Minimal Residual Disease (MRD)

Application of LymphoTrack® Assays

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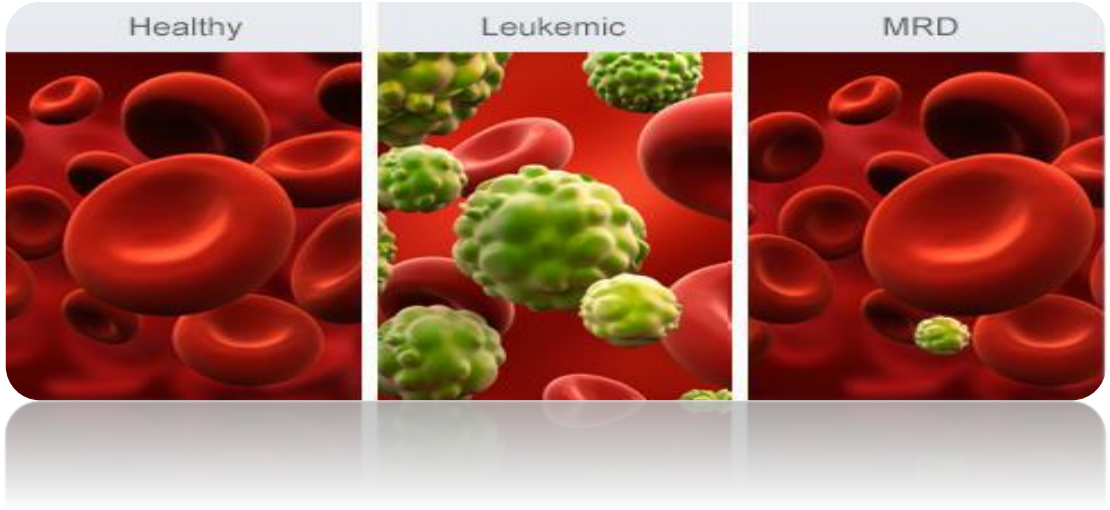
Improving Lives with Precision Diagnostics®

MRD Applications are Research Use Only; not for use in diagnostic procedures

Background

Definition

- Minimal Residual Diseases (**MRD**) refers to Leukemic cells that remain during or after treatment
- Considered a major cause of **relapse**
- Assessment of MRD can help develop new and improved clinical pathways



E Paietta. Assessing minimal residual disease (MRD) in leukemia, *Bone Marrow Transplantation* (2002) **29**, 459-465
Campana D. Minimal residual disease in acute lymphoblastic leukemia, Hematology Am Soc Hematol Educ Program (2010)**10**:7-12
Mailankody, *et al*. Minimal residual disease in multiple myeloma. *Nature Reviews Clinical Oncology* (2015) **12**, 286–295

The Study of MRD

LymphoTrack® MRD studies may be used to determine

- If subject treatment has eradicated clonal cells
- The efficacy of various treatments on subjects
- To monitor subject remission status
- To detect subject relapse and refractory disease

Clinical laboratories

Hospitals

Research laboratories

Potentially useful in the study of a number of malignancies

- Acute Lymphoblastic Leukemia (ALL)
- Chronic Lymphocytic Leukemia (CLL)
- Follicular Lymphoma (FL)
- Mantle Cell Lymphoma (MCL)
- Acute Myeloid Leukemia (AML)
- Multiple Myeloma (MM)

E Paietta. Assessing minimal residual disease (MRD) in leukemia, *Bone Marrow Transplantation* (2002) **29**, 459-465
Campana D. Minimal residual disease in acute lymphoblastic leukemia, Hematology Am Soc Hematol Educ Program (2010)**10**:7-12
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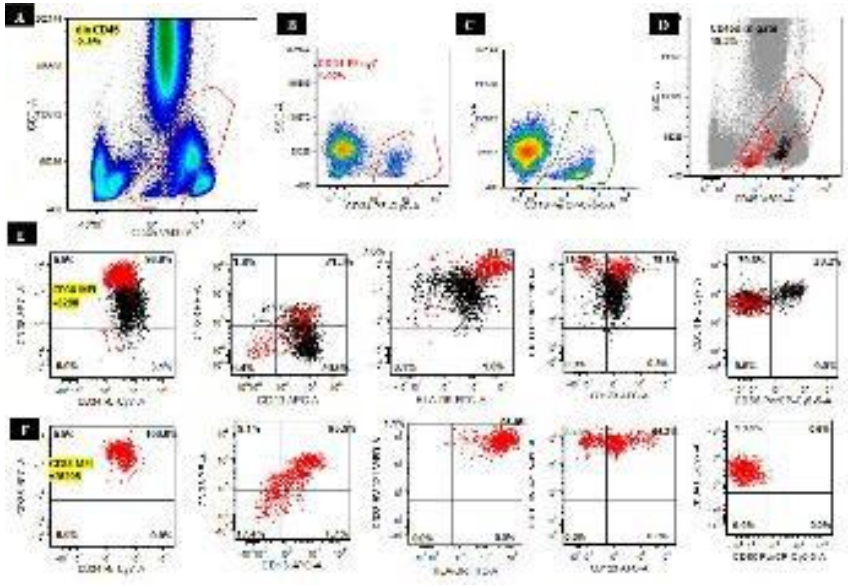
3 Main Types of MRD testing

Flow Cytometry

- Based upon cell surface markers – clonal shift/subclones may not be detected
- Relatively quick, but requires a large amount of **fresh** sample material
- Some amount of false negative results possible depending on methodology used and expertise of the laboratory
- Very **subjective** and difficult to compare across different labs

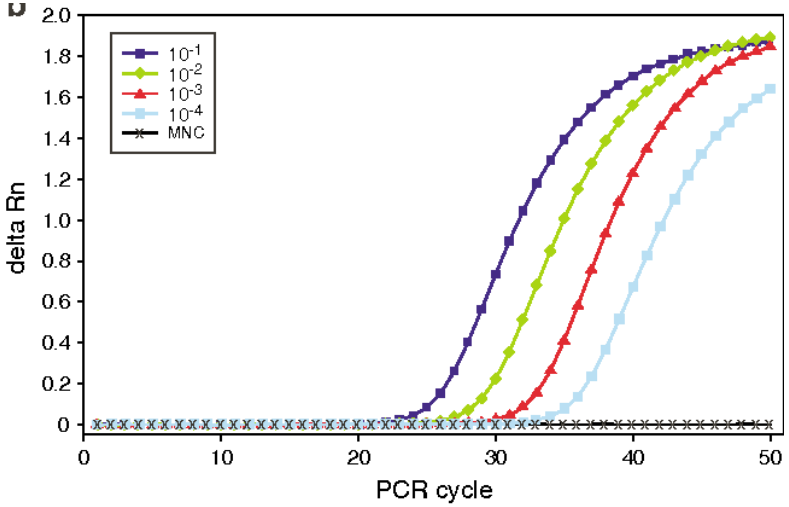


Multi-Color Flow Cytometry for Minimal Residual Disease Detection in Acute Myeloid Leukemia, MD Anderson Cancer Center Experience by Jesse M. Jaso and Sa A. Wang, ICCS Newsletter VOL. V No. 3, Summer 2014

3 Main Types of MRD testing

ASO PCR

- Allele-Specific Oligonucleotide RT-PCR (ASO PCR)
- Requires extraction of identified clone followed by Sanger sequencing
- Subject specific PCR assay is developed to track the specific clone(s) in subsequent samples
  - This takes a **long time** to develop
- Fast and relatively inexpensive, but not appropriate for diseases such as myeloma due to high degree of **False Negative results**
  - **Up to 25% of myeloma cases are not detectable***

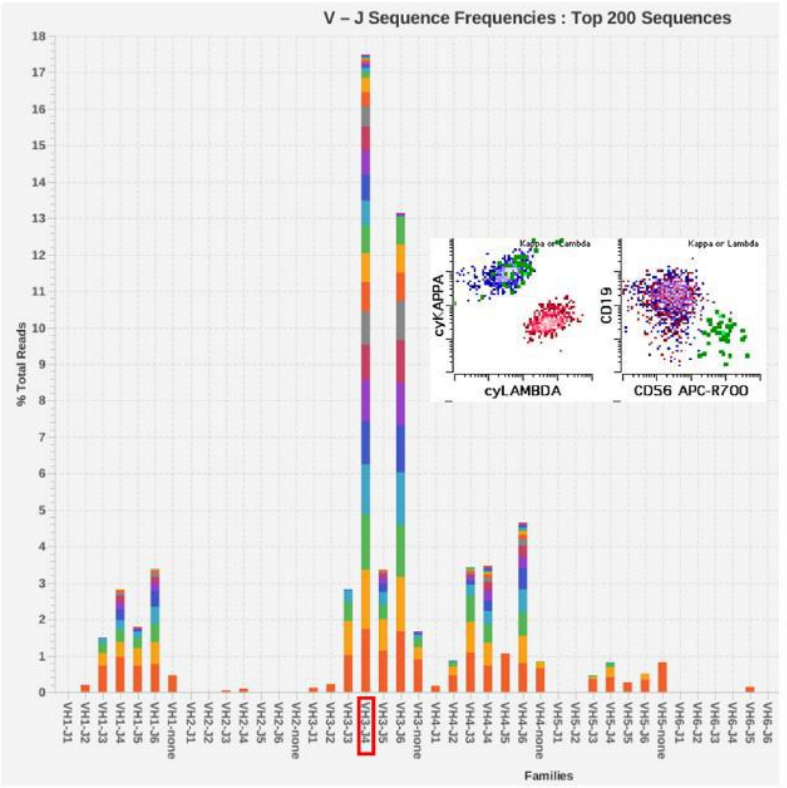


*Bai et. al, "Molecular detection of minimal residual disease in multiple myeloma" British Journal of Haematology, Volume181, Issue1, April 2018, Pages 11-26

3 Main Types of MRD testing

Next Generation Sequencing

- Identify low levels of malignant cells at **sensitivities** down to $1x10^{-6}$ with sufficient DNA input
- Consistently assess and **track** residual clonal populations
- **Multiplex** NGS assays are subject independent
- Also useful for detecting **new** clonal **development**
- **Independent** of changes in cell-surface protein expression

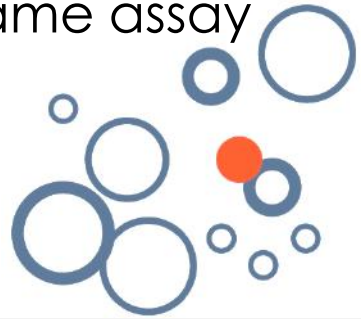


Ho et. al "Minimal residual disease detection of myeloma using sequencing of immunoglobulin heavy chain gene VDJ regions", Seminars in Hematology.

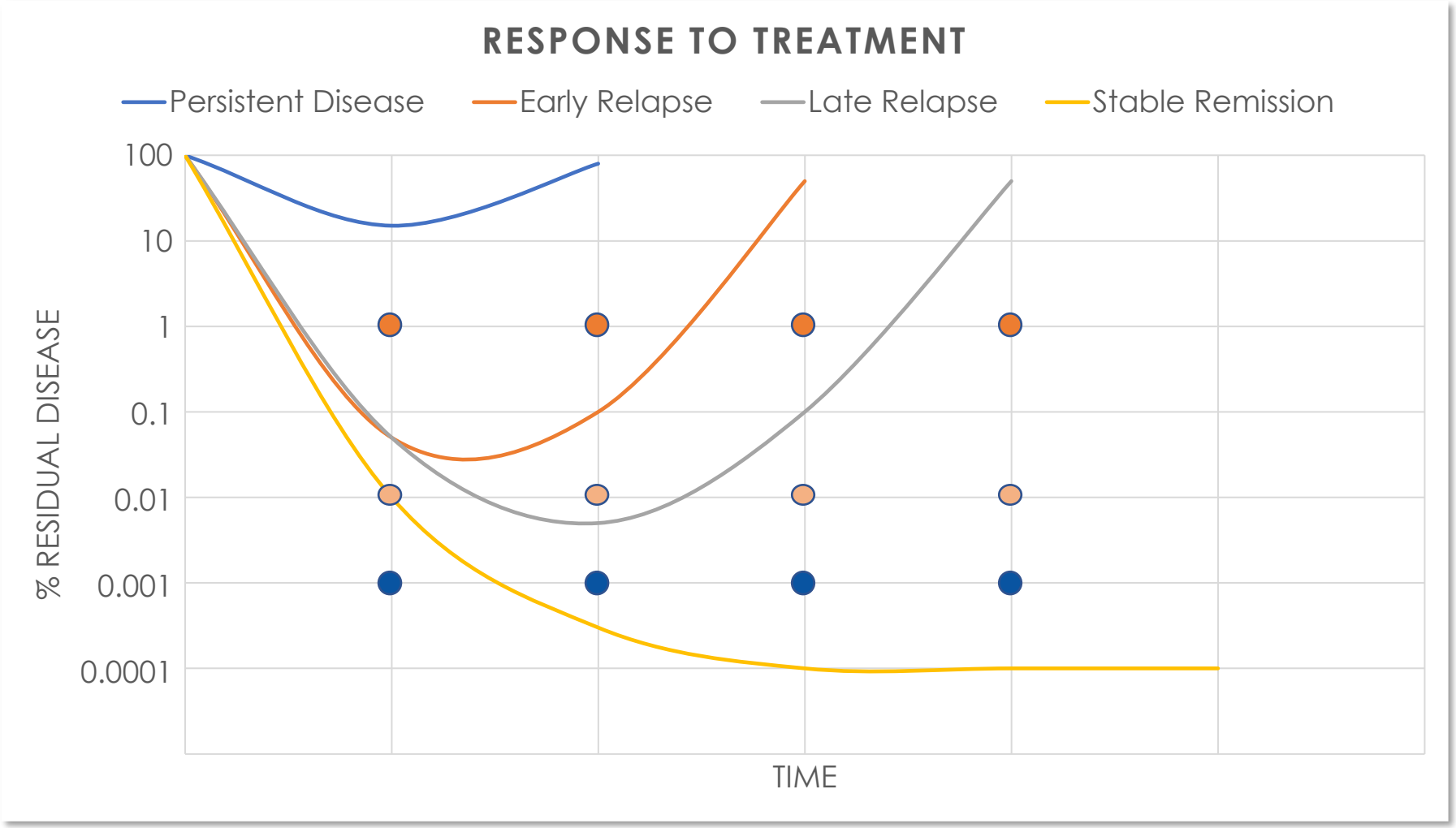
NGS MRD Analysis is Versatile

Many advantages and applications

- Sensitive Detection – Detect clonal rearrangements as low as 10^{-6}
- Flexibility – Test samples based on available DNA input
- Trace identified clonal sequences & monitor development over time
  - Plot time courses – Observe changes in clonal burden over time
  - Test for an expected decline in frequency
  - Detect the resurgence of a clone after its absence
  - Identify the emergence of new clonal rearrangements – all with the same assay
- Determine all clonal populations in one analysis



