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Personalized Molecular Medicine®

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

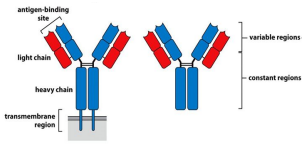
Presha Shah, Ph.D.
Development Scientist

Outline

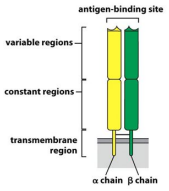
- ❑ Part I: LymphoTrack Products
  - What are different types clonality targets?
  - How diversity is generated?
  - What is LymphoTrack Assay used for?
  - Why test clonality?
  - How clonality was tested?
  - Overview of *TRB* gene, Multiplexing and Workflow
  - LymphoTrack Assay for MRD
- ❑ Part II: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

Clonality Targets

B-Cell Receptors (BCR)/Immunoglobulins (Ig)

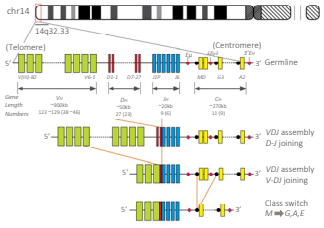


T-Cell Receptor (TCR)



The immune System, 4th ed (Garland Science 2015).

Human *IGH* locus

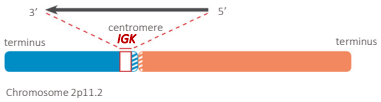


Dyer, M. et al., *Blood* 115:1490-1499(2010).

Human *IGK* locus

IGKC: 1
IGKJ: 5
IGKV: 40 or 67

1820 kb
(46-82 genes)



Chromosome 2p11.2

Lefranc, M.-P., *Exp. Clin. Immunogenet.*, 18, 161-174 (2001).

Human *TRB* locus

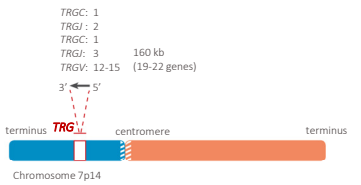
TRBV: 63-66
TRBD : 1
TRBJ: 6
TRBC: 1
TRBD: 1
TRBJ: 8
TRBC: 6
TRBV: 1

620 kb
(82-85 genes)

Chromosome 7q34

Ginestoux, C. et al. (2011, Feb 8). *IMGT Repertoire (IG and TR)*. Retrieved from http://www.imgt.org

Human *TRG* locus



Ginestoux, C. et al. (2011, Feb 8). *IMGT Repertoire (IG and TR)*. Retrieved from http://www.imgt.org.

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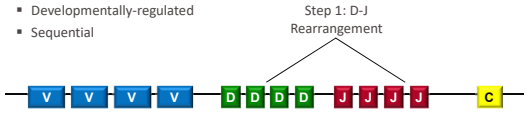
Generation of Diversity: *IGH* Rearrangement

Gene Rearrangements Are

- Lymphoid-specific
- Developmentally-regulated
- Sequential



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

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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

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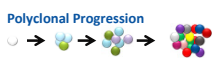
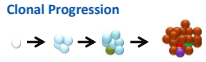
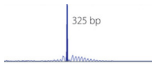
Polyclonal vs. Clonal Progression

Polyclonal Progression



Clonal Progression





Highly indicative of B- or T-cell malignancy

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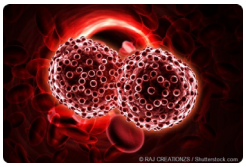
Why Test for B- and T-Cell Clonality?

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.

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Our Focus

PCR-based molecular testing for B- and T-cell malignancies associated with leukemias and lymphomas

- Gene Clonality / Rearrangements
- Chromosome Translocations
- Gene Mutations

RUO (Research Use Only)

CE Marked IVD (Clinical Diagnostic Utility)

GPR controls (General Purpose Reagents (DNA and RNA))

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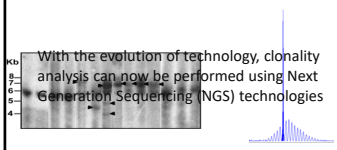
Detection Methods

Gel
IdentiClone™ Assays

ABI
IdentiClone™ Assays

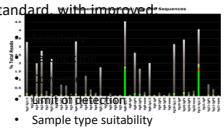
NGS
LymphoTrack® (Dx) Assays

Evolution of Clonality Testing



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

PCR-based clonality assays are now widely accepted as the gold standard, with improved:



- Limit of detection
- Sample type suitability

Southern Blot → Fragment Analysis → NGS

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

Ion PGM™

MiSeq®

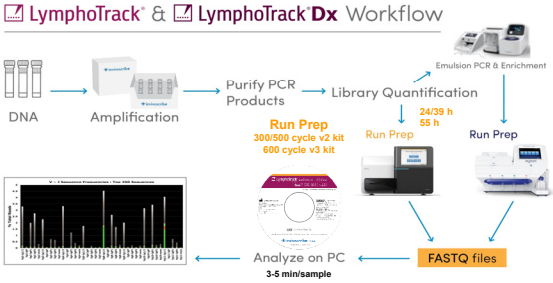
Available Assays & Software

- *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

LymphoTrack® & LymphoTrack® Dx Workflow



One assay – Multiple applications!

