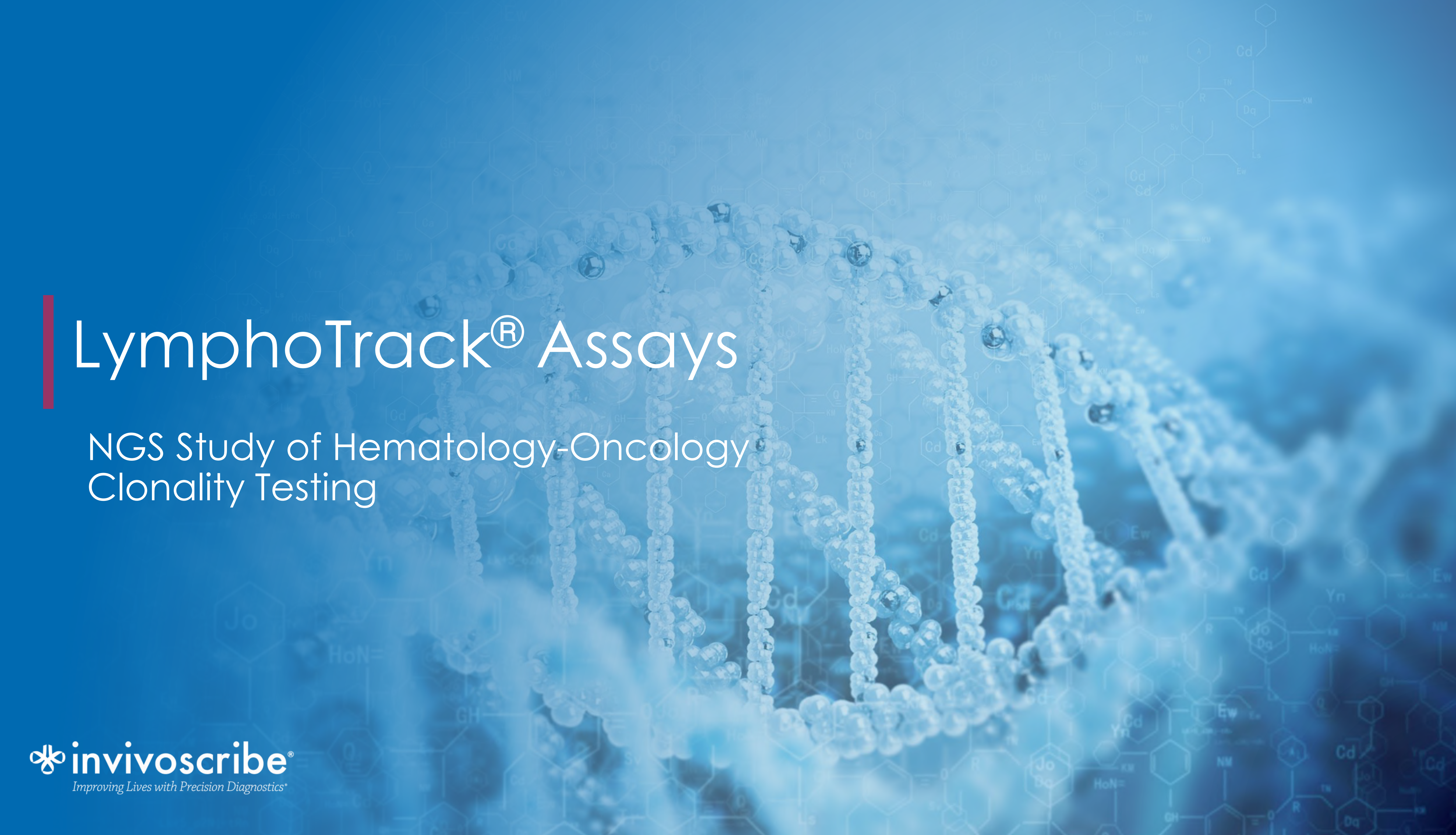

LymphoTrack® Assays
NGS Study of Hematology-Oncology Clonality Testing
invivoscribe®
Improving Lives with Precision Diagnostics®

Molecular testing of clonality

Current methods are effective, but limited

- Low throughput
- Low sensitivity
- Difficult to interpret
- Cannot readily differentiate between clonal populations that have the same PCR product
- Not appropriate for MRD research

There is a need for improvement

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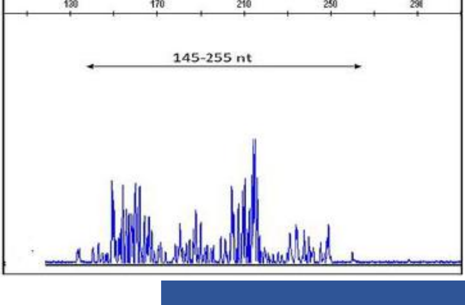
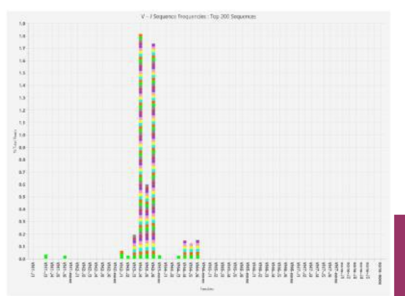
The Next Step in Clonality Assessment



Fragment Analysis

- **Reduced testing time** (1-2 days)
- **Base pair resolution** Sequences obtained by cloning & Sanger sequencing
- **Custom assay required to track clones** (MRD)
- **Widely used method** many references available
- **Can be hard to interpret and is subjective**



NGS

- **Increased objectivity** specific sequence provided
- **Reduced false positives** non-specific bands filtered
- **Increased sensitivity**
- **Same reagents & workflow** for the study of clonality, SHM, & MRD
- **Less analysis time required**

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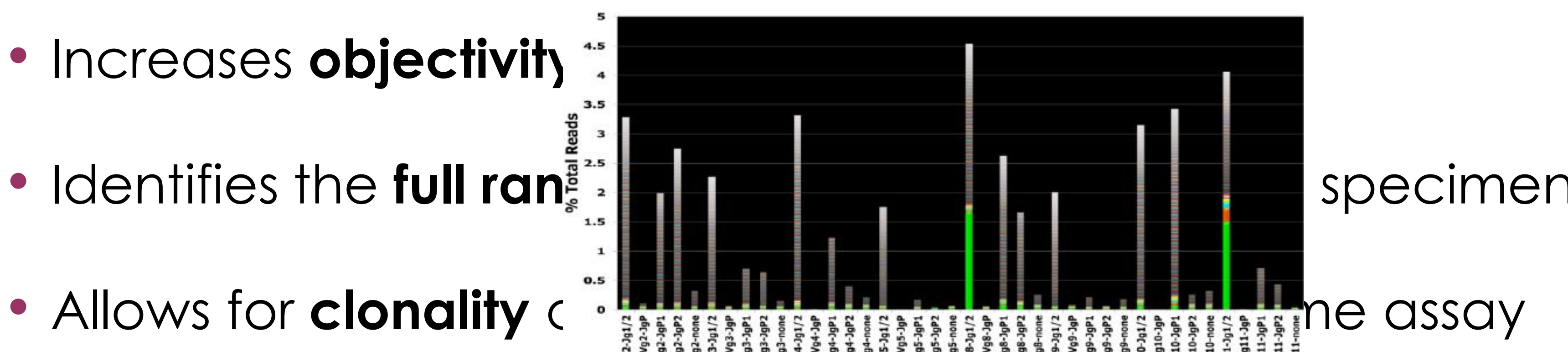
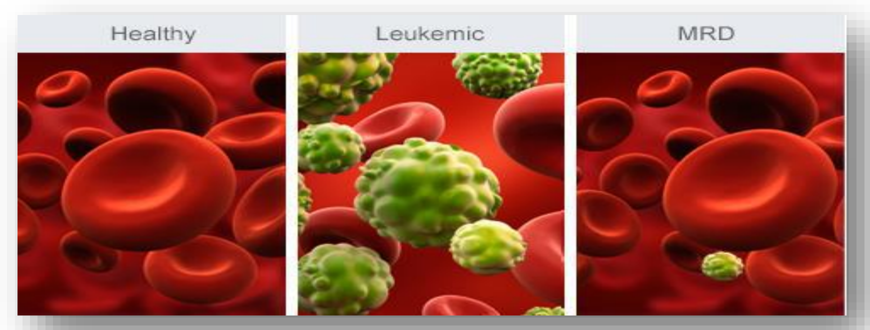
Advantages of NGS testing

