Complete Suite of NGS Clonality Assays with Bioinformatics - Identification and Tracking Patient-Specific Clones

Outline
- Part I: LymphoTrack Products
  - What are different types clonality targets?
  - How diversity is generated?
  - What is LymphoTrack Assay used for?
  - Why test clonality?
  - How clonality was tested?
  - Overview of TRB gene, Multiplexing and Workflow
  - LymphoTrack Assay for MRD
- Part II: LymphoTrack Data Analysis – Identification and Tracking Patient-specific Clones

Clonality Targets

Human IGH locus

Human IGK locus

Human TRB locus
Human TRG locus

TRGC: 1
TRGC: 2
TRGC: 3
TRGJ: 1
TRGJ: 2
TRGJ: 3
TRGV: 12-15
160 kb (19-22 genes)

Terminus: TRGC
Antiparallel: TRGC
Terminus: TRGJ
Chromosome 7p14


Generation of Diversity: IGH Rearrangement

Gene Rearrangements Are
- Lymphoid-specific
- Developmentally-regulated
- Sequential

Step 1: D-J Rearrangement

IGH, TRB & TRD V, D, and J
IGH, IGK, and TRG V and J regions

B- and T-Cell Gene Rearrangements
- During B- and T-Cell Development and Maturation
- Genetic Recombination Process at the DNA Level
- Each of the V, D and J Gene Segments are Randomly Recombined
- Encode Unique Antigen Receptors up to 10^14
- Sources of Diversity:
  - Gene Rearrangements
  - N-region Diversity
  - Somatic Hypermutations

Polyclonal vs. Clonal Progression

Polyclonal Progression
- Highly indicative of B- or T-cell malignancy

Clonal Progression
- RUO (Research Use Only)
- CE Marked IVD (Clinical Diagnostic Utility)
- GPR controls (General Purpose Reagents (DNA and RNA))

Why Test for B- and T-Cell Clonality?

Leukemias and lymphomas can be challenging to diagnose by
- morphology
- immunohistochemistry
- flow cytometry

Using these methods, 5-15% of cases result in inconclusive diagnoses.

Diagnosis of lymphoid malignancies is greatly supported and facilitated by clonality testing.
- Being adopted in routine diagnostics specifically to track disease with MRD testing.

Our Focus

PCR-based molecular testing for B- and T-cell malignancies associated with leukemias and lymphomas
- Gene Clonality / Rearrangements
- Chromosome Translocations
- Gene Mutations
Detection Methods

- PCR-based clonality assays are now widely accepted as the gold standard, with improved:
  - Sensitivity
  - Testing time
  - Coverage
  - Limit of detection
  - Sample type suitability

Evolution of Clonality Testing

With the evolution of technology, clonality analysis can now be performed using Next Generation Sequencing (NGS) technologies.

Advantages of NGS Clonality Testing

- Determines the DNA sequence of clonal rearrangements
- Reduces subjectivity in interpretation
- Identifies the full range of clonal populations in a specimen
- Allows both identification and tracking of clonal populations with the same reagents and workflow
- Can be standardized between labs and cleared or approved by Regulatory Agencies Worldwide

LymphoTrack Dx Portfolio

Available Assays & Software

- TRG
- TRB - New! MiSeq
- TRB - Ion PGM/S5 in development
- IGHV Leader
- IGH FR1/2/3 Combo
- IGH FR1
- IGH FR2
- IGH FR3
- IgK

- LymphoTrack® Dx Software
- LymphoTrack® MRD Software - Research Use Only

One assay – Multiple applications!

- Clonality
- Somatic Hypermutation
- Minimal Residual Disease*
- CAR-T Cell & Immunotherapy Tracking

*LymphoTrack® is registered trademark of Invivoscribe Technologies, Inc.
Advantages

- Tests Compatible with Range of Specimen Types
- ONE-STEP PCR Master Mixes
- MULTIPLEXING – Reduces Costs
  - Combine Multiple Samples - up to 12 (Ion PGM) & 24 (MiSeq)
  - Combine Multiple Assays - up to 72 or 168 samples
- Unparalleled Sensitivity
  - Unparalleled clonality detection with the ability to identify and track the specific sequence of clonal populations for MRD studies.
- INCLUDED Analysis SOFTWARE Package

LymphoTrack®Dx TRB Assay - MiSeq®

- T-cell malignancies arise from clonal expansion of single cell.
- During early T-cell development, somatic rearrangements occur within T cell receptor beta (TRB) locus that bring together, sequentially, the joining of D and J gene segments, followed by joining of a V segment to the DJ pair.
- Next-generation sequencing (NGS) based gene rearrangement methods may improve the sensitivity of clonal detection and identify the specific V(D)J DNA sequences.
- T cell receptor gamma (TRG) locus rearranges prior to T cell receptor beta (TRB) locus and testing both loci can identify the vast majority of T-cell malignancies.

| 24 indices for Panel and 8 indices for Kit A
| Positive and negative control

| 1 step/PCR
| Specific target

<table>
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<th>Name</th>
<th>No.</th>
<th>Template</th>
<th>Template</th>
<th>PCR</th>
<th>No.</th>
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<td>22</td>
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Minimal Residual Disease (MRD)

Leukemic cells that remain during or after treatment when a patient is in remission

**Major cause of relapse; Use to determine:**
- Has treatment eradicated clonal cells?
- Efficacy of different treatments
- Monitor patient remission status
- Detect recurrence

