Somatic Hypermutation Analysis

Invivoscribe Offerings

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Improving Lives with Precision Diagnostics®

Invivoscribe Products for SHM - RUO

Gel & Capillary

IGH Somatic Hypermutation Assay v2.0
Gel Detection

IGH Somatic Hypermutation Assay v2.0
ABI Fluorescence Detection

NGS

LymphoTrack® *IGHV* Leader SHM Assay - MiSeq®

LymphoTrack® *IGH* FR1 Assay – MiSeq®

LymphoTrack® *IGH* FR1 Assay – S5/PGM™

Invivoscribe Products for SHM – CE-IVD

NGS

LymphoTrack® Dx
IGHV Leader SHM Assay - MiSeq®

LymphoTrack® Dx
IGH FR1 Assay – MiSeq®

LymphoTrack® Dx
IGH FR1 Assay – S5/PGM™

Invivoscribe Products for SHM

Gel & Capillary

RUO Products	Catalog #	
	Kit (33 rxn)	MegaKit (330 rxn)
Gel Detection		
IGH Somatic Hypermutation Assay v2.0	5-101-0030	5-101-0040
ABI Fluorescence Detection		
IGH Somatic Hypermutation Assay v2.0	5-101-0031	5-101-0041

Invivoscribe RUO Products for SHM

NGS

RUO Products	Catalog #	
	Kit A 8 indices (40 rxn)	Panel 24 indices (120 rxn)
MiSeq®		
LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay	7-121-0059	7-121-0069
LymphoTrack® *IGH* FR1 Assay	7-121-0009	7-121-0039
LymphoTrack® Software - MiSeq®	7-500-0009	
	12 indices (60 rxn)	Panel
S5/PGM™		
LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay	X	X
LymphoTrack® *IGH* FR1 Assay	7-121-0007	X
LymphoTrack® Software – S5/PGM™	7-500-0007	X

Up to 48 indices available

Invivoscribe CE-IVD Products for SHM

NGS

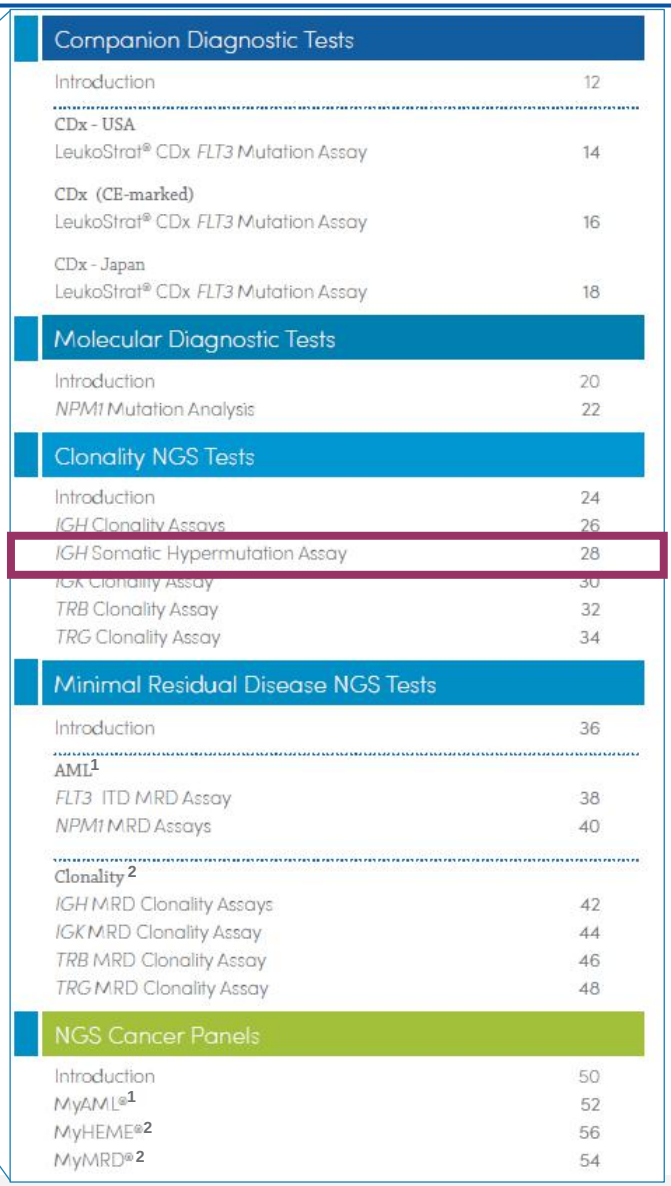
CE-IVD Products	Catalog #	
	Kit A 8 indices (40 rxn)	Panel 24 indices (120 rxn)
MiSeq®		
LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay	9-121-0059	9-121-0069
LymphoTrack® Dx *IGH* FR1 Assay	9-121-0009	9-121-0039
LymphoTrack® Dx Software - MiSeq®	9-500-0009	
	12 indices (60 rxn)	Panel
S5/PGM™		
LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay	X	X
LymphoTrack® Dx *IGH* FR1 Assay	9-121-0007	X
LymphoTrack® Dx Software – S5/PGM™	9-500-0007	X

LabPMM Clonality and SHM Testing

Services Catalog

Available services include:

- CDx *FLT3*
- NGS Gene Panels
- Clonality testing (*IGH, IGK, TRG & TRB*)
- MRD assays
- Custom assays



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[1]Testing is CLIA validated; [2]RUO Service

Quiz

Which options does Invivoscribe offer for SHM testing ?

- Assays kits compatible with Gel, Capillary or NGS methods
- Controls
- Service
- All of the above

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Gel and Capillary assays for SHM analysis

IGH Somatic Hypermutation Assay v2.0 (RUO)

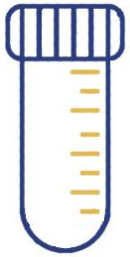
IGH Somatic Hypermutation Assay v2.0

Kit Contents

Reagent	Reagent Components		Unit Quantity	Kit # of Units	MegaKit # of Units
	Gel Detection	ABI Detection			
Master Mixes	Hypermutation Mix 1 v2.0 - Unlabeled	Hypermutation Mix 1 v2.0 – 6-FAM	1500µL	1	10
	Hypermutation Mix 2 v2.0 - Unlabeled	Hypermutation Mix 2 v2.0 – 6-FAM	1500µL	1	10
Template Amplification Control Master Mix	Specimen Control Size Ladder - Unlabeled	Specimen Control Size Ladder – 6-FAM	1500µL	1	10
Positive Control DNA and RNA	IVS-0013 Clonal Control DNA		100µL	1	5
	IVS-0013 Clonal Control RNA		100µL	1	5
Negative (Normal) Control DNA	IVS-0000 Polyclonal Control DNA		100µL	1	5
Sequencing Primer	*IGH* JH Primer - Unlabeled		10µL	1	5
	Primer – Hypermutation - Unlabeled		10µL	1	5

IGH Somatic Hypermutation Assay v2.0

Gel and ABI Fluorescence Detection

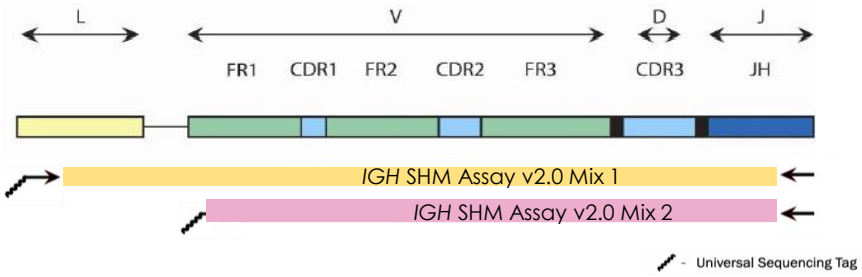


Mix 1

- Contains forward primers that target the **Leader (L) region**
- Sequence upstream of the *IGHV* gene
- Allows a complete analysis of the *IGHV* gene

Mix 2

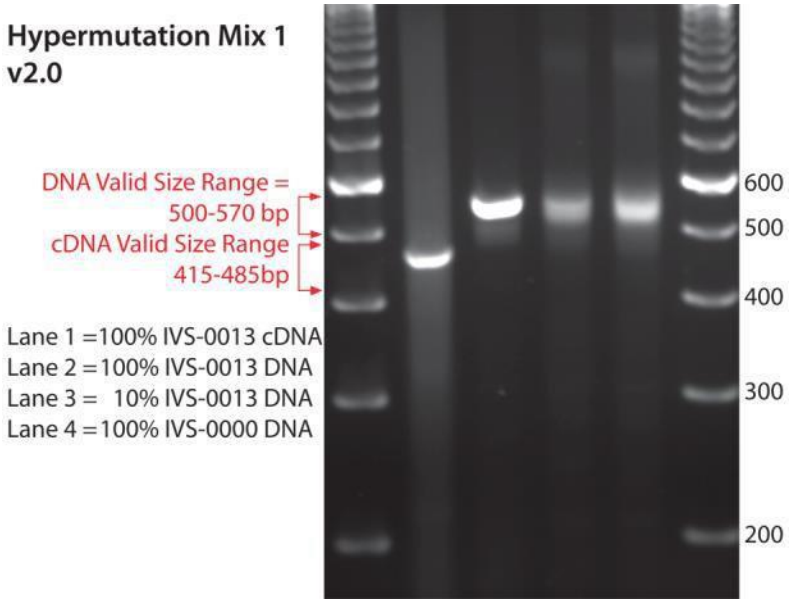
- Contains forward primers that target the **Framework Region 1 (FR1)**
- Allow an analysis of sequence between FR1 and the downstream joining (J) region of the *IGHV* gene