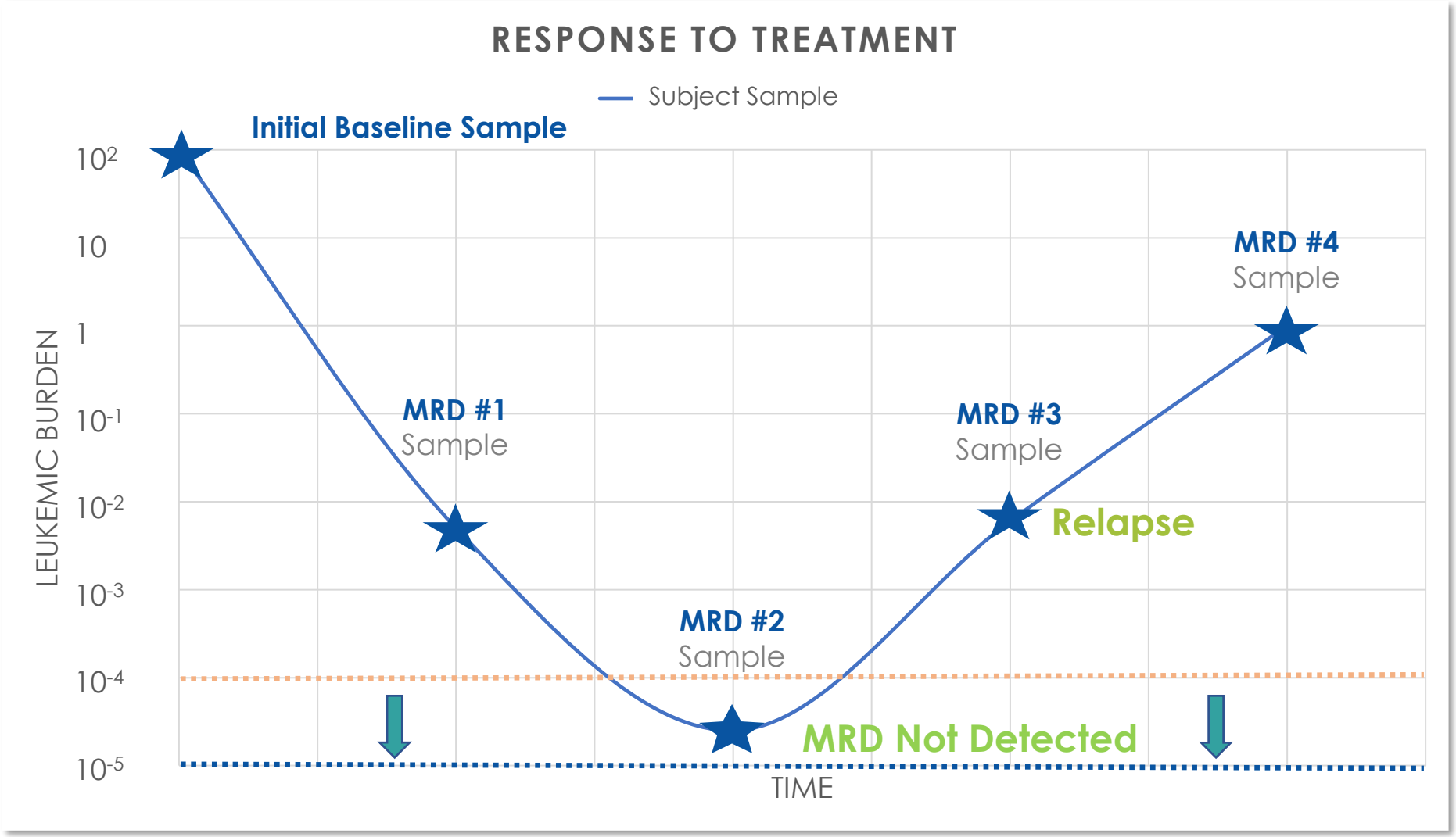
Methods of Detection



- Cytomorphological Detection Limit (10^{-2})
- Flow Cytometry (10^{-4})
- NGS (10^{-5})

Adapted from presentation by Dr. Maria Arcila, from MSKCC at EHA 2020 Invivoscribe's Symposium

Methods and MRD Sensitivity



Cytomorphological Detection Limit (10^{-2})

Flow Cytometry (10^{-4})

NGS (10^{-5})

Adapted from:
Szczepanski et al. *Minimal residual disease in leukaemia patients* The Lancet Oncology (2001). 2, 409-17

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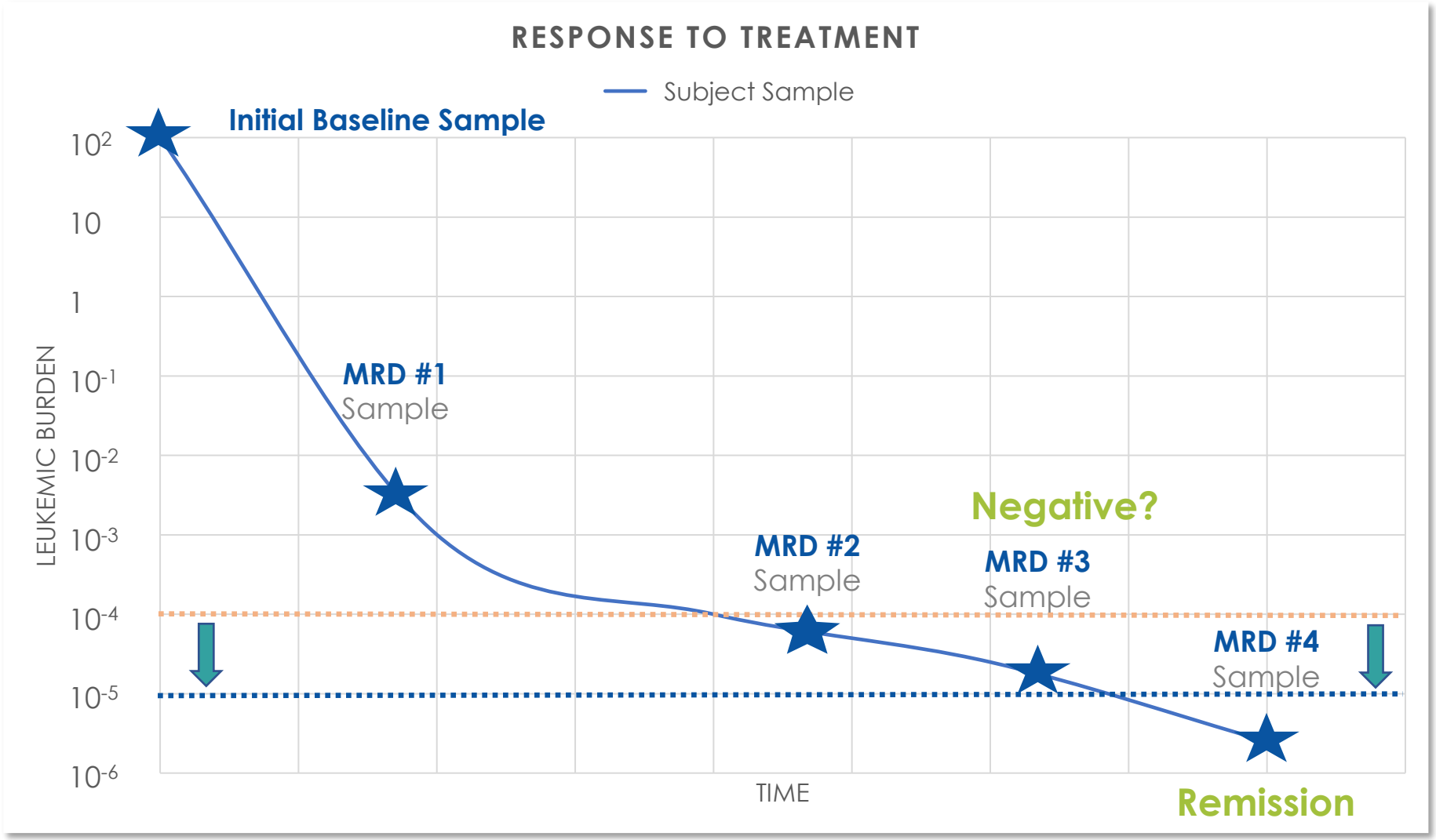
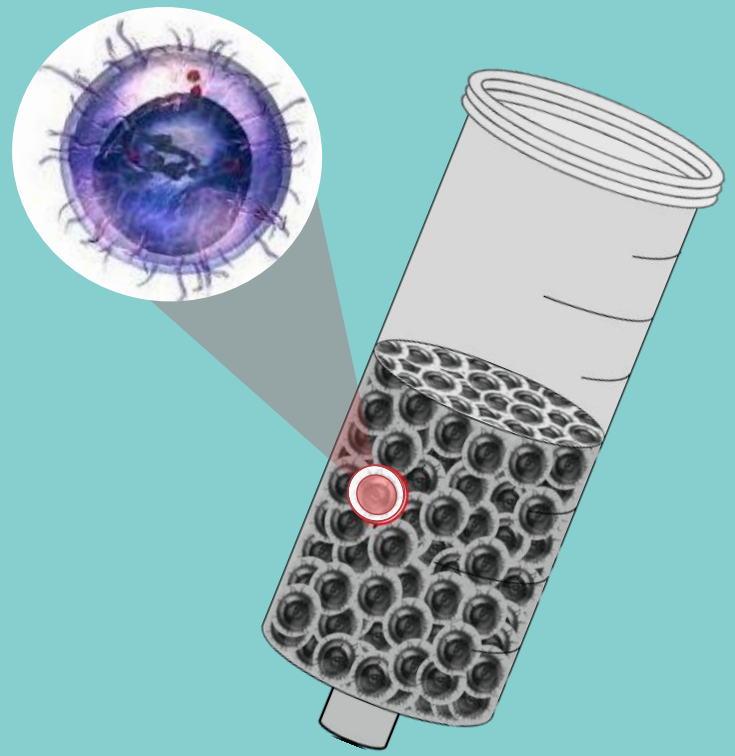
Methods and MRD Sensitivity



- Cytomorphological Detection Limit (10^{-2})
- Flow Cytometry (10^{-4})
- NGS (10^{-5})

Adapted from:
Szczepanski et al. *Minimal residual disease in leukaemia patients* The Lancet Oncology (2001). 2, 409-17

Finding the Right Level of Confidence



6.5 pg of DNA per cell

Sensitivity of up to 10^{-6}

X	10^6 cells	=	6.5 µg of DNA	→	66% confidence
X	2 x 10^6 cells	=	13 µg of DNA	→	88% confidence
X	3 x 10^6 cells	=	20 µg of DNA	→	95% confidence

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DNA Input & Read Depths Requirements for MRD

95% Confidence of a True MRD Negative Sample at Various Sensitivity Levels*		
Sensitivity	Total DNA	Total Read Depth
$1x10^{-4}$	200ng	500,000
$1x10^{-5}$	4µg	2,200,000
$1x10^{-6}$	20µg	44,000,000

*The optimal sample for MRD assessment is the first pull or early pull of the bone marrow aspirate.

Desired Sensitivity of MRD Analysis

International Recommendations and Standards

- Sensitivity (target disease cells / total nucleated cells)

Disease	Recommended Level of Sensitivity
Multiple Myeloma[1]	10^{-5}
Acute Lymphoblastic Leukemia[2]	10^{-4}
Chronic Lymphocytic Leukemia[3]	10^{-4}
Non-Hodgkin's Lymphoma[4]	10^{-4}

1 Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Annals of Oncology28 (Supplement 4): iv52–iv61, 2017
2 Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Annals of Oncology27 (Supplement 5): v69–v82, 2016
3 iwCLL Guidelines for Diagnosis, Indications for Treatment, Response Assessment, and Supportive Management of CLL, 10.1182/blood-2017-09-806398
4 The Minimal Residual Disease in Non-Hodgkin's Lymphomas: From the Laboratory to the Clinical Practice, doi: 10.3389/fonc.2019.00528