- Determines the **DNA sequence** of clonal rearrangements
- Increases **objectivity**
- Identifies the **full ran** specimen
- Allows for **clonality** ne assay
- Allows **tracking** of clonal populations with the **same reagents** and **workflow**





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Next Generation Sequencing

A molecular analysis revolution

- **Next Generation Sequencing**
  - Sequence millions of nucleic acid strands simultaneously
  - Very sensitive
  - High degree of multiplexing – look at many targets simultaneously
- Impressive **data analysis requirements**
  - Has rapidly increased the field of bioinformatics
  - Is typically highly complex
- Has touched all areas of life and medical sciences and changed how we understand the world

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Next Generation Sequencing

Vocabulary

- **Adapters** – short nucleotide sequences
  - Allow amplicons to bind to sequencing component (bead of flow cell)
  - Use to amplify adapter-ligated DNA fragments only
- **Indexes/Indices**
  - Each DNA fragment in a sample is ‘tagged’ with a short sequence specific to that sample
  - Indexes/Indices are used after sequencing to separate fragments into different samples
- **Library**
  - Collection of amplicons with indexed adapters
  - Can be ‘pooled’ with other libraries for multiplex sequencing

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NGS Workflow

"Typical" workflow

A
Library Preparation

B
Pool

C
Sequence

D
Demultiplex

E
Align

Index 1
(CATTCG)

Index 2
(AACTGA)

Sequence Output to Data File

CATTCGACGGATCG
AACTGAGTCCGATA
AACTGATCGGATCC
CATTCGTGGCAGTC
AACTGAACCTGATG
AACTGAGATTACAA
CATTCGCAGTTCATT
CATTCGAACTTCGA

CATTCGACGGATCG
CATTCGTGGCAGTC
CATTCGCAGTTCATT
CATTCGAACTTCGA

AACTGAGTCCGATA
AACTGATCGGATCC
AACTGAACCTGATG
AACTGAGATTACAA

- Library 1 Barcode
- Library 2 Barcode
- Sequencing Reads
- DNA Fragments
- Reference Genome

"An introduction to Next-Generation Sequencing Technology", Illumina®, https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf (Accessed July 2020)

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Available Assays & Software

B-Cell

- *IGHV* Leader SHM*
- *IGH* FR1/2/3 Combo
- *IGH* FR1
- *IGH* FR2
- *IGH* FR3
- *IGK*

T-Cell

- *TRG*
- *TRB**

Software

- LymphoTrack® Software
- MRD Software - Research Use Only (RUO)

*MiSeq Only

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LymphoTrack® Assays

- **One-Step PCR** Master Mixes
- **Multiplexing** – Reduces Costs by Combining:
  - Multiple Samples - up to 12 (Ion S5/PGM™) & 24 (MiSeq®)
  - Multiple Invivoscribe Assays - up to 72 or 168 Samples
  - Other Platform-Specific Assays
- **Unparalleled Sensitivity**
  - Unparalleled clonality detection with the ability to identify and track the specific sequence of clonal populations for MRD research studies.
- **Analysis Software Package - Included**

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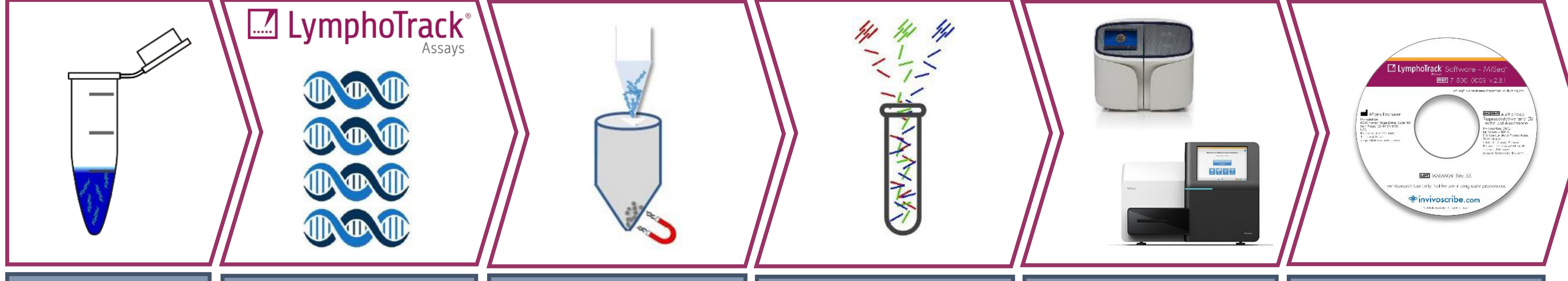
LymphoTrack® Workflow

Easy Workflow



Extracted DNA	Generate libraries using the **one-step PCR LymphoTrack® Kits**	**Purify** PCR products using AMPure XP beads	**Quantify** libraries, **pool** and check concentration (e.g. qPCR or BioAnalyzer)	**Sequence** libraries using Ion Torrent™ or Illumina® MiSeq® technology	**Analyze** results locally with included **LymphoTrack® Software**

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Comprehensive menu

Available Sequencing Platforms	MiSeq®	Ion S5™	Ion PGM™
Menu	**B-Cell** *IGHV (Leader)* *IGH FR1* *IGH FR2* *IGH FR3* *IGK* **T-Cell** *TRG* *TRB*	**B-Cell** *IGH FR1* *IGH FR2* *IGH FR3* *IGK* **T-Cell** *TRG*	**B-Cell** *IGH FR1* *IGH FR2* *IGH FR3* *IGK* **T-Cell** *TRG*
Kit size(s)	8-index or 24-index kits	12 barcodes	12 barcodes
Validated Sequencing Kits	V2 (2 x 150 bp) V2 (2 x 250 bp) V3 (2 x 300 bp)	Ion 520™ Ion 530™	Ion 316™ v2 BC Ion 318™ v2 BC

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Included in the LymphoTrack® Kits

Library preparation and analysis

- Kit contents
  - 1 **mastermix** per index/barcode
  - Required **controls**
  - LymphoTrack® analysis **software**