IGH Somatic Hypermutation Assay v2.0

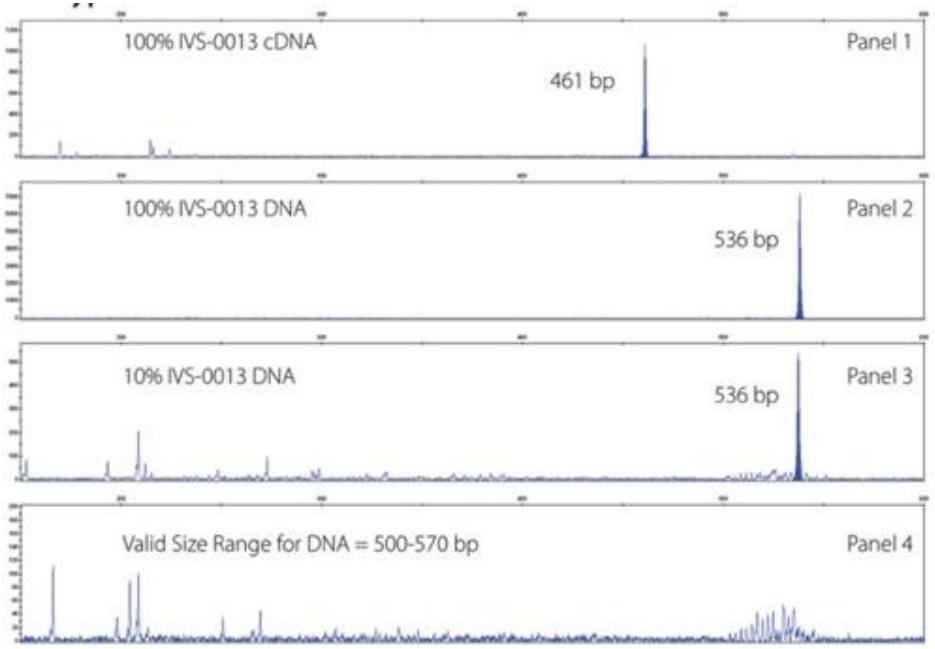
Mix 1 Clonality Assessment

Gel Detection



- Mix 1 generates large product sizes which have low resolution when run on a gel
- For higher resolution, decrease voltage and run for a longer duration

ABI Fluorescence Detection

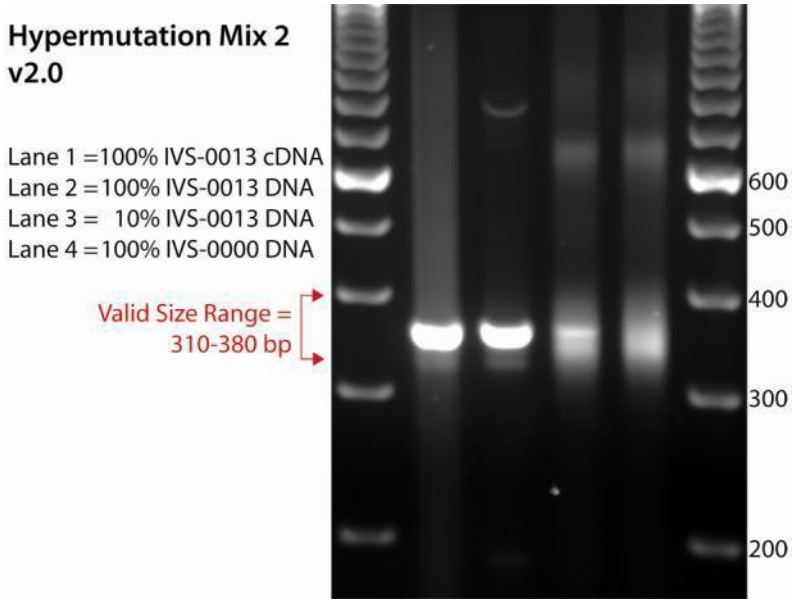


- The resolution is enhanced by the fluorescence label and peak detection software

IGH Somatic Hypermutation Assay v2.0

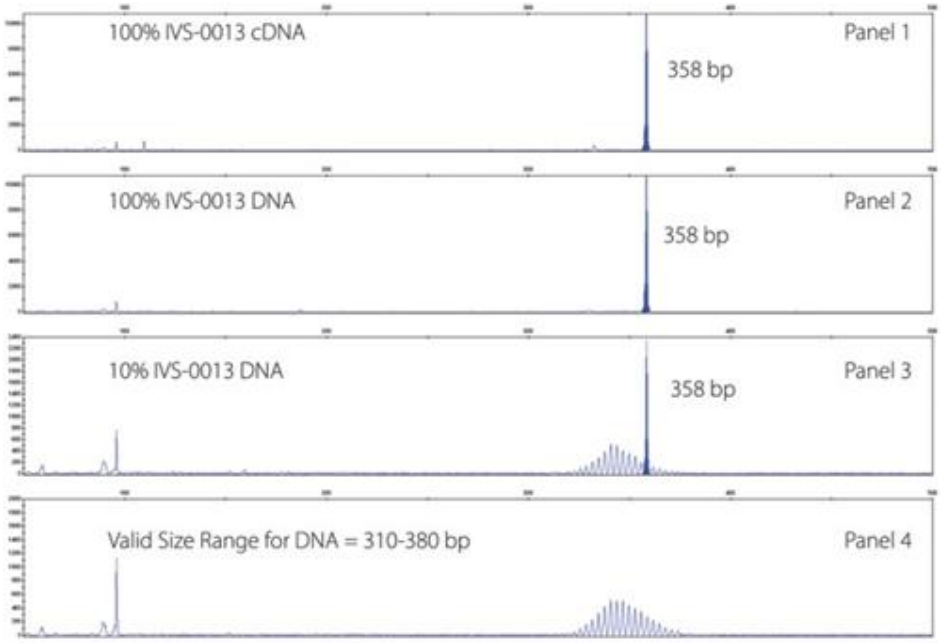
Mix 2 Clonality Assessment

Gel Detection



- Mix 2 generates smaller product sizes and has higher resolution, as compared to mix 1
- Mutation coverage by this primer set may be decreased relative to Mix 1 as products do not include the complete FR1 sequence

ABI Fluorescence Detection

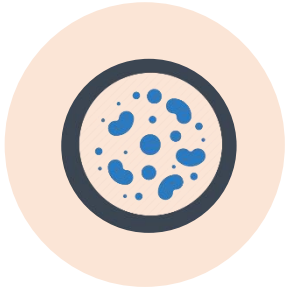


- The resolution is enhanced by the fluorescence label and peak detection software

IGH Somatic Hypermutation Assay v2.0

Sequencing of PCR product

Cloning into a vector



- Only use with unlabeled amplicons
- Works best with weak clonal bands or if there is more than one clonal band

Sanger Sequencing

Gel Extraction

- Works best with weak clonal bands or if there is more than one clonal band

Direct Sanger Sequencing

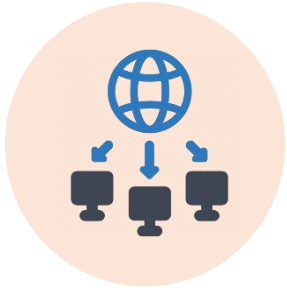
- Works best with little to no background amplification and only one clonal product
- Risk of Sequencing Failures / Presence of multiple PCR products

Time Consuming and Labor Intensive

IGH Somatic Hypermutation Assay v2.0

Data bank selection & Data analysis

Data Bank Selection



Find and align the germline V region sequence that best corresponds to the sample sequence.

Sequence Data Analysis

Determine the number of mismatched bases and the total number of bases that are being compared.