Assays for Clonality and MRD Assessment

LymphoTrack® Assays

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

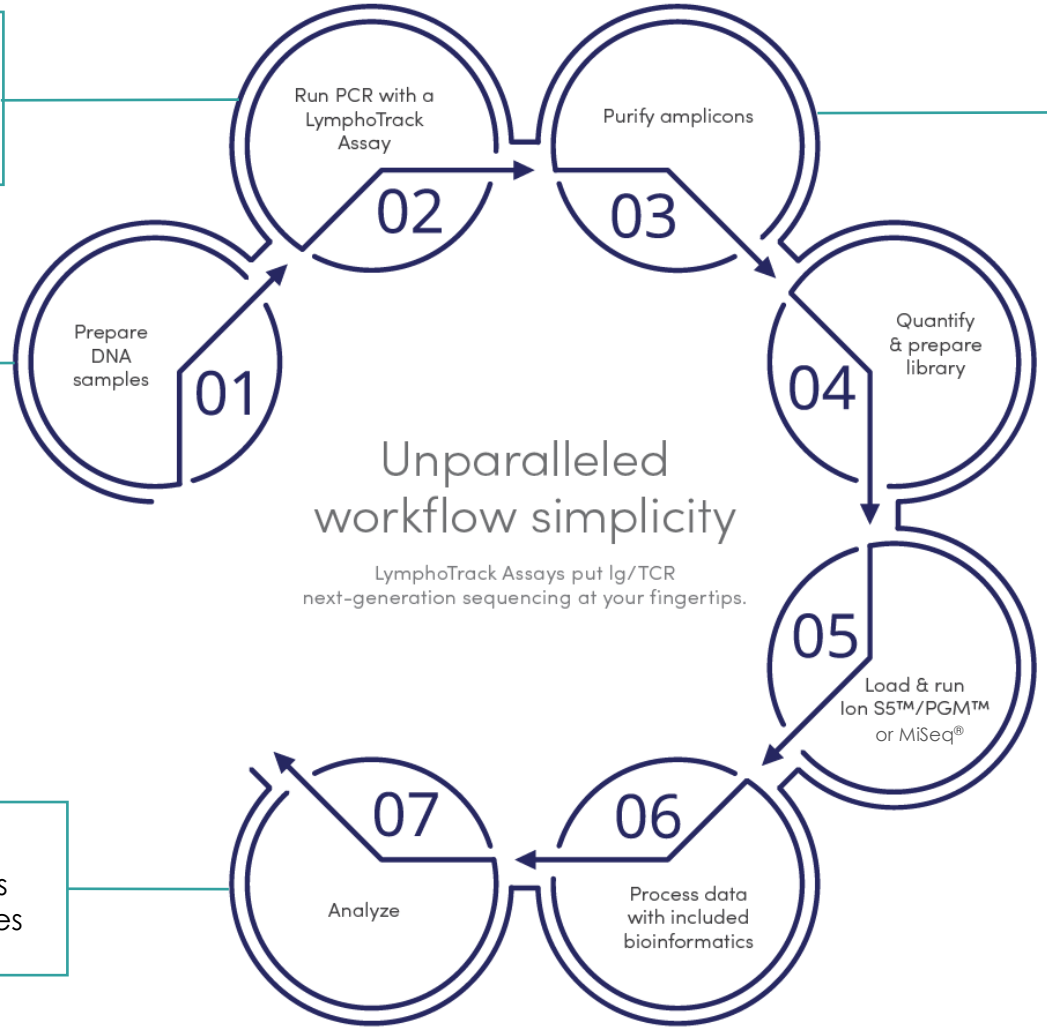
Same Assays and Workflow

Add 2 µL LQIC to Low Positive Control and each sample PCR

Use Experimental Planner to determine DNA input to attain desired sensitivity

Monitor Normalized Clonal Cell Equivalents and Clonal Frequencies

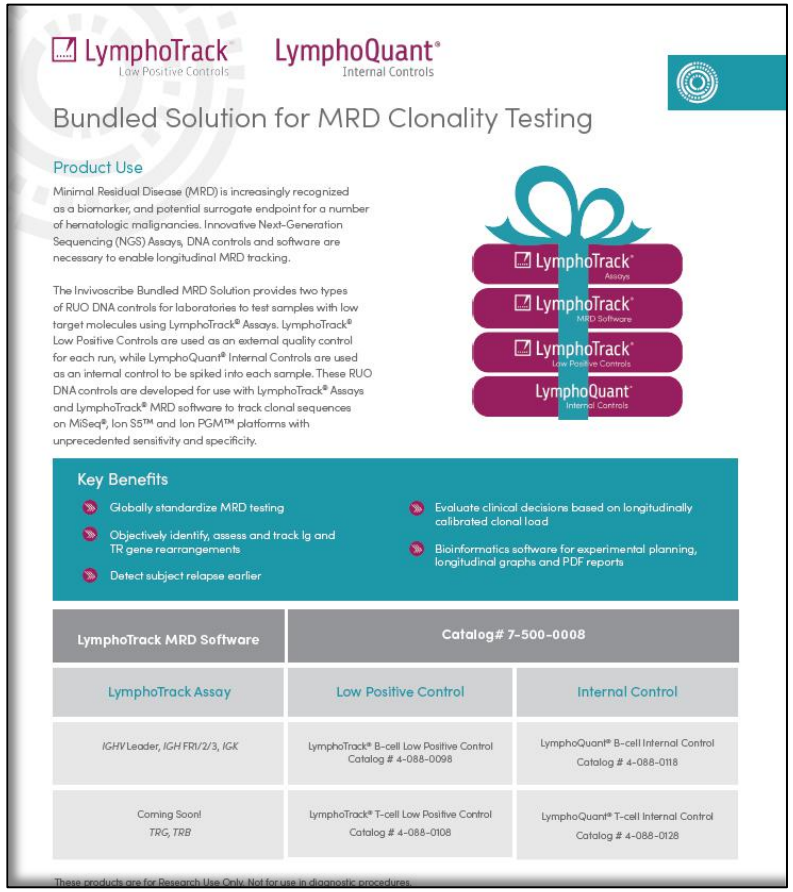
Adjust bead volume to keep bead ratio constant (55uL)



LymphoTrack MRD Solutions

A Comprehensive Solution for MRD Clonality Tests

- LymphoTrack® Assays
- B- and T-cell Controls
  - LymphoTrack® Low Positive Control
  - LymphoQuant® Internal Control
- MRD Software for Seamless Sample Analysis



LymphoTrack Low Positive Controls

LymphoQuant Internal Controls

Bundled Solution for MRD Clonality Testing

Product Use

Minimal Residual Disease (MRD) is increasingly recognized as a biomarker, and potential surrogate endpoint for a number of hematologic malignancies. Innovative Next-Generation Sequencing (NGS) Assays, DNA controls and software are necessary to enable longitudinal MRD tracking.

The Invivoscribe Bundled MRD Solution provides two types of RUO DNA controls for laboratories to test samples with low target molecules using LymphoTrack® Assays. LymphoTrack® Low Positive Controls are used as an external quality control for each run, while LymphoQuant® Internal Controls are used as an internal control to be spiked into each sample. These RUO DNA controls are developed for use with LymphoTrack® Assays and LymphoTrack® MRD software to track clonal sequences on MiSeq®, Ion S5™ and Ion PGM™ platforms with unprecedented sensitivity and specificity.

LymphoTrack Assays

LymphoTrack MRD Software

LymphoTrack Low Positive Controls

LymphoQuant Internal Controls

Key Benefits

- Globally standardize MRD testing
- Objectively identify, assess and track Ig and TR gene rearrangements
- Detect subject relapse earlier
- Evaluate clinical decisions based on longitudinally calibrated clonal load
- Bioinformatics software for experimental planning, longitudinal graphs and PDF reports

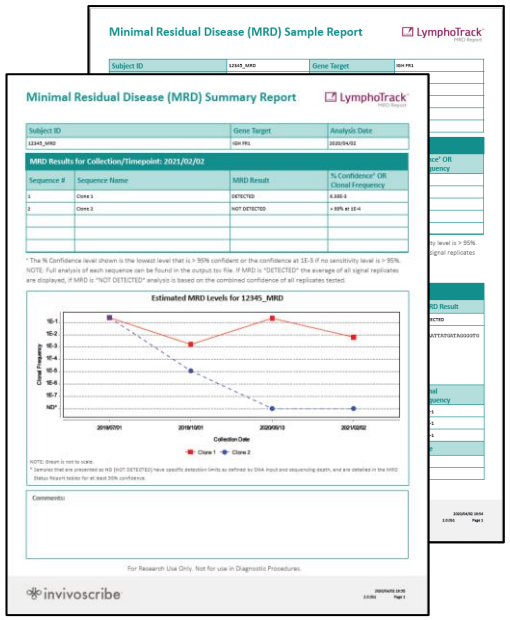
LymphoTrack MRD Software	Catalog# 7-500-0008	
LymphoTrack Assay	Low Positive Control	Internal Control
IGHV Leader, *IGH* FR1/2/3, *IGK*	LymphoTrack® B-cell Low Positive Control Catalog # 4-088-0098	LymphoQuant® B-cell Internal Control Catalog # 4-088-0118
Coming Soon! *TRG, TRB*	LymphoTrack® T-cell Low Positive Control Catalog # 4-088-0108	LymphoQuant® T-cell Internal Control Catalog # 4-088-0128

These products are for Research Use Only. Not for use in diagnostic procedures.

LymphoTrack® MRD Software

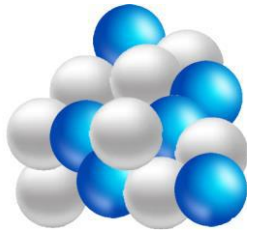
LymphoTrack® MRD Products help

- Identify low levels of clonal cells
- Consistently assess and track residual clonal populations
- Simultaneously track **up to 5 clonotype sequences**
  - Evaluate the effectiveness of treatment
  - Identify returning clones
- Offer **objective and standardized testing** worldwide by tracking sequence specific DNA targets
- **Calculate cell equivalents** for MRD level assessment over time



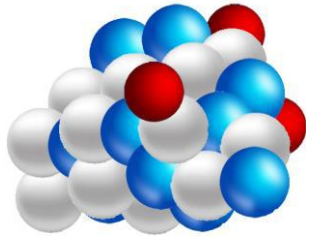
LymphoQuant® Internal Controls

Sample without LymphoQuant®



Output: % Total reads

Sample with LymphoQuant®



Output: Clonal cell equivalents

Non-clonal Cells

Clonal Cells

LymphoQuant®

$$\frac{\%Reads \text{ for MRD Sample}}{\%Reads \text{ for Internal Control}} \times 100\ cells = \text{Estimated Clonotype Cell Equivalents}$$

LymphoQuant® Internal Controls

Principle behind the control: Convert % reads into cell equivalents

Baseline Test **Follow Up Test**

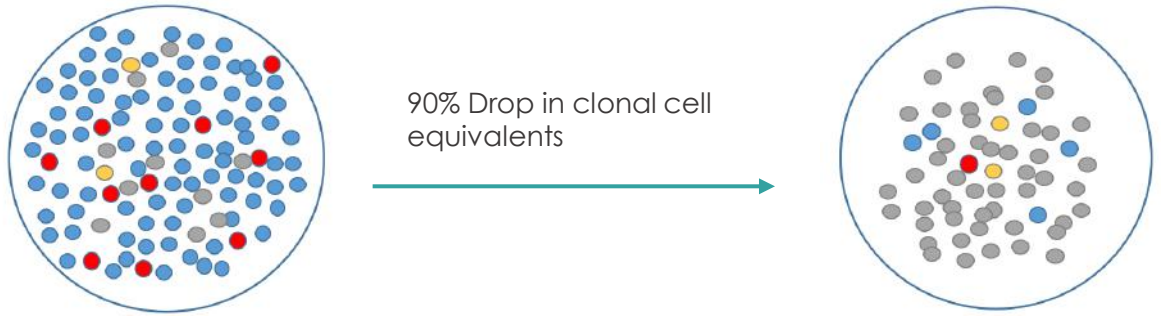
LymphoTrack® without LQIC

10 clonal / 100 lymphocytes
10% clonal reads

1 clonal / 5 lymphocytes
20% clonal reads

Lymphocyte
Clonal Cells
Other Cells
LQIC

LymphoTrack® with LQIC



10 clonal cell equivalents

1 clonal cell equivalent

LymphoQuant® Internal Controls adds 100 cell equivalents to any B- or T-cell sample

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Setting up LymphoTrack® for MRD

Intuitive Software can guide setup

- Creation of libraries is almost identical to Clonality testing
- 1st use Project Planner tool to design experiment with needed sensitivity
- Then set up the LymphoTrack® PCR reactions:

Component	Volume (µl)
LymphoTrack® PCR Master Mix	45.0
DNA from MRD sample	**8.0**
LymphoQuant Internal Control	**2.0**
EagleTaq™ DNA Polymerase	0.2
Total	**55.2**

For full setup please refer to the instructions for use (IFU)

LymphoTrack® Low Positive Control

Replaces the LymphoTrack® Positive Control

- Typically at a level of 10^{-4}
- Can be diluted further in IVS-0000 negative control if greater sensitivity is desired

LymphoTrack Assay	Catalog No.	Description	Notes
IGHV, IGH FR1/2/3 and *IGK*	4-088-0098	LymphoTrack® B-cell Low Positive Control	Average expected read frequency is 10^{-4}*
TRG, TRB	4-088-0108	LymphoTrack® T-cell Low Positive Control	Average expected read frequency is 10^{-4}*

*The *IGK* and *TRB* loci may generate a read frequency of 10^{-3}

Clonality and MRD Controls

Baseline Sample Controls	MRD Follow-Up Sample Controls
1. Positive 2. Negative 3. Non Template (water)	1. LymphoTrack® Low Positive 2. LymphoQuant® Internal Control 3. Negative 4. Non Template (water)
Positive and Negative controls are included with LymphoTrack Kits	Low Positive and Internal Controls can be purchased separately

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LymphoTrack® MRD Software

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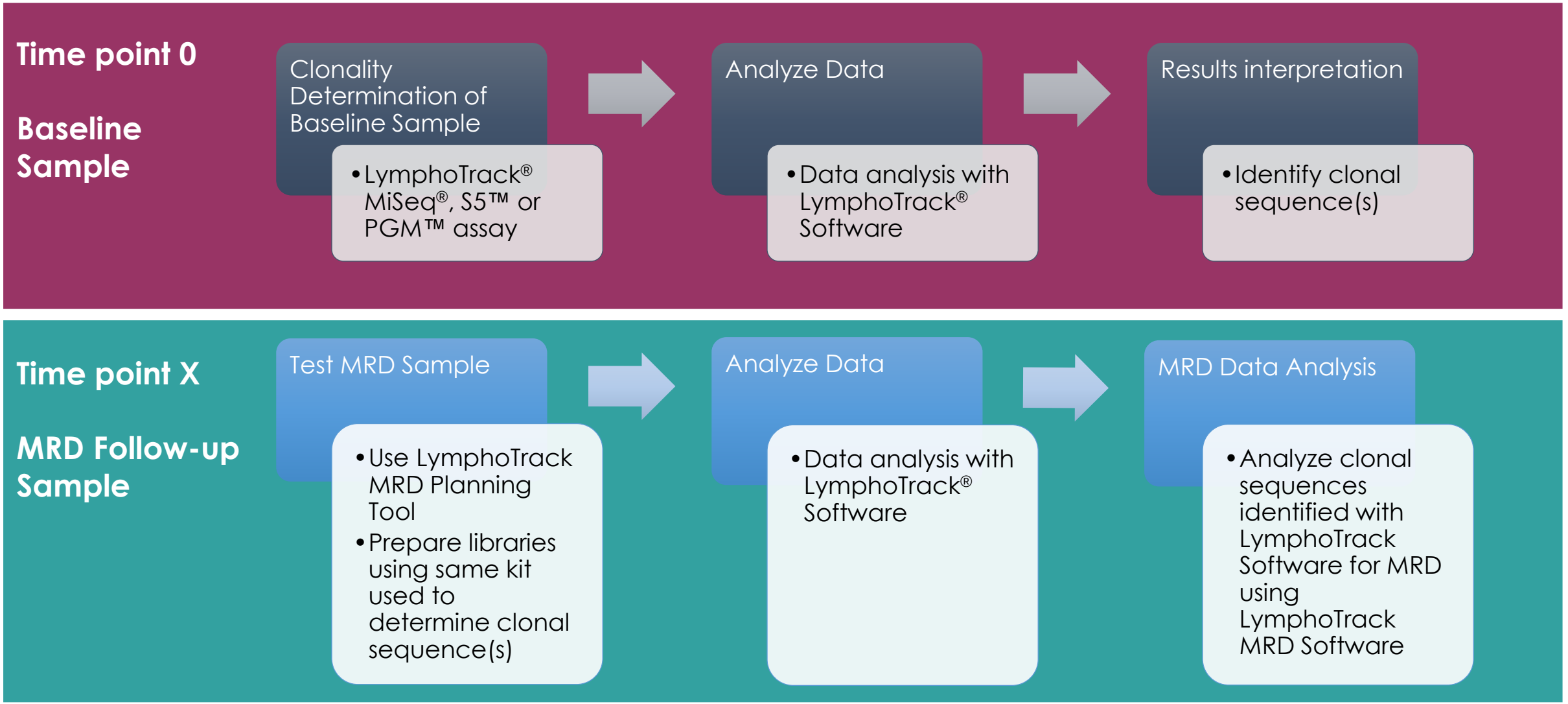
Data Analysis Workflow