- Kit sizes:

Platform	Kit Type	# of indices/ barcodes	Reactions per mastermix	Reactions per kit
MiSeq®	Kit A	8	5	40
	Panel	24	5	120
	Panel B	24	5	120
S5/PGM™	All	12	5	60

*Only available for *IGH* FR1

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LymphoTrack® Software

Local, offline and easy to use

- Included with each LymphoTrack® kit
- Available for both Ion S5/PGM™ and Illumina® MiSeq® platforms
- Does not require bioinformatics personnel
- Can run on most standard windows platforms
- Utilizes FASTQ files
- Fast results reported in individual PDF reports or in Excel

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Summary of Sequencing Workflows

Summary of LymphoTrack* Assays – S5/PGM

	IGH FR1	*IGH* FR2	*IGH* FR3	*IGK*	*TRG*
Average Target Size (bp)	295	243	103	222	147
Average Amplicon Size Including Target, Index, and Adaptors (bp)	430	370	240	390	280
DNA Input (ng/PCR)	50				
Validated PCR Cycles	29				
Purification Method	AMPure XP Beads (1.8:1 ratio)				
Quantification Method	Agilent 2100 Bioanalyzer or Perkin Elmer LabChip® GX				
Planned Run Setting for Flows**	850				500 or 850
Recommended Sequencing Kit**	For Ion S5™ use: Ion 520™ & Ion 530™ Kit – OT2 or Ion 510™ & 520™ & Ion 530™ Kit – Chef For Ion PGM™ use: Ion PGM™ Hi-Q™ View OT2 Kit & Ion PGM™ Hi-Q™ View Sequencing Kit & Ion PGM™ Wash 2 Bottle Kit				

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**When multiplexing amplicons of different gene targets it is important to use the appropriate sequencing chemistry. When selecting a sequencing kit, ensure that it offers a sufficient number of number of sequencing cycles (flows) for valid interpretation of the largest amplicon and depth of coverage for each sample in the multiplex.

Summary of Sequencing Workflows

Summary of LymphoTrack* Assays - Miseq

	IGHV Leader SHM	IGH FR1	IGH FR2	IGH FR3	IGK	TRG	TRB
Target Size (bp)	483	295	243	104	222	147	290
Amplicon Size Including Target, Index, and Adaptors (bp)	660	450	390	260	410	300	400
DNA Input (ng/PCR)	50						
Validated PCR Cycles	32	29					
Purification Method	AMPure XP Beads (1:1 ratio)						AMPure XP Beads (0.7:1 ratio)
Quantification Method	KAPA qPCR						
Sample Sheet Settings**	Cycles Read1: 301 Cycles Read2: 301	Cycles Read1: 251 Cycles Read2: 251		Cycles Read1: 151 Cycles Read2: 151	Cycles Read1: 251 Cycles Read2: 251	Cycles Read1: 151 Cycles Read2: 151	Cycles Read1: 251 Cycles Read2: 251
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)

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**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.

Sequencing Instruments

LymphoTrack® Assays

Available Sequencing Platforms	MiSeq®	Ion S5™	Ion PGM™
Run Time	**36-56 Hours**	**6-17.5 Hours**	**5-8 Hours**
Reads Per Run	15 million (V2 standard) 25 million (V3)	3-5 million (Ion 520 chip) 15-20 million (Ion 530 chip)	2-3 million (316 chip) 4-5.5 million (318 chip)
Read Lengths	V2 (2 x 150 bp) V2 (2 x 250 bp) V3 (2 x 300 bp)	Ion 520™ (400 bp) Ion 530™ (400 bp)	Ion 316™ v2 BC (400 bp) Ion 318™ v2 BC (400 bp)

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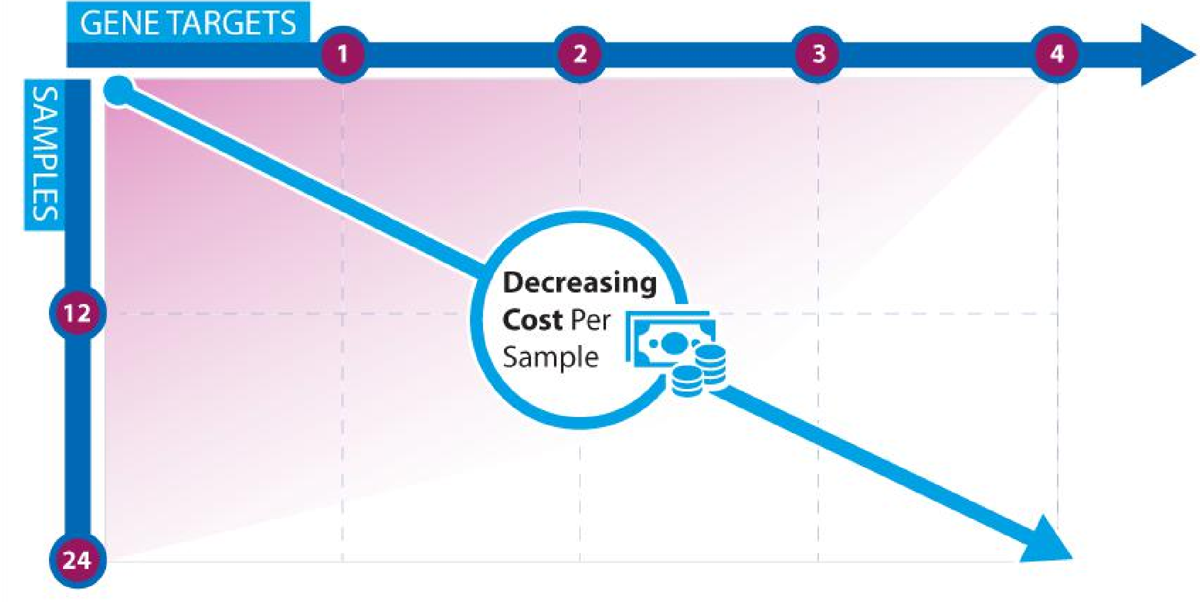
LymphoTrack® – Experiment Planning

Key Factors

- How many samples?
- How many targets?
- Which targets?

These will determine:

- # of indices/barcodes needed
- MiSeq® or Ion Torrent™ chemistry
- Cost per target and per sample



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Increasing Capture Rate

Easy to combine:

- *IGH* and *IGK*
- *TRB* and *TRG*

Advantages of Combining Targets:

- Highest capture rate
- Higher confidence

B-Cell Targets

	IGH (FR1, 2 & 3)	*IGK (Vk – Jk & Kde)*	*IGH+IGK*
MCL%)	100	100	100
B-CLL/SLL(%)	100	100	100
FL(%)	84	84	100
MZL(%)	87	83	97
DLBCL(%)	79	80	96
Total(%)	88	88	98

PAS Evans et al., Leukemia. 2006 21:201-206.

T-Cell Targets

	TRB	*TRG*	*TRB+TRG*
T-PLL(%)	100	94	100
T-LGL(%)	96	96	100
PTCL-U(%)	98	94	100
AILT(%)	89	92	95
ALCL(%)	74	74	79*
Total(%)	91	89	94 (99)*

J.J.M. van Krieken et al. Leukemia. 2007 21:201-206.

Testing Complementary Gene Targets in Parallel Improves Confidence!