Hypermutation Reporting

$$\% \text{ divergence} = \frac{\text{N (mismatched bases)}}{\text{total N bases}}$$

$$\% \text{ homology} = 100\% - \% \text{ divergence}$$

IMGT – The International ImMunoGeneTics information system
- Analysis tools : IMGT/V-QUEST and IMGT/Junction Analysis

V BASE – The MRC Centre for Protein Engineering's Database of human antibody genes
- Analysis tools : DNAPLOT

NCBI – National Center for Biotechnology Information
- Analysis tool : IgBLAST (Basic Local Alignment Search Tool)

Clonal Control: Sequence data obtained from the positive IVS-0013 Clonal Control DNA or RNA should correspond to an unmutated, in-frame VH1-46 to JH4 rearrangement.

SHM status*:
- % divergence ≥ 2% : Presence of *IGH* SHM
- % divergence <2% : Absence of *IGH* SHM

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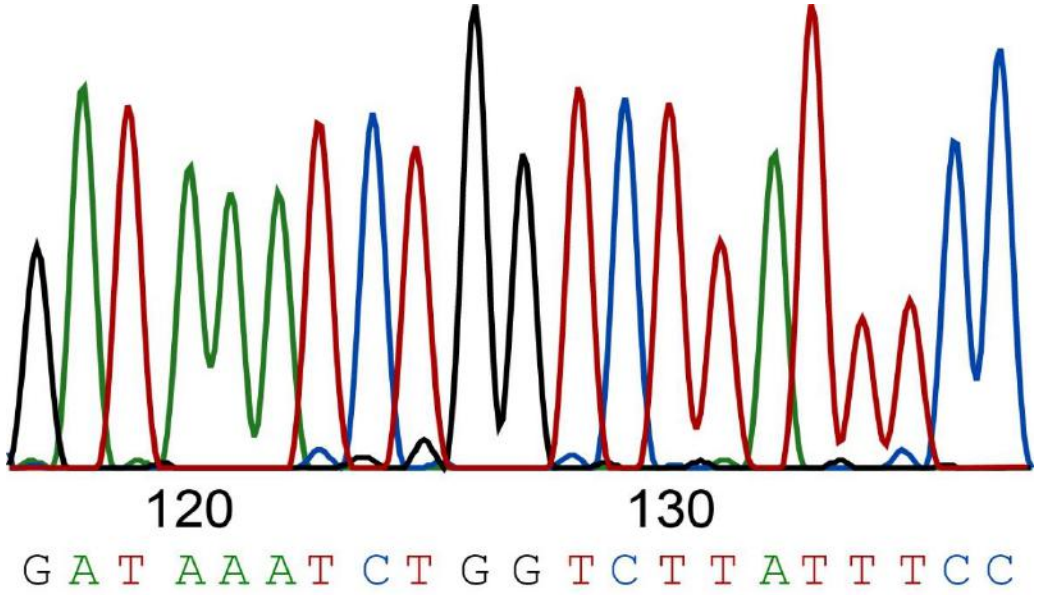
*Gupta et. al "Evaluation of Somatic Hypermutation Status in Chronic Lymphocytic Leukemia (CLL) in the Era of Next Generation Sequencing" Front Cell Dev Biol. 2020; 8: 357.

Traditional Method for *IGHV* Analysis

Sanger Sequencing is standard, BUT it has caveats

- May not differentiate different clonal populations in a sample
- Lower sensitivity
- Failure rate is 9-18%



Krober A, et al. Blood 2002; 100: 1410–1416.
Austen B, et al. Blood 2005; 106: 3175–3182.
Burger JA, et al. Lancet Oncol 2014; 15:1090–1099

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NGS Assays for SHM Analysis

LymphoTrack® SHM Assays

Widely Available Sequencing Platforms

Ion PGM™

Ion S5™

MiSeq®

NGS Assays for SHM Analysis:

- LymphoTrack® *IGHV* Leader SHM Assay - MiSeq®
- LymphoTrack® *IGH* FR1 Assay – MiSeq®
- LymphoTrack® *IGH* FR1 Assay – S5/PGM™

Same kits as for Clonality Assessment!

LymphoTrack® Dx SHM Assays

LymphoTrack® Dx Assays

Widely Available Sequencing Platforms

Ion PGM™

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MiSeq®

NGS Assays for SHM Analysis:

- LymphoTrack® Dx *IGHV* Leader SHM Assay - MiSeq®
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Same kits as for Clonality Assessment!

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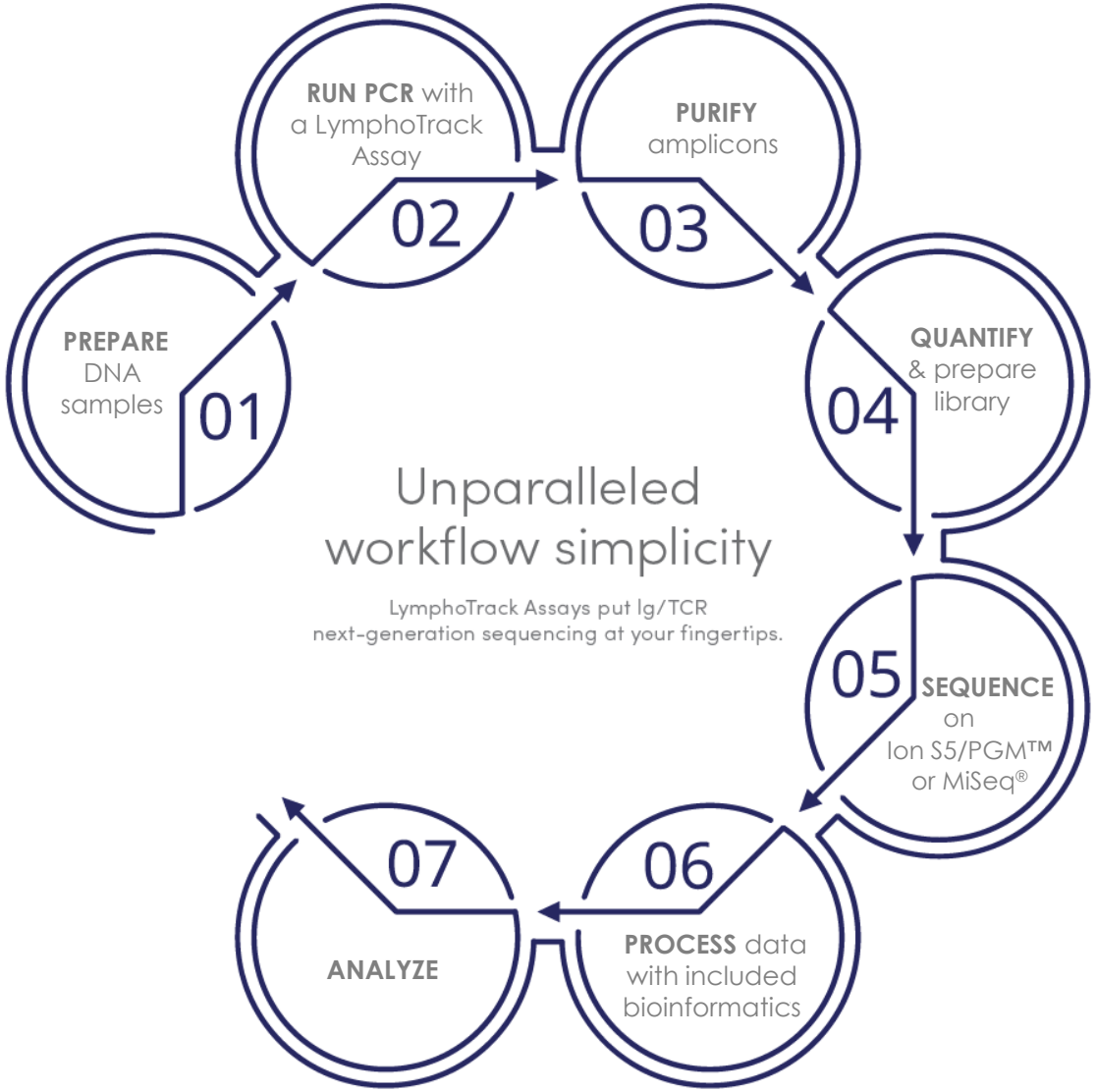
Same Assays and Workflow for Clonality & SHM

LymphoTrack® Dx Assays



Comprehensive Menu

LymphoTrack® Assays

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

SHM Analysis

Comprehensive Menu

LymphoTrack® Dx Assays

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

SHM Analysis

LymphoTrack® SHM Assays

LymphoTrack® Assays

Kit Contents

Reagent	Reagent Components		Unit Quantity	Kit A # of Units	Panel # of Units
	IGHV Leader Hypermutation Somatic Assay - MiSeq®	*IGH* FR1 Assay - MiSeq®			
MiSeq®					
Master Mixes	*IGH* Leader MiSeq 08 indices	*IGH* FR1 MiSeq 08 indices	250µl	8	24
	IGH Leader MiSeq 16 additional indices	*IGH* FR1 MiSeq 16 additional indices		8	24
Positive Control DNA	*IGH* SHM POS (+)	X	45µl	1	3
	IGH POS (+)				
Negative Control DNA	NGS NEG (-)				
S5/PGM™					
Master Mixes	*IGH FR1 S5/PGM* 12 indices		250µl	12	X
Positive Control DNA	*IGH* POS (+)		45µl	2	X
Negative Control DNA	NGS NEG (-)				

