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A Case for Precision MRD Assessments: Clonal Rearrangement Detection in Hematologic Malignancies

Presented by

Dr. Yury Monczak, PhD
Molecular Pathology Center
Jewish General Hospital
McGill University

Tuesday, May 6 | 10:00 AM PST

A Case for Precision MRD Assessments: Clonal Rearrangement Detection in Hematologic Malignancies

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May 6, 2025

“Clonal expansion 101”

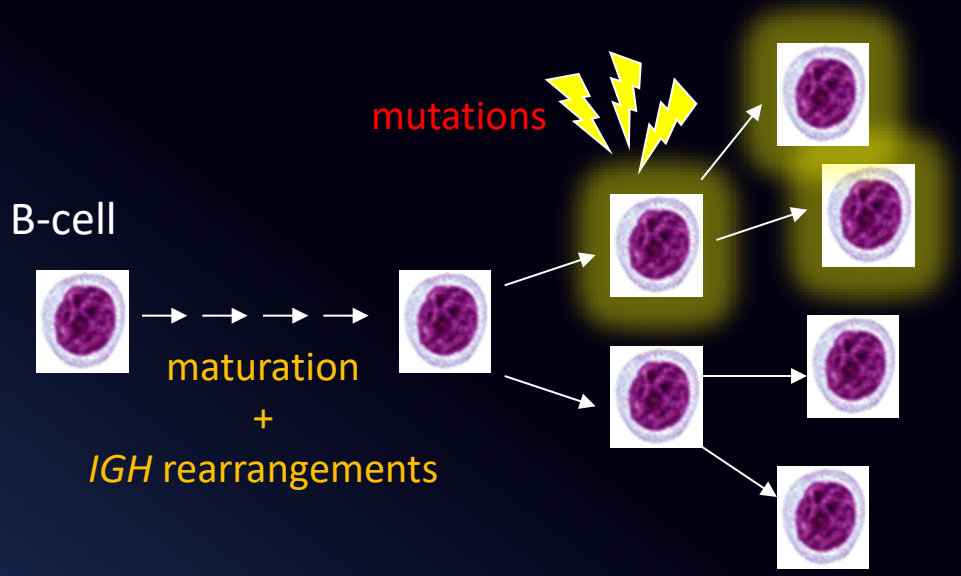
B-cell

maturation

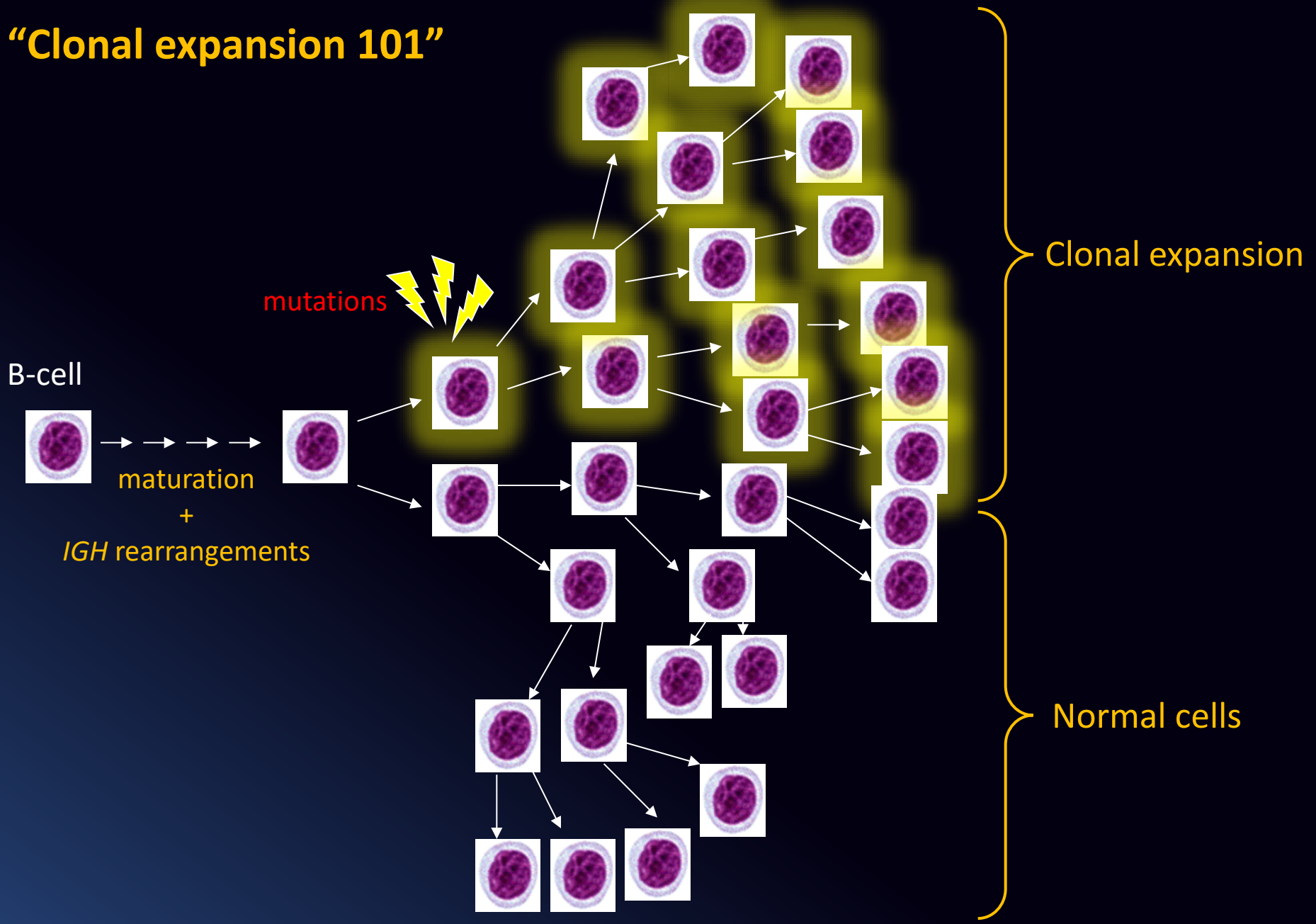
+

IGH rearrangements

“Clonal expansion 101”



“Clonal expansion 101”



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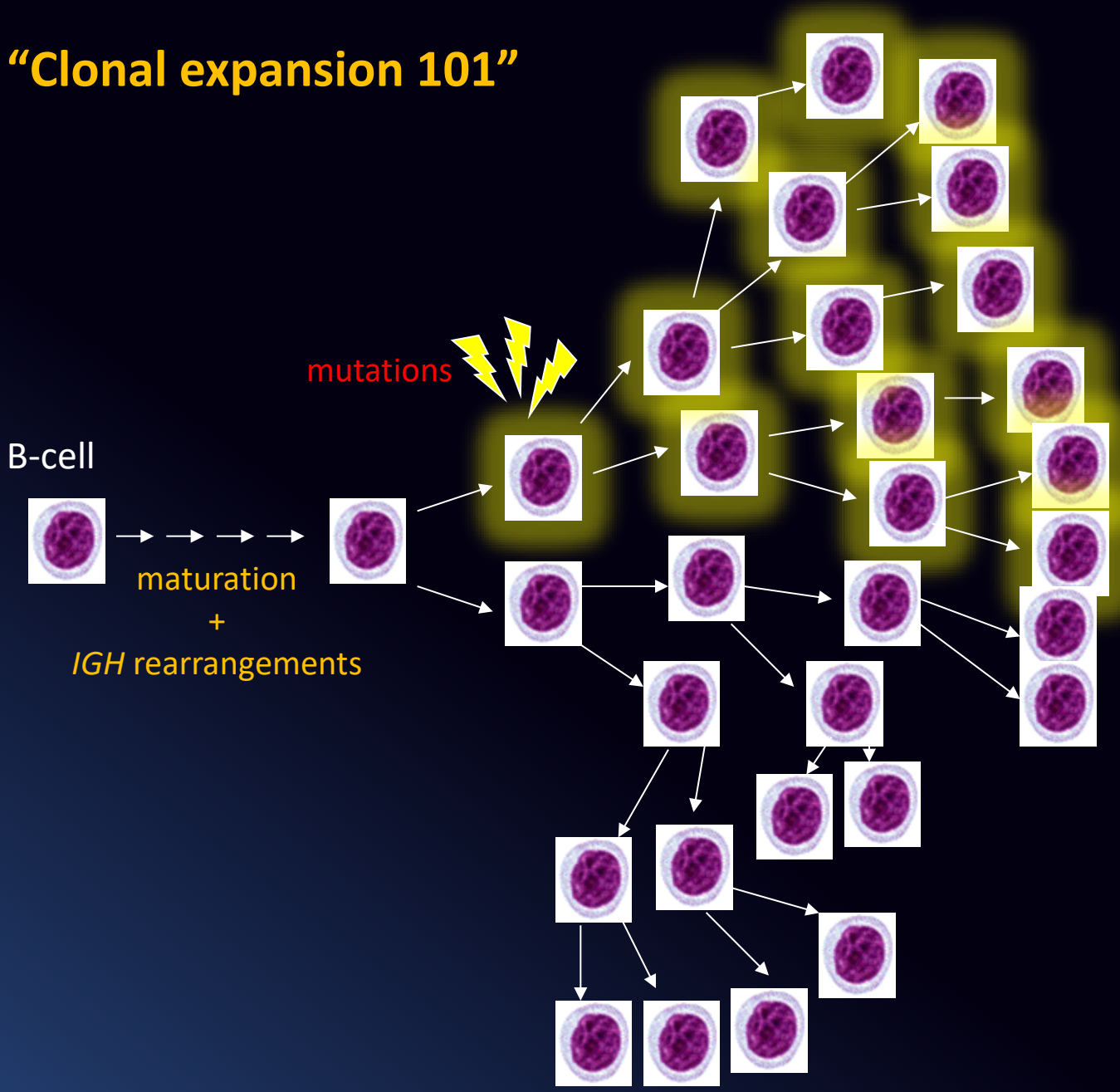
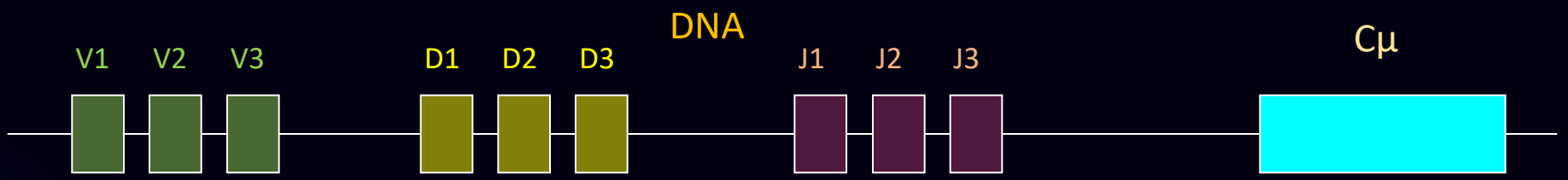


But how to differentiate clones from normal cells?

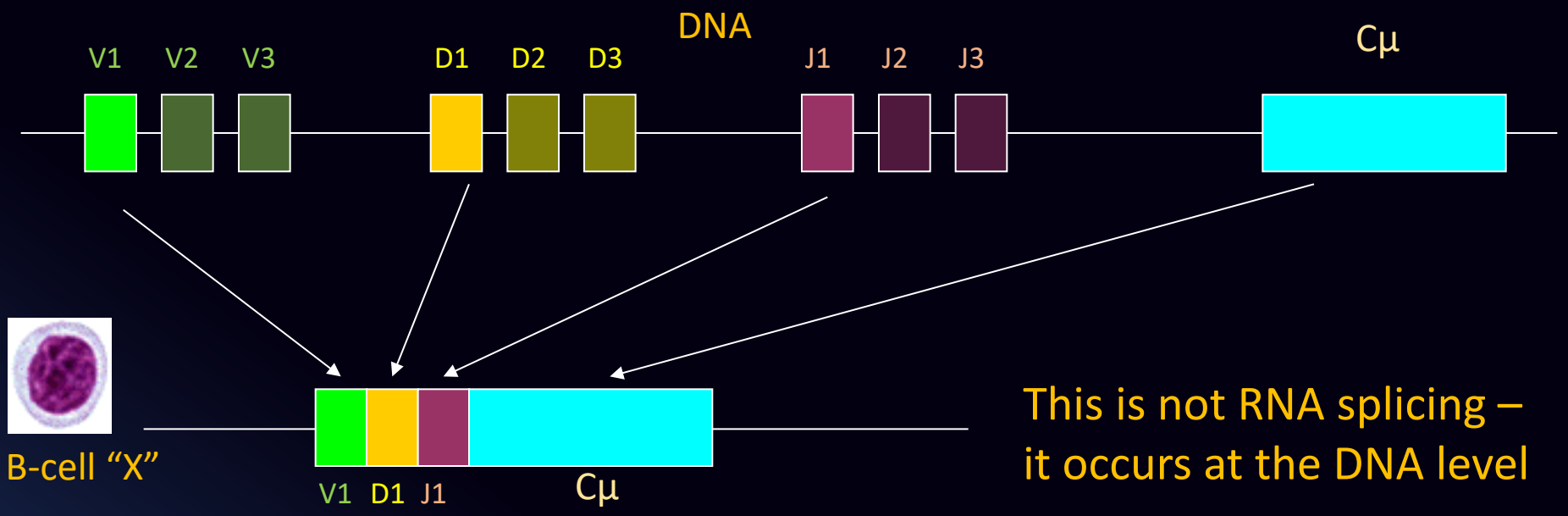
A unique marker for each B-cell is necessary

***IGH* gene**

IGH locus (chromosome 14)



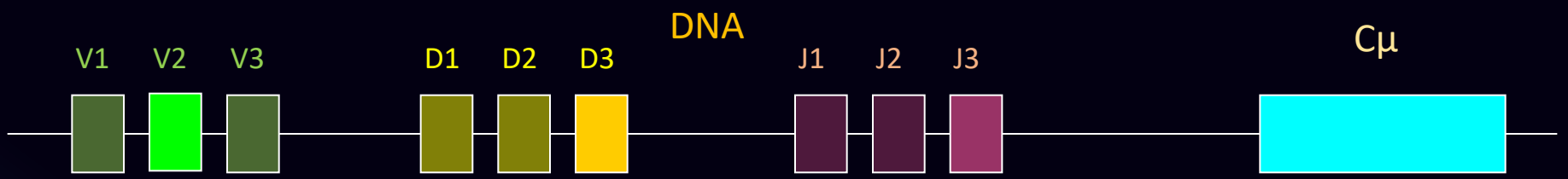
IGH locus (chromosome 14)



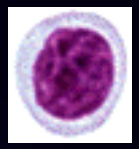
This is not RNA splicing – it occurs at the DNA level

Combinatorial diversity + imprecise joining of gene segments create unique clonal rearrangements and sequences.