*Approximately 20–25% of ALCL are known to have no TCR gene rearrangements and are defined as null ALCL; J.J.M. van Krieken et al. *Leukemia*. 2007 21:201-206.

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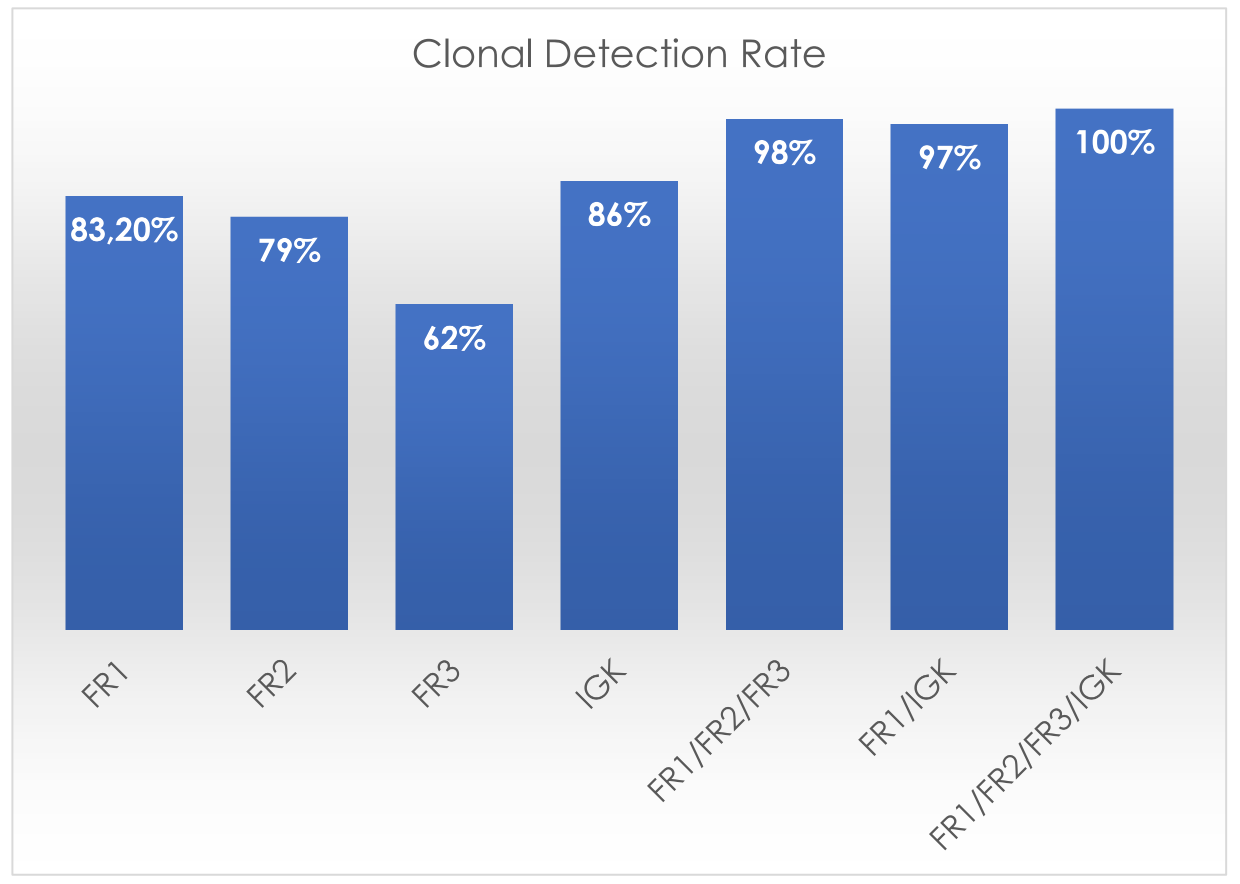
Increasing Detection Rate

Example of how using multiple targets can increase the detection rate in clonality in multiple myeloma samples



"Comparison of LymphoTrack® Assays - MiSeq® and Flow Cytometry for Clonality and Minimum Residual Disease Assessment in Multiple Myeloma", InvivoScribe, EHA Meeting 2018

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Multiplexing

LymphoTrack® Assays

Platform	Sequencing Kit	LymphoTrack® Kits	Number of Targets
Illumina® MiSeq®	V2 (2 x 150 bp)	*FR3* and *TRG*	Up to 2 targets
	V2 (2 x 250 bp)	*FR1*, *FR2*, *FR3*, *IGK*, *TRB* and *TRG*	Up to 4 targets*
	V3 (2 x 300 bp)	*IGHV*, *FR1*, *FR2*, *FR3*, *IGK*, *TRB* and *TRG*	Up to 7 targets
Ion S5™ and Ion PGM™	Ion 520	*FR1*, *FR2*, *FR3*, *IGK* and *TRG*	Up to 5 targets
	Ion 530	*FR1*, *FR2*, *FR3*, *IGK* and *TRG*	Up to 5 targets
	Ion 316	*FR1*, *FR2*, *FR3*, *IGK* and *TRG*	Up to 3 targets
	Ion 318	*FR1*, *FR2*, *FR3*, *IGK* and *TRG*	Up to 5 targets

* Due to capacity of the V2 Sequencing Kit it is possible to sequence only up to 4 targets.

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Multiplexing

1 **Determine the Targets to be tested & necessary Sequencing Kit**

- **Multiple Targets** can be multiplexed from any LymphoTrack® B- and T-cell kit
- Select the MiSeq® or S5/PGM™ Sequencing kit compatible with the **largest amplicon** in the multiplex

2 **Calculate the # of Reads available per Sample**

= # Total reads available
÷ # Samples
÷ # Targets per sample

Reads per Target, Per Sample

3 **Compare with the minimum # of Reads desired**

- # of Reads per Target

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Multiplexing

4 Assign one Index per Sample

- Assign the **same index per Sample across different Targets**
  - e.g. Sample A - Index 01 for *IGH FR1*, Index 01 for *TRG*, etc.
  - e.g. Sample B - Index 02 for *IGH FR1*, Index 02 for *TRG*, etc.

- A **unique index** should also be assigned to the **Positive and Negative Controls**
  - e.g. Positive Control - Index 08 for *IGH FR1*, Index 08 for *TRG*, etc.
  - e.g. Negative Control - Index 09 for *IGH FR1*, Index 09 for *TRG*, etc.

- **NTC** does not need to be sequenced so **any index can be used**
  - e.g. NTC – Index 08 for *IGH FR1*, Index 08 for *TRG*, etc.

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Multiplexing Example

1 Determine the Targets to be tested & necessary Sequencing Kit

- **Targets**: We will multiplex *IGH* Leader, *IGH* FR1, FR2, FR3, *IGK*, *TRG* and *TRB*
- Select **Sequencing kit**: *IGH* Leader is the largest target and requires as minimum a MiSeq® v3 Flow Cell (25 Million reads available).