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LymphoTrack® Dx SHM Assays

LymphoTrack® Dx Assays

Kit Contents

Reagent	Reagent Components		Unit Quantity	Kit A # of Units	Panel # of Units
	IGHV Leader Hypermutation Somatic Assay - MiSeq®	*IGH* FR1 Assay - MiSeq®			
MiSeq®					
Master Mixes	*IGH* Leader MiSeq 08 indices	*IGH* FR1 MiSeq 08 indices	250µl	8	24
	IGH Leader MiSeq 16 additional indices	*IGH* FR1 MiSeq 16 additional indices		8	24
Positive Control DNA	*IGH* SHM POS (+)	X	45µl	1	3
	IGH POS (+)				
Negative Control DNA	NGS NEG (-)				
S5/PGM™					
Master Mixes	*IGH FR1 S5/PGM* 12 indices		250µl	12	X
Positive Control DNA	*IGH* POS (+)		45µl	2	X
Negative Control DNA	NGS NEG (-)				

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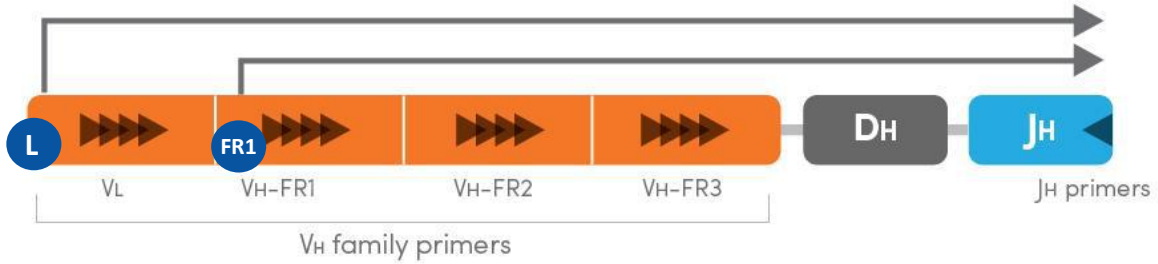
LymphoTrack® SHM Assays

Two primary options:

Schematic of the *IGH* Gene Locus :



MiSeq® – 2 Options:

- *IGHV Leader* Somatic Hypermutation Assay
  - Primers target the *IGHV* Leader sequence
- *IGH* FR1 Assay
  - Primers target the *IGH* FR1

Ion Torrent™ – 1 Option:

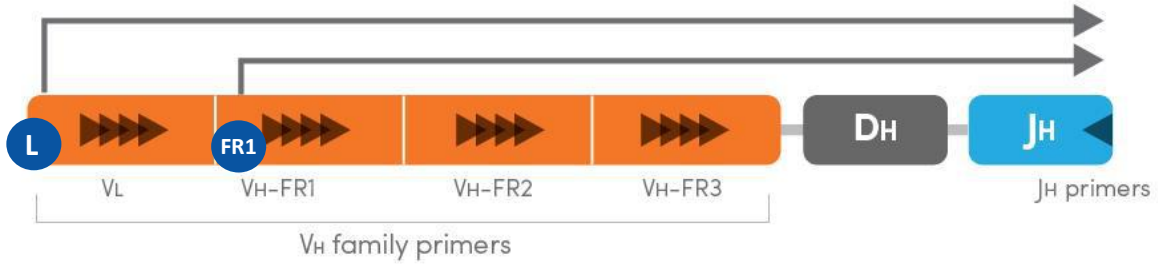
- *IGH* FR1 Assay
  - Primers target the *IGH* FR1

LymphoTrack® Dx SHM Assays

LymphoTrack® Dx Assays

Two primary options:

Schematic of the *IGH* Gene Locus :



MiSeq® – 2 Options:

- *IGHV Leader* Somatic Hypermutation Assay
  - Primers target the *IGHV* Leader sequence
- *IGH* FR1 Assay
  - Primers target the *IGH* FR1

Ion Torrent™ – 1 Option:

- *IGH* FR1 Assay
  - Primers target the *IGH* FR1

SHM LymphoTrack® Data

LymphoTrack® Assays

LymphoTrack® *IGH* FR1 and *IGHV* Leader Somatic Hypermutation Assays

Sample Name

Total reads = 32,458

Easy identification of specific types of gene rearrangements such as *IGHV*3-21.

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	*IGHV*4-59_08	*IGHJ*4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	*IGHV*5-51_01	*IGHJ*4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	*IGHV*3-7_01	*IGHJ*4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	*IGHV*5-51_01	*IGHJ*5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	*IGHV*5-51_01	*IGHJ*4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	*IGHV*5-51_01	*IGHJ*4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	*IGHV*2-5_10	*IGHJ*5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	*IGHV*3-48_02	*IGHJ*6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	*IGHV*5-51_02	*IGHJ*2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	*IGHV*5-51_02	*IGHJ*2_01	0.05	10.44	3.38	Y	N	26.01

SHM LymphoTrack® Dx Data

LymphoTrack® Dx Assays

LymphoTrack® Dx *IGH* FR1 and *IGHV* Leader Somatic Hypermutation Assays

Sample Name

Total reads = 32,458

Easy identification of specific types of gene rearrangements such as *IGHV*3-21.

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	*IGHV*4-59_08	*IGHJ*4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	*IGHV*5-51_01	*IGHJ*4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	*IGHV*3-7_01	*IGHJ*4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	*IGHV*5-51_01	*IGHJ*5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	*IGHV*5-51_01	*IGHJ*4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	*IGHV*5-51_01	*IGHJ*4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	*IGHV*2-5_10	*IGHJ*5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	*IGHV*3-48_02	*IGHJ*6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	*IGHV*5-51_02	*IGHJ*2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	*IGHV*5-51_02	*IGHJ*2_01	0.05	10.44	3.38	Y	N	26.01