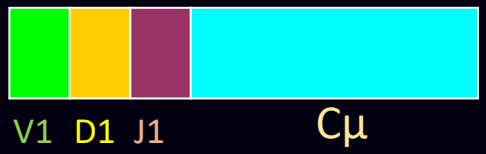
IGH locus (chromosome 14)

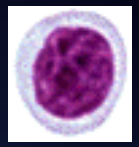


Unique sequences = Unique nucleic acid fingerprints

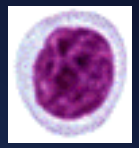


B-cell "X"





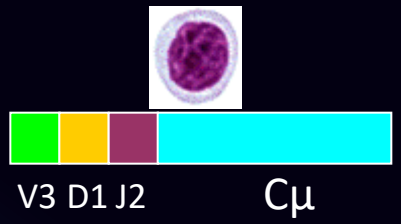
B-cell "Y"

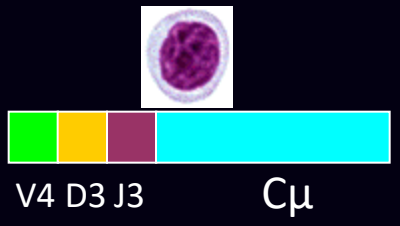


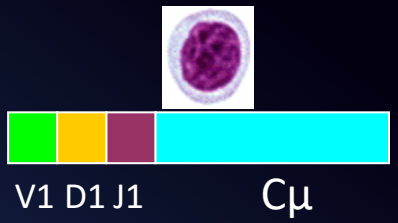
B-cell "Z"

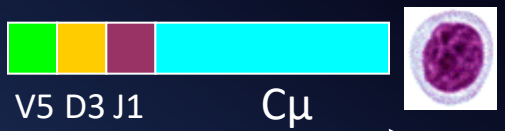
Polyclonal population – each V-D-J molecule differs in sequence and length

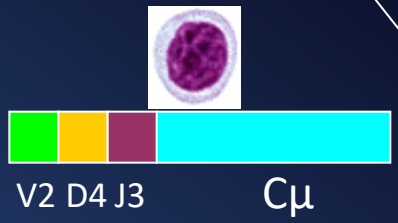
“Clonal expansion 101”

