POSTER # 6304 LymphoTrack® Enterprise Software v3.0.1: A High-Throughput Software Solution for Immunogenomic Analysis

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INTRODUCTION

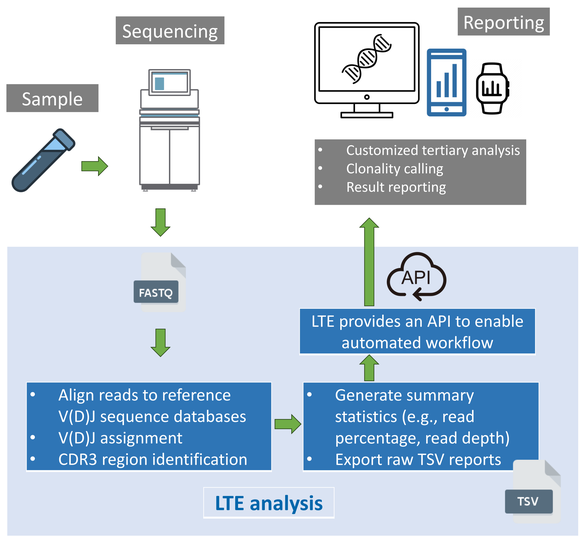
LymphoTrack® Assays - MiSeq™ are set of Research Use Only (RUO) next-generation sequencing (NGS) assays designed for immunogenomic applications, including clonality detection and measurable residual disease (MRD) tracking. These assays target immunoglobulin (Ig) and T-cell receptor (TCR) genes, helping researchers analyze immune repertoires with high sensitivity and specificity. LymphoTrack Enterprise Software v3.0.1 (LTE) is a powerful, high-throughput Research Use Only (RUO) software suite designed to analyze RUO LymphoTrack Assays designed for Illumina and Complete Genomics sequencing instruments.

- LTE is a bioinformatics solution that addresses the growing demand and needs of high-volume sequencing labs which are involved in increasingly important immunogenomic sequencing.
- LTE allows for broad sequencing platform compatibility and enhanced automation to support diverse experimental designs and large-scale studies for both clonality detection and MRD tracking in follow-up samples.
- Unlike the desktop RUO LymphoTrack Software – MiSeq, which is optimized for smaller-scale users, LTE introduces enterprise-level architecture with:
  - Containerization for efficient deployment and scalability.
  - Integration via a rest API for Laboratory Information Management Systems (LIMS).
  - Utilization on premise solutions or other scalable infrastructure.
- New features of LTE v 3.0.1 include:
  - Support for multiple sequencing technologies (e.g., Illumina, Complete Genomics), ensuring flexibility in assay development.
  - Speed and accuracy– optimized algorithms reduce data processing time and enhance V-gene and J-gene assignment.
  - Interoperability with LIMS to facilitate sample tracking and automated notifications.
  - References IgBlast and IMGT databases.

METHODS & WORKFLOW

- **Software Capabilities:**
  - Containerized architecture for scalability.
  - Allows users to choose between hands-on analysis or automation-enabled workflows.
  - Parallelized data processing for increased speed.
  - Reads compressed FASTQ files directly without decompression, significantly improving speed and reducing I/O overhead.
  - New algorithm increases accuracy and speed of merging similar reads by filtering previously merged sequences and optimizing subsequence matching.
  - Improved CDR3 identification by incorporating V and J gene alignments and reference anchor positions.
- **Supported Sequencing Platforms:**
  - Illumina® MiSeq™
  - Illumina® NextSeq™1000
  - Complete Genomics: DNBSEQ-G99
- **Data Processing Steps:**
  - FASTQ recognition & adapter trimming (for DNBSEQ-G99 only); identifies clonal expansions within immune repertoires.
  - Detects low-frequency MRD clones with high sensitivity, supporting disease monitoring and relapse prediction analysis.
  - CDR3 region identification using updated immunogenetic databases.
- **API Integration**
  - Can be accessed through an API to facilitate automated result retrieval and reporting. This allows seamless integration with LIMS.
  - The API supports custom queries, enabling users to filter, extract, and format data in a way that best fits their reporting and downstream analysis needs.
  - Automation reduces processing bottlenecks, allowing for high-throughput sequencing workflows to run more efficiently.

Workflow diagram of an automated LTE processing pipeline



RESULTS & PERFORMANCE METRICS

Processing Time Improvements

- LTE significantly reduced MiSeq processing time approximately 14-fold, from 30.3 ± 1.50 min to 2.2 ± 0.34 min (n=3 runs, 24 samples each)
- Number of runs and samples in platform-specific analysis (Table 1):
  - NextSeq1000: n=4 runs, 96 samples.
  - DNBSEQ-G99: n=3 runs, 24 samples.

CDR3 Identification Accuracy (Table 2)

- LTE achieved a CDR3 identification rate of 98.6%, compared to 58.4% in the previous software version.

Broad Platform Compatibility

- LTE processes DNBSEQ-G99 FASTQ files, trims adapters, and performs clonality analysis efficiently.

Table 1: Runtime Performance between softwares

Sequencers	LTE v3.0.1	RUO LymphoTrack 2.4.6
MiSeq	2.2 ± 0.34 min	30.3 ± 1.50 min
NextSeq1000	8.98 ± 2.23 min	NA
DNBSEQ-G99	4.6 ± 9.49 min (*)	NA

(*) The adapter trimming step before analysis increases the total run time by 65.7 ± 7.06 minutes.

Table 2: Runtime Performance between algorithms

Sequences Analyzed	LT-CDR3	LTE-CDR3
Top 200	307 ms	5 ms
Top 10,000	N/A	64 ms

Table 3: Total Number of CDR3 Sequences Identified in top 200 sequences

Target	LT-CDR3		LTE-CDR3	
	Proportion	Percent	Proportion	Percent
IGH FR1	65542/ 107864	60.8%	105207/ 107781	97.6%
IGHV Leader	44108/ 56480	78.1%	54431/ 56474	96.4%
TRG	19416/ 56600	34.3%	54007/ 56525	95.5%
Total	129066/ 220944	58.4%	213645/ 220780	98.6%

CONCLUSIONS & FUTURE DIRECTIONS

- LTE v3.0.1 improves processing **speed, automation, and platform compatibility,** streamlining clonality and MRD analyses for high-throughput NGS workflows.
- Achieving a **98.6% CDR3 identification rate**, LTE significantly improves the precision of immune receptor profiling, critical for clinical diagnostics, immune monitoring, and biomarker discovery.
- Upcoming updates will extend LTE's compatibility to additional sequencing platforms, including **MiSeq i100,** ensuring adaptability to evolving sequencing technologies.
- LTE is a top-tier solution for large-scale clonality and MRD analyses, making it a key tool in immunogenomics research and automated testing environments.

ACKNOWLEDGEMENTS & REFERENCES

- Supported by Invivoscribe, Inc.
- Ye, J., Ma, N., Madden, T.L., & Ostell, J.M. (2013). IgBLAST: an immunoglobulin variable domain sequence analysis tool. *Nucleic Acids Research*, 41(W1), W34–W40.
- Brochet, X., Lefranc, M.-P., & Giudicelli, V. (2008). IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Research*, 36(Web Server issue), W503–W508.
- Giudicelli, V., Chaume, D., & Lefranc, M.-P. (2005). IMGT/GENE-DB: a comprehensive database for human and mouse immunoglobulin and T cell receptor genes. *Nucleic Acids Research*, 33(Database issue), D256–D261.

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