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

*For Research Use Only. Not for use in Diagnostic Procedures. 18

LymphoTrack® & LymphoTrack® Dx Advantages

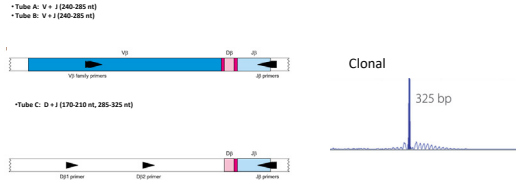
- **Tests Compatible with Range of Specimen Types**
- **ONE-STEP PCR Master Mixes**
- **MULTIPLEXING – Reduces Costs**
  - Combine Multiple Samples - up to 12 (Ion PGM) & 24 (MiSeq)
  - Combine Multiple Assays - up to 72 or 168 samples
- **Unparalleled Sensitivity**
  - Unparalleled clonality detection 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®

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.

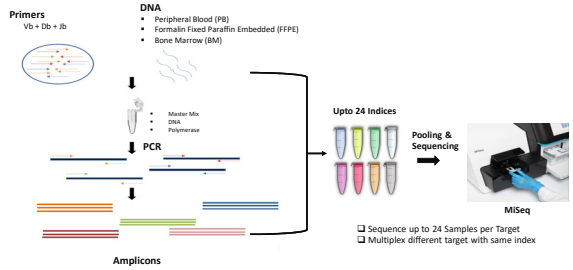
IdentiClone® *TCRB* Gene Clonality Assays Capillary Electrophoresis (Size)



- 24 indices for Panel and 8 indices for Kit A
- Positive and Negative control

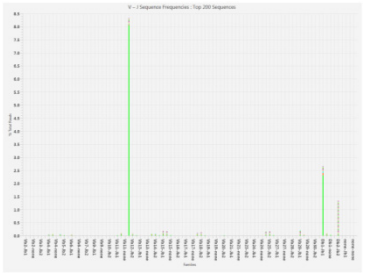
Kit	Name	# of Indices/MM	Indices:	# of Rxns	# of Controls	# of Boxes
Panel	LymphoTrack® Dx *TRB* Assay Panel – MiSeq®	24	1 - 27	5	3 + 3	1
Kit A	LymphoTrack® Dx *TRB* Assay Kit A – MiSeq®	8	1 - 8	5	3 + 3	1

Multiplex- One Step PCR



- Sequence up to 24 Samples per Target
- Multiplex different target with same index

TRB Positive (Clonal)



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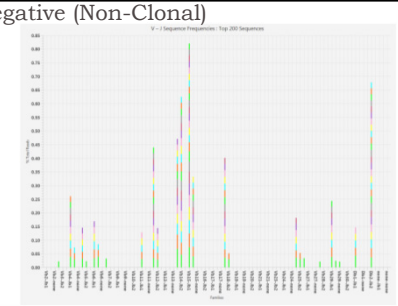
TRB Negative (Non-Clonal)



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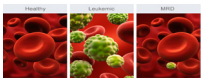
Minimal Residual Disease (MRD)

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

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LymphoTrack® Assays & MRD

- Same LymphoTrack Assay Kits As *IGH*, *IGK*, *TRG*, *TRB* Clonality
- Same Library Preparation
- Sensitivity Only Limited By DNA Input In PCR Amplification Step

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MRD Applications are For Research Use Only. Not for use in Diagnostic Procedures.

Summary of All LymphoTrack Assays - MiSeq

	MiSeq Assays						
	IGH/V Leader SHM	*IGH FR1*	*IGH FR2*	*IGH FR3*	*IGK*	*TRG*	*TRB*
Target Size (bp)	483	295	243	104	222	147	290
Amplicon Size Inc. Target, Index, and Adaptors (bp)	660	450	390	260	410	300	400
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 v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)	MiSeq v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)	MiSeq v2 Reagent (300-cycle) or MiSeq v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)	MiSeq v2 Reagent (500-cycle) or MiSeq v3 Reagent (600-cycle)

When multiplexing amplicons of different gene targets it is important to use the appropriate sequencing chemistry. The number of sequencing cycles must be sufficient to sequence the largest amplicon in the multiplex.