1. Baseline Sample Analysis (Time Point 0)

Identify Clonal Sequence(s) with LymphoTrack Assays

In Merged Read Summary

- Full sequence can be copied from PDF report

LymphoTrack Report for assay LEADER

Sample name: Leader_positive_S23_L001_001_combined

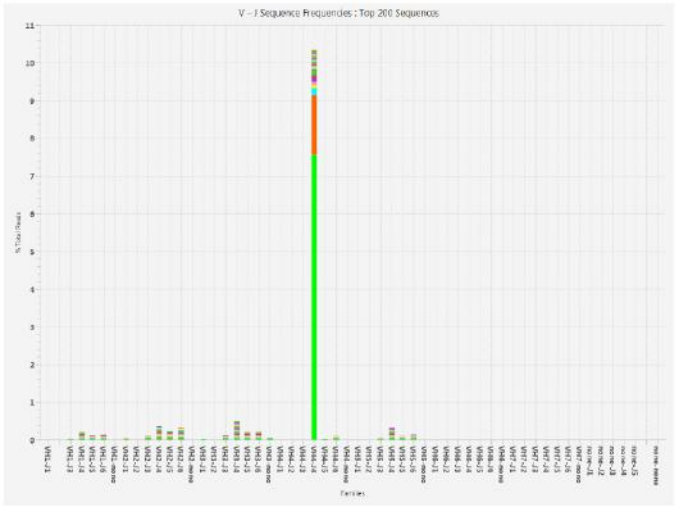
Total Read Count: 474947

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	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			
3	CTGCTGCTGACCA	466	175	IGHV2-5_01	IGHJ5_01	0.04	10.66	6.62	Y			
4	CTGCTGCTGACCA	457	162	IGHV2-5_05	IGHJ6_02	0.03	10.69	2.99	Y			
5	CTGCTGCTGACCA	474	154	IGHV2-5_05	IGHJ4_02	0.03	10.72	3.99	Y			
6	CTGCTGCTGACCA	454	150	IGHV2-5_10	IGHJ5_02	0.03	10.76	11.78	Y			
7	CTGCTGCTGACCA	469	139	IGHV2-5_01	IGHJ4_02	0.03	10.78	1.32	Y			
8	CTCGCCCTCCTCC	466	139	IGHV5-51_01	IGHJ4_02	0.03	10.81	7.09	Y			
9	CTGCTACTGACTG	490	137	IGHV2-70_10	IGHJ3_02	0.03	10.84	0.66	Y			
10	CTGCTGCTGACCA	478	135	IGHV2-5_10	IGHJ6_02	0.03	10.87	3.70	Y			



TTCTCTGGGTTCTCACTCACCACTAGGGGATTGGGTGTGGCCTGGATCCGTCAGCCCCCAGGAAAGGCCCTGGAGTGGCTTGCACTCATTTTTTGGGATGATGATA
AACGCTACAGCCCATCTCTGAAGAGCAGACTCACCATCACCAAGGACGCCTCCAAGAACCAGGTGGTCCTTACAATGACCAACATGGACCCTGTAGACACAGCCAC
CTATTACTGTGCACACAGCGGGAGCTACCAAGGTGGGACTACCTATTACCCACACTACTATTTTGACTACTGGGGCCAGGGAACCCT

2. MRD Sample Analysis (Time Point X)

Analyze clonal sequence(s) that were identified in baseline sample

- Possibly identify newly emerging clones
- Search for target sequence identified in baseline sample

LymphoTrack Report for assay LEADER

Sample name: Leader_negative_S24_L001_001_combined

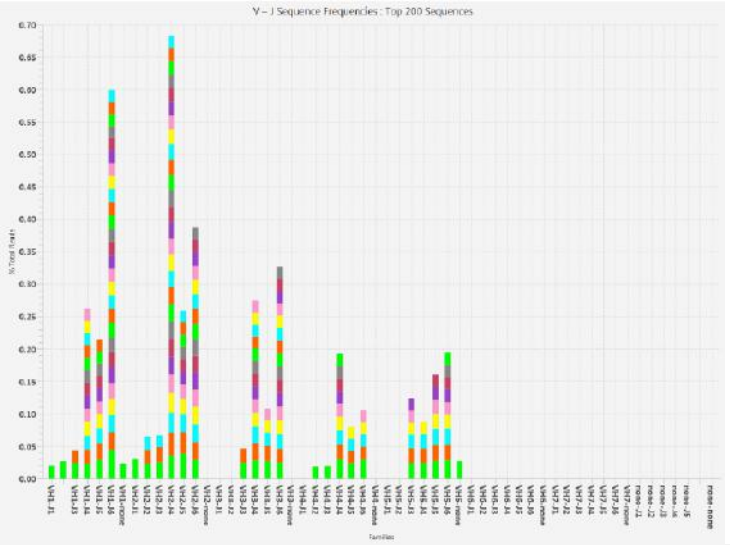
Total Read Count: 653015

IndexQ30: 88.17

Caution: Do not edit fields and save.

Top 10 Merged Read Summary

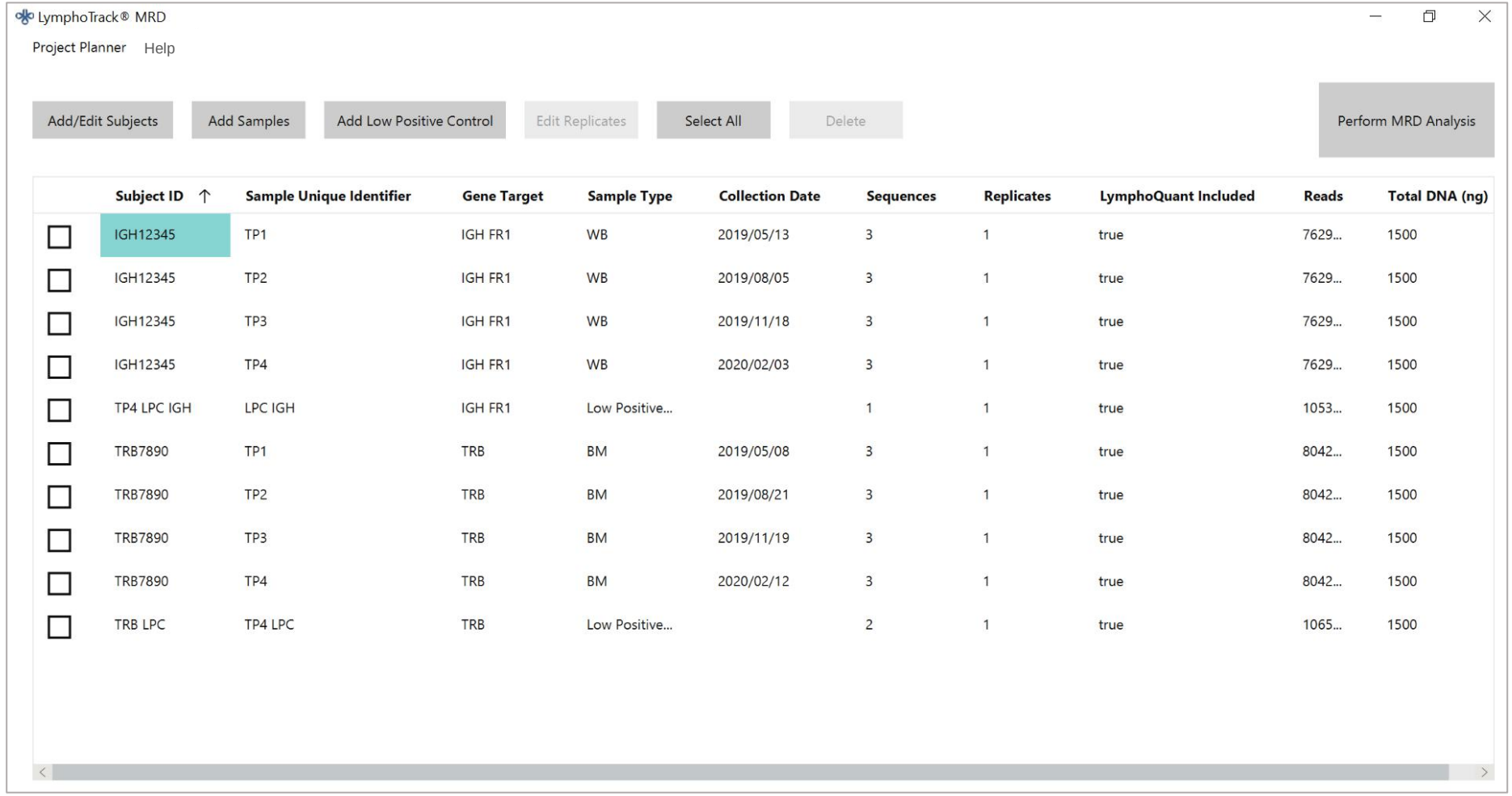
Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 Seq
1	GTTCCTCTTTGTGC	312	287	IGHV1-69_06	IGHJ6_02	0.04	0.04	1.35	n/a	N	52.36	not found
2	CTGCTACTGACTG	391	253	IGHV2-70_13	IGHJ5_02	0.04	0.08	1.99	n/a	N	31.89	not found
3	CTGCTGCTGACCA	454	233	IGHV2-5_10	IGHJ4_02	0.04	0.12	2.02	Y	Y	98.32	not found
4	CTGCTGCTGACCA	460	227	IGHV2-5_10	IGHJ4_02	0.03	0.15	3.03	Y	Y	98.99	GCATACAGAAAGA
5	CTGCTGCTGACCA	475	212	IGHV2-5_05	IGHJ5_02	0.03	0.19	0.33	Y	Y	99.34	GCACACAGCATCG
6	CTGCTGCTGACCA	466	203	IGHV2-5_05	IGHJ4_02	0.0						
7	CTGCTGCTGACCA	478	196	IGHV2-5_10	IGHJ4_02	0.0						
8	CTGCTGCTGACCA	462	193	IGHV2-5_05	IGHJ1_01	0.0						
9	TCCTCCTGGTGGC	459	191	IGHV4-34_01	IGHJ4_02	0.0						
10	TCCTCCTGGTGGC	439	188	IGHV4-34_06	IGHJ6_02	0.0						



TTCTCTGGGTTCTCACTCACCACTAGGGGATTGGGTGTGGCCTGGATCCGTCAGCCCCCAGGAAAGGCCCTGGAGTGGCTTGCACTCATTTTTTGGGATG
ATGATAAACGCTACAGCCCATCTCTGAAGAGCAGACTCACCATCACCAAGGACGCCTCCAAGAACCAGGTGGTCCTTACAATGACCAACATGGACCCTGT
AGACACAGCCACCTATTACTGTGCACACAGCGGGAGCTACCAAGGTGGGACTACCTATTACCCACACTACTATTTTGACTACTGGGGCCAGGGAACCCT