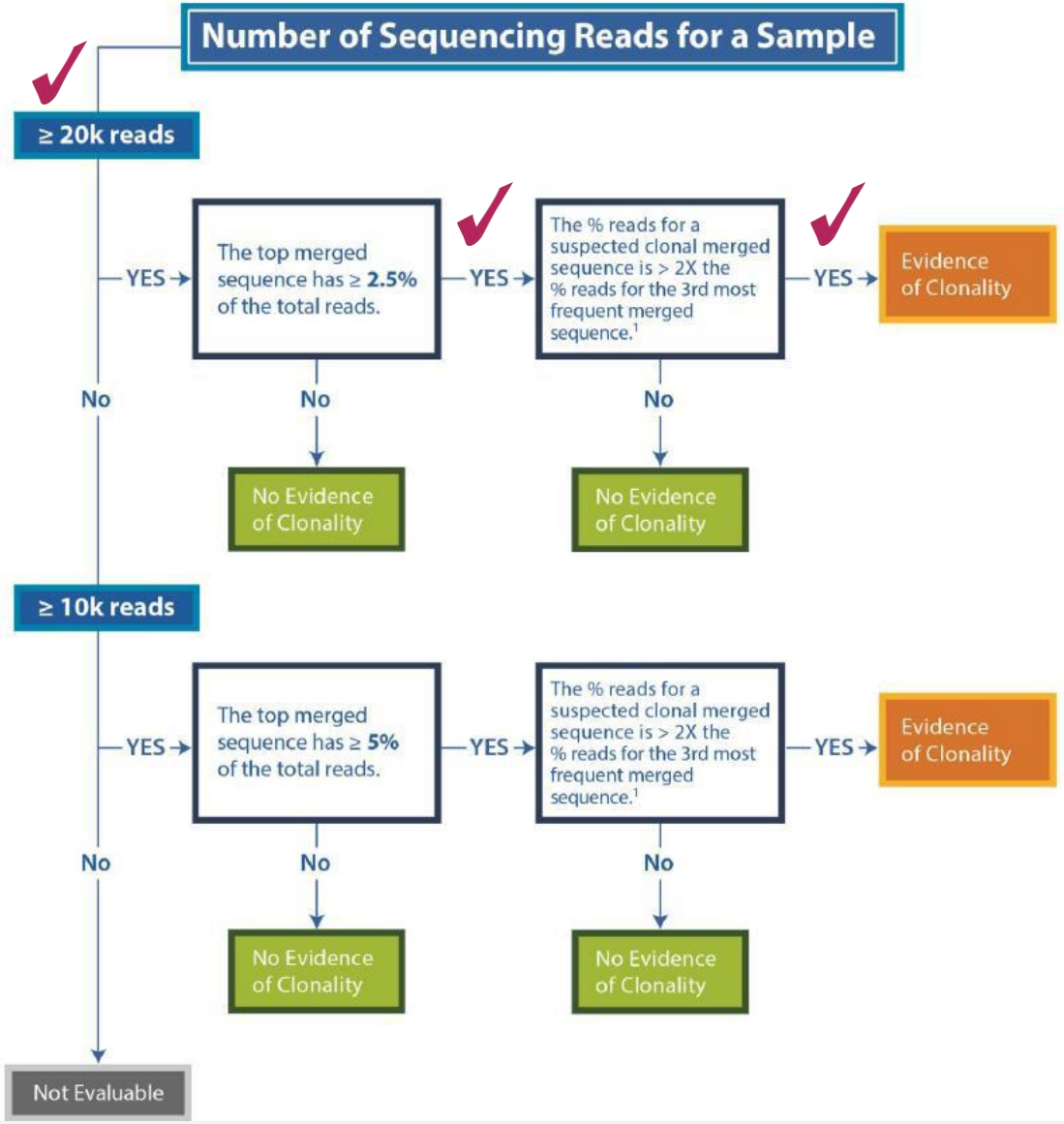
Interpreting SHM – Step 1

LymphoTrack Dx Assays

1/2 - Determine Clonality

Total Read Count 474947

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %
1	TTCTCGTGGTGGC	455	50248	IGHV4-59_08	IGHJ4_02	10.58	10.58
2	CTGCTACTGACTG	319	192	IGHV2-70_10	IGHJ4_02	0.04	10.62
3	CTGCTGCTGACCA	466	175	IGHV2-5_01	IGHJ5_01	0.04	10.66
4	CTGCTGCTGACCA	457	162	IGHV2-5_05	IGHJ6_02	0.03	10.69
5	CTGCTGCTGACCA	474	154	IGHV2-5_05	IGHJ4_02	0.03	10.72
6	CTGCTGCTGACCA	454	150	IGHV2-5_10	IGHJ5_02	0.03	10.76



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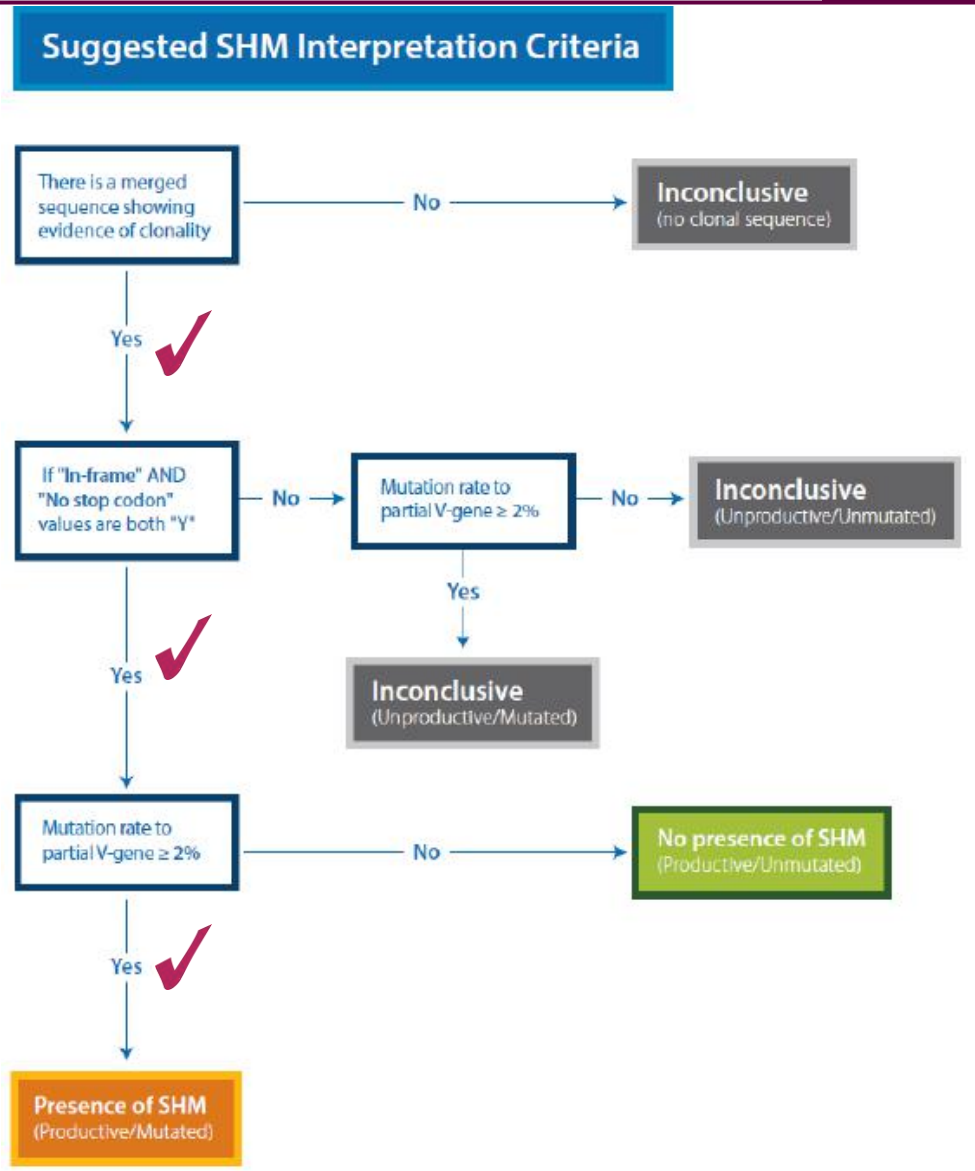
Interpreting SHM – Step 2

LymphoTrack Dx Assays

2/2 - Determine SHM Status

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	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



Data Interpretation – *IGHV* and FR1

LymphoTrack Dx Assays

1/2 - Determine Clonality

1. Ensure Total Read Count is >20000 (or 10000)
2. Check Q30 Score
3. Top ranked sequence should account for at least 2.5% (or 5%) of total reads
4. Top ranked sequence should be >2x the 3rd ranked sequence

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	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	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

Data Interpretation – *IGHV SHM*

LymphoTrack Dx Assays

2/2 - Determine SHM Status

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	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	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

5. If sample is clonal, check whether clone is productive (Y+Y = Productive)

6. Identify whether mutation rate is >2%

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

Case Study – Oxford Univ. Hospitals

LymphoTrack Dx Assays

Leukemia (2016), 1–9

www.nature.com/leu

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]

The immunoglobulin heavy-chain variable region gene (IgHV) mutational status is considered the gold standard of prognostication in chronic lymphocytic leukemia (CLL) and is currently determined by Sanger sequencing that allows the analysis of the major clone. Using next-generation sequencing (NGS), we sequenced the *IgHV* gene from two independent cohorts: (A) 270 consecutive patient samples obtained at diagnosis and (B) 227 patients from the UK ARCTIC-AdMIRe clinical trials. Using complementary DNA from purified CD19+CD5+ cells, we demonstrate the presence of multiple rearrangements in independent experiments and showed that 24.4% of CLL patients express multiple productive clonally unrelated IgHV rearrangements. On the basis of IgHV-NGS subclonal profiles, we defined five different categories: patients with (a) multiple hypermutated (M) clones, (b) 1 M clone, (c) a mix of M-unmutated (UM) clones, (d) 1 UM clone and (e) multiple UM clones. In population A, IgHV-NGS classification stratified patients into five different subgroups with median treatment-free survival (TFS) of >280(a), 131(b), 94(c), 29(d), 15(e) months ($P < 0.0001$) and a median OS of >397(a), 292(b), 196(c), 137(d) and 100(e) months ($P < 0.0001$). In population B, the poor prognosis of multiple UM patients was confirmed with a median TFS of 2 months ($P = 0.0038$). In conclusion, IgHV-NGS highlighted one quarter of CLL patients with multiple productive IgHV subclones and improves disease stratification and raises important questions concerning the pre-leukemic cellular origin of CLL.