2 Calculate the # of Reads available per Sample

= # Total reads available
÷ # Samples
÷ # Targets per sample

Reads per Target, Per Sample

20,000,000 Reads*
÷ 24 Samples
÷ 7 Targets

~119,000 Reads per Target, per Sample

3 Compare with the minimum # of Reads desired

- # of Reads per Target

* Only 80% of 25 Million reads are considered = 20 Million reads as loading might not be perfect

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Multiplexing Example

Assign one Index per Sample

- Assign the **same index per Sample across different Targets**
- A **unique index** should also be assigned to the **Positive and Negative Controls**
- **NTC** does not need to be sequenced so **any index can be used**

Index	***IGH* FR1**	***TRG***	***Target X …***
1	Index 01 Sample A	Index 01 Sample A	Index 01 Sample A
2	Index 02 Sample B	Index 02 Sample B	Index 02 Sample B
3	Index 03 Sample C	Index 03 Sample C	Index 03 Sample C
4	Index 04 PosCtrl	Index 04 PosCtrl	Index 04 PosCtrl
5	Index 05 NegCtrl	Index 05 NegCtrl	Index 05 NegCtrl
6	Index 05 NTC	Index 05 NTC	Index 05 NTC
…	…	…	…

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Multiplexing - Summary

Easily maximize run efficiency

- Use same index/barcode across **multiple targets**
- The LymphoTrack® software can assign all reads to the correct target
- MiSeq® example

Index	*IGHV*	*FR1*	*FR2*	*FR3*	*IGK*	*TRG*	*TRB*
1	NC	NC	NC	NC	NC	NC	NC
2	PC	PC	PC	PC	PC	PC	PC
3	SHM PC	Sample 1	Sample 1	Sample 1	Sample 1	Sample 2	Sample 2
4	Sample 3	Sample 4	Sample 4	Sample 4	Sample 4	Sample 5	Sample 5

Up to 24 indices

- Possible to generate up to 153 sample results in one run (v3 flow cell)

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The following NGS instruments are suitable for LymphoTrack® except,

1. Illumina® MiSeq®
2. Ion PGM™
3. Illumina® NextSeq®
4. Ion S5™
5. Illumina® MiniSeq™

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Quiz

Somatic hypermutation analysis can be done using the Ion Torrent™ platform?

1. Yes, using *FR1*
2. Yes, using *IGK*
3. No

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Which kits can be analyzed on the Illumina® MiSeq® using the v2 (2x250) flow cell?

1. *TRB* and *TRG*
2. *FR1*, *FR2*, *FR3*, and *IGK*
3. *FR3* and *TRG*
4. *FR1*, *FR2*, *FR3*, *IGK*, *TRG* and *TRB*

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Quiz

Which of the following flow cells has not been validated for the Illumina® MiSeq® instrument by Invivoscribe?

1. V3, 2x300 bp
2. V2, 2x250 bp
3. V2, 2x150 bp
4. V2 nano, 2x250 bp

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Quiz

Which library quantitation methods are validated for the LymphoTrack® MiSeq® kits?

1. Bioanalyzer
2. Kapa qPCR kits
3. Qubit
4. Tape station

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LymphoTrack® Software

Data analysis and Interpretation

Analysis Workflow

FASTQ Files from MiSeq®, S5™ or PGM™	LymphoTrack® Data Analysis Software	LymphoTrack® Data Visualization	Data Interpretation
Save FASTQ files in a designated folder	Open LymphoTrack® software and select analysis to be carried out	All results are summarized in PDF files or can be viewed in Excel	Interpret each report for both controls and samples

- Computer requirements
  - **64 bit** Windows, 4GB RAM (or more), Intel Core Duo 2 or newer CPU
  - Java 8 **(64 bit)** or newer
  - PDF reader
  - CD-ROM drive to access the program on supplied CD

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Analyze Data in Just 4 Steps

1. Select target(s) analyzed
2. Choose decimal mark
3. Browse for data folder
4. Begin automated analysis

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Data Output – PDF Report

Merged Read Summary

- All sequences that differ by only 1 or 2 bp are merged
  - Accounts for library prep and sequencing errors
- Shows read count after final analysis
- Full sequence can be copied from PDF report

LymphoTrack Report for assay LEADER

Sample name: Leader_positive_S23_L001_001_combined

Total Read Count: 474947

IndexQ30: 87.88

Caution: Do not edit fields and save.

Top 10 Merged Read Summary

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 Seq
1	TTCTCGTGGTGG	455	50248	IGHV4-59_08	IGHJ4_02	10.58	10.58	11.26	Y	Y	98.63	GCGAGACGGAG
2	CTGCTACTGACTG	319	192	IGHV2-70_10	IGHJ4_02	0.04	10.62	4.32	n/a	N	35.55	not found
3	CTGCTGCTGACCA	466	175	IGHV2-5_01	IGHJ5_01	0.04	10.66	6.62	Y	Y	100.00	GCACACAGACCG
4	CTGCTGCTGACCA	457	162	IGHV2-5_05	IGHJ6_02	0.03	10.69	2.99	Y	Y	99.67	GCACACAGATACT
5	CTGCTGCTGACCA	474	154	IGHV2-5_05	IGHJ4_02	0.03	10.72	3.99	Y	Y	99.67	GCACACAGATACT
6	CTGCTGCTGACCA	454	150	IGHV2-5_10	IGHJ5_02	0.03	10.76	11.78	Y	Y	98.99	GCATATGGTGTAA
7	CTGCTGCTGACCA	469	139	IGHV2-5_01	IGHJ4_02	0.03	10.78	1.32	Y	Y	97.68	GCACTCGCGACA
8	CTCGCCCTCCTCC	466	139	IGHV5-51_01	IGHJ4_02	0.03	10.81	7.09	Y	Y	99.32	GCGAGATACTATT
9	CTGCTACTGACTG	490	137	IGHV2-70_10	IGHJ3_02	0.03	10.84	0.66	Y	Y	99.34	GCACGGATTCCTG
10	CTGCTGCTGACCA	478	135	IGHV2-5_10	IGHJ6_02	0.03	10.87	3.70	Y	Y	98.99	GCATACACTTGTT

TTCTCTGGGTTCTCACTCACCACTAGGGGATTGGGTGTGGCCTGGATCCGTCAGCCCCCAGGAAAGGCCCTGGAGTGGCTTGCACTCATTTTTTGGGATGATGATA
AACGCTACAGCCCATCTCTGAAGAGCAGACTCACCATCACCAAGGACGCCTCCAAGAACCAGGTGGTCCTTACAATGACCAACATGGACCCTGTAGACACAGCCAC
CTATTACTGTGCACACAGCGGGAGCTACCAAGGTGGGACTACCTATTACCCACACTACTATTTTGACTACTGGGGCCAGGGAACCCT

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Data Output – PDF Report

Sequence Frequency Graph

- Displays rearrangements organized by families
- Based on top 200 sequences
- Solid color bar pieces represent one clonal sequence
- Stacked bars are from the same family, but may or may not be the same clone

