---|
| Clonal | 19/60 (32%) | 16/60 (27%) | 20/60 (33%) |
| Non-Clonal | 41/60 (68%) | 43/60 (73%) | 39/60 (65%) |

n=44

| *TRB + TRG* MiSeq® | *TRB +TRG* IdentiClone®: Clonal | *TRB +TRG* IdentiClone®: Non-Clonal |
|---|---|---|
| Clonal | 18 | 0 |
| Non-Clonal | 7 | 19 |

| *TRB + TRG* MiSeq® vs. IdentiClone® | |
|---|---|
| Concordance (%) | 84.1 |
| Sensitivity (%) | 72.0 |
| Specificity (%) | 100.0 |
| PPV (%) | 100.0 |
| NPV (%) | 73.1 |



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