Leukemia advance online publication, 9 December 2016; doi:10.1038/leu.2016.307

Stamatopoulos, B., Timbs, A., Bruce, D. et al. Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. *Leukemia* 31, 837–845 (2017). https://doi.org/10.1038/leu.2016.307

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Improving Lives with Precision Diagnostics

Sample Types – 2 Independent Cohorts

LymphoTrack® Dx Assays

Cohort 1 (n=270)

- Consecutive subject samples obtained during baseline testing
- Peripheral blood, CD19+ cells were selected
- RNA extracted & cDNA were tested

Cohort 2 (n=227)

- Subjects from the UK ARCTIC-AdMIRe clinical trials
- Peripheral blood, no selection
- gDNA tested

LymphoTrack® Dx
IGHV Leader Somatic Hypermutation Assay

Stamatopoulos, B., Timbs, A., Bruce, D. et al. Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. *Leukemia* 31, 837–845 (2017). https://doi.org/10.1038/leu.2016.307

Results

LymphoTrack® Dx Assays

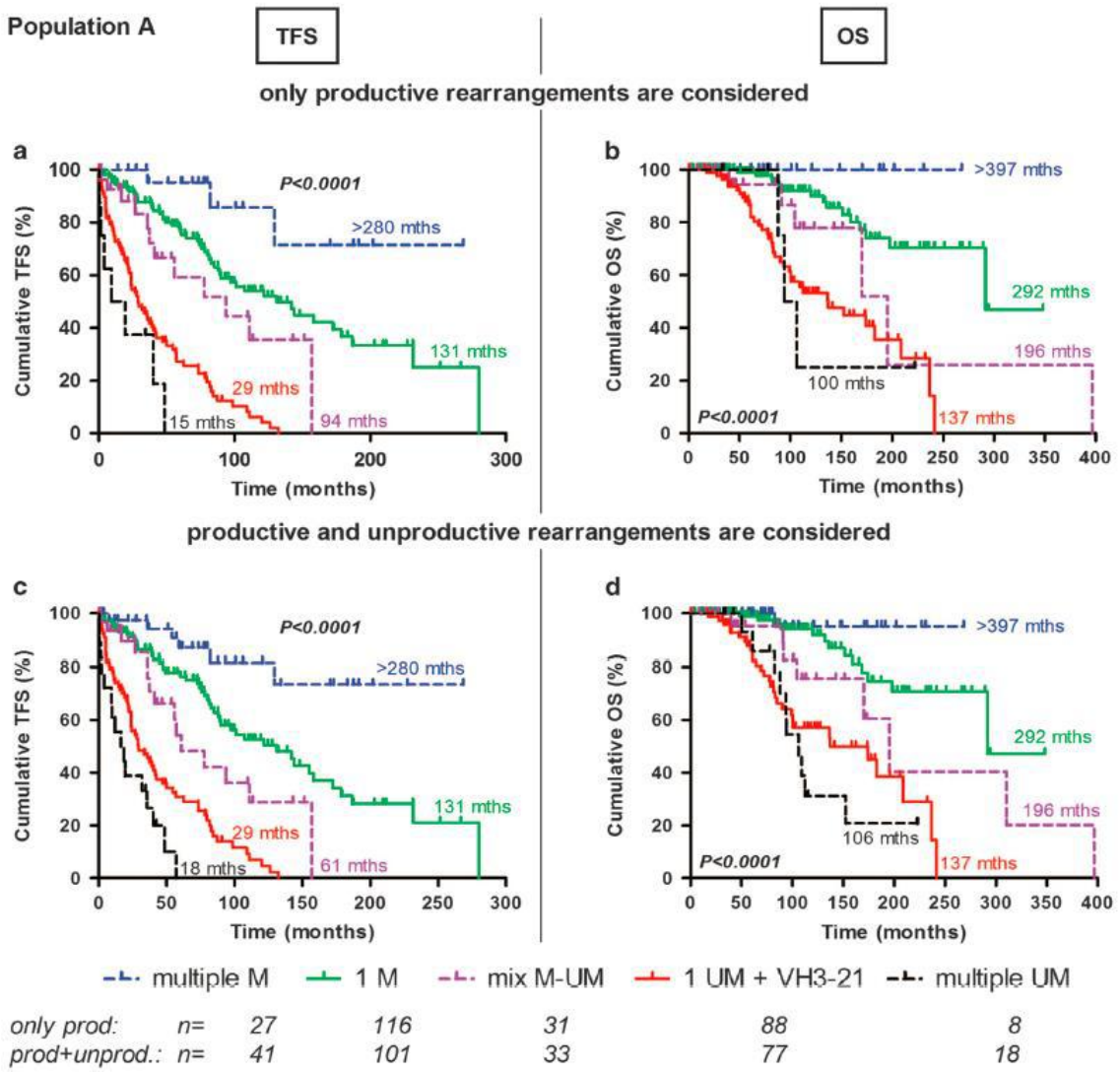
In 24.4% of CLL samples tested multiple productive subclones are identified

- 2 studies for a total of 497 samples

Five categories

1. Multiple mutated clones
2. One mutated clone
3. Mix of mutated and unmutated clones
4. One unmutated clone, but the presence of a VH3-21 clone
5. Multiple unmutated clones

In this study the prognostic value of productive vs. unproductive rearrangements was the same



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Stamatopoulos, B., Timbs, A., Bruce, D. et al. Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. *Leukemia* 31, 837–845 (2017). https://doi.org/10.1038/leu.2016.307

NGS vs. Sanger Sequencing

LymphoTrack® Dx Assays

Sequence and mutation rate for most abundant clone compared

- *IGHV* mutation status concordance = **99.6%** (235/236)
  - Discordance in biclonal sample, small unmutated clone identified by Sanger
- Same sequence identified in **97.9%** of samples (231/236)
  - 5 cases the discordance was 1 bp and in 4 of these cases NGS sequence was closest to germline **suggesting sequencing error with Sanger**

Stamatopoulos, B., Timbs, A., Bruce, D. et al. Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. *Leukemia* 31, 837–845 (2017). https://doi.org/10.1038/leu.2016.307

Conclusions

LymphoTrack® Dx Assays

- *IgHV* mutational status by NGS refines *IgHV* Sanger Sequencing classification and can be used to define five different prognostic subgroups in an unselected population

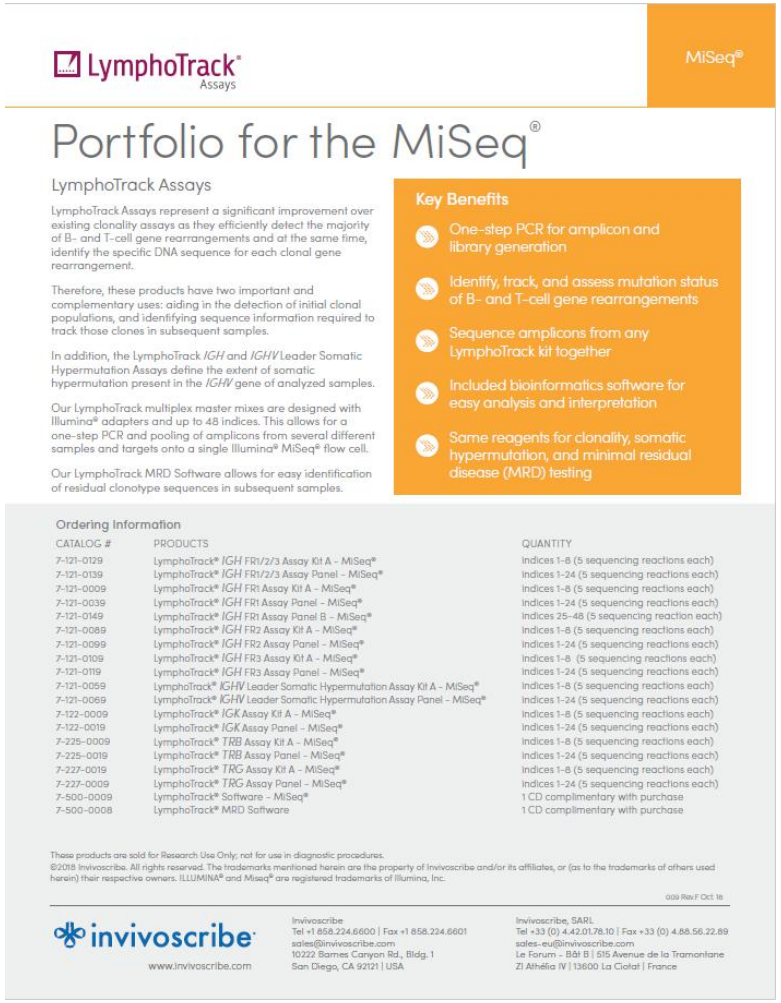
- NGS-*IgHV* classification was able to precisely classify subjects with multiple *IgHV* rearrangements for which Sanger Sequencing was inconclusive and improved prognostication for 92 out of 270 cases

Stamatopoulos, B., Timbs, A., Bruce, D. et al. Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. *Leukemia* 31, 837–845 (2017). https://doi.org/10.1038/leu.2016.307

Promotional Tools - Flyers

LymphoTrack® Assays

LymphoTrack® Flyers (RUO) – MiSeq® and Ion S5/PGM™



LymphoTrack® Assays

MiSeq®

Portfolio for the MiSeq®

LymphoTrack Assays

LymphoTrack Assays represent a significant improvement over existing clonality assays as they efficiently detect the majority of B- and T-cell gene rearrangements and at the same time, identify the specific DNA sequence for each clonal gene rearrangement.