LymphoTrack® MRD Software

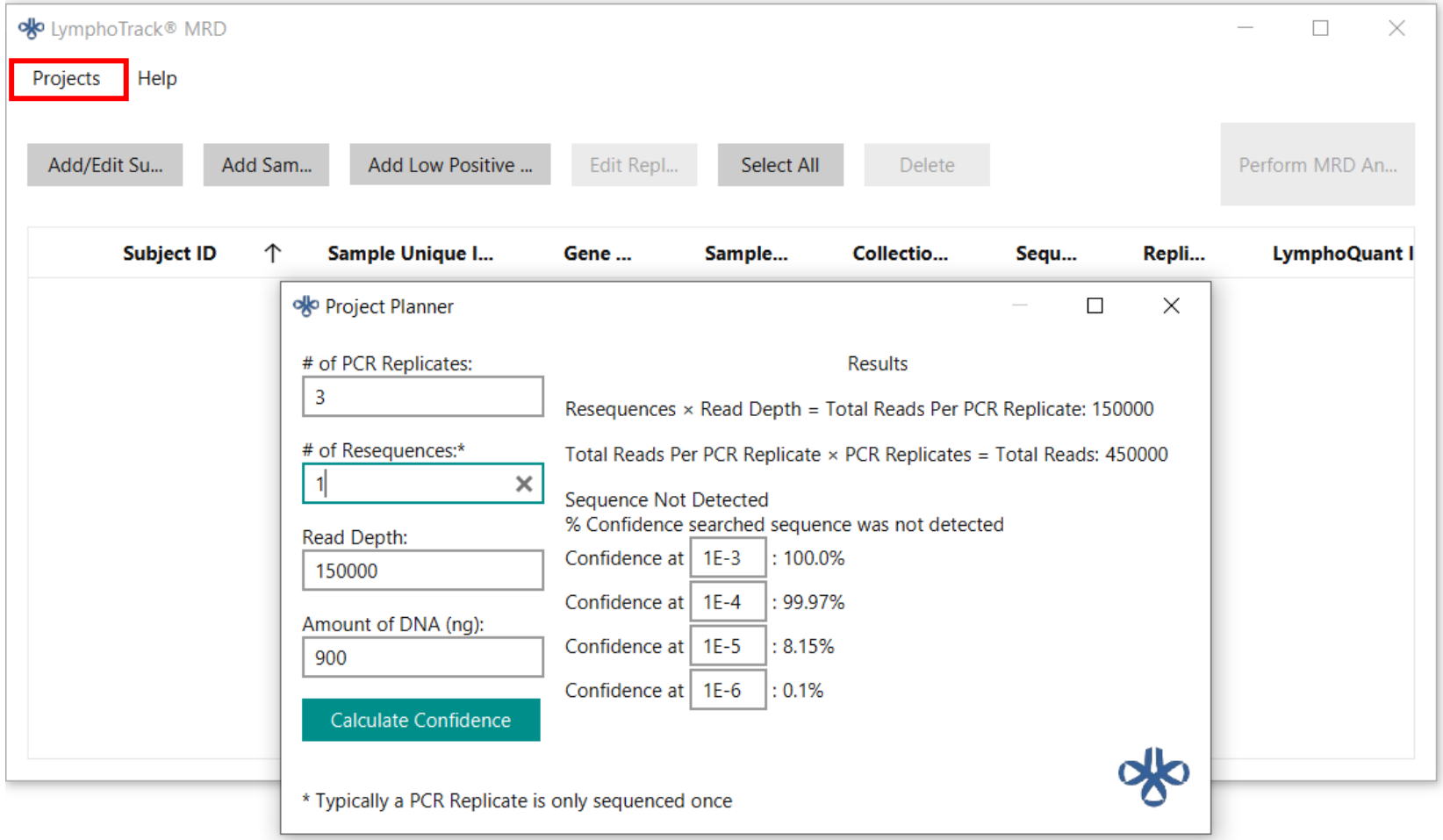
Simple User Interface



Subject ID	Sample Unique Identifier	Gene Target	Sample Type	Collection Date	Sequences	Replicates	LymphoQuant Included	Reads	Total DNA (ng)
IGH12345	TP1	IGH FR1	WB	2019/05/13	3	1	true	7629...	1500
IGH12345	TP2	IGH FR1	WB	2019/08/05	3	1	true	7629...	1500
IGH12345	TP3	IGH FR1	WB	2019/11/18	3	1	true	7629...	1500
IGH12345	TP4	IGH FR1	WB	2020/02/03	3	1	true	7629...	1500
TP4 LPC IGH	LPC IGH	IGH FR1	Low Positive...		1	1	true	1053...	1500
TRB7890	TP1	TRB	BM	2019/05/08	3	1	true	8042...	1500
TRB7890	TP2	TRB	BM	2019/08/21	3	1	true	8042...	1500
TRB7890	TP3	TRB	BM	2019/11/19	3	1	true	8042...	1500
TRB7890	TP4	TRB	BM	2020/02/12	3	1	true	8042...	1500
TRB LPC	TP4 LPC	TRB	Low Positive...		2	1	true	1065...	1500

Project Planner

Determine how to reach the desired level of sensitivity

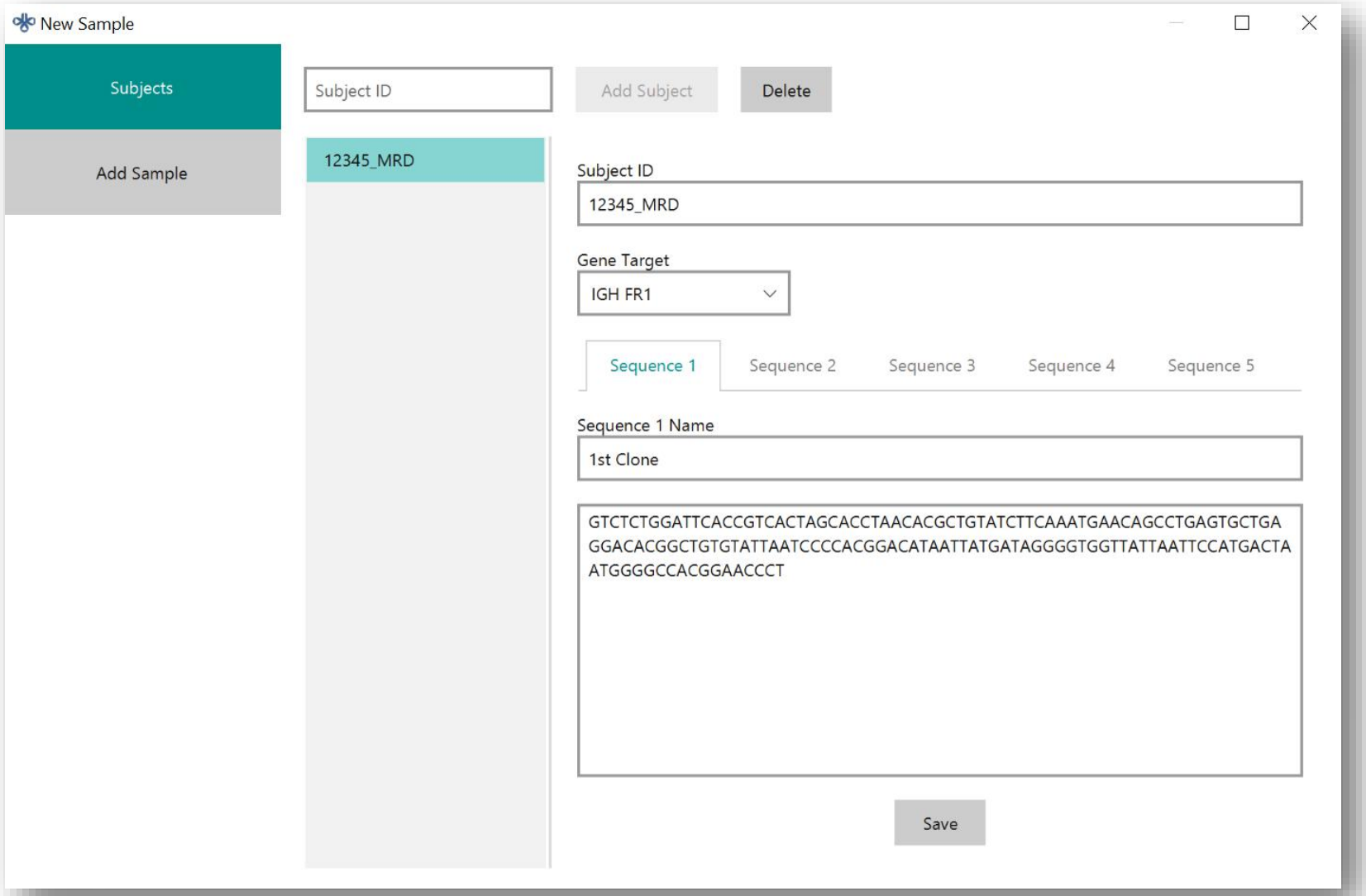


Design your experiment according to your (detection) needs with the included experiment planner.

MRD Analysis Setup

Add subjects – Track up to 5 sequences

- Enter clonal sequences from LymphoTrack® baseline run
- Clones identified from subsequent MRD runs can also be entered



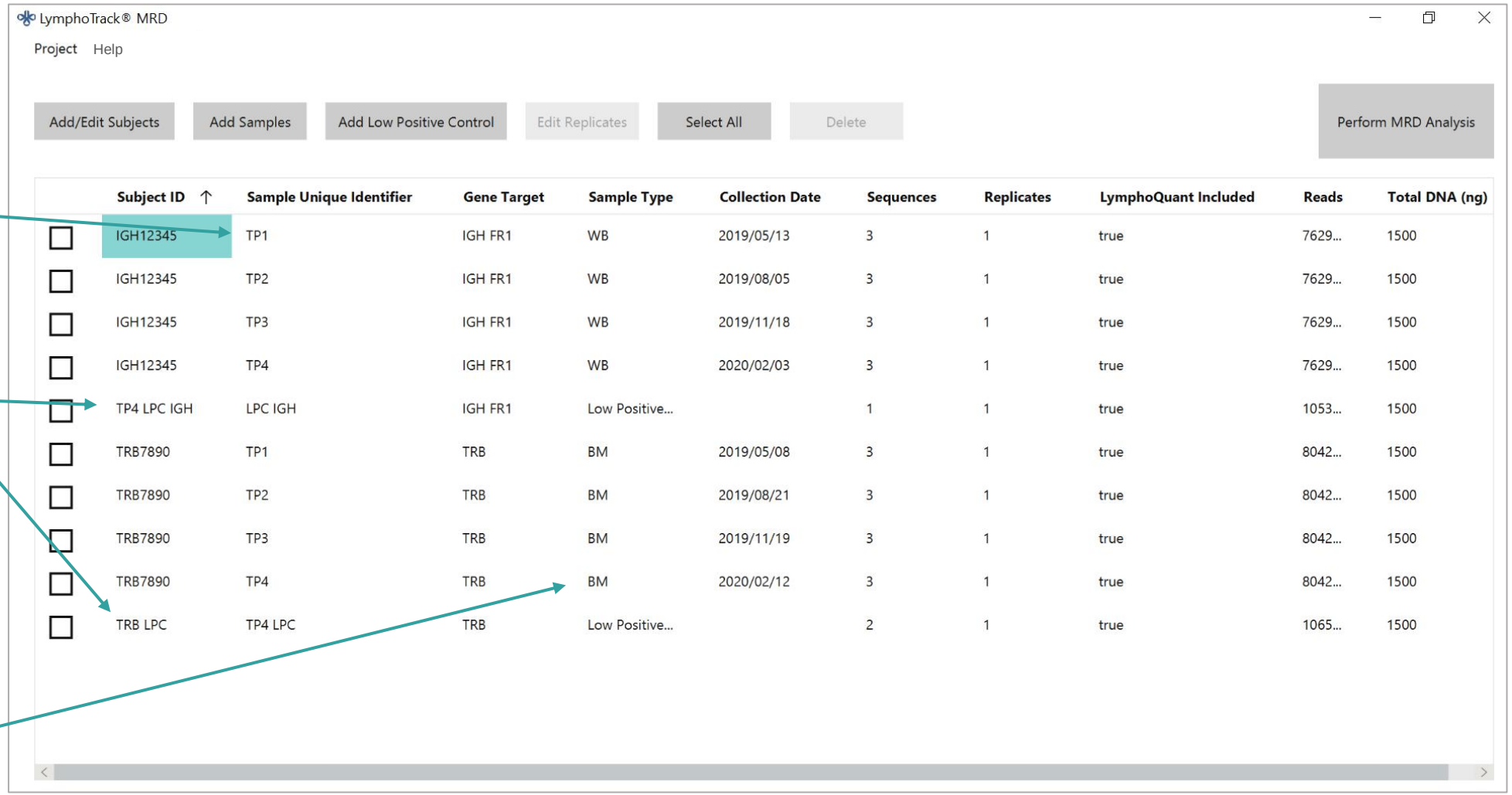
MRD Analysis Setup

Add samples – Multiple samples, time points and targets

Sample results from different time points

Low Positive Control for each target

Keep track of sample types

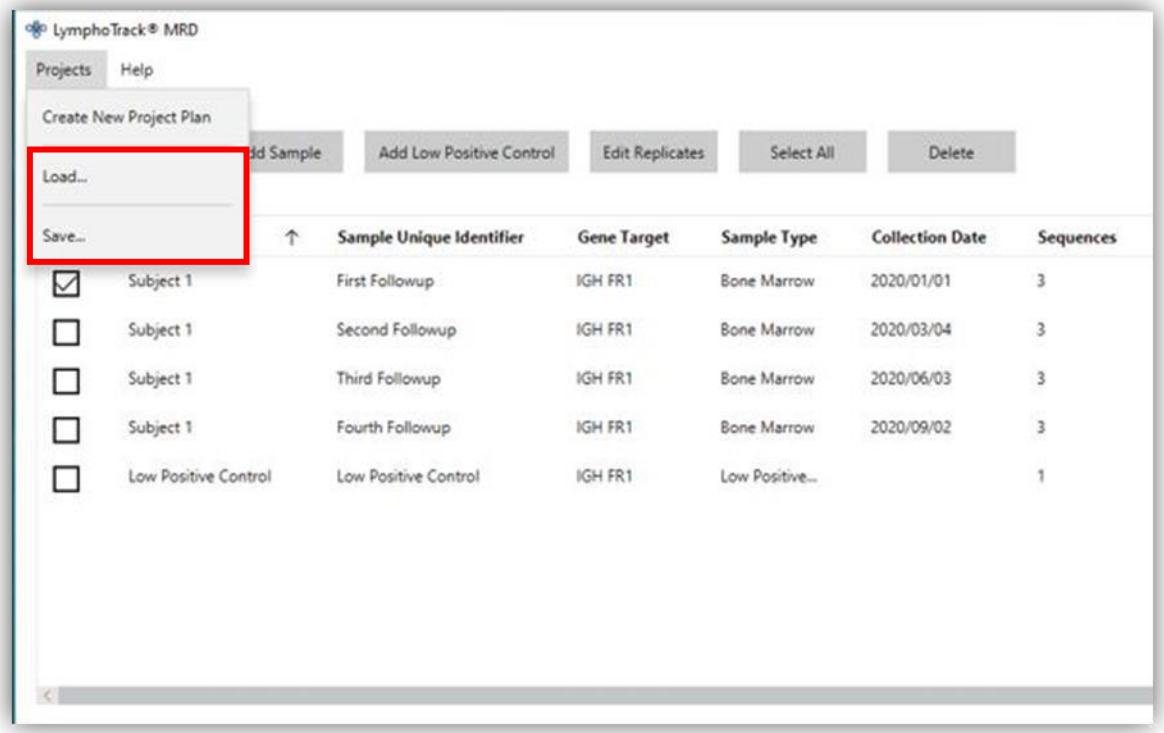


Subject ID	Sample Unique Identifier	Gene Target	Sample Type	Collection Date	Sequences	Replicates	LymphoQuant Included	Reads	Total DNA (ng)
IGH12345	TP1	IGH FR1	WB	2019/05/13	3	1	true	7629...	1500
IGH12345	TP2	IGH FR1	WB	2019/08/05	3	1	true	7629...	1500
IGH12345	TP3	IGH FR1	WB	2019/11/18	3	1	true	7629...	1500
IGH12345	TP4	IGH FR1	WB	2020/02/03	3	1	true	7629...	1500
TP4 LPC IGH	LPC IGH	IGH FR1	Low Positive...		1	1	true	1053...	1500
TRB7890	TP1	TRB	BM	2019/05/08	3	1	true	8042...	1500
TRB7890	TP2	TRB	BM	2019/08/21	3	1	true	8042...	1500
TRB7890	TP3	TRB	BM	2019/11/19	3	1	true	8042...	1500
TRB7890	TP4	TRB	BM	2020/02/12	3	1	true	8042...	1500
TRB LPC	TP4 LPC	TRB	Low Positive...		2	1	true	1065...	1500