**Benefits of NGS vs. Flow Cytometry & ASO-PCR:**
- Better sensitivity & specificity
- Can be standardized across treatment centers
- No custom assay development required
- Sequence identity / frequency distribution of all alleles
- Track multiple clones

**Applications**
-Minimal Residual Disease (MRD)
-Leukemic cells that remain during or after treatment when a patient is in remission
-Uses to determine:
  - Has treatment eradicated clonal cells?
  - Efficacy of different treatments
  - Monitor patient remission status
  - Detect recurrence
-**Benefits of NGS vs. Flow Cytometry & ASO-PCR:**
  - Better sensitivity & specificity
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  - No custom assay development required
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**Summary of All LymphoTrack Assays - MiSeq**

<table>
<thead>
<tr>
<th>Assay</th>
<th>IGHV Leader SHM</th>
<th>IGH FR1</th>
<th>IGH FR2</th>
<th>IGH FR3</th>
<th>IGK</th>
<th>TRG</th>
<th>TRB</th>
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<tr>
<td><strong>Target Size (bp)</strong></td>
<td>483</td>
<td>295</td>
<td>243</td>
<td>104</td>
<td>222</td>
<td>147</td>
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<td><strong>Amplicon Size</strong></td>
<td>660</td>
<td>450</td>
<td>390</td>
<td>260</td>
<td>410</td>
<td>300</td>
<td>400</td>
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</table>

**Recommended Sequencing Kit**
- MiSeq v3 Reagent (600-cycle)
- MiSeq v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)
- MiSeq v2 Reagent (300-cycle) or MiSeq v3 Reagent (600-cycle)
- MiSeq v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)

**Multiplexing Can Reduce Run Cost**

- **# of Gene Targets Analyzed**
  - 12
  - 24

When multiplexing analyses of different gene targets, it is important to use the appropriate sequencing chemistry. The number of sequencing cycles must suffice to sequence the largest amplicon of the multiplex.
LymphoTrack® Data Analysis – Identification and Tracking Patient-Specific Clones

Kasey Hutt, Ph.D.
Staff Scientist, Bioinformatics

Can we include both logs as workflow applies to both LymphoTrack and LymphoTrack Dx Assays. Don’t think you should include Assays as part of the logo here as it doesn’t read right.

LymphoTrack® (Dx) Software Features

• Simultaneously analyze multiple targets
• Rich data
• Free software upgrades
• Clear, exportable data/graphics
• For MiSeq®, PGM™, S5 FASTQ files
• Free of charge
• Easy to run
• Local analysis/no internet
• Low computer requirements

Software Workflow

Copy the LymphoTrack.zip file to a local drive
Extract the.zip file
Run the Analysis Software
Open Excel Visualizations

LymphoTrack® Dx Software – CD Content

LymphoTrack® Dx Software – Processing Data

LymphoTrack Assays are for Research Use Only (RUO)
LymphoTrack Dx are CE-marked; Available Outside of North America

Open the startLymphoTrackMiSeq.jar or PGM.jar file
LymphoTrack® Dx Software – License Agreement

LymphoTrack Assays are for Research Use Only (RUO)
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LymphoTrack® Dx Software – Processing Data

Select target(s) represented in pooled library

Data Output – TRB Assay

Importance of Merge Read Summaries

Amplification & Sequencing May Cause Artificial Nucleotide Changes

Merged Read Summary:
The Read Summary tab only shows the top 10 reads after merging with the top 500 reads that differ in 1 or 2 nucleotides

Where to Find SHM Data?

LymphoTrack® Dx Visualization Automation

PDF Report Automatically Generated by Analysis Software for Each Target and Sample

Report will include:
- Top 10 merged read summary
- Top 200 sequence frequency graph
- Top 200 gene usage graph
- Top 200 read summary
Top 200 Read Summary
Unmerged Raw Data – Can Be Several Pages

Top 10 Merged Read Summary
- Identical Sequences Within 1-2 bp are Merged to Improve Accuracy and Simplify Analysis
- Sequence Can Easily Be Copied and Pasted for MRD Tracking

New LymphoTrack® MRD Software
Includes:
- Project Planner
- Replicate Analysis
- Bi-allelic Tracking
- Local Installation
- PDF Output

LymphoTrack® MRD Software
Project Planner
- Calculate confidence based on read depth, replicate count, and DNA input
Simple, Efficient Bioinformatics
- Bi-allelic tracking enables simultaneous tracking of 2 sequences
- Track across multiple replicates which allows determinations of much greater sensitivity
Automated Report Output
- Previous outputs required manual generation of each index sample, resulting in 20+ minutes of overhead, per target. Automated PDF report output cuts this time down to a few seconds.

MRD Software - Data Analysis
Input Data:
1. Select Gene Target
2. Name the Project
3. Paste Sequence of Interest
4. Enter Sequence Name
5. Enter Replicate Information

MRD Software - Data Input
Step 5: Entering Replicates

5.1 Select Unique Reads File
5.2 Entering Replicate Information:
   - DNA Amount,
   - Number of Replicates and
   - Save Replicates

Step 6: Save Replicates
6.1 Click “Back”

Perform Analysis

MRD Software – Detected Sequence

MRD Software – Not Detected Sequence

Project Planner
PDF Output

Future Additions

- Better quantification
- Optimized data flow between Clonality and MRD analysis
- Timepoint aggregation

Thank You Everyone at IVS!

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- Maggie Kaminsky
- Presha Shah
- Ying Huang

For more information on our LymphoTrack Clonality Suite please contact us at: sales@invivoscribe.com or sales-eu@invivoscribe.com if located outside of North America.

Thank you very much!

Questions?