V – J Sequence Frequencies : Top 200 Sequences

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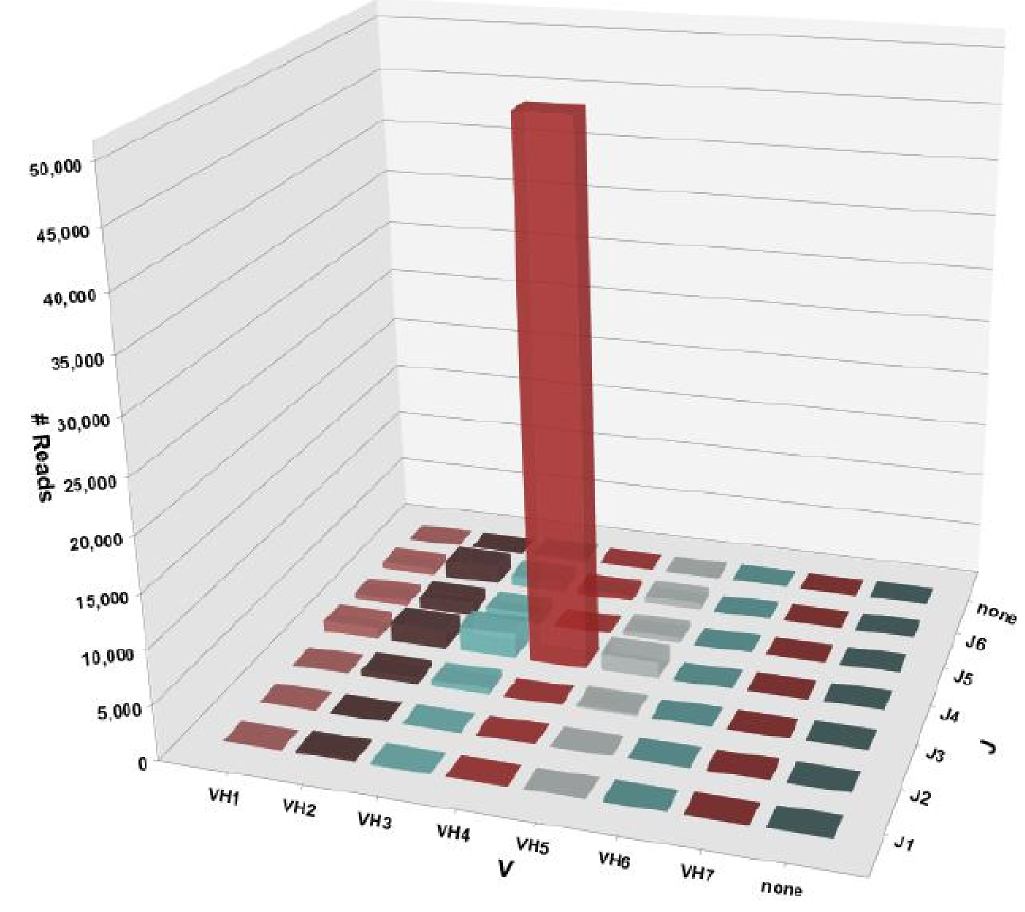
Data Output – PDF Report

V-J Usage Graph

- Displays V-gene and J-gene recombinations
- Bar is not based on a particular clone
- Frequency of a pair of families based on amount of reads

V-J Usage: Top 200 Sequences



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Data Output – PDF Report

Top 200 Read Summary

- Unmerged read summary of top 200 sequences
- Top sequences may represent same clone and are merged together downstream
- Fewer than 200 reads may be displayed depending on the sample

Top 200 Read Summary

Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 seq
1	TTCTCGTGGTGGC	455	35909	IGHV4-59_08	IGHJ4_02	7.56	7.56	11.26	Y	Y	98.63	GCGAGACGGAGC
2	TTCTCGTGGTGGC	455	7484	IGHV4-59_08	IGHJ4_02	1.58	9.14	11.60	Y	Y	98.63	GCGAGACGGAGC
3	TTCTCGTGGTGGC	455	911	IGHV4-59_08	IGHJ4_02	0.19	9.33	11.60	Y	Y	98.63	GCGAGACGGAGC
4	TTCTCGTGGTGGC	455	395	IGHV4-59_08	IGHJ4_02	0.08	9.41	11.60	Y	Y	98.63	GCGAGACGGAGC
5	TTCTCGTGGTGGC	455	386	IGHV4-59_08	IGHJ4_02	0.08	9.49	11.26	Y	Y	98.63	not found
6	TTCTCGTGGTGGC	455	370	IGHV4-59_08	IGHJ4_02	0.08	9.57	11.26	Y	Y	98.63	not found
7	TTCTCGTGGTGGC	455	366	IGHV4-59_08	IGHJ4_03	0.08	9.65	11.26	Y	Y	98.63	GCGAGACGGAGC
8	TTCTCGTGGTGGC	455	318	IGHV4-59_08	IGHJ4_02	0.07	9.71	11.60	Y	Y	98.63	GCGAGACGGAGC
9	TTCTCGTGGTGGC	455	310	IGHV4-59_08	IGHJ4_02	0.07	9.78	11.26	Y	Y	98.63	GCGAGACGGAGC
10	TTCTCGTGGTGGC	455	194	IGHV4-59_08	IGHJ4_02	0.04	9.82	11.60	Y	Y	98.63	GCGAGACGGAGC
11	CTGCTACTGACTG	319	192	IGHV2-70_10	IGHJ4_02	0.04	9.86	4.32	n/a	N	35.55	not found
12	TTCTCGTGGTGGC	455	183	IGHV4-	IGHJ4_02	0.04	9.90	11.95	Y	Y	98.63	GCGAGACGGAGC

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Data Output – *IGHV*

Top 10 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

Note: This analysis is also possible with *IGH FR1*

LymphoTrack Report for assay LEADER

Sample name: Leader_positive_S23_L001_001_combined

Total Read Count: 474947

IndexQ30: 87.88

Caution: Do not edit fields and save.

Top 10 Merged Read Summary

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 Seq
1	TTCTCGTGGTGGC	455	50248	IGHV4-59_08	IGHJ4_02	10.58	10.58	11.26	Y	Y	98.63	GCGAGACGGAGC
2	CTGCTACTGACTG	319	192	IGHV2-70_10	IGHJ4_02	0.04	10.62	4.32	n/a	N	35.55	not found
3	CTGCTGCTGACCA	466	175	IGHV2-5_01	IGHJ5_01	0.04	10.66	6.62	Y	Y	100.00	GCACACAGACCGC
4	CTGCTGCTGACCA	457	162	IGHV2-5_05	IGHJ6_02	0.03	10.69	2.99	Y	Y	99.67	GCACACAGATACT
5	CTGCTGCTGACCA	474	154	IGHV2-5_05	IGHJ4_02	0.03	10.72	3.99	Y	Y	99.67	GCACACAGATACT
6	CTGCTGCTGACCA	454	150	IGHV2-5_10	IGHJ5_02	0.03	10.76	11.78	Y	Y	98.99	GCATATGGTGTAA
7	CTGCTGCTGACCA	469	139	IGHV2-5_01	IGHJ4_02	0.03	10.78	1.32	Y	Y	97.68	GCACTCGCGACAC
8	CTCGCCCTCCTCC	466	139	IGHV5-51_01	IGHJ4_02	0.03	10.81	7.09	Y	Y	99.32	GCGAGATACTATT
9	CTGCTACTGACTG	490	137	IGHV2-70_10	IGHJ3_02	0.03	10.84	0.66	Y	Y	99.34	GCACGGATTCCTG
10	CTGCTGCTGACCA	478	135	IGHV2-5_10	IGHJ6_02	0.03	10.87	3.70	Y	Y	98.99	GCATACACTTGTT

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Expected Values *IGHV SHM*

LymphoTrack Report for assay LEADER

Sample name: Leader_positive_S23_L001_001_combined

Total Read Count: 474947

IndexQ30: 87.88

Caution: Do not edit fields and save.