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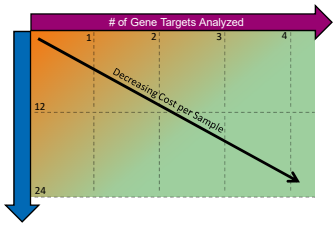
Multiplexing Can Reduce Run Cost



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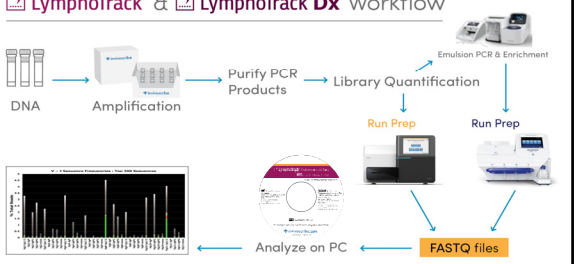
Personalized Molecular Medicine

LymphoTrack® Data Analysis – Identification and Tracking Patient-Specific Clones

Kasey Hutt, Ph.D.
Staff Scientist,
Bioinformatics

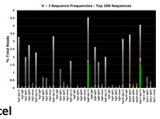
LymphoTrack® & LymphoTrack® Dx Workflow



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

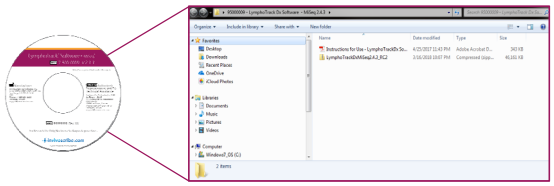
Run the Analysis Software

Open Excel Visualizations

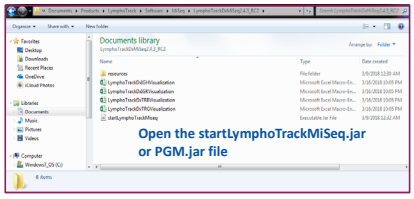
Software (MiSeq) includes an installer to walk customers through process and avoid issues with unzipping files.

Or PDF summaries (MiSeq) that are now generated by analysis software.

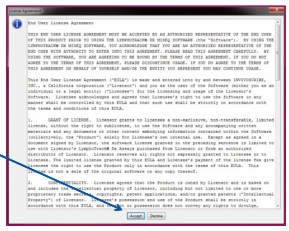
LymphoTrack® Dx Software – CD Content



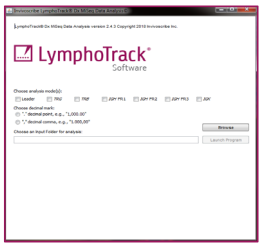
LymphoTrack® Dx Software – Processing Data



LymphoTrack® Dx Software – License Agreement

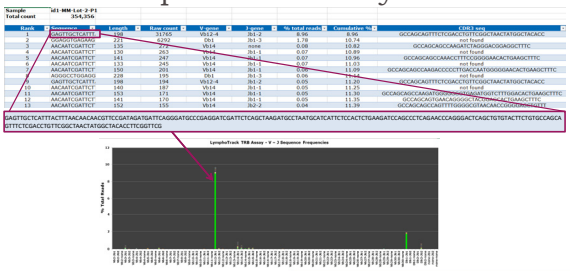


LymphoTrack® Dx Software – Processing Data



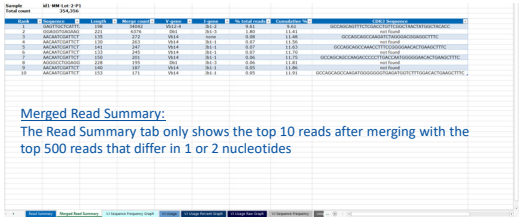
Select target(s) represented in pooled library

Data Output – *TRB* Assay



Importance of Merge Read Summaries

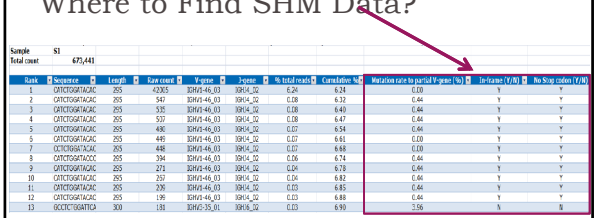
Amplification & Sequencing May Cause Artificial Nucleotide Changes