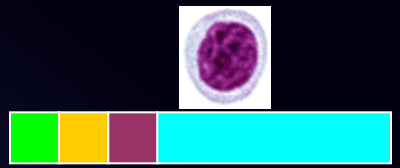






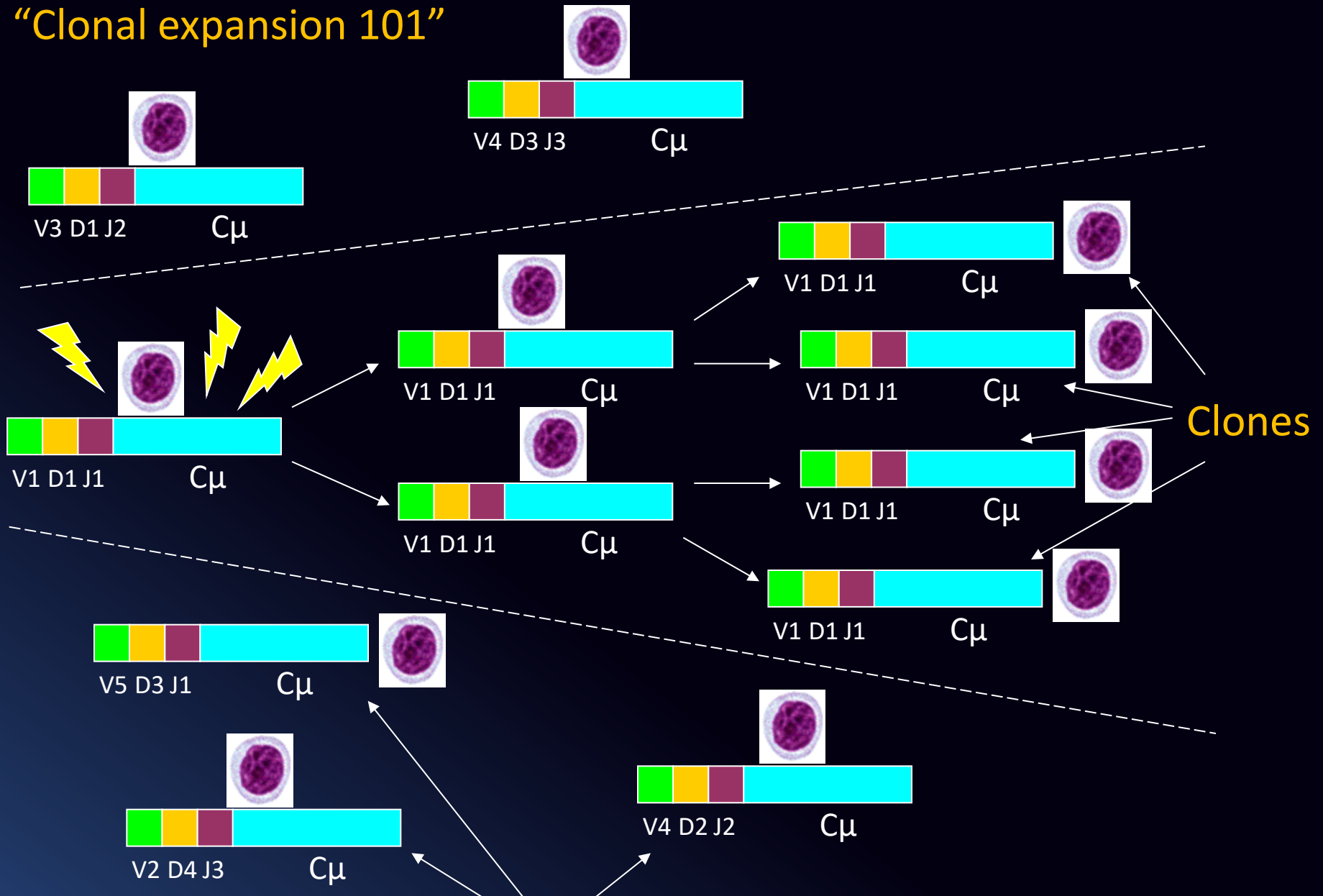




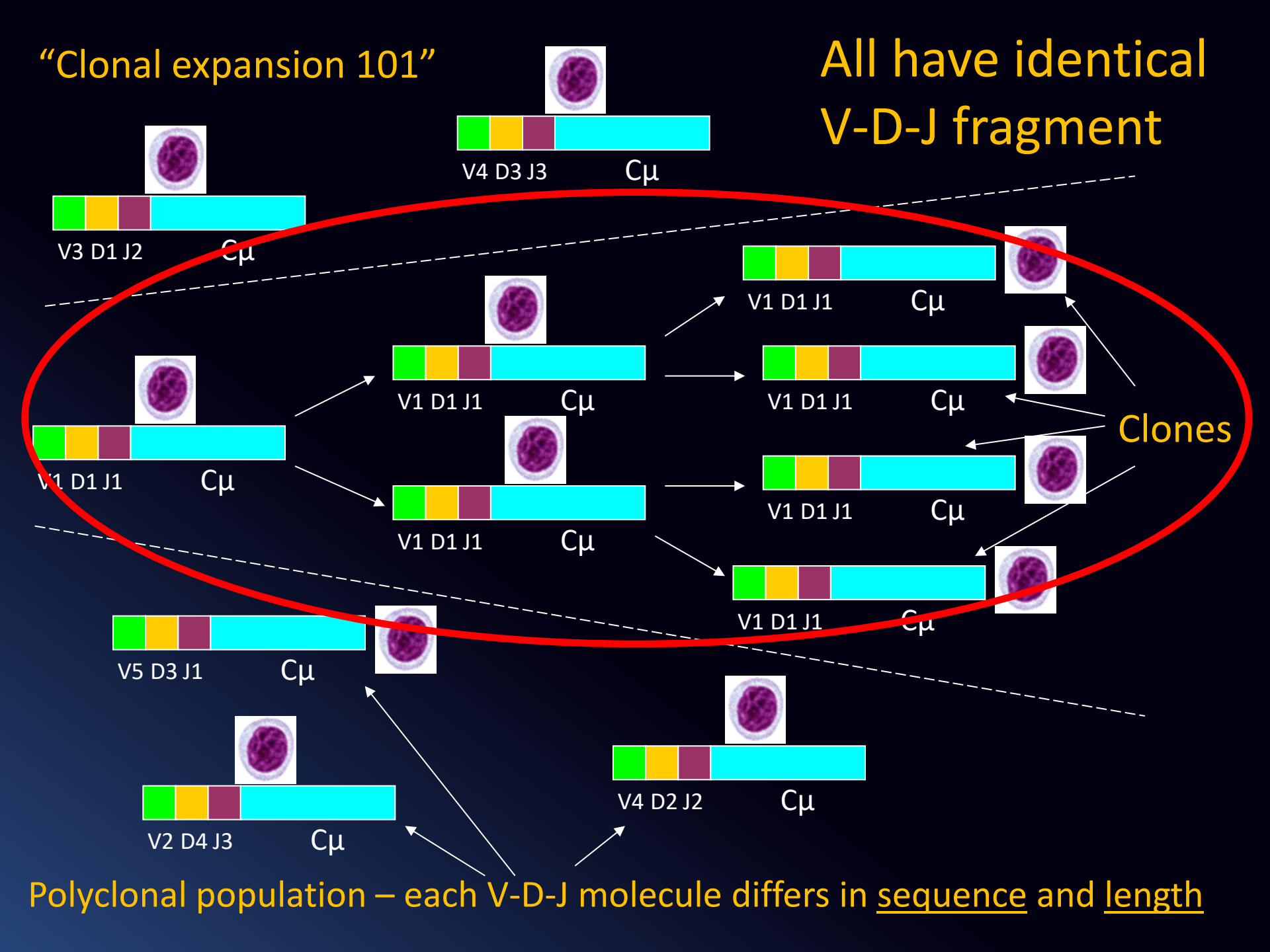


Polyclonal population – each V-D-J molecule differs in sequence and length

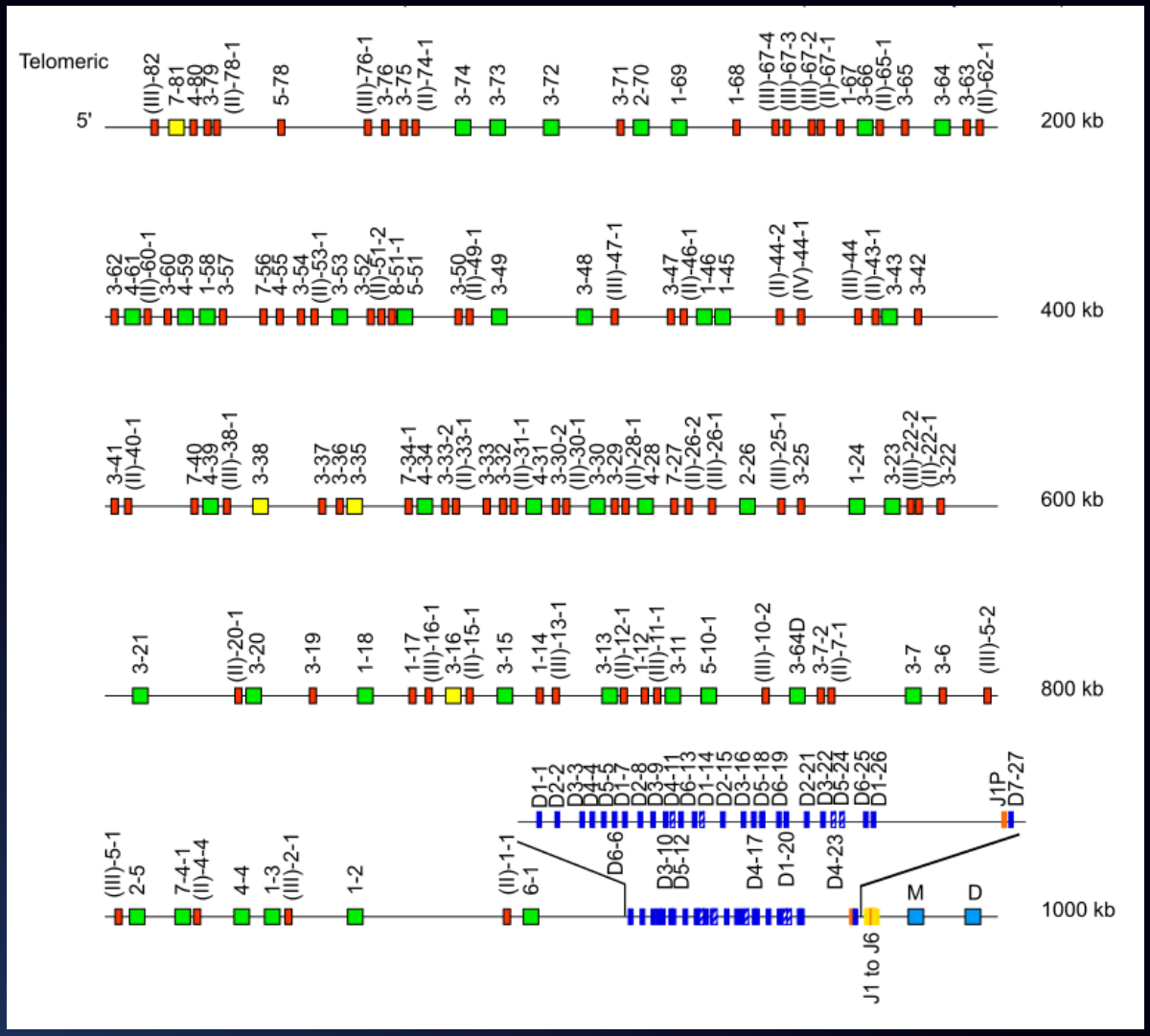
“Clonal expansion 101”



Polyclonal population – each V-D-J molecule differs in sequence and length

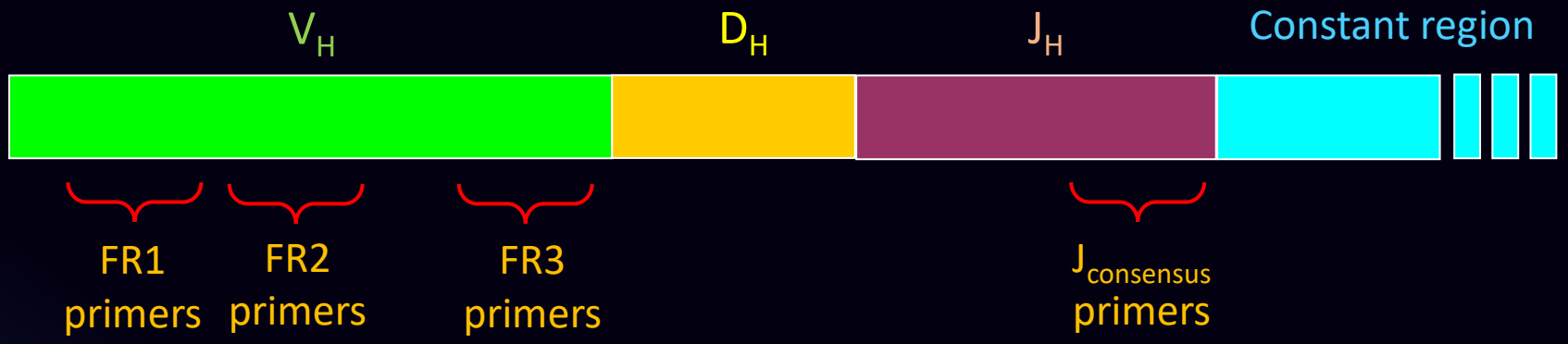

"Clonal expansion 101"
All have identical V-D-J fragment
V4 D3 J3
Cμ
V3 D1 J2
Cμ
V1 D1 J1
Cμ
V1 D1 J1
Cμ
V1 D1 J1
Cμ
V1 D1 J1
Cμ
V1 D1 J1
Cμ
V1 D1 J1
Cμ
Clones
V5 D3 J1
Cμ
V4 D2 J2
Cμ
V2 D4 J3
Cμ
Polyclonal population – each V-D-J molecule differs in sequence and length

Sequencing of entire *IGH* (and *IGK*, *IGL*, *TRG*, *TRB*, *TRD*) loci

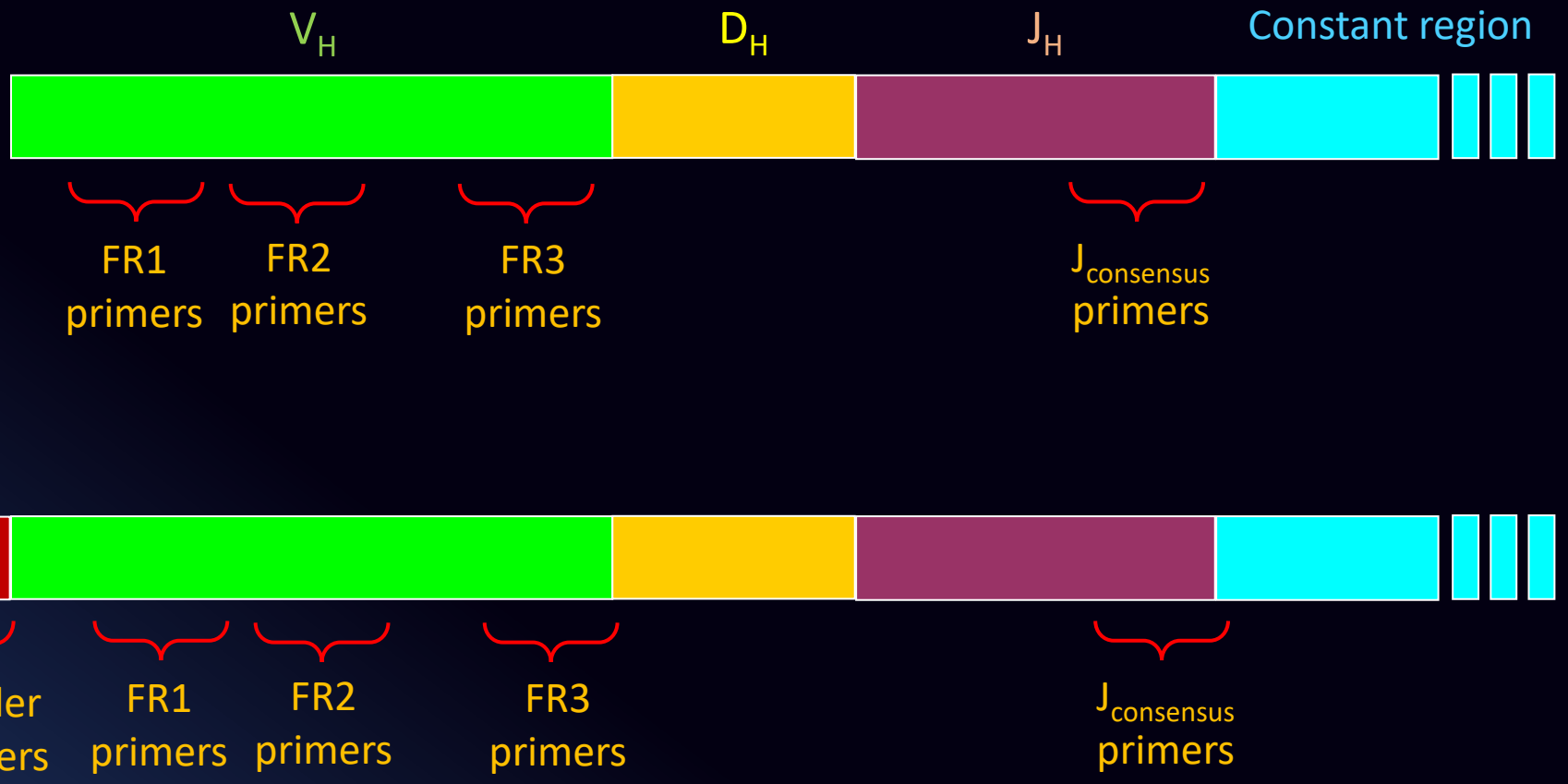


IMGT/LIGM-DB: BK063801 (1259983 bp), Human (*Homo sapiens*) IGH locus on chromosome 14.