Therefore, these products have two important and complementary uses: aiding in the detection of initial clonal populations, and identifying sequence information required to track those clones in subsequent samples.

In addition, the LymphoTrack *IGH* and *IGHV* Leader Somatic Hypermutation Assays define the extent of somatic hypermutation present in the *IGHV* gene of analyzed samples.

Our LymphoTrack multiplex master mixes are designed with Illumina® adapters and up to 48 indices. This allows for a one-step PCR and pooling of amplicons from several different samples and targets onto a single Illumina® MiSeq® flow cell.

Our LymphoTrack MRD Software allows for easy identification of residual clonotype sequences in subsequent samples.

Key Benefits

- One-step PCR for amplicon and library generation
- Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack kit together
- Included bioinformatics software for easy analysis and interpretation
- Same reagents for clonality, somatic hypermutation, and minimal residual disease (MRD) testing

Ordering Information

CATALOG #	PRODUCTS	QUANTITY
7-121-0129	LymphoTrack® *IGH* FR1/2/3 Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-121-0139	LymphoTrack® *IGH* FR1/2/3 Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-121-0009	LymphoTrack® *IGH* FR1 Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-121-0039	LymphoTrack® *IGH* FR1 Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-121-0149	LymphoTrack® *IGH* FR1 Assay Panel B - MiSeq®	Indices 25-48 (5 sequencing reaction each)
7-121-0089	LymphoTrack® *IGH* FR2 Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-121-0099	LymphoTrack® *IGH* FR2 Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-121-0109	LymphoTrack® *IGH* FR3 Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-121-0119	LymphoTrack® *IGH* FR3 Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-121-0059	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-121-0069	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-122-0009	LymphoTrack® *IGK* Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-122-0019	LymphoTrack® *IGK* Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-225-0009	LymphoTrack® *TRB* Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-225-0019	LymphoTrack® *TRB* Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-227-0019	LymphoTrack® *TRG* Assay Kit A - MiSeq®	Indices 1-8 (5 sequencing reactions each)
7-227-0009	LymphoTrack® *TRG* Assay Panel - MiSeq®	Indices 1-24 (5 sequencing reactions each)
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software	1 CD complimentary with purchase

These products are sold for Research Use Only; not for use in diagnostic procedures.

009 Rev F Oct. 18

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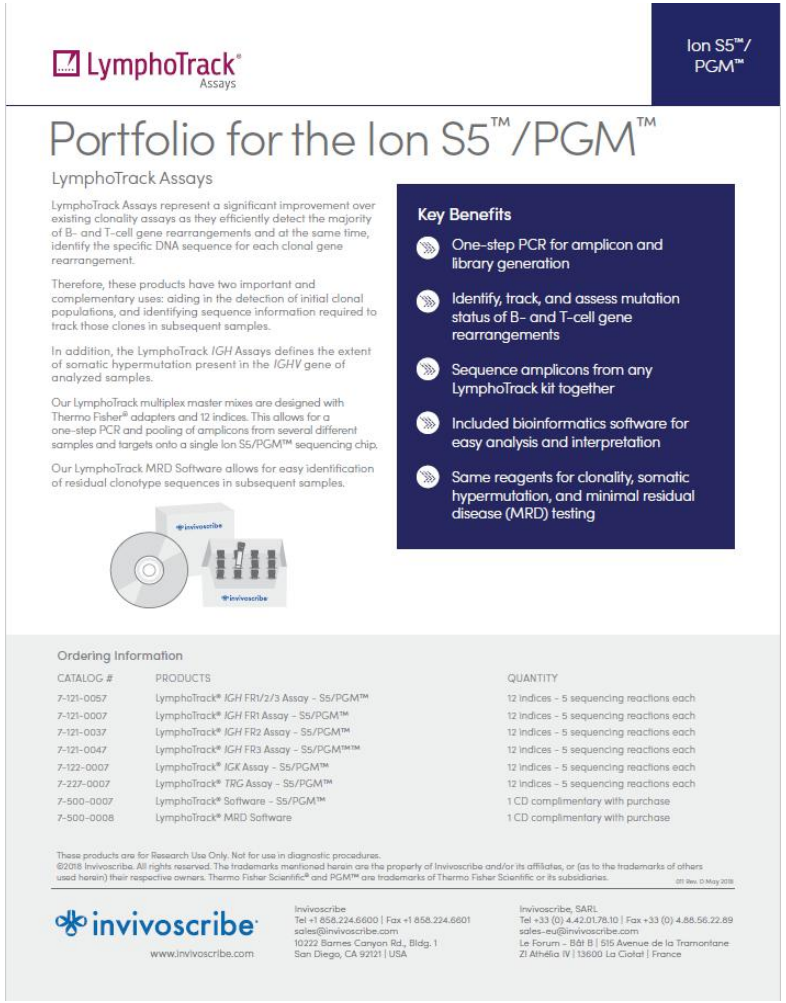

LymphoTrack® Assays

Ion S5™/ PGM™

Portfolio for the Ion S5™/PGM™

LymphoTrack Assays

LymphoTrack Assays represent a significant improvement over existing clonality assays as they efficiently detect the majority of B- and T-cell gene rearrangements and at the same time, identify the specific DNA sequence for each clonal gene rearrangement.

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In addition, the LymphoTrack *IGH* Assays defines the extent of somatic hypermutation present in the *IGHV* gene of analyzed samples.

Our LymphoTrack multiplex master mixes are designed with Thermo Fisher® adapters and 12 indices. This allows for a one-step PCR and pooling of amplicons from several different samples and targets onto a single Ion S5/PGM™ sequencing chip.

Our LymphoTrack MRD Software allows for easy identification of residual clonotype sequences in subsequent samples.

Key Benefits

- One-step PCR for amplicon and library generation
- Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack kit together
- Included bioinformatics software for easy analysis and interpretation
- Same reagents for clonality, somatic hypermutation, and minimal residual disease (MRD) testing

Ordering Information

CATALOG #	PRODUCTS	QUANTITY
7-121-0057	LymphoTrack® *IGH* FR1/2/3 Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-121-0007	LymphoTrack® *IGH* FR1 Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-121-0037	LymphoTrack® *IGH* FR2 Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-121-0047	LymphoTrack® *IGH* FR3 Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-122-0007	LymphoTrack® *IGK* Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-227-0007	LymphoTrack® *TRG* Assay - S5/PGM™	12 Indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software	1 CD complimentary with purchase

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011 Rev. D May 2018

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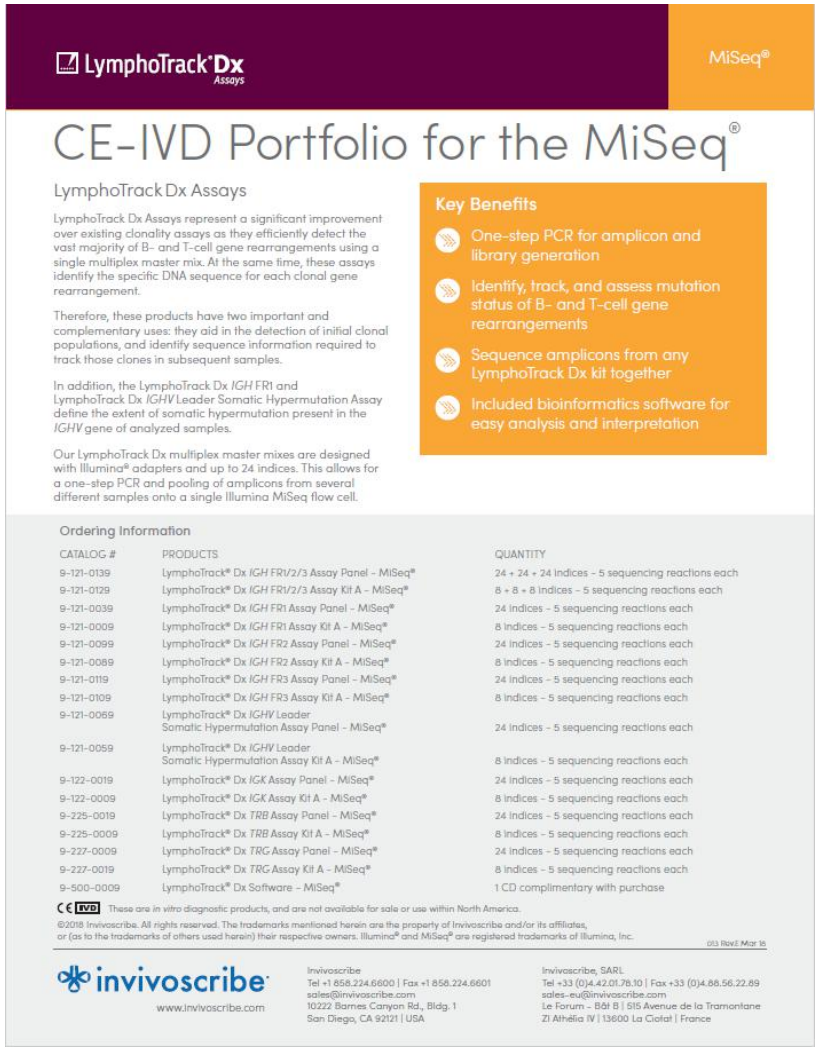
Promotional Tools - Flyers

LymphoTrack® Flyers (CE-IVD) – MiSeq® and Ion S5/PGM™



LymphoTrack Dx Assays

MiSeq®

CE-IVD Portfolio for the MiSeq®

LymphoTrack Dx Assays represent a significant improvement over existing clonality assays as they efficiently detect the vast majority of B- and T-cell gene rearrangements using a single multiplex master mix. At the same time, these assays identify the specific DNA sequence for each clonal gene rearrangement.