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 200 Read Summary

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	CDR3 seq
1	GAGTTGCTCATTTACTTT	198	51905	Vb12.4	Jb1-2	9.98	9.98	GCCAGCAGTTTCTCGAC
2	GGAGCGTGAGAAGGAAAC	221	6092	Db1	Jb1-3	1.78	10.74	not found
3	AACAATCGATTCTTAGCC	135	272	Vb14	none	0.08	10.82	GCCAGCAGCCAAGATTC
4	AACAATCGATTCTTAGCC	130	263	Vb14	Jb1-1	0.07	10.89	not found
5	AACAATCGATTCTTAGCC	141	247	Vb14	Jb1-1	0.07	10.96	GCCAGCAGCCAAACCTT
6	AACAATCGATTCTTAGCC	133	245	Vb14	Jb1-1	0.07	11.03	not found
7	AACAATCGATTCTTAGCC	150	201	Vb14	Jb1-1	0.06	11.09	GCCAGCAGCCAAGACCC
8	AGGGCCTGGAGGTGAAG	228	195	Db1	Jb1-3	0.06	11.14	not found
9	GAGTTGCTCATTTACTTT	140	187	Vb12.4	Jb1-2	0.05	11.20	GCCAGCAGTTTCTCGAC
10	AACAATCGATTCTTAGCC	153	187	Vb14	Jb1-1	0.05	11.25	not found
11	AACAATCGATTCTTAGCC	153	171	Vb14	Jb1-1	0.05	11.30	GCCAGCAGCCAAGATTC
12	AACAATCGATTCTTAGCC	151	170	Vb14	Jb1-1	0.05	11.35	GCCAGCAGTGAACAGGC
13	AACAATCGATTCTTAGCC	157	150	Vb14	Jb1-2	0.04	11.39	GCCAGCAGCCAGTTTTC
14	GAGTTGCTCATTTACTTT	157	151	Vb12.4	Jb1-2	0.04	11.43	GCCAGCAGTTTCTCGAC
15	AACAATCGATTCTTAGCC	135	141	Vb14	Jb1-1	0.04	11.47	GCCAGCAGCCAAGATTC
16	AAAGCTCACTTAAATCT	138	140	Vb3.1	Jb1-1	0.04	11.51	GCCAGCAGCGGTGGGAGG
17	AAAGCTCACTTAAATCT	135	138	Vb3.1	Jb1-1	0.04	11.55	GCCAGCAGTCGGAGTCC
18	GAGTTGCTCATTTACTTT	156	133	Vb12.4	Jb1-2	0.04	11.59	GCCAGCAGTTTCTCGAC
19	AACAATCGATTCTTAGCC	130	129	Vb14	Jb1-2	0.04	11.62	not found
20	CAAAGCTGCTGTTCCAG	134	126	Vb15	Jb1-1	0.04	11.66	GCCACCAGCAGCGACAG
21	CCTTCCATTTTAATTCA	159	126	Db2	Jb2-7	0.04	11.69	not found
22	ATCCATTATTCAAATAC	165	124	Vb6.4	Jb1-1	0.03	11.73	GCCAGCAGTGAATCCGC
23	ATCCATTATTCAAATAC	163	117	Vb6.4	Jb1-1	0.03	11.76	GCCAGCAGTGTAAGAGT
24	AACAATCGATTCTTAGCC	141	117	Vb14	Jb2-1	0.03	11.80	GCCAGCAGCCTTTACGC
25	GAGTTGCTCATTTACTTT	154	116	Vb12.4	Jb1-2	0.03	11.83	not found
26	AAAGCTCACTTAAATCT	135	115	Vb3.1	none	0.03	11.86	GCCAGCAGCTTCGCTGT
27	AAAGCTCACTTAAATCT	138	113	Vb3.1	Jb1-1	0.03	11.89	GCCAGCAGCGAGCTAG
28	AACAATCGATTCTTAGCC	154	112	Vb14	Jb2-7	0.03	11.92	GCCAGCAGCCAAGATTC
29	CCTTCCATTTTAATTCA	165	112	Db2	Jb1-1	0.03	11.96	not found
30	AACAATCGATTCTTAGCC	145	108	Vb14	Jb2-7	0.03	11.99	GCCAGCAACCCCCTGGG