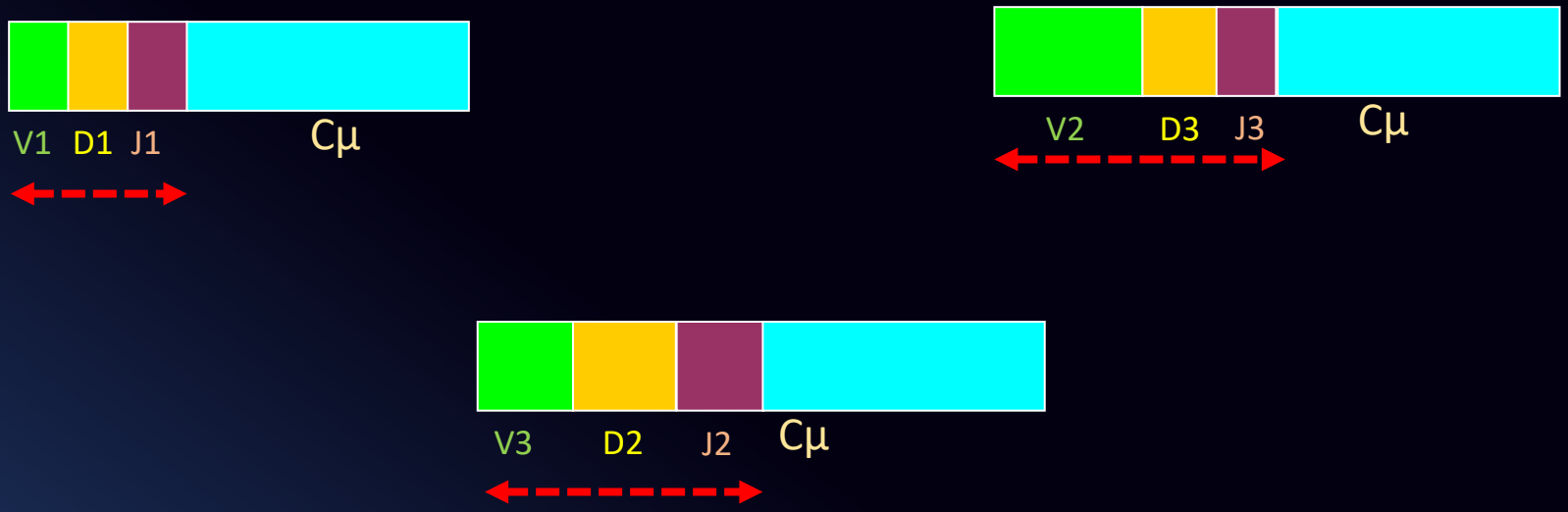
Rearranged *IGH* gene



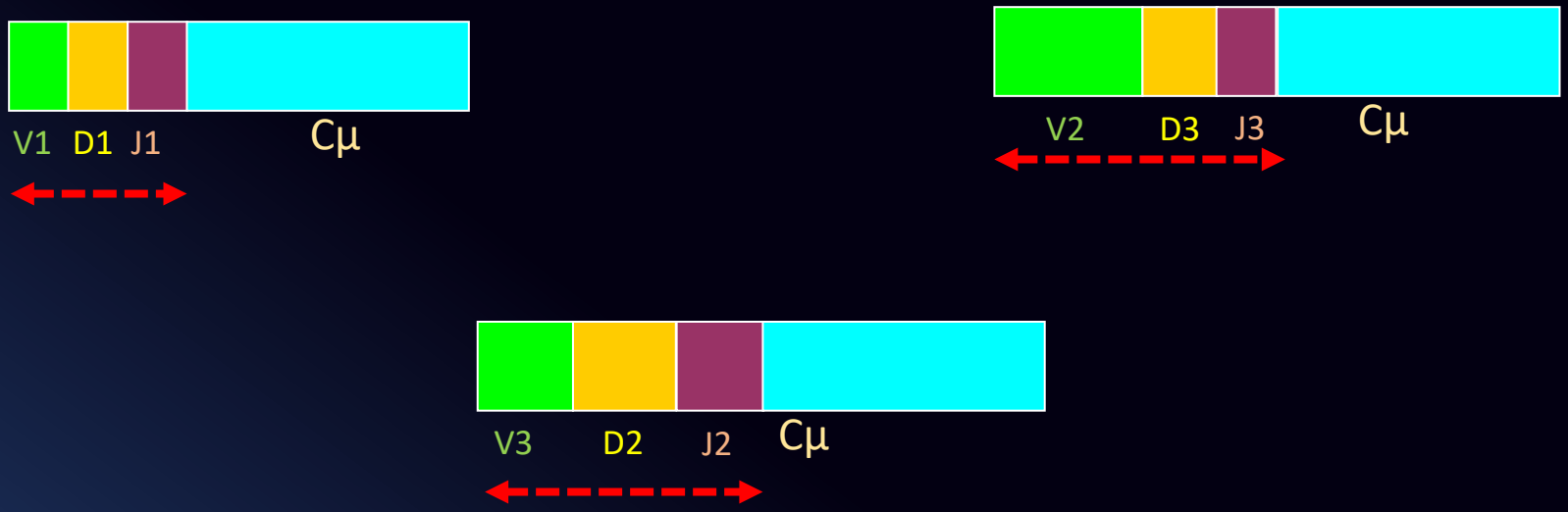
Rearranged *IGH* gene



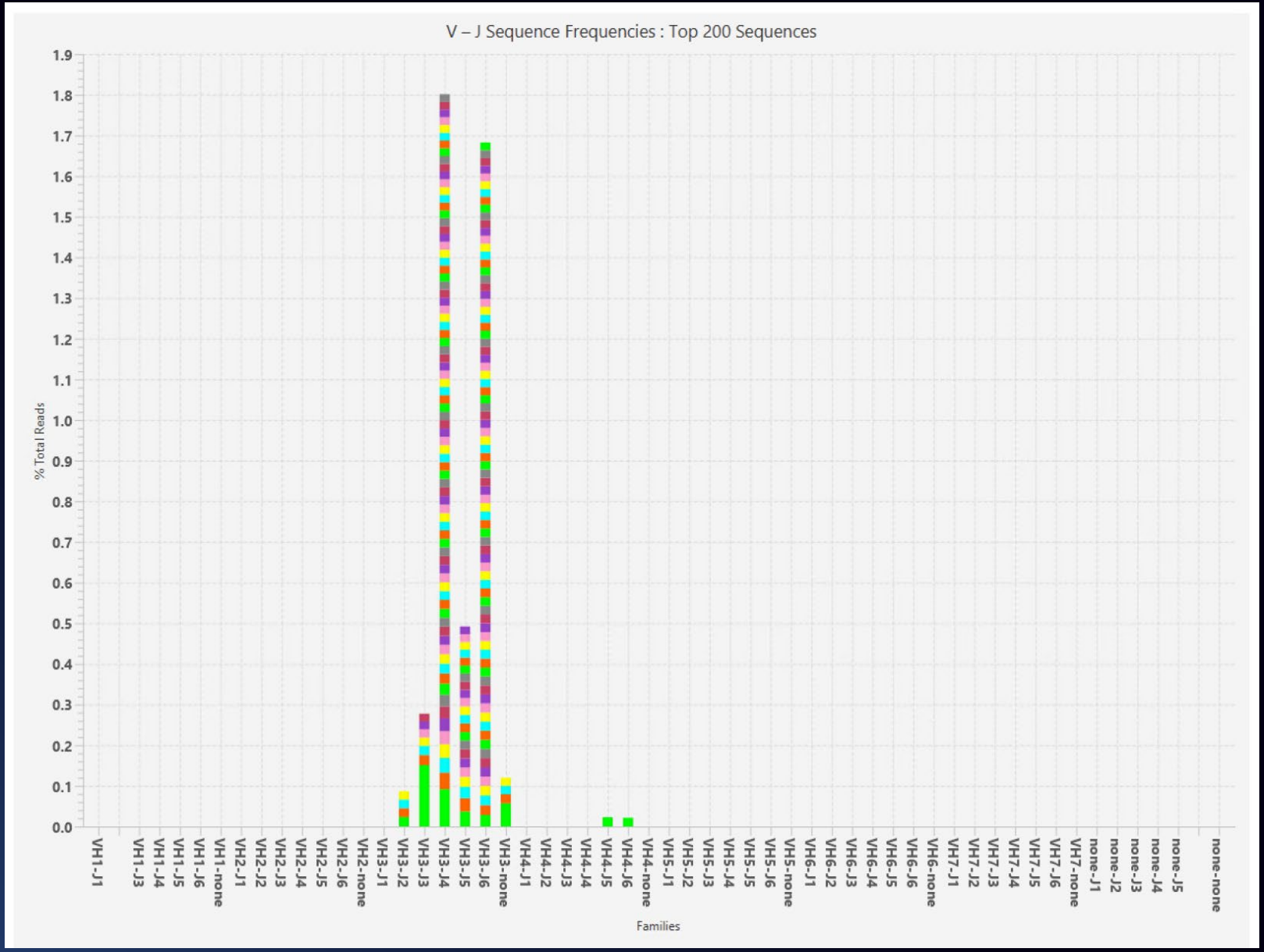
Next-generation sequencing allows us to "read" each *IGH* molecule present in the specimen – millions of molecules!



… and thus recognize one specific predominant (clonal) sequence among millions of different (non-clonal) sequences



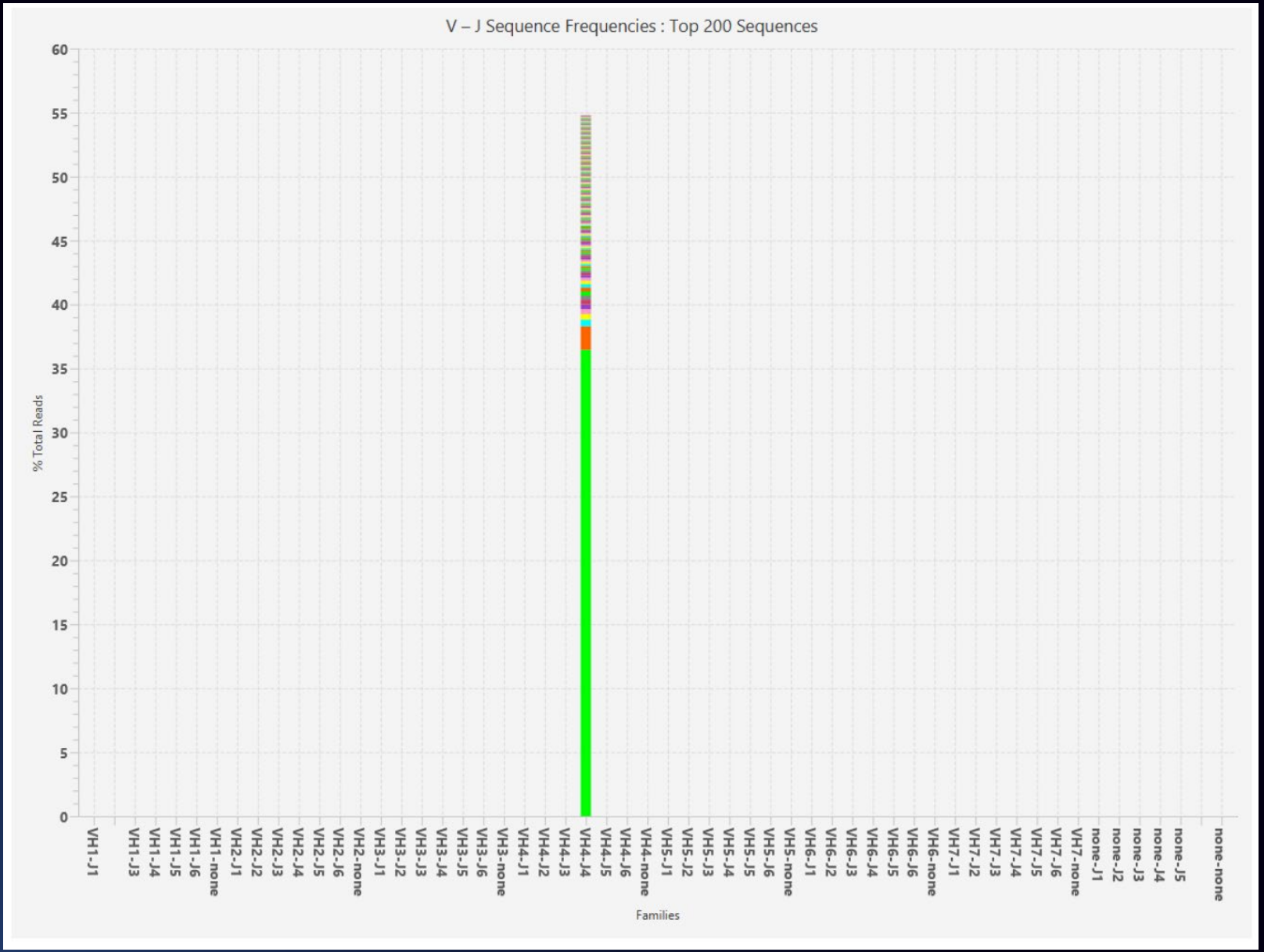
Polyclonal population in graphic form



% total "reads" (molecules)

Various *IGH* rearrangements ("families")

Clonal population in graphic form



% total "reads" (molecules)

Various *IGH* rearrangements ("families")

Results of *IGH* NGS sequencing in table form

IGH gene clonality analysis using “leader” panel

Total Read Count: 134496

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	TCCTGCTGGTGGC	504	89440	IGHV4-39_01	IGHJ6_04	66.50	66.50	0.00	Y	Y	99.67	not found
2	TGTGAGTGTTTCTA	481	2056	IGHV4-39_01	IGHJ6_04	1.53	68.03	0.00	Y	Y	99.67	not found
3	TCCTGCTGGTGGC	501	844	IGHV4-39_01	IGHJ6_04	0.63	68.66	0.00	Y	Y	99.67	not found
4	TCCTGCTGGTGGC	507	62	IGHV4-39_01	IGHJ6_04	0.05	68.70	0.00	Y	Y	99.67	not found

Number of *IGH* molecules sequenced (134,496)

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66.5% of all *IGH* molecules sequenced have unique “clonal” sequence

Total Read Count: 134496

Top 10 Merged Read Summary

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4	TCCTGCTGGTGGC	507	62	IGHV4-39_01	IGHJ6_04	0.05	68.70	0.00	Y	Y	99.67	not found

Sequence of each *IGH* fragment

Total Read Count: 134496

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
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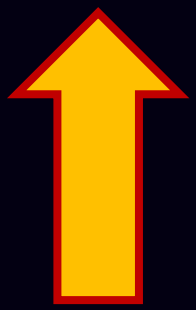
4	TCCTGCTGGTGGC	507	62	IGHV4-39_01	IGHJ6_04	0.05	68.70	0.00	Y	Y	99.67	not found

TCCTGCTGGTGGCGGCTCCCAGATGTGAGTGTTTCTAGGATGCAGACATGGAGATATGGGAGGCTG
CCTCTGATCCCAGGGCTCACTGTGGGTTTTTCTGTTCACAGGGGTCCTGTCCCAGCTGCAGCTGCAG
GAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGGC
TCCATCAGCAGTAGTAGTTACTACTGGGGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG
ATTGGGAGTATCTATTATAGTGGGAGCACCTACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATC
CGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCAGACACGGCTGT
GTATTACTGTGCGAGACGGGCGAGTATTACGATTTTTGGAGTGGTTATTATACGGGAAGAAGACTAC
TACTACTACGGTATGGACGTCTGGGGCAAAGGGACCAC

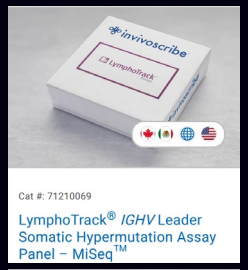
And this becomes the “probe” – the unique fingerprint of the B-cell (or T-cell) clone



TCCTGCTGGTGGCGGCTCCCAGATGTGAGTGTTTCTAGGATGCAGACATGGAGATATGGGAGGCTG
CCTCTGATCCCAGGGCTCACTGTGGGTTTTTCTGTTCACAGGGGTCCTGTCCCAGCTGCAGCTGCAG
GAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGGC
TCCATCAGCAGTAGTAGTTACTACTGGGGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG
ATTGGGAGTATCTATTATAGTGGGAGCACCTACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATC
CGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCAGACACGGCTGT
GTATTACTGTGCGAGACGGGCGAGTATTACGATTTTTGGAGTGGTTATTATACGGGAAGAAGACTAC
TACTACTACGGTATGGACGTCTGGGGCAAAGGGACCAC

So how far can you push this whole NGS clonality detection?