Therefore, these products have two important and complementary uses: they aid in the detection of initial clonal populations, and identify sequence information required to track those clones in subsequent samples.

In addition, the LymphoTrack Dx *IGH* FR1 and LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay define the extent of somatic hypermutation present in the *IGHV* gene of analyzed samples.

Our LymphoTrack Dx multiplex master mixes are designed with Illumina® adapters and up to 24 indices. This allows for a one-step PCR and pooling of amplicons from several different samples onto a single Illumina MiSeq flow cell.

Key Benefits

- One-step PCR for amplicon and library generation
- Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack Dx kit together
- Included bioinformatics software for easy analysis and interpretation

Ordering Information

CATALOG #	PRODUCTS	QUANTITY
9-121-0139	LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel – MiSeq®	24 + 24 + 24 Indices – 5 sequencing reactions each
9-121-0129	LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A – MiSeq®	8 + 8 + 8 Indices – 5 sequencing reactions each
9-121-0039	LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-121-0009	LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-121-0099	LymphoTrack® Dx *IGH* FR2 Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-121-0089	LymphoTrack® Dx *IGH* FR2 Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-121-0119	LymphoTrack® Dx *IGH* FR3 Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-121-0109	LymphoTrack® Dx *IGH* FR3 Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-121-0069	LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-121-0059	LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-122-0019	LymphoTrack® Dx *IGK* Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-122-0009	LymphoTrack® Dx *IGK* Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-225-0019	LymphoTrack® Dx *TRB* Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-225-0009	LymphoTrack® Dx *TRB* Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-227-0009	LymphoTrack® Dx *TRG* Assay Panel – MiSeq®	24 Indices – 5 sequencing reactions each
9-227-0019	LymphoTrack® Dx *TRG* Assay Kit A – MiSeq®	8 Indices – 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software – MiSeq®	1 CD complimentary with purchase

CE IVD These are *in vitro* diagnostic products, and are not available for sale or use within North America.

013 Rev.E Mar 16

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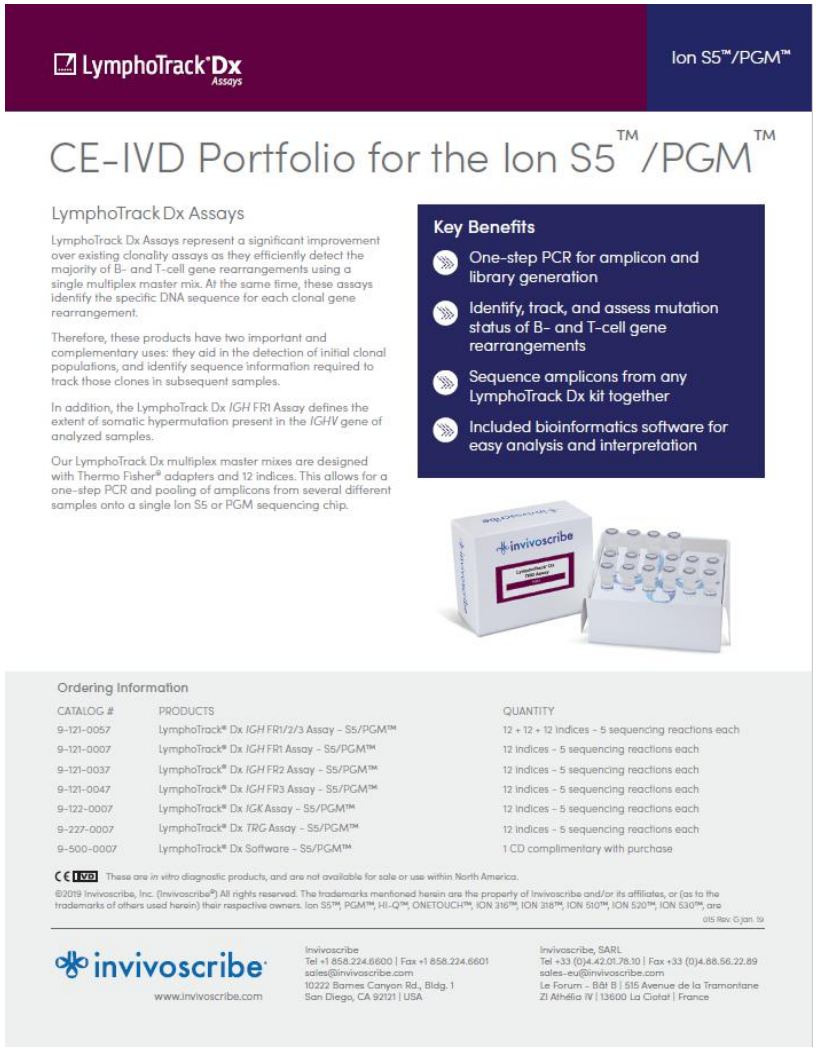

LymphoTrack Dx Assays

Ion S5™/PGM™

CE-IVD Portfolio for the Ion S5™/PGM™

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In addition, the LymphoTrack Dx *IGH* FR1 Assay defines the extent of somatic hypermutation present in the *IGHV* gene of analyzed samples.

Our LymphoTrack Dx multiplex master mixes are designed with Thermo Fisher® adapters and 12 indices. This allows for a one-step PCR and pooling of amplicons from several different samples onto a single Ion S5 or PGM sequencing chip.

Key Benefits

- One-step PCR for amplicon and library generation
- Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack Dx kit together
- Included bioinformatics software for easy analysis and interpretation

Ordering Information

CATALOG #	PRODUCTS	QUANTITY
9-121-0057	LymphoTrack® Dx *IGH* FR1/2/3 Assay – S5/PGM™	12 + 12 + 12 Indices – 5 sequencing reactions each
9-121-0007	LymphoTrack® Dx *IGH* FR1 Assay – S5/PGM™	12 Indices – 5 sequencing reactions each
9-121-0037	LymphoTrack® Dx *IGH* FR2 Assay – S5/PGM™	12 Indices – 5 sequencing reactions each
9-121-0047	LymphoTrack® Dx *IGH* FR3 Assay – S5/PGM™	12 Indices – 5 sequencing reactions each
9-122-0007	LymphoTrack® Dx *IGK* Assay – S5/PGM™	12 Indices – 5 sequencing reactions each
9-227-0007	LymphoTrack® Dx *TRG* Assay – S5/PGM™	12 Indices – 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software – S5/PGM™	1 CD complimentary with purchase

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015 Rev. G Jan. 19

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Promotional Tools - Postcards

LymphoTrack® Dx Assays

Services Postcards

Take Home Message

- Invivoscribe offers solutions for SHM assessment for both **Leader and FR1 assays**
- The **same NGS kits and software used for clonality** can be used for SHM assessment
- NGS assays are easy, less time consuming and **more sensitive** than the traditional Sanger sequencing method

Quiz

LymphoTrack® Assays

The *IGH* SHM Pos (+) Control DNA is included in all of the NGS kits that can be used to determine SHM status.

- True
- False

What are the advantages of testing SHM on NGS platforms compared to testing with the gel method?

- Same kits than for clonality
- Easier, less time consuming, less labor intensive
- More sensitive
- All of the above

Quiz

LymphoTrack® Assays

Somatic Hypermutation status needs to be assessed before determining Clonality.

- True
- False

The LymphoTrack® (Dx) software automatically calculates the SHM status by comparing the identified sequence with a germline sequence.

- True
- False

Quiz

LymphoTrack® Assays

Which assay(s) are available for the PGM and S5 platforms ?

- LymphoTrack (Dx) *IGH* FR1 Assay
- LymphoTrack (Dx) *IGHV* Leader Assay
- All of the above

Which products would you promote to a customer interested in assessing SHM status using FR1 primers on the MiSeq ?

- LymphoTrack (Dx) *IGH* FR1 Assay - MiSeq
- Lymphotrack (Dx) Software – MiSeq
- *IGH* SHM Pos (+) Control DNA
- Specimen Control Size Ladder
- All of the above


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