Top 10 Merged Read Summary

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 Seq
1	TTCTCGTGGTGGC	455	50248	IGHV4-59_08	IGHJ4_02	10.58	10.58	11.26	Y	Y	98.63	GCGAGACGGAGC
2	CTGCTACTGACTG	319	192	IGHV2-70_10	IGHJ4_02	0.04	10.62	4.32	n/a	N	35.55	not found
3	CTGCTGCTGACCA	466	175	IGHV2-5_01	IGHJ5_01	0.04	10.66	6.62	Y	Y	100.00	GCACACAGACCGC
4	CTGCTGCTGACCA	457	162	IGHV2-5_05	IGHJ6_02	0.03	10.69	2.99	Y	Y	99.67	GCACACAGATACT
5	CTGCTGCTGACCA	474	154	IGHV2-5_05	IGHJ4_02	0.03	10.72	3.99	Y	Y	99.67	GCACACAGATACT
6	CTGCTGCTGACCA	454	150	IGHV2-5_10	IGHJ5_02	0.03	10.76	11.78	Y	Y	98.99	GCATATGGTGTAA
7	CTGCTGCTGACCA	469	139	IGHV2-5_01	IGHJ4_02	0.03	10.78	1.32	Y	Y	97.68	GCACTCGCGACAC
8	CTCGCCCTCCTCC	466	139	IGHV5-51_01	IGHJ4_02	0.03	10.81	7.09	Y	Y	99.32	GCGAGATACTATT
9	CTGCTACTGACTG	490	137	IGHV2-70_10	IGHJ3_02	0.03	10.84	0.66	Y	Y	99.34	GCACGGATTCCTC
10	CTGCTGCTGACCA	478	135	IGHV2-5_10	IGHJ6_02	0.03	10.87	3.70	Y	Y	98.99	GCATACACTTGTT

- *IGH* Positive Control top % reads ≥ 2.5%
- NGS Negative Control top % reads < 1.0%
- *IGH* SHM Positive Control (4-088-0008, can be purchased separately) top % reads ≥ 2.5%
- *IGH* SHM Positive Control mutation rate ≥ 2.0%
- MiSeq Run Validity Q30 > 70% for v3 (2x301)

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LymphoTrack® FAQ

- Will the software work on my computer?
  - The software requires Microsoft Windows 64 bit and Excel
- Do I always need to run the positive & negative controls?
  - Yes, these controls are always recommended & they are the only way our support team can guarantee the assay preformed correctly and provide troubleshooting tips to customers
- Two top reasons users have software difficulties:
  - Files named incorrectly. For the MiSeq® our software only recognizes filenames that contain the following characters: A-Z, a-z, 0-9, _ (underscore), - (hyphen)
  - Files from software CD were not copied on the computer or files are zipped
- Reason for low Q30 score on the MiSeq®
  - The Q30 score assesses the flow cell loading. Low scores likely indicate overloading or insufficient DNA quality
  - Q30 ≥ 75% for v2 (2x250); Q30 ≥ 70% for v3 (2x300)

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Take Home Message

- Broad menu of standardized kits for both B- and T-cell analysis with included bioinformatics package
- CE-IVD marked kits available
- Higher sensitivity and greater accuracy than traditional methods
- Be part of the community of labs and benefit from great publications and knowhow
- LymphoTrack® kits are a great solution for labs that wish to take the next step in their clonality analysis

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LymphoTrack® Assays

Full featured solution

- Designed for MiSeq®, Ion PGM™ & Ion S5™
- One-Step PCR Master Mixes – low contamination risk
- Easy Workflow for the study of Clonality, MRD and SHM analysis
- Flexibility/Scalability
- Multiplexing Targets & Applications
- Included Bioinformatics Software Package
- Comprehensive Technical Support

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Quiz

Name the 4 steps needed to start the analysis in the LymphoTrack® software:

1. Log in with username and password
2. Select targets
3. Choose decimal format
4. Browse to BAM files
5. Browse to FASTQ files
6. Select 'Launch Program'

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Quiz

Which of the following are requirements for running the LymphoTrack® software:

1. Windows 64 bit
2. Java 8, 64 bit or newer
3. PDF Reader
4. CD-Rom Drive
5. All of the above

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LymphoTrack® Related Publications

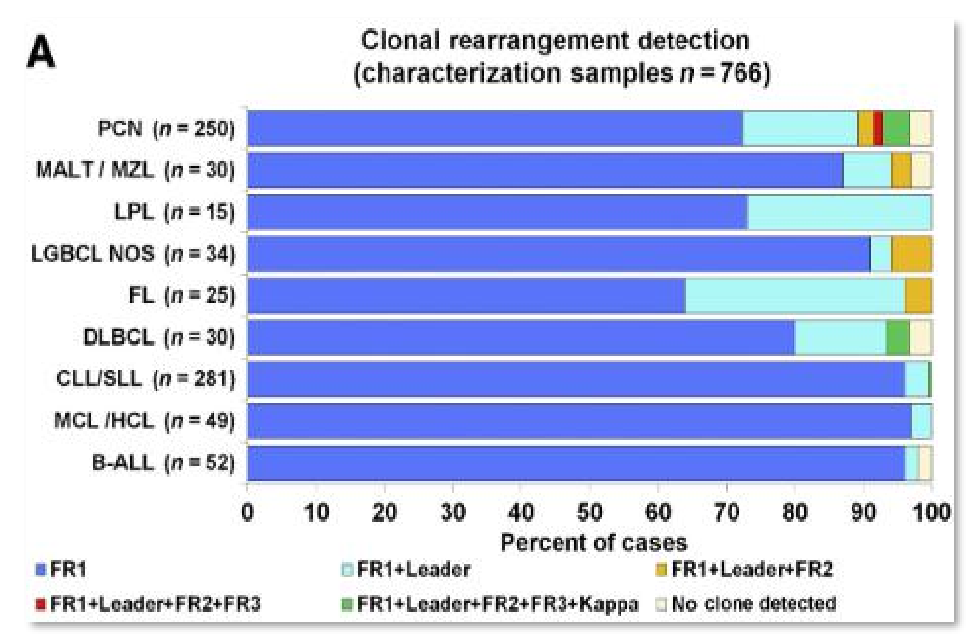
LymphoTrack® Related Publications

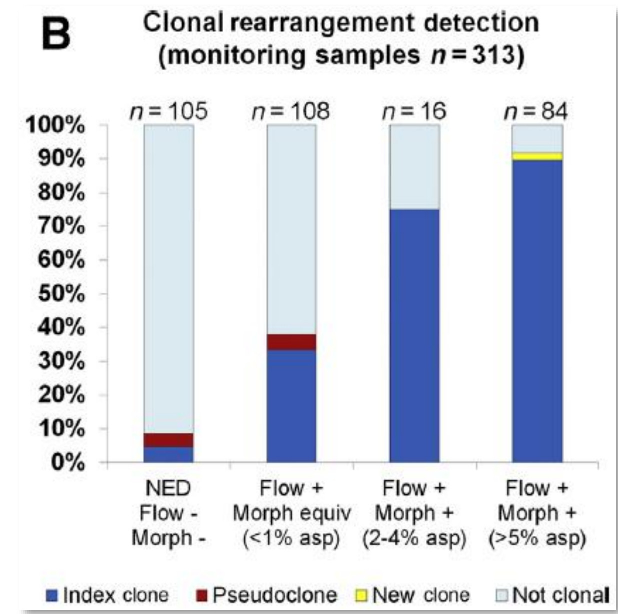
Highlights

- Large study with more than 1189 clinical samples
- Great publication for those considering LymphoTrack®
- NGS demonstrates superior performance compared with CE assays
  - higher sensitivity/resolution
  - improved detection
- Good notes with respect to implementation