IGH leader

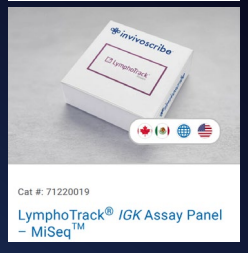


IGH FR1

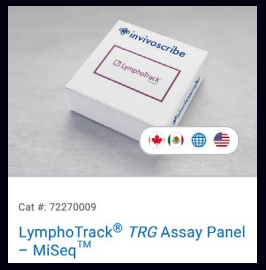
IGH FR2

IGH FR3

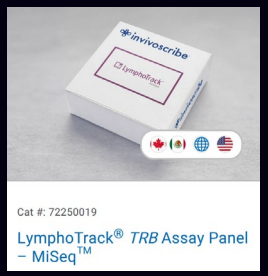
IG kappa



TCR gamma



TCR beta



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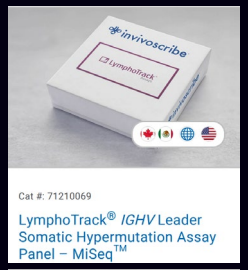
IGH leader

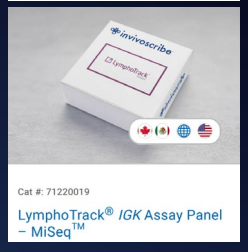


IGH FR1

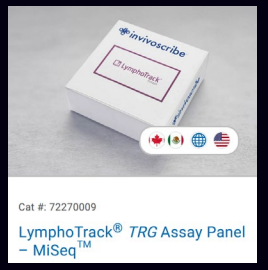
IGH FR2

IGH FR3

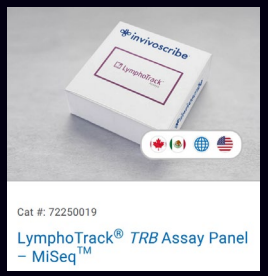
IG kappa



TCR gamma



TCR beta



x 24 indices each panel

=

154 samples (+ controls) / flow-cell (technically)

Done on Illumina MiSeq with V3 flow-cell

But there must be a limit to the capacity of a single flow-cell (“read depth”) with so many specimens…

… which will impact the sensitivity

We tested a set of specimens with all 7 panels on one flowcell

index	MDL #
A001	
A002	
A003	
A004	
A005	25.1974
A006	25.1977
A007	25.1987
A008	25.2020
A009	25.2035
A010	25.2036
A011	25.1981
A012	25.2007
A013	25.2008
A014	25.2009
A015	25.1946
A016	25.1956
A018	25.1969
A019	25.1970
A020	25.1982
A021	25.1991
A022	25.1992
A023	25.1994
A025	25.2013
A027	25.0000

19 specimens for *IGH* leader panel

index	MDL #
A001	
A002	
A003	25.1981
A004	25.2007
A005	25.2008
A006	25.2009
A007	25.1946
A008	25.1956
A001	
A002	
A003	25.1981
A004	25.2007
A005	25.2008
A006	25.2009
A007	25.1946
A008	25.1956
A001	
A002	
A003	25.1981
A004	25.2007
A005	25.2008
A006	25.2009
A007	25.1946
A008	25.1956
A001	
A002	
A003	25.1981
A004	25.2007
A005	25.2008
A006	25.2009
A007	25.1946
A008	25.1956

6 specimens for each of FR1, FR2, FR3 and *IG* kappa panels

A018	
A019	25.1993
A020	25.2001
A021	25.1981
A022	25.2007
A023	25.2008
A025	25.2009
A027	25.0000
A018	
A019	25.1993
A020	25.2001
A021	25.1981
A022	25.2007
A023	25.2008
A025	25.2009
A027	25.0000

6 specimens for each of *TRG* and *TRB* panels

31 samples / V3 flowcell (Illumina MiSeq)

Each sample in each panel had at least 100,000 reads

How about “more is better”:
moving from the MiSeq to the NextSeq 2000

MDL #	Replicate	Primer set	Total reads
24.3633	1 (10E-3)	FR1	2,564,084
	2 (10E-3)	FR1	4,903,685
	3 (10E-3)	FR1	3,388,958
	4 (10E-3)	FR1	5,991,333
			16,848,060
	1 (10E-4)	FR1	8,599,957
	2 (10E-4)	FR1	3,606,248
	3 (10E-4)	FR1	3,305,999
	4 (10E-4)	FR1	3,124,012
			18,636,216
	1 (10E-5)	FR1	2,406,009
	2 (10E-5)	FR1	3,360,993
	3 (10E-5)	FR1	4,455,488
	4 (10E-5)	FR1	2,594,690
			12,817,180
	1 (10E-6)	FR1	2,533,631
	2 (10E-6)	FR1	2,912,782
	3 (10E-6)	FR1	4,618,804
	4 (10E-6)	FR1	7,505,885
			17,571,102
	1 (10E-7)	FR1	9,896,854
	2 (10E-7)	FR1	4,803,691
	3 (10E-7)	FR1	48,110
	4 (10E-7)	FR1	2,323,174
			17,071,829

- Same *IGH* FR1 library prep (no modifications)
- 1,200 ng DNA for each “specimen” (dilution)
- Loaded on an Illumina P2 XLEAP

How about “more is better”:
moving from the MiSeq to the NextSeq 2000

MDL #	Replicate	Primer set	Total reads
24.3633	1 (10E-3)	FR1	2,564,084
	2 (10E-3)	FR1	4,903,685
	3 (10E-3)	FR1	3,388,958
	4 (10E-3)	FR1	5,991,333
			16,848,060
	1 (10E-4)	FR1	8,599,957
	2 (10E-4)	FR1	3,606,248
	3 (10E-4)	FR1	3,305,999
	4 (10E-4)	FR1	3,124,012
			18,636,216
	1 (10E-5)	FR1	2,406,009
	2 (10E-5)	FR1	3,360,993
	3 (10E-5)	FR1	4,455,488
	4 (10E-5)	FR1	2,594,690
			12,817,180
	1 (10E-6)	FR1	2,533,631
	2 (10E-6)	FR1	2,912,782
	3 (10E-6)	FR1	4,618,804
	4 (10E-6)	FR1	7,505,885
			17,571,102
	1 (10E-7)	FR1	9,896,854
	2 (10E-7)	FR1	4,803,691
	3 (10E-7)	FR1	48,110
	4 (10E-7)	FR1	2,323,174
			17,071,829

- Same *IGH* FR1 library prep (no modifications)
- 1,200 ng DNA for each “specimen” (dilution)
- Loaded on an Illumina P2 XLEAP

But if good data is obtained from 100,000 sequences, why do I need 10-20 million reads?

It’s a question of sensitivity and statistical significance:

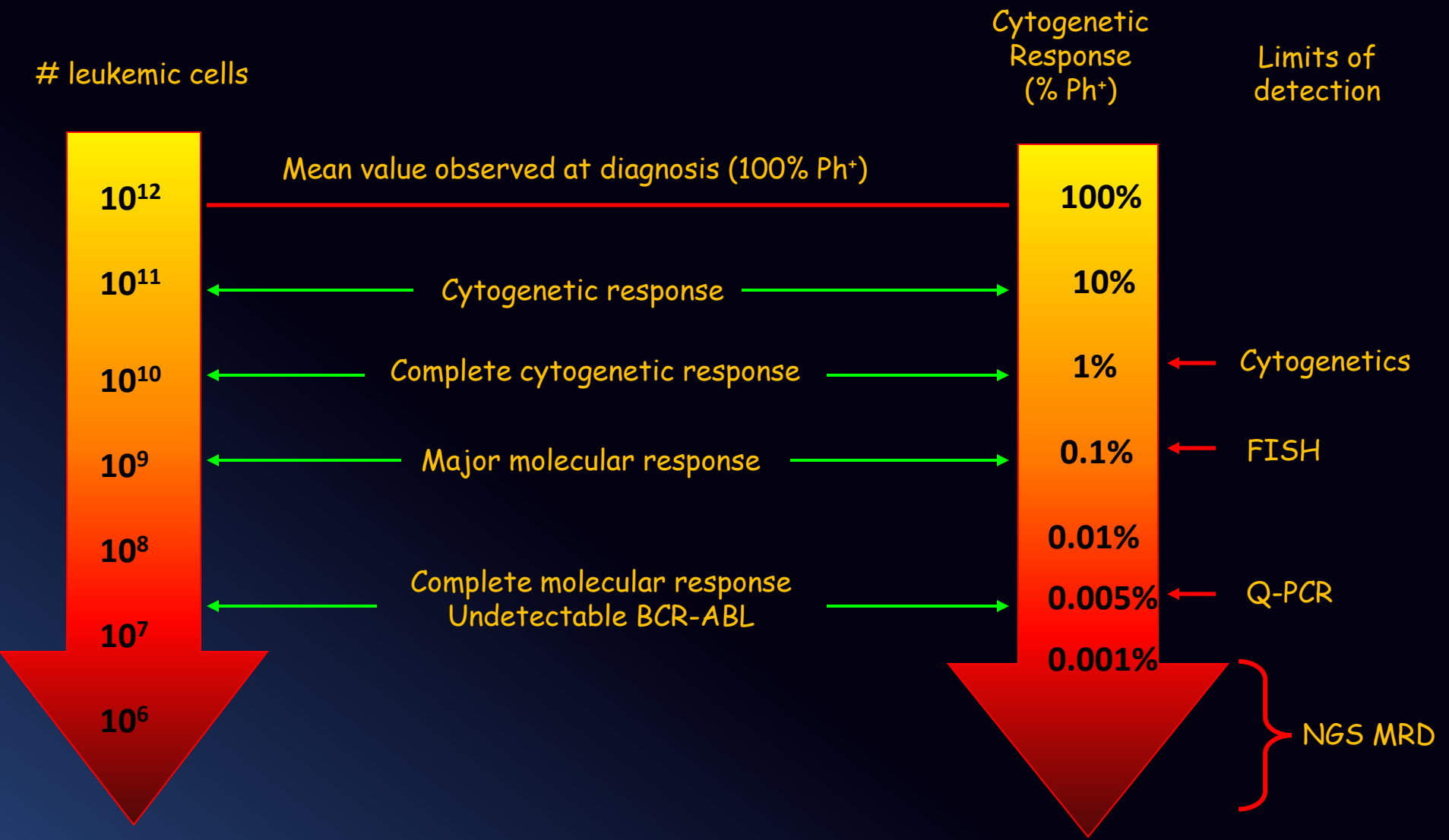
Small lake + one line = one fish

versus

Big lake + fishnet = many fish

… and MRD requires high sensitivities and good statistics

Leukemic cells vs. MRD assay and cytogenetic response:
CML example



(adapted from Baccarani et al, 2008)

Molecular detection:

- Highest sensitivity of any technique (can detect a unique clonal sequence in 10,000 to 1,000,000 cells)
- Targets genetic sequence
- Rapid, inexpensive
- Highly specific
- “Marker” (gene) usually not lost during treatment

MRD level (sensitivity) is determined by two things:

1. Number of normal (background) cells/genes counted

2. Number of target cells/genes present in sample

To achieve more sensitive MRD levels, Poisson sampling and generating more reads are key (interrogating a bigger haystack results in better sensitivity):

1. If target cells/gene numbers are high, then background cells/gene numbers can be low

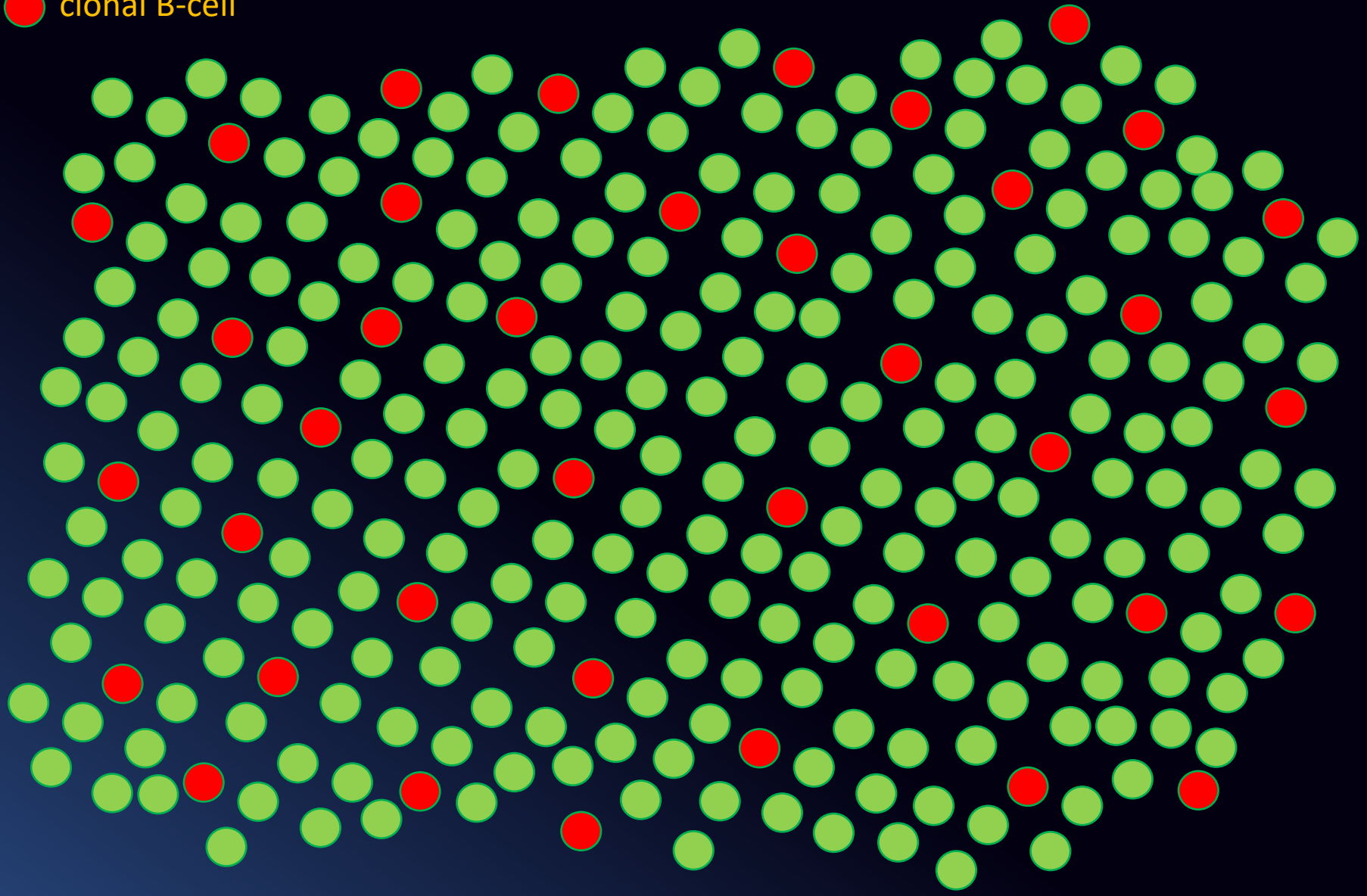
2. If target cells/gene numbers are low, then background cells/gene numbers must be high