Saving and Loading Projects

MRD Projects

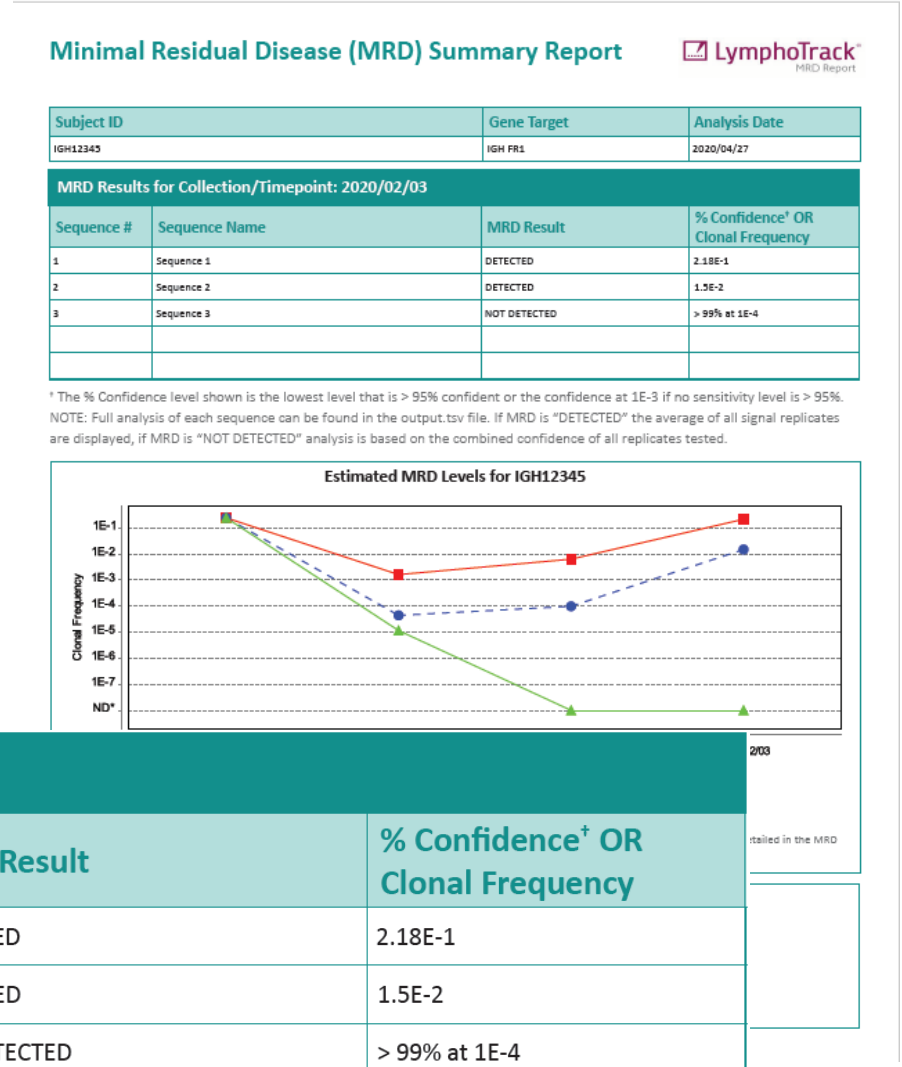
- Once clonal sequences are associated with a Subject and Samples, a Project can be Saved for future use
- Saved Projects can be Loaded when additional time points are added to a study.



MRD Reporting

Comprehensive Summary Report

- Tracking of up to 5 clones simultaneously
- Displays MRD levels over time
- % confidence in a negative result automatically displayed
- Easy to investigate effectiveness of treatment with clonal levels



Minimal Residual Disease (MRD) Summary Report

LymphoTrack MRD Report

Subject ID	Gene Target	Analysis Date
IGH12345	IGH FR1	2020/04/27

MRD Results for Collection/Timepoint: 2020/02/03

Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
1	Sequence 1	DETECTED	2.18E-1
2	Sequence 2	DETECTED	1.5E-2
3	Sequence 3	NOT DETECTED	> 99% at 1E-4

† The % Confidence level shown is the lowest level that is > 95% confident or the confidence at 1E-3 if no sensitivity level is > 95%.
NOTE: Full analysis of each sequence can be found in the output.tsv file. If MRD is "DETECTED" the average of all signal replicates are displayed, if MRD is "NOT DETECTED" analysis is based on the combined confidence of all replicates tested.

Estimated MRD Levels for IGH12345

2/03

tailed in the MRD

MRD Results for Collection/Timepoint: 2020/02/03

Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
1	Sequence 1	DETECTED	2.18E-1
2	Sequence 2	DETECTED	1.5E-2
3	Sequence 3	NOT DETECTED	> 99% at 1E-4

MRD Reporting

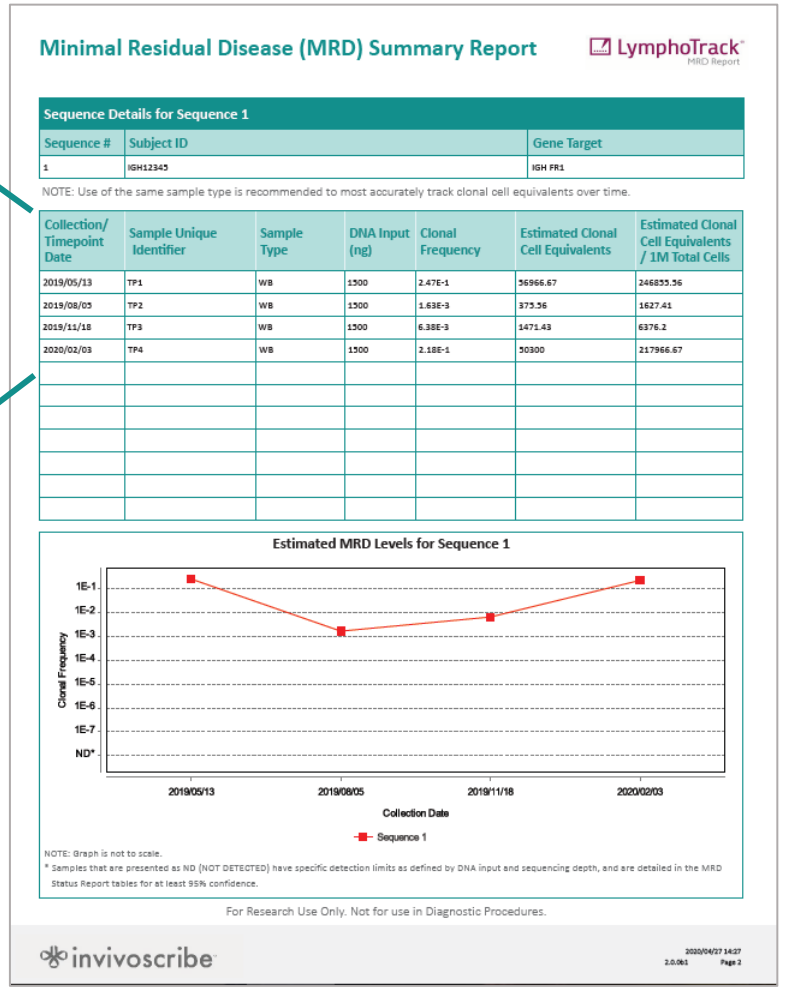
Individual Sequence Details

Sequence Details for Sequence 1

Sequence #	Subject ID	Gene Target
1	IGH12345	IGH FR1

NOTE: Use of the same sample type is recommended to most accurately track clonal cell equivalents over time.

Collection/ Timepoint Date	Sample Unique Identifier	Sample Type	DNA Input (ng)	Clonal Frequency	Estimated Clonal Cell Equivalents	Estimated Clonal Cell Equivalents / 1M Total Cells
2019/05/13	TP1	WB	1500	2.47E-1	56966.67	246855.56
2019/08/05	TP2	WB	1500	1.63E-3	375.56	1627.41
2019/11/18	TP3	WB	1500	6.38E-3	1471.43	6376.2
2020/02/03	TP4	WB	1500	2.18E-1	50300	217966.67

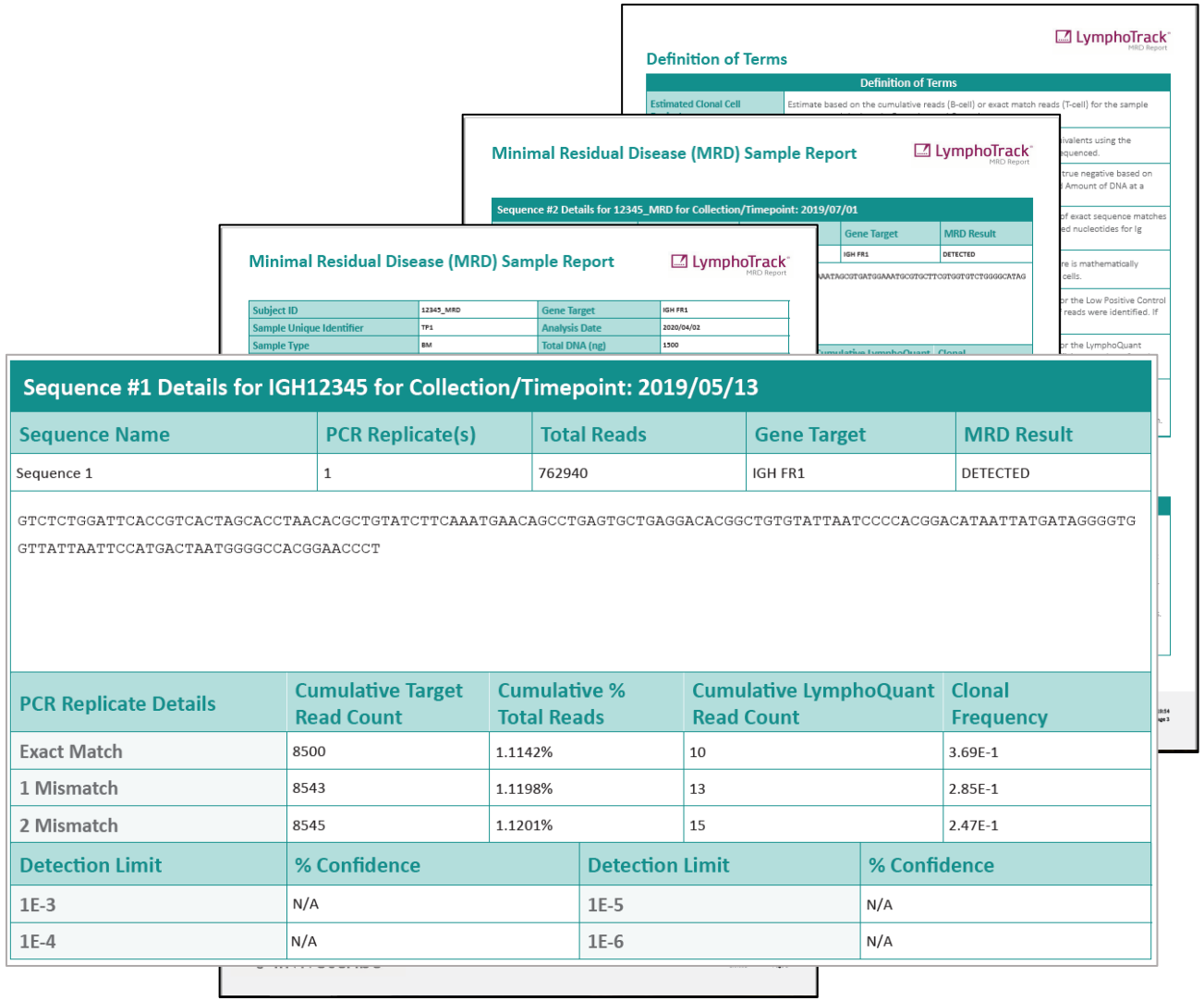


- Automated calculation of **clonal cell equivalents** (with LymphoQuant)
- Listing of separate time points
- Lists sample date and amount of DNA input

MRD Reporting

Individual time point reports

- Detailed information for the laboratory
- Levels of confidence at various detection levels
- Number of reads with exact match, 1 mismatch and 2 mismatches



LymphoTrack MRD Report

Definition of Terms

Definition of Terms	
Estimated Clonal Cell	Estimate based on the cumulative reads (B-cell) or exact match reads (T-cell) for the sample

Minimal Residual Disease (MRD) Sample Report

Sequence #2 Details for 12345_MRD for Collection/Timepoint: 2019/07/01

Gene Target	MRD Result
IGH FR1	DETECTED

Minimal Residual Disease (MRD) Sample Report

Subject ID	12345_MRD	Gene Target	IGH FR1
Sample Unique Identifier	TP1	Analysis Date	2020/04/02
Sample Type	BM	Total DNA (ng)	1500

Sequence #1 Details for IGH12345 for Collection/Timepoint: 2019/05/13

Sequence Name	PCR Replicate(s)	Total Reads	Gene Target	MRD Result
Sequence 1	1	762940	IGH FR1	DETECTED

GTCTCTGGATTCACCGTCACTAGCACCTAACACGCTGTATCTTCAAATGAACAGCCTGAGTGCTGAGGACACGGCTGTGTATTAATCCCCACGGACATAATTATGATAGGGGTG
GTTATTAATTCCATGACTAATGGGGCCACGGAACCCT

PCR Replicate Details	Cumulative Target Read Count	Cumulative % Total Reads	Cumulative LymphoQuant Read Count	Clonal Frequency
Exact Match	8500	1.1142%	10	3.69E-1
1 Mismatch	8543	1.1198%	13	2.85E-1
2 Mismatch	8545	1.1201%	15	2.47E-1