Establishment of Immunoglobulin Heavy (IGH) Chain Clonality Testing by Next-Generation Sequencing for Routine Characterization of B-Cell and Plasma Cell Neoplasms

Maria E. Arcila,* Wayne Yu,† Mustafa Syed,† Hannah Kim,† Lidia Maciag,† JinJuan Yao,† Caleb Ho,† Kseniya Petrova,† Christine Moung,† Paulo Salazar,† Ivelise Rijo,† Tessara Baldi,† Ahmet Zehir,† Ola Landgren,† Jae Park,† Mikhail Roshal,† Ahmet Dogan,† and Khedoudja Nafa†





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LymphoTrack® Related Publications

ORIGINAL ARTICLE

Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia

B Stamatopoulos[1,2,3,7], A Timbs[1,7], D Bruce[1], T Smith[4], R Clifford[1,3], P Robbe[1,3], A Burns[1,3], DV Vavoulis[3], L Lopez[5], P Antoniou[3], J Mason[1], H Dreau[1] and A Schuh[1,6]

Highlights

- Demonstrates advantage of NGS as compared to Sanger sequencing for determining *IgHV* mutational status and identify the presence of multiple subclones.
- The additional information provided through NGS testing improves disease stratification and prognostication significantly

Population A

TFS | OS

only productive rearrangements are considered

a
Cumulative TFS (%)
P<0.0001
>280 mths
131 mths
29 mths
94 mths
15 mths
Time (months)

b
Cumulative OS (%)
>397 mths
292 mths
196 mths
100 mths
137 mths
P<0.0001
Time (months)

productive and unproductive rearrangements are considered

c
Cumulative TFS (%)
P<0.0001
>280 mths
131 mths
29 mths
61 mths
18 mths
Time (months)

d
Cumulative OS (%)
>397 mths
292 mths
196 mths
106 mths
137 mths
P<0.0001
Time (months)

		multiple M	1 M	mix M-UM	1 UM + VH3-21	multiple UM
only prod:	*n=*	*27*	*116*	*31*	*88*	*8*
prod+unprod.:	*n=*	*41*	*101*	*33*	*77*	*18*

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LymphoTrack® Related Publications

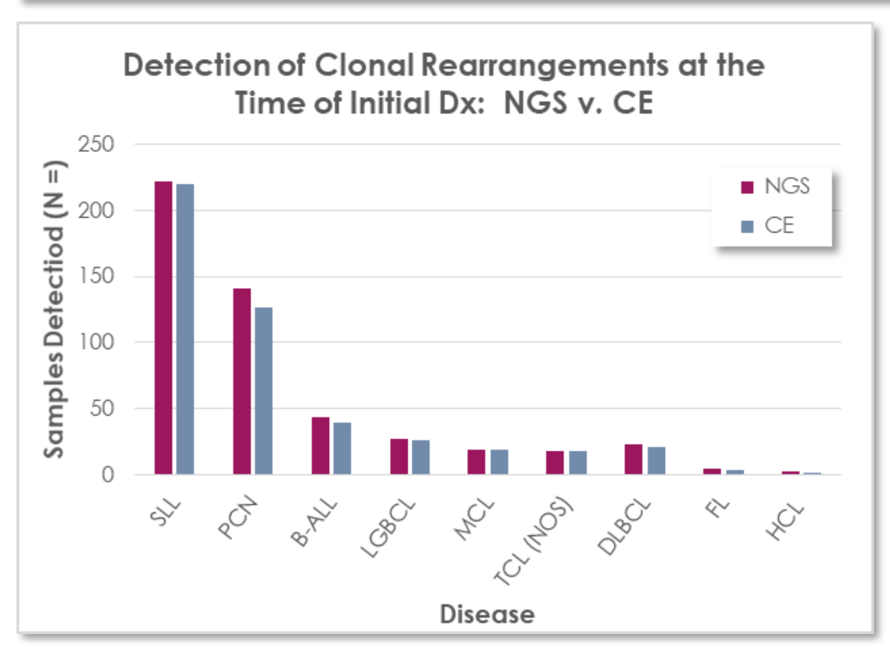
LymphoTrack® Assays

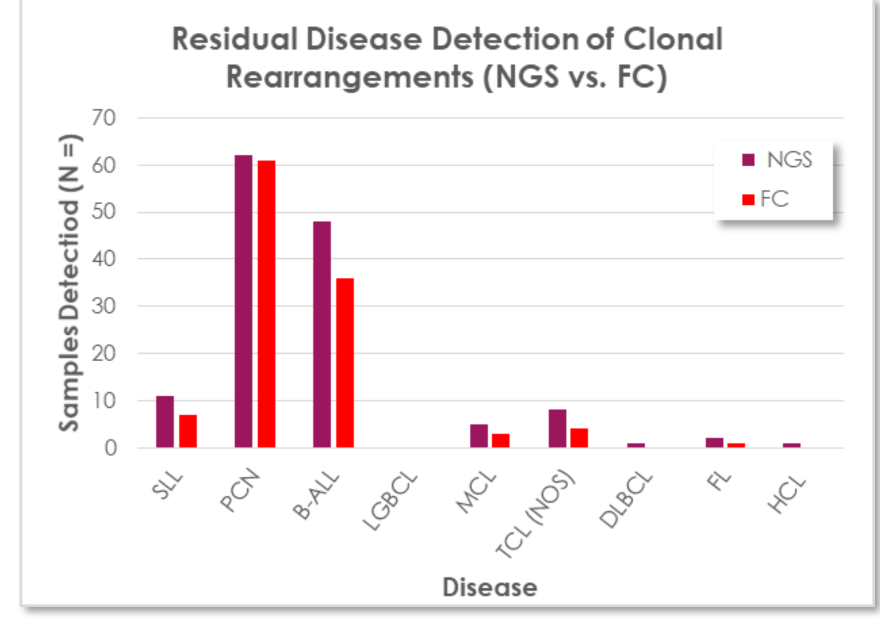
Highlights

- LymphoTrack® detected clonal rearrangements in 94% of Dx cases vs. 89% by CE.
- NGS was equivalent to FC for detection of plasma cell neoplasms (PCN) but showed advantages in disease monitoring for B-ALL, B and T cell lymphomas.

Next Generation Sequencing (NGS) Based IGH and TCR Clonality Assays Provide Excellent Specificity and Sensitivity for Routine Clonal Characterization and Monitoring of Lymphoproliferative Disorders

Maria E. Arcila, Mustafa Syed, Wayne Yu, Hannah Kim, JinYuan Yao, Caleb Ho, Kseniya Petrova-Drus, Mikhail Roshal, Jae H. Park, Ola Landgren, Ahmet Dogan, and Khedoudja Nafa





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