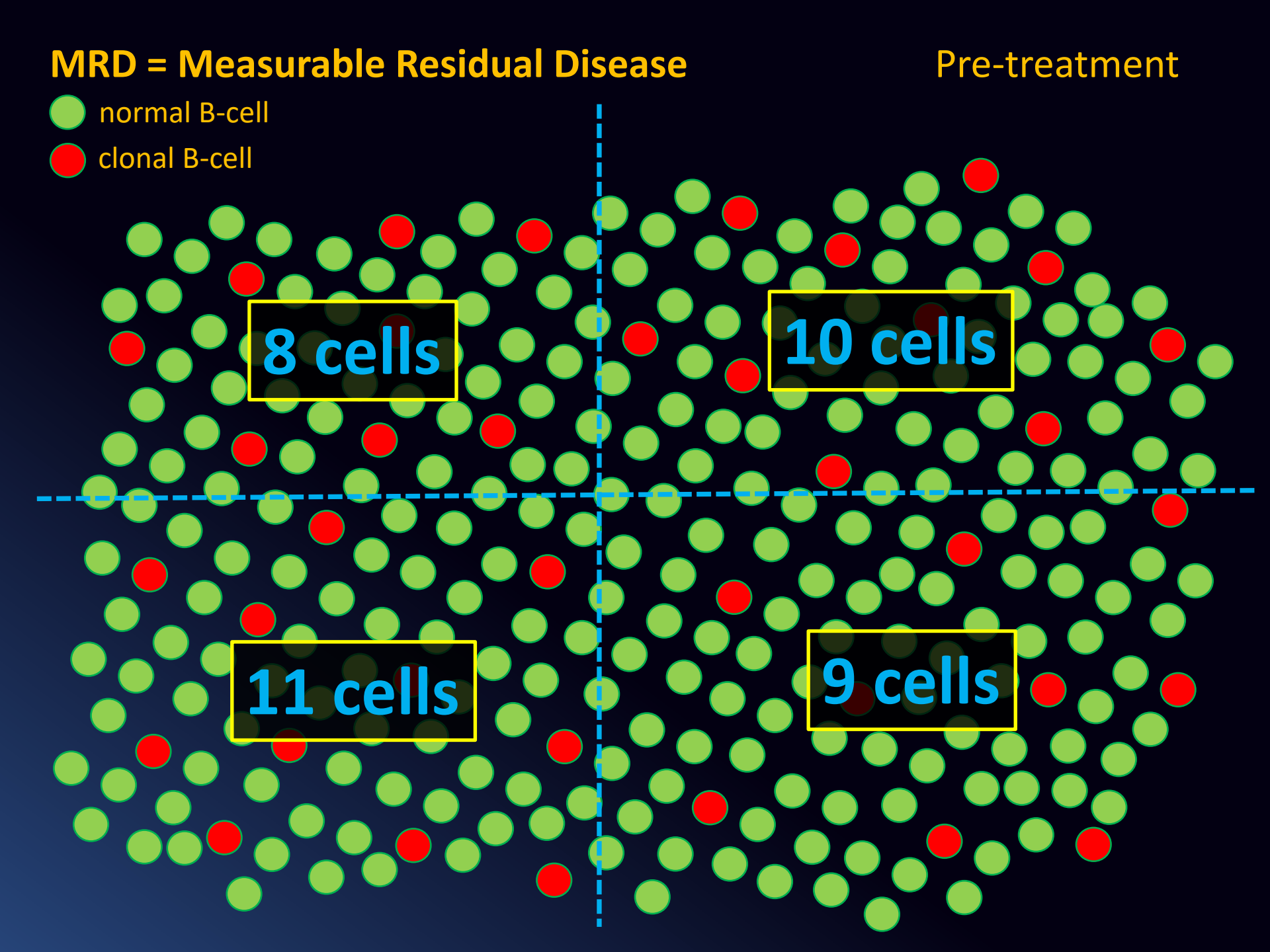
MRD = Measurable Residual Disease

Pre-treatment

normal B-cell

clonal B-cell



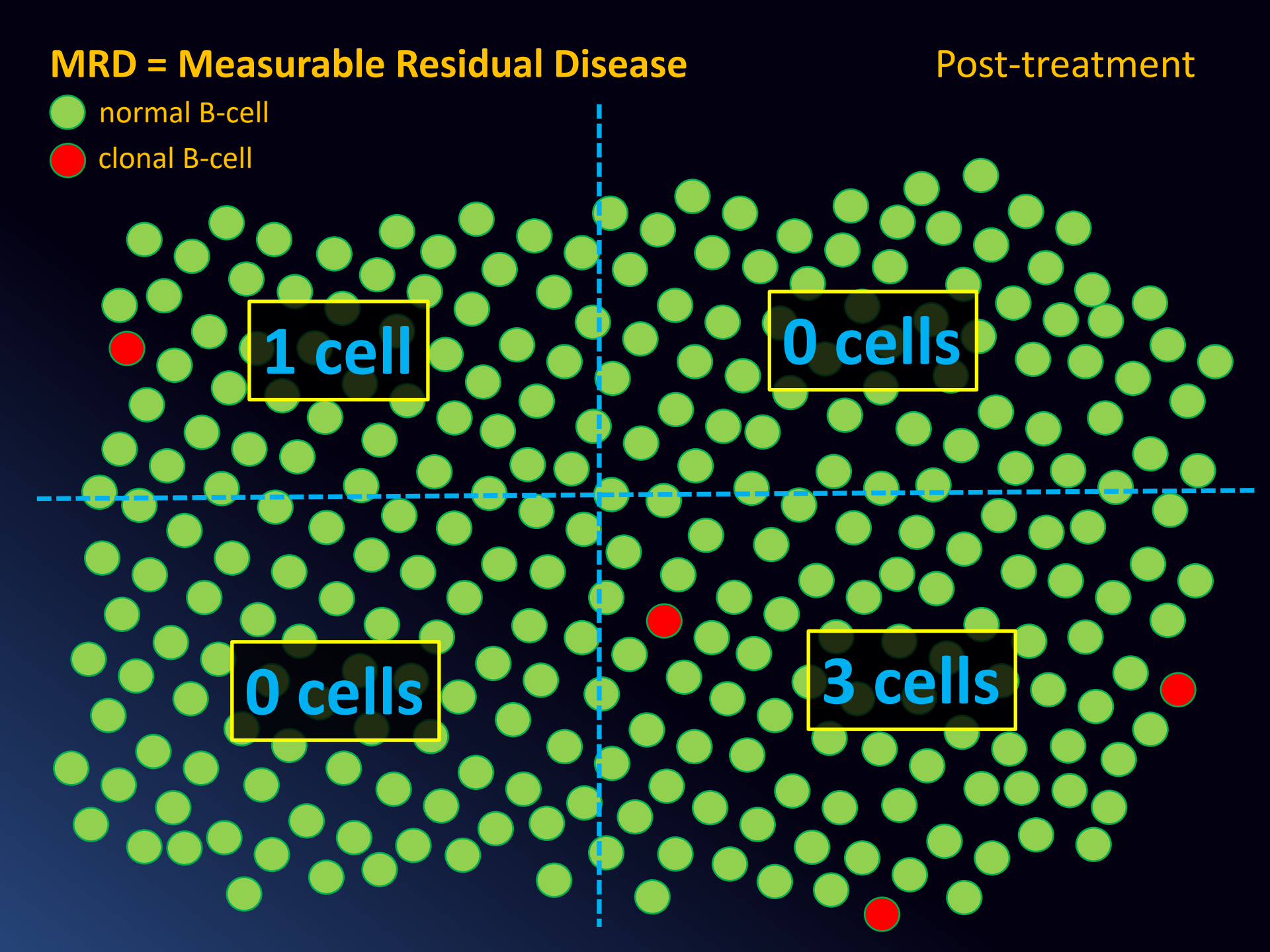

MRD = Measurable Residual Disease
Pre-treatment
normal B-cell
clonal B-cell
8 cells
10 cells
11 cells
9 cells

MRD = Measurable Residual Disease

Post-treatment

normal B-cell

clonal B-cell

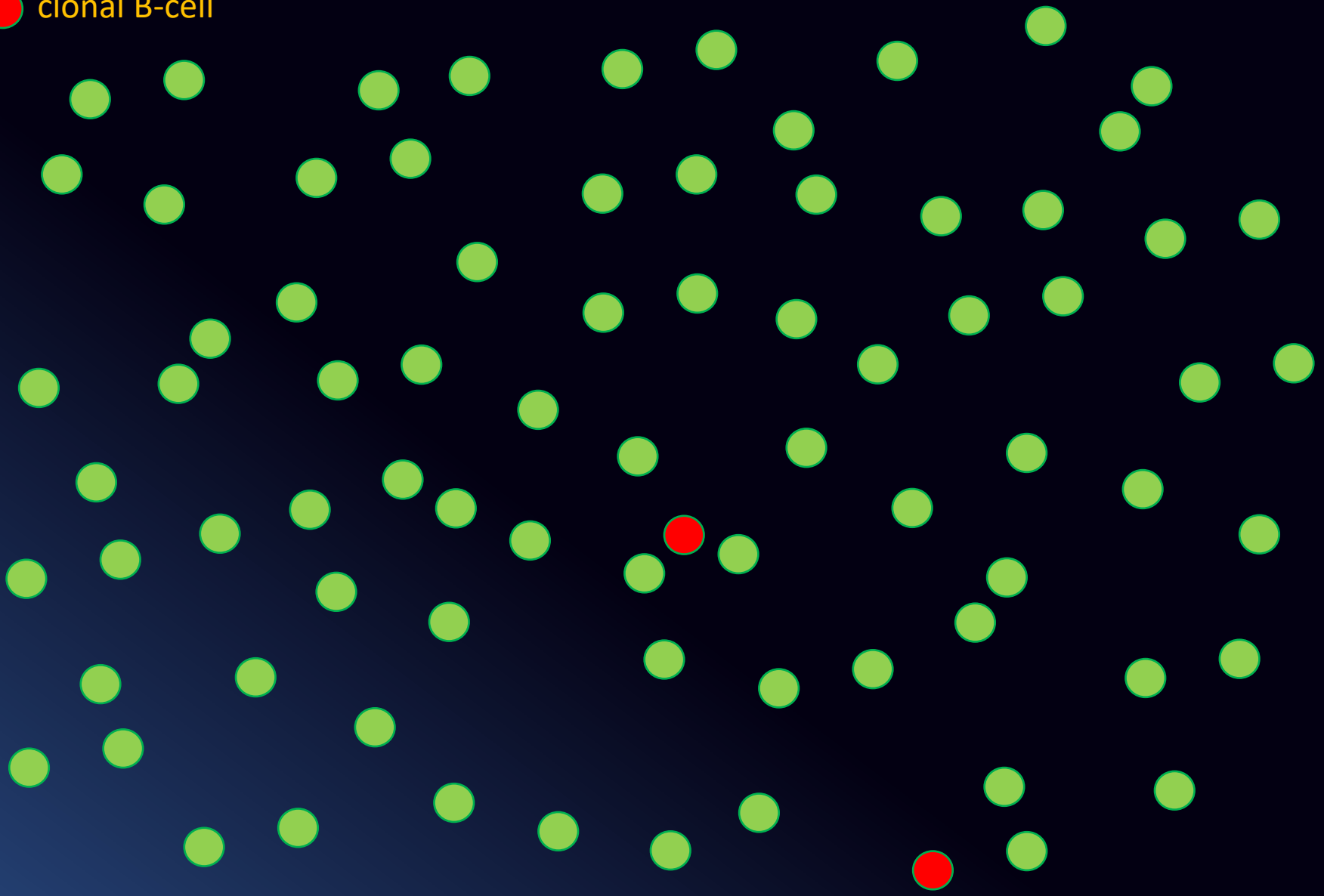

MRD = Measurable Residual Disease
Post-treatment
normal B-cell
clonal B-cell
1 cell
0 cells
0 cells
3 cells