Detection Limit	% Confidence	Detection Limit	% Confidence
1E-3	N/A	1E-5	N/A
1E-4	N/A	1E-6	N/A

Considerations for MRD Testing

Use Project Planner tool in the MRD Software to design experiment

Mitigate contamination risk

- Never use the same barcode for a sample from run-to-run
- Use different barcodes for each replicate, and only use a barcode once within an MRD run
- Avoid running known high-positive samples together with follow-up samples screened at high-read depths on the same chip or flow cell
- Conduct an Illumina® 'Template Line Wash' with bleach after each MiSeq® run (or run a non-MRD/LymphoTrack® run between runs)

The LymphoTrack® Assays use one-step PCR which reduces contamination risk

Considerations for MRD Testing

IGK

- There are three common rearrangements that are not suitable for MRD analysis due to the high frequency in which they occur. As a result, any clonotype sequence that is listed below should *not* be used for minimal residual disease analysis:
  - **Intron-Kdel**
  - **V3D-20 with any J or Kdel**
  - **V3-11 with any J or Kdel**

Define MRD

- Always be cautious about 'MRD NEGATIVITY'
- Sensitivity level should be noted for each sample based on DNA input

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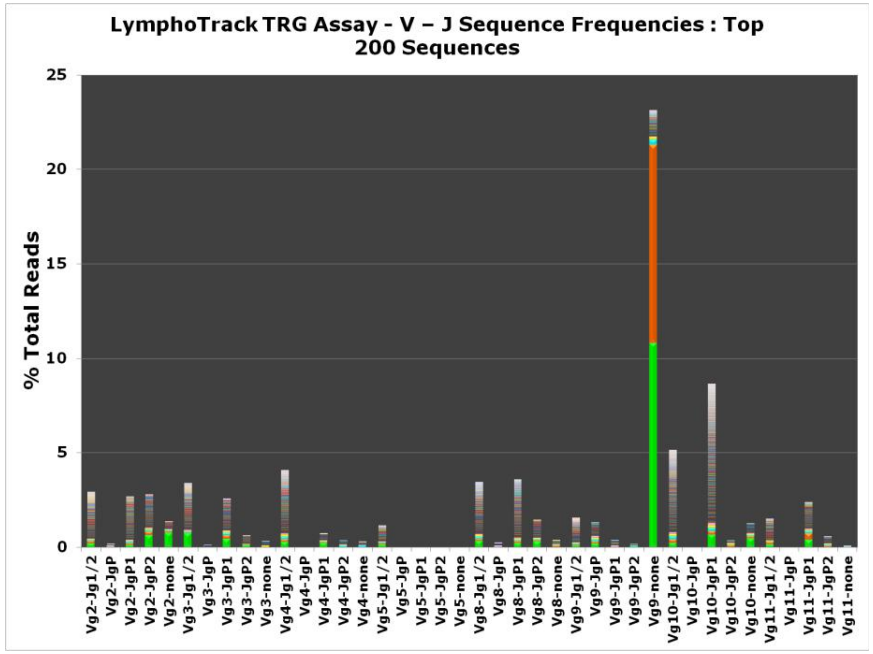
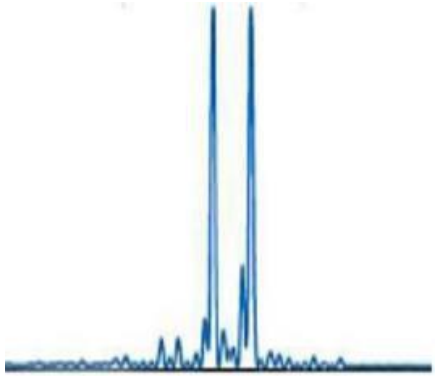
MRD Case Study

TRG Case Study: First Time Point

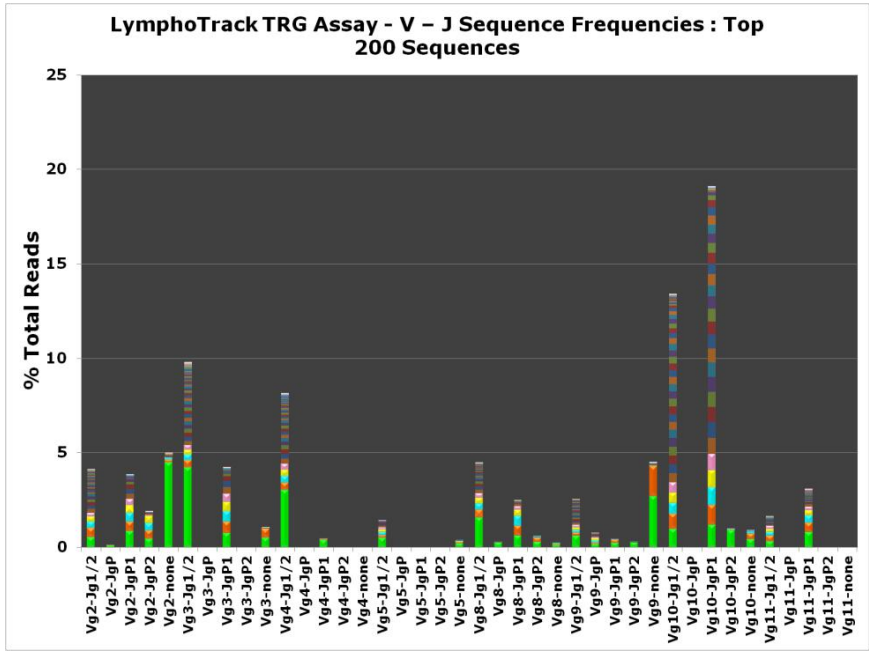


Total Read Count > 1.5 M

Rank	Sequence	Length	Merge Count	V-gene	J-gene	% total reads	Cumulative %
1	CGGCATTCCGTCAGGCAAATTTGAGGT	137	171284	Vg9	none	10.8329296	10.8329296
2	CGGCATTCCGTCAGGCAAATTTGAGGT	140	170093	Vg9	none	10.7576043	21.5905339
3	GAGTCAGTCCAGGGAAGTATTATACTTAC	126	12344	Vg2	none	0.7807015	22.3712355
4	AGAATCAGTAGAGGAAAGTATTTTACTTAT	149	11708	Vg3	Jg1/2	0.7404775	23.1117129
5	TGGGTAAGACAAGCAACAAAGTGGAGGC	158	10570	Vg10	JgP1	0.6685042	23.7802171



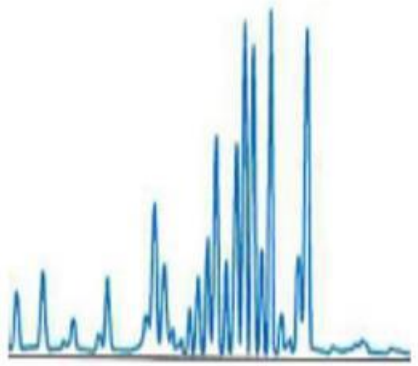
TRG Case Study: Second Time Point



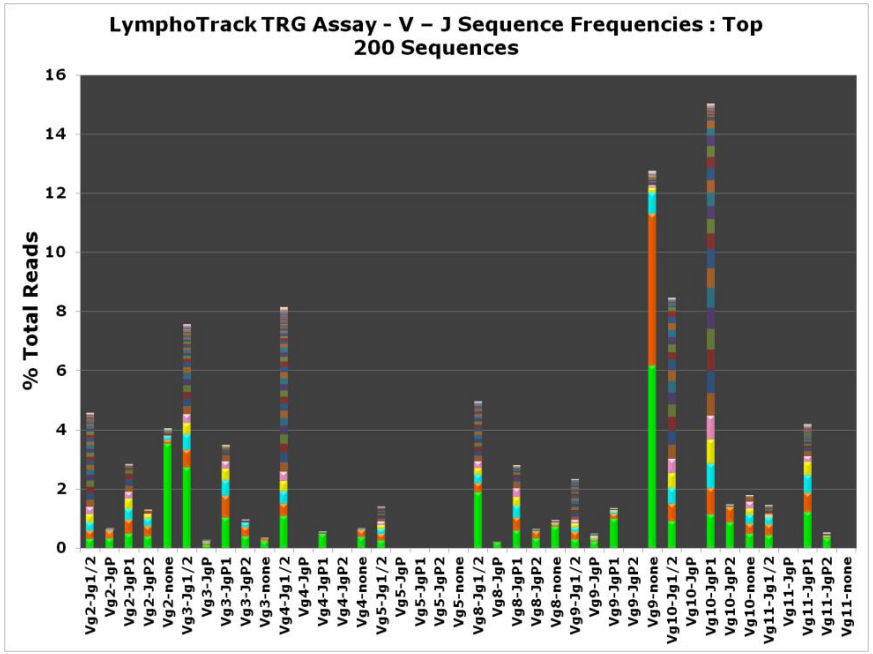
Total Read Count > 439k

Rank	Sequence	Length	Merge Count	V-gene	J-gene	% total reads	Cumulative %
1	GGAGTCAGTCCAGGGAA GTATTATAC	126	20659	Vg2	none	4.7045937	4.7045937
2	AGAATCAGTAGAGGAAA GTATTTTACT	149	20487	Vg3	Jg1/2	4.6654248	9.3700185
3	GGAATCAGCCCAGGGAA GTATGATAC	142	13935	Vg4	Jg1/2	3.1733633	12.5433818
4	CGGCATTCCGTCAGGCAA ATTTGAGG	137	12005	Vg9	none	2.7338519	15.2772338
5	TGGGTAAGACAAGCAACA AAGTGGAG	146	7184	Vg10	JgP1	1.6359844	16.9132181

Clone Not Identified

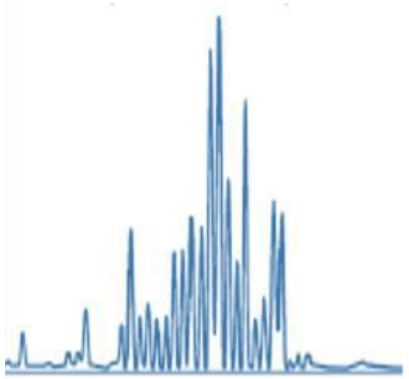


TRG Case Study: Third Time Point



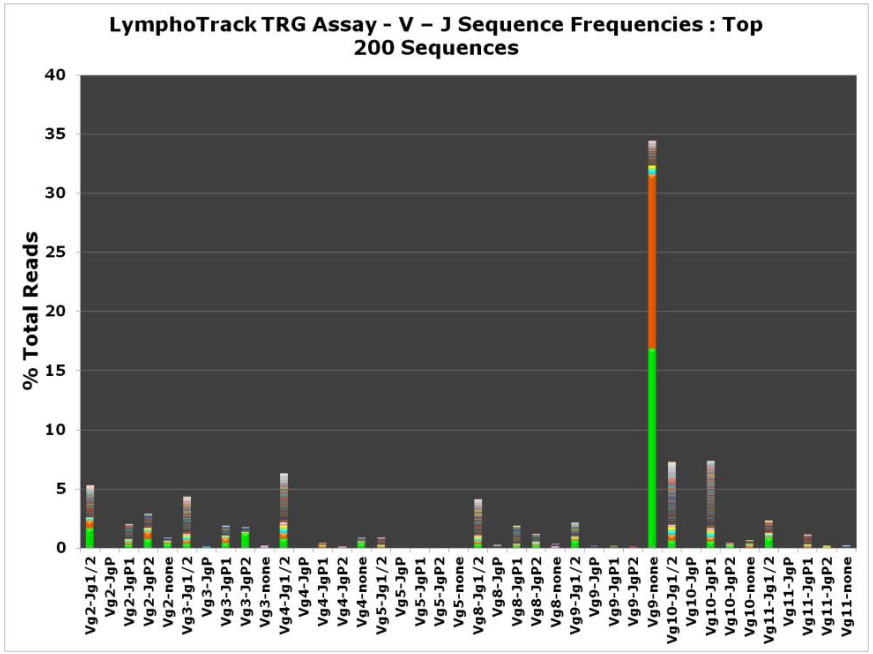
Total Read Count > 471k

Rank	Sequence	Length	Merge Count	V-gene	J-gene	% total reads	Cumulative %
1	CGGCATTCCGTCAGGCAA ATTTGAGG	137	29481	Vg9	none	6.2550524	6.2550524
2	CGGCATTCCGTCAGGCAA ATTTGAGG	140	25060	Vg9	none	5.3170385	11.5720909
3	GGAGTCAGTCCAGGGAA GTATTATAC	126	17596	Vg2	none	3.7333843	15.3054751
4	AGAATCAGTAGAGGAAA GTATTTTACT	149	13689	Vg3	Jg1/2	2.9044270	18.2099021
5	GGAATCAGTCGAGAAAA GTATCATAC	141	9259	Vg8	Jg1/2	1.9645036	20.1744057



Peaks Re-Emerge, But Not Above Background

TRG Case Study: Fourth Time Point



Total Read Count > 1.5M

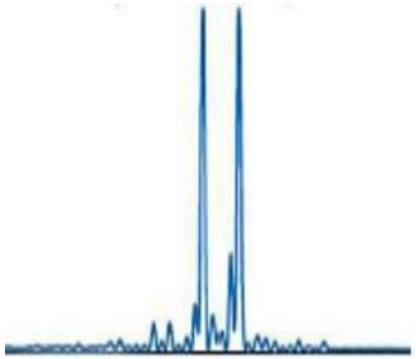
Rank	Sequence	Length	Merge Count	V-gene	J-gene	% total reads	Cumulative %
1	CGGCATTCCGTCAGGCAAATTTGAGG	137	195616	Vg9	none	16.9726438	16.9726438
2	CGGCATTCCGTCAGGCAAATTTGAGG	140	176466	Vg9	none	15.3110920	32.2837358
3	GGAGTCAGTCCAGGGAAGTATTATAC	144	19216	Vg2	Jg1/2	1.6672784	33.9510142
4	AGAATCAGTAGAGGAAAGTATTTTACT	129	14813	Vg3	JgP2	1.2852516	35.2362657
5	GAAGACTAAGAAACTTGAGGTAAGTA	136	12056	Vg11	Jg1/2	1.0460402	36.2823059

Sequence ID: Query_174715 Length: 137 Number of Matches: 1

Range 1: 1 to 137 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
254 bits(137)	5e-73	137/137(100%)	0/137(0%)	Plus/Plus