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

Top 10 Merged Read Summary

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	CDR3 Sequence
1	GAGTTGCTCATTTACTTT	198	34042	Vb12.4	Jb1-2	9.61	9.61	GCCAGCAGTTTCTCGAC
2	GGAGCGTGAGAAGGAAAC	221	6376	Db1	Jb1-3	1.80	11.41	not found
3	AACAATCGATTCTTAGCC	135	272	Vb14	none	0.08	11.48	GCCAGCAGCCAAGATTC
4	AACAATCGATTCTTAGCC	130	263	Vb14	Jb1-1	0.07	11.56	not found
5	AACAATCGATTCTTAGCC	141	247	Vb14	Jb1-1	0.07	11.63	GCCAGCAGCCAAACCTT
6	AACAATCGATTCTTAGCC	133	245	Vb14	Jb1-1	0.07	11.70	not found
7	AACAATCGATTCTTAGCC	150	201	Vb14	Jb1-1	0.06	11.75	GCCAGCAGCCAAGACCC
8	AGGGCCTGGAGGTGAAG	228	195	Db1	Jb1-3	0.06	11.81	not found
9	AACAATCGATTCTTAGCC	140	187	Vb14	Jb1-1	0.05	11.86	not found
10	AACAATCGATTCTTAGCC	153	171	Vb14	Jb1-1	0.05	11.91	GCCAGCAGCCAAGATTC

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

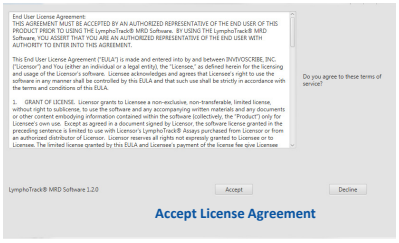
Simple, Efficient Bioinformatics

- Bi-allelic tracking enables simultaneous tracking of 2 sequences
- Track across multiple replicates which allows determinations of much greater sensitivity

Automated Report Output

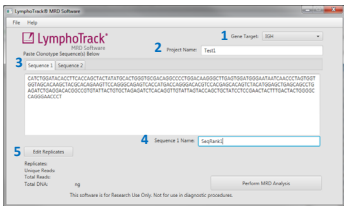
- Previous outputs required manual generation of each index sample, resulting in 20+ minutes of overhead, per target. Automated PDF report output cuts this time down to a few seconds.

MRD Software - Data Analysis



Accept License Agreement

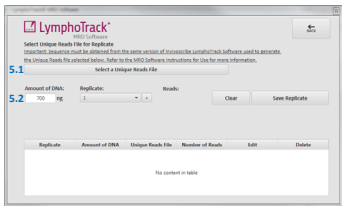
MRD Software - Data Input



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

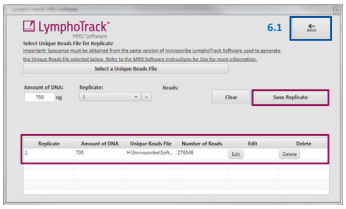


Step 5: Entering Replicates

5.1 Select Unique Reads File

5.2 Entering Replicate Information: DNA Amount, Number of Replicates and Save Replicates

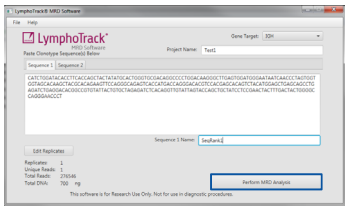
MRD Software - Data Input



Step 6: Save Replicates

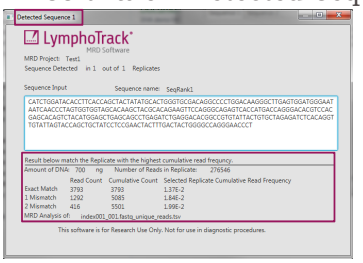
6.1 Click "Back"

MRD Software - Data Input

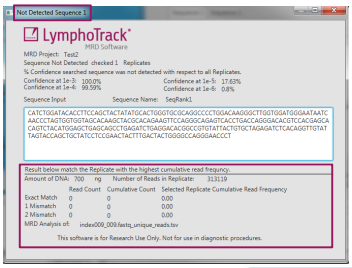


Perform Analysis

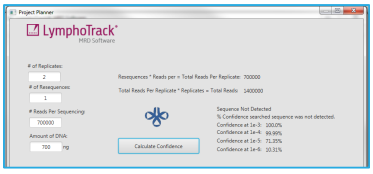
MRD Software – Detected Sequence



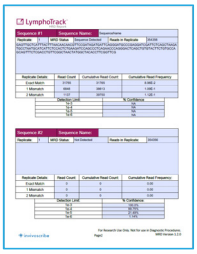
MRD Software – Not Detected Sequence



Project Planner



PDF Output



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Future Additions

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

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Thank You Everyone at IVS!

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Personalized Molecular Medicine

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Thank you very much!

Questions?

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