What if the normal B-cells are depleted post-therapy?

normal B-cell

clonal B-cell

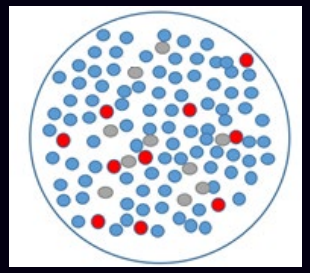
High read depth becomes important



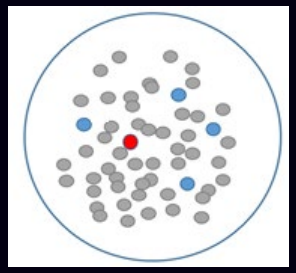
LymphoQuant® Internal Controls:

- Spiked into a specimen
- Converts “reads” into cell-equivalents
- Standardized estimate of percent clonotype

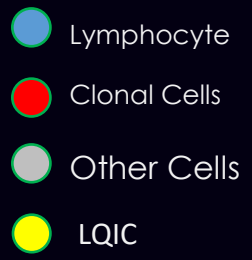
Analysis without LymphoQuant internal controls



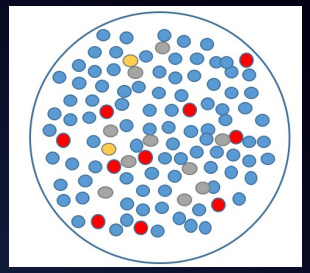
10 clonal / 100 lymphocytes
10% clonal reads



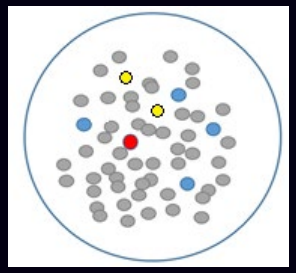
1 clonal / 5 lymphocytes
20% clonal reads



Analysis with LymphoQuant internal controls



10 clonal cell equivalents



1 clonal cell equivalent

Three major MRD technologies [1]

Assay type	Sensitivity [2]	Target	Standardization
Flow cytometry	MRD4 - MRD5	CD19, CD20, CD43, CD79b, CD81	Poor standardization across laboratories (except LabPMM)

1. Benintende *et al,* Frontiers in Oncology, 2023

2. MRD4 = 1 clonal cell in 10,000
   MRD5 = 1 clonal cell in 100,000
   MRD6 = 1 clonal cell in 1,000,000

Three major MRD technologies [1]

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Flow cytometry	MRD4 - MRD5	CD19, CD20, CD43, CD79b, CD81	Poor standardization across laboratories (except LabPMM)
ASO-PCR	~ MRD6	IGHV	Patient specific assays

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Three major MRD technologies [1]

Assay type	Sensitivity [2]	Target	Standardization
Flow cytometry	MRD4 - MRD5	CD19, CD20, CD43, CD79b, CD81	Poor standardization across laboratories (except LabPMM)
ASO-PCR	~ MRD6	IGHV	Patient specific assays
NGS	> MRD5 (> MRD6)[3]	*IGH* CDR3	Internationally standardized with commercially available kits and bioinformatics software

1. Benintende *et al,* Frontiers in Oncology, 2023

2. MRD4 = 1 clonal cell in 10,000
   MRD5 = 1 clonal cell in 100,000
   MRD6 = 1 clonal cell in 1,000,000

3. Hengeveld et al, Blood, 2023

Three major MRD technologies

Assay type	Sensitivity	Advantages	Challenges
Flow cytometry	MRD4 - MRD5	- rapid - cost-efficient	- fresh specimens - (24 hr TAT from sample reception)

Three major MRD technologies

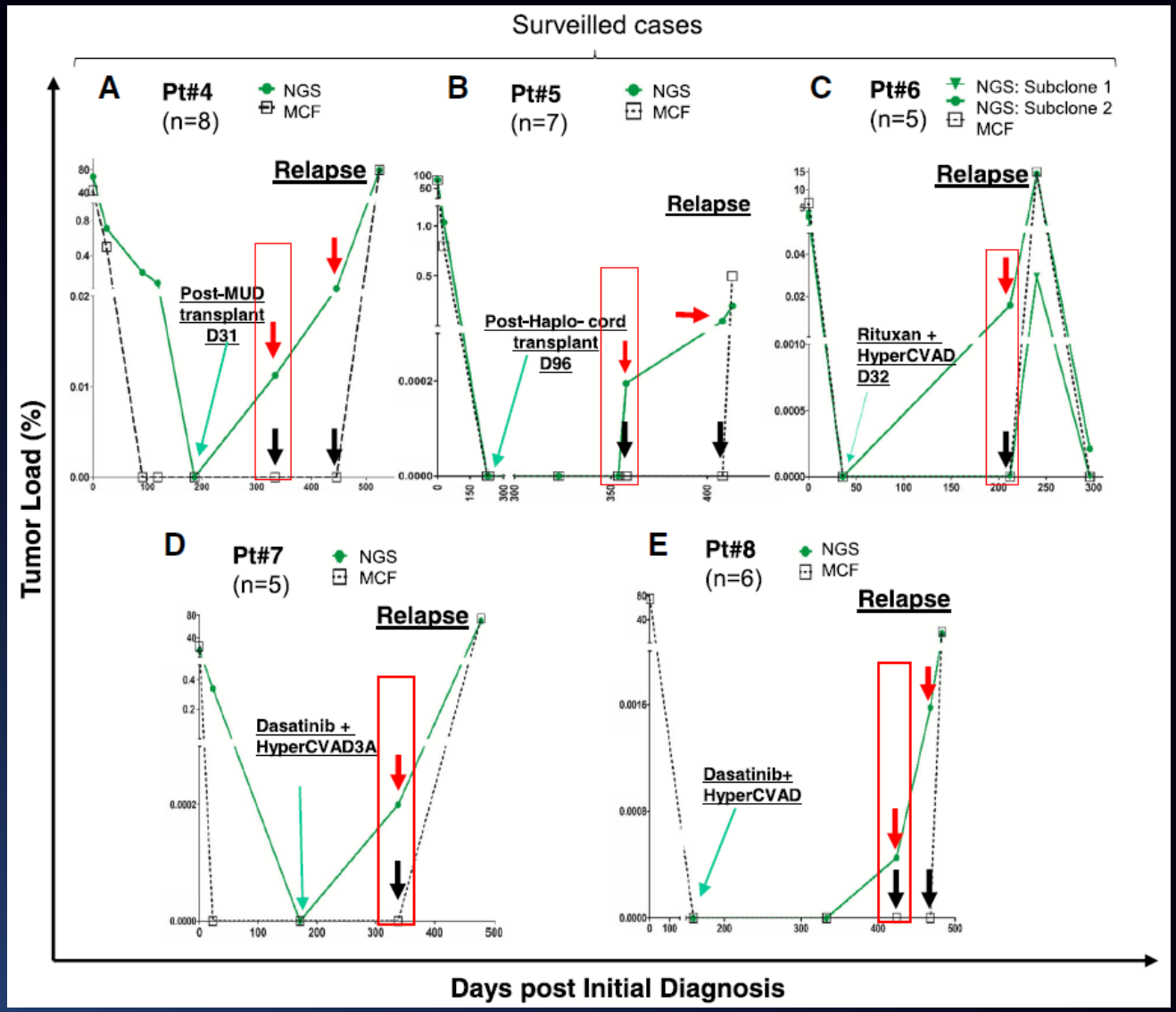
Assay type	Sensitivity	Advantages	Challenges
Flow cytometry	MRD4 - MRD5	- rapid - cost-efficient	- fresh specimens - (24 hr TAT from sample reception)
ASO-PCR	~ MRD6	- very sensitive	- patient-specific PCR primers needed - SHM hinders PCR

Three major MRD technologies

Assay type	Sensitivity	Advantages	Challenges
Flow cytometry	MRD4 - MRD5	- rapid - cost-efficient	- fresh specimens - (24 hr TAT from sample reception)
ASO-PCR	~ MRD6	- very sensitive	- Patient specific PCR primers needed - SHM hinders PCR
NGS	MRD5 (>MRD6)	- very sensitive - commercial kits available	- A bit more expensive - longer (TAT ~ 5-6 days) - up to 1-8 µg of DNA for MRD5 - 18µg for MRD6(*)

* 6 pg DNA = 1 cell
18µg DNA = 3.0 X 10^6 cells
(1 NGS reaction can accept up to 2µg DNA)
(9 replicates)

MRD monitoring predicts clinical relapse in B-ALL:
NGS vs. MCF



- Sensitivity = 10E-5
- Earlier relapse prediction than MCF
- Conversion to positive MRD detected sooner than MCF

Cheng *et al*, J Hematol & Oncol (2018)

Setting up MRD testing in our lab:

- B-cell lymphoma specimen
- Two clonal IGH gene rearrangements (major and minor)
- DNA diluted in tonsil DNA (polyclonal, rich in normal B-cells)

Specimen #1:
B-cell lymphoma – original clonality analysis:

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	GCCTCTGGATTCA	279	343525	IGHV3-7_01	IGHJ4_02	69.40	69.40	0.88	N	N	100.00	not found
2	GCCTCTGGATACA	221	83974	IGHV3-48_04	IGHJ5_02	16.96	86.36	12.33	n/a	N	80.62	not found
3	GCCTCTGGATTCA	285	181	IGHV3-13_01	IGHJ4_02	0.04	86.40	0.00	N	N	100.00	not found

clone 1 (69%)
clone 2 (17%)

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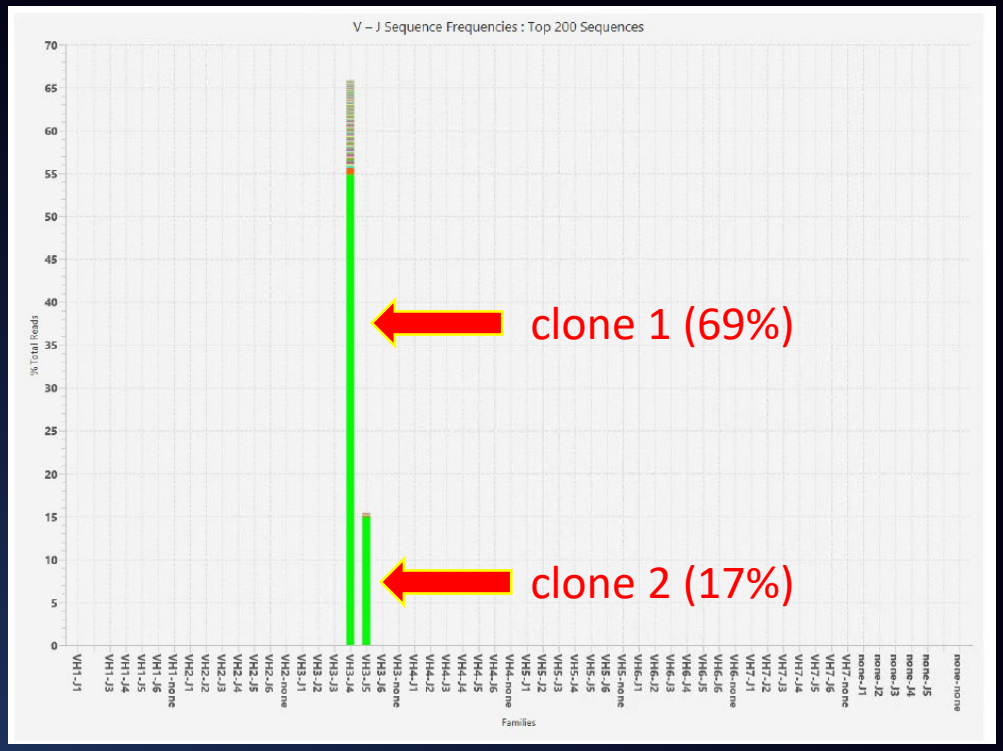

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IGH sequence of clone #1 used as "probe" in first MRD analysis

Specimen #1:
B-cell lymphoma – original clonality analysis:

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1	GCCTCTGGATTCA	279	343525	IGHV3-7_01	IGHJ4_02	69.40	69.40	0.88	N	N	100.00	not found
2	GCCTCTGGATACA	291	83974	IGHV3-48_04	IGHJ5_02	16.96	86.36	12.33	n/a	N	80.62	not found
3	GCCTCTGGATTCA	285	181	IGHV3-13_01	IGHJ4_02	0.04	86.40	0.00	N	N	100.00	not found

clone 2 (17%)

IGH sequence of clone #2 used as "probe" in second MRD analysis

Specimen #1:

MDL #	Replicate	Primer set	Total reads
24.3118	1 (10E-3)	FR1	1,442,044
	2 (10E-3)	FR1	1,118,121
	3 (10E-3)	FR1	1,103,231
	4 (10E-3)	FR1	1,065,234
			4,728,630
	1 (10E-4)	FR1	1,161,710
	2 (10E-4)	FR1	1,084,255
	3 (10E-4)	FR1	1,097,901
	4 (10E-4)	FR1	1,093,620
			4,437,486
	1 (10E-5)	FR1	750,548
	2 (10E-5)	FR1	1,269,882
	3 (10E-5)	FR1	1,164,742
	4 (10E-5)	FR1	1,549,740
			4,734,912

- 3 dilutions in tonsil DNA:
  - 10E-3, 10E-4, 10E-5

  Each dilution represents an MRD timepoint after treatment

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- 3 dilutions in tonsil DNA:
  - 10E-3, 10E-4, 10E-5

  Each dilution represents an MRD timepoint after treatment

- 4 replicates / dilution

- 1,200 ng DNA / replicate = 4.8 µg total DNA for each dilution (timepoint)

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	4 (10E-4)	FR1	1,093,620
			4,437,486
	1 (10E-5)	FR1	750,548
	2 (10E-5)	FR1	1,269,882
	3 (10E-5)	FR1	1,164,742
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			4,734,912

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  - 10E-3, 10E-4, 10E-5

  Each dilution represents an MRD timepoint after treatment

- 4 replicates / dilution

- 1,200 ng DNA / replicate = 4.8 µg total DNA for each dilution (timepoint)

- ~ 1 million reads per replicate
  > 4 million total per dilution

- Illumina MiSeq with V3 flowcell

Specimen #1:

MRD Results for Collection/Timepoint: 2024/12/01			
Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
1	Seq1	DETECTED	2.19E-4
2	Seq2	DETECTED	3.38E-5

clone 1 (69%)

clone 2 (17%)

Clone 1 = positive at 2.19E-4

Clone 2 = positive at 3.38E-5

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MRD Results for Collection/Timepoint: 2024/12/01			
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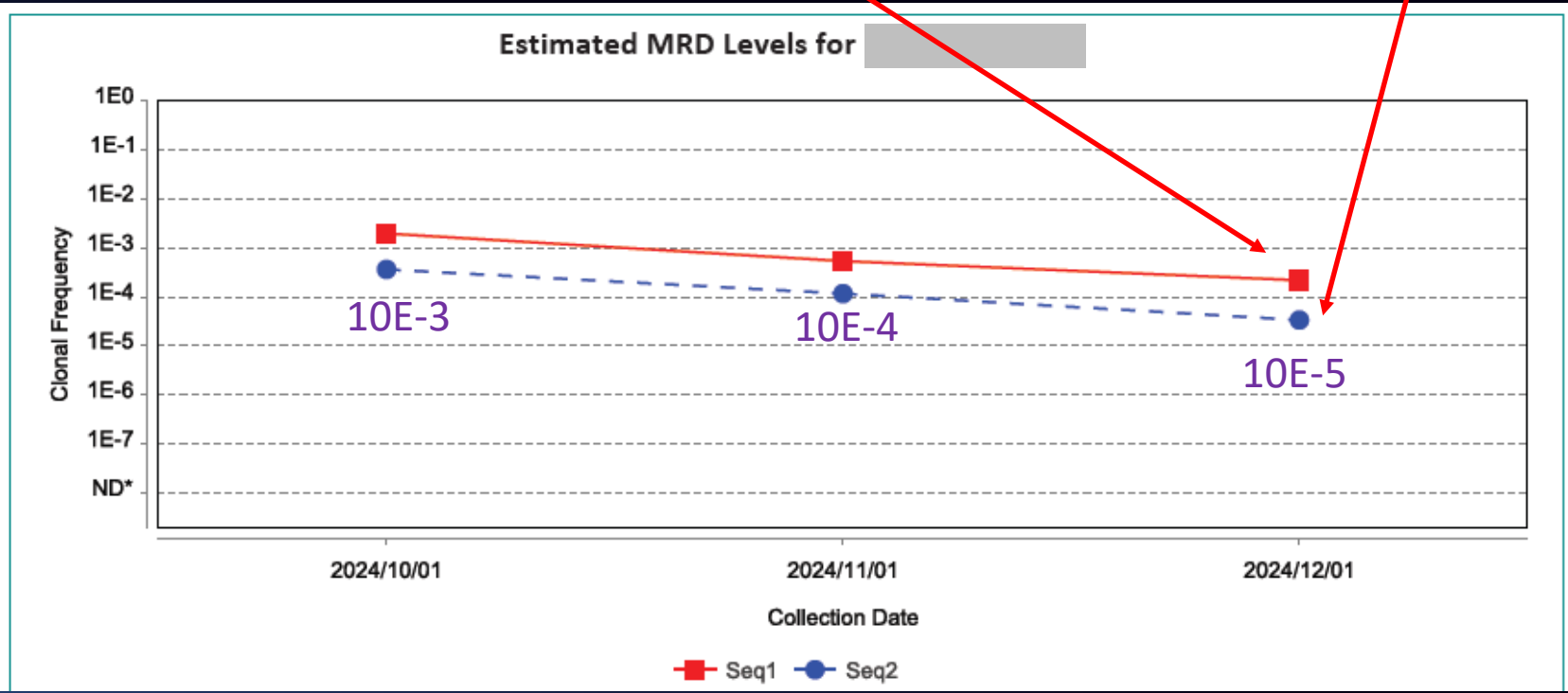

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			4,728,630
	1 (10E-4)	FR1	1,161,710
	2 (10E-4)	FR1	1,084,255
	3 (10E-4)	FR1	1,097,901
	4 (10E-4)	FR1	1,093,620
			4,437,486
	1 (10E-5)	FR1	750,548
	2 (10E-5)	FR1	1,269,882
	3 (10E-5)	FR1	1,164,742
	4 (10E-5)	FR1	1,549,740
			4,734,912

Original data from first run

MDL #	Replicate	Primer set	Total reads
24.3118	1 (10E-6)	FR1	1,278,050
	2 (10E-6)	FR1	1,718,762
	3 (10E-6)	FR1	1,761,400
	4 (10E-6)	FR1	1,648,937
			6,407,149
	1 (10E-7)	FR1	1,947,207
	2 (10E-7)	FR1	1,537,717
	3 (10E-7)	FR1	1,607,656
	4 (10E-7)	FR1	1,784,318
			6,876,898

- 2 additional dilutions in tonsil DNA:
  - 10E-6, 10E-7
- Data from run combined with previous run (initial three dilutions) for analysis

Specimen #1:

MRD Results for Collection/Timepoint: 2025/02/01			
Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
1	Seq1	NOT DETECTED	> 99% at 1E-5
2	Seq2	NOT DETECTED	> 99% at 1E-5

clone 1 (69%)

clone 2 (17%)

Both clones “negative”, but
with 99% confidence at 10E-5

Specimen #1:

MRD Results for Collection/Timepoint: 2025/02/01			
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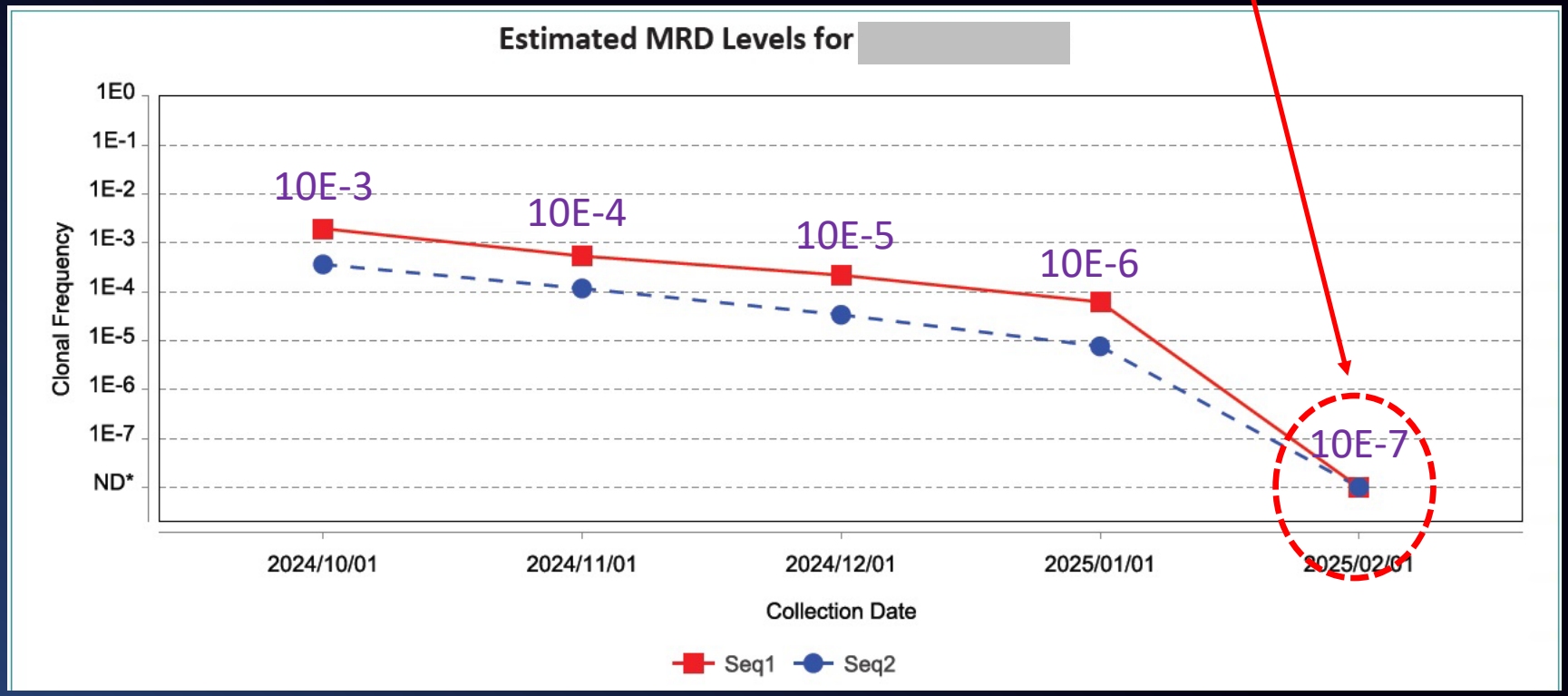

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Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
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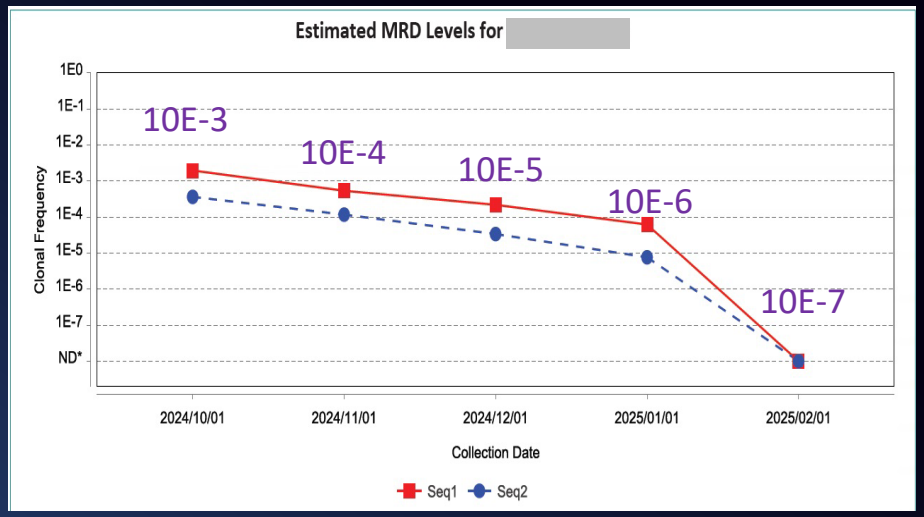
Both clones “negative”, but with 99% confidence at 10E-5



But why negative at 10E-5, when the negative dilution is 10E-7?

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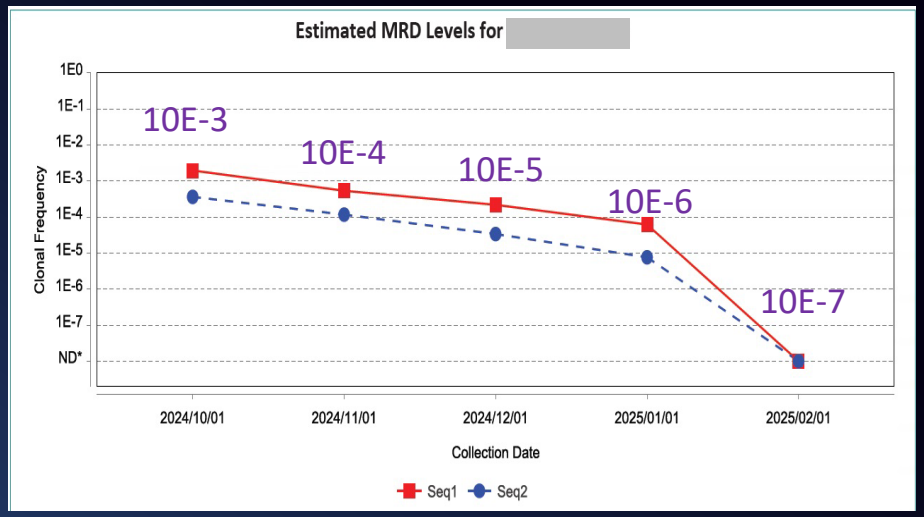
1	Seq