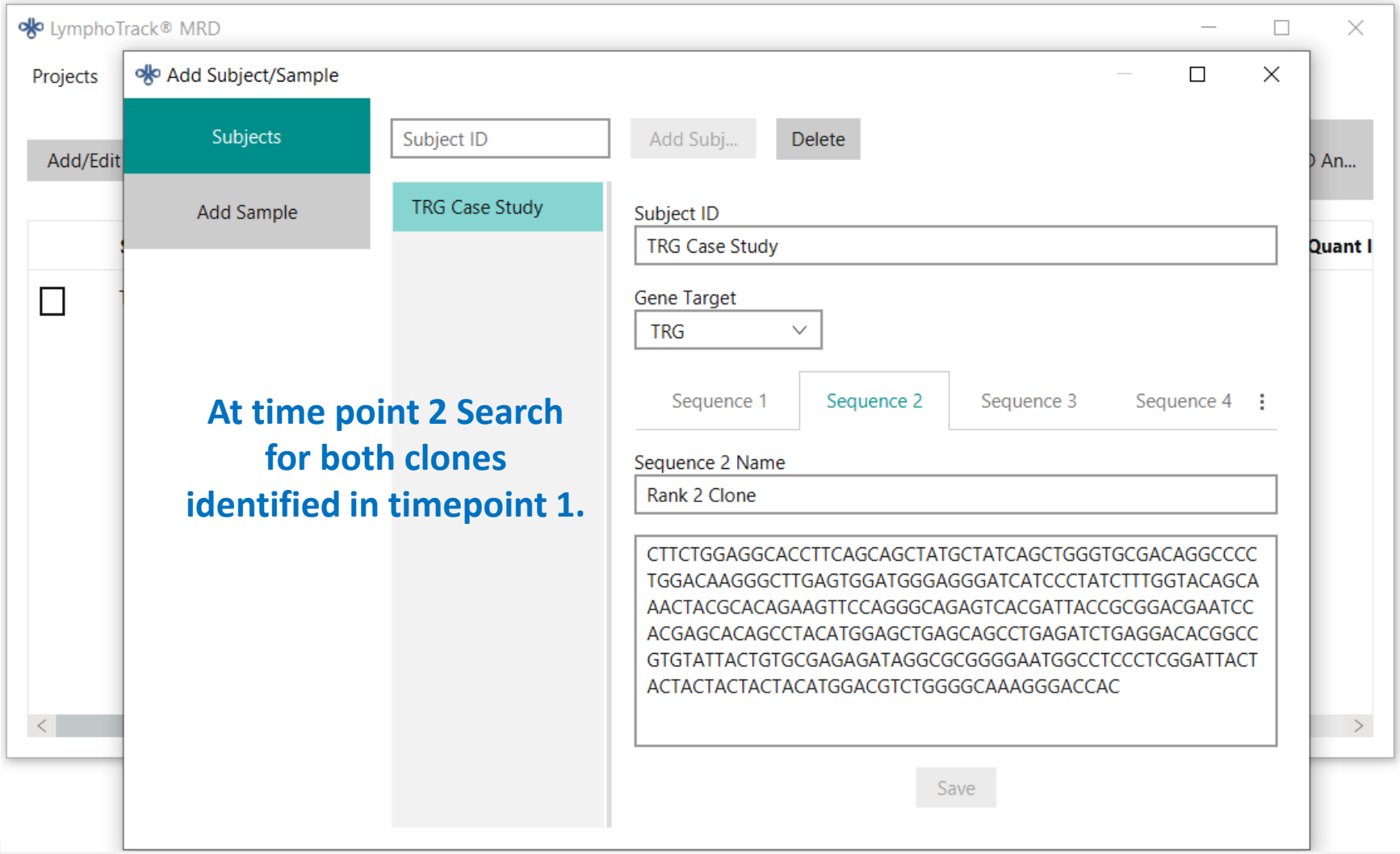
```
Query  1    CGGCATTCCGTCAGGCAAATTTGAGGTGGATAGGATACCTGAAACGTCTACATCCACTCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CGGCATTCCGTCAGGCAAATTTGAGGTGGATAGGATACCTGAAACGTCTACATCCACTCT  60

Query  61   CACCATTCACAATGTAGAGAAACAGGACATAGCTACCTACTACTGTGCCTTGTGGGAGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CACCATTCACAATGTAGAGAAACAGGACATAGCTACCTACTACTGTGCCTTGTGGGAGGT  120

Query  121  GCGGGGTTTTGGCAGTG  137
            |||||||||||||||||
Sbjct  121  GCGGGGTTTTGGCAGTG  137
```



Relapsed: Original Clones Confirmed

TRG Case Study:

Could the clones resurgence have been detected earlier?



Take Home Message

LymphoTrack® MRD Advantages

- Establish NGS MRD testing in-house quickly and easily
- Works with broad menu of targets for B- and T-cell analysis
- Same LymphoTrack® kits for Clonality and MRD testing
- One-Step PCR reduces contamination risk
- Track up to 5 sequences simultaneously
- Analyze and compare multiple time points with Saved Projects
- Track normalized MRD levels with LymphoQuant spike-in control
- Intuitive analysis tools and PDF Reports

Quiz

What is the lowest practical level of detection with LymphoTrack® MRD

1. 10^{-3}
2. 10^{-4}
3. 10^{-5}
4. 10^{-6}

Quiz

Which samples types are typically used for MRD analysis?

1. FFPE
2. Whole Blood
3. Bone marrow
4. Sorted cells

Quiz

Which of the following are not part of the MRD Analysis

1. LymphoTrack Assays
2. LymphoQuant Internal Controls
3. LymphoTrack Low Positive Controls
4. LymphoTrack MRD Software
5. LymphoQuest Internal Controls

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LymphoTrack® Publications

LymphoTrack® MRD Publications

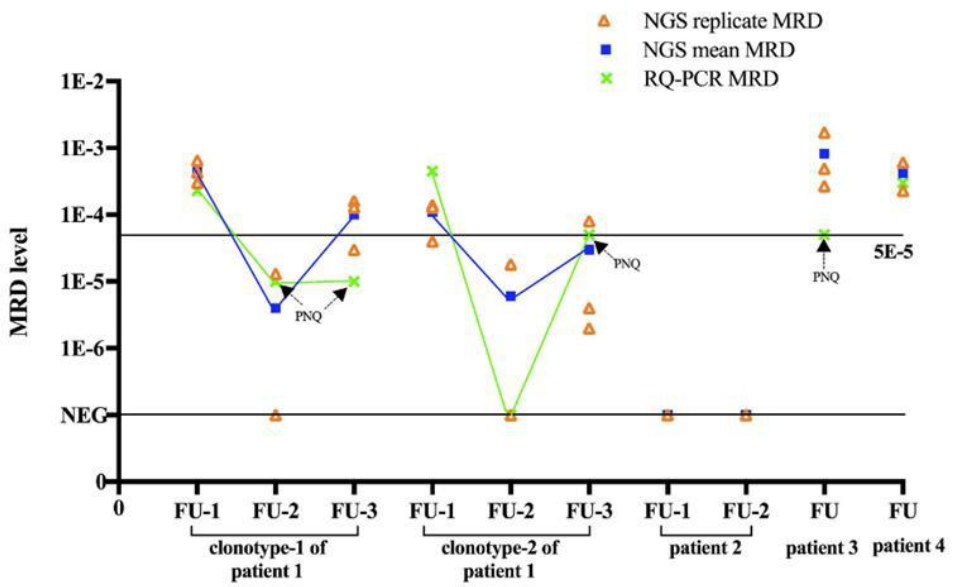
Highlights

- Establishes a standardized experimental design to track MRD, verifying a sensitivity of 10^{-5}.
- Comparison to ASO RQ-PCR, NGS demonstrates enhanced sensitivity and quantification.
- Determines that the LymphoTrack® MiSeq® method is an effective tool for MRD monitoring in MM.

Standardized Minimal Residual Disease Detection by Next-Generation Sequencing in Multiple Myeloma

*Qiumei Yao [1], Yinlei Bai [2], Alberto Orfao [3] and Chor Sang Chim [1]**

FIGURE 2 | Comparison between NGS and RQ-PCR



LymphoTrack® MRD Publications

Highlights

- Defines the factors influencing the identification of clonal V(D)J sequences.
- Uses logistic regression analysis to determine which factors have independent effects on clonality detection.
- Demonstrates effectiveness of using NGS-based assays to overcome the limitations imposed by SHM.

Baseline identification of clonal V(D)J sequences for DNA-based minimal residual disease detection in multiple myeloma

Even H. Rustad[1,2], Malin Hultcrantz[1], Venkata D. Yellapantula[3], Theresia Akhlaghi[1], Caleb Ho[4], Maria E. Arcila[4], Mikhail Roshal[4], Akshar Patel[5], Denise Chen[6], Sean M. Devlin[3], Austin Jacobsen[7], Ying Huang[7], Jeffrey E. Miller[7], Elli Papaemmanuil[3], Ola Landgren[1]*

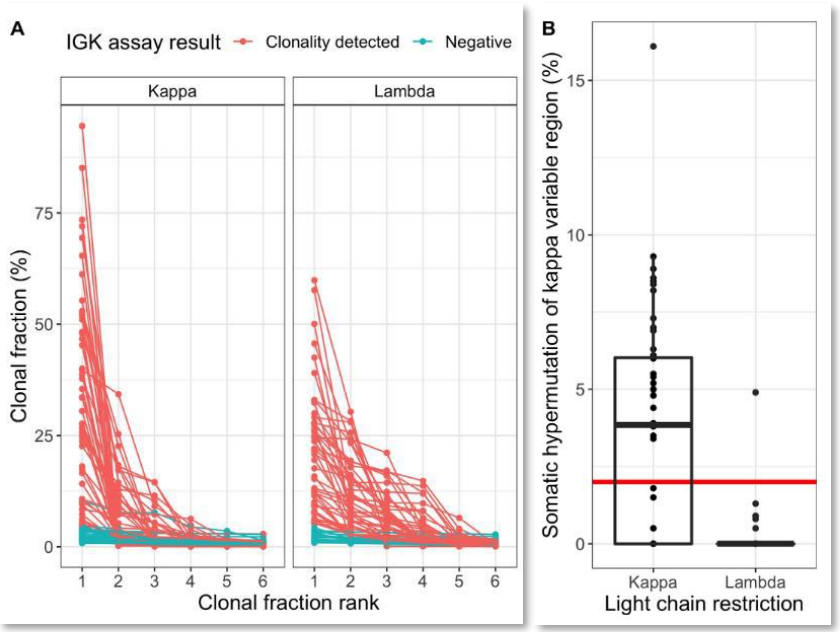


FIGURE (above)**: More *IGK* rearrangements and minimal somatic hypermutation of clonal VK-sequences in lambda-restricted multiple myeloma.**

LymphoTrack® MRD Publications

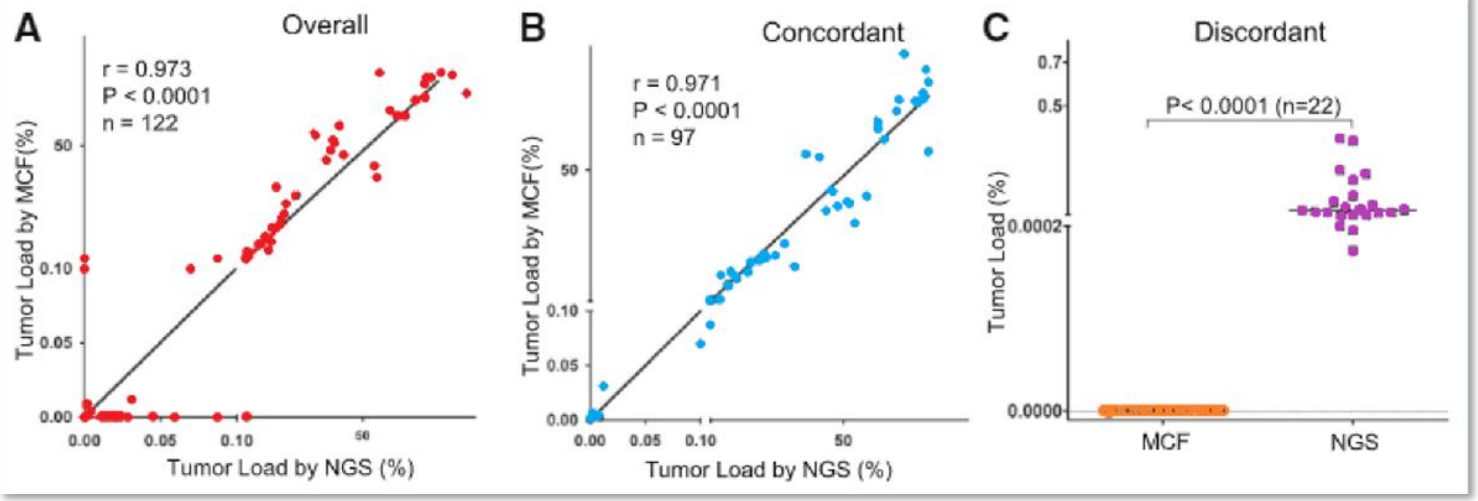
Highlights

- Compared the performance of LymphoTrack® to detect MRD in B-ALL samples vs. MCF
- Demonstrates that NGS performs better than MCF
- Enables better risk stratification and earlier preemptive therapies against impending relapse, thus potentially improving outcome for B-ALL cases.
- Interesting for labs looking to establish MRD with NGS

Simple deep sequencing-based post-remission MRD surveillance predicts clinical relapse in B-ALL

Shuhua Cheng[1], Giorgio Inghirami[1], Shuo Cheng[2] and Wayne Tam[1*]

Comparison of tumor load determined by the NGS and MCF assays.



LymphoTrack® MRD Publications

Highlights

- Review paper which provides a good case for updating MRD detection technology for myeloma, comparing:
  - MCF and NGS to
  - ASO-RQ PCR and F-PCR
- Both technologies provide a high level of sensitivity, enabling prognostic significance in stratifying cases into different levels of MRD.

Minimal residual disease detection of myeloma using sequencing of immunoglobulin heavy chain gene VDJ regions

Caleb Ho, MD[a,b,1], Maria E. Arcila, MD[a,b,*,2]

Results from MRD detection in post-treatment marrow sample of a myeloma patient, including 10-color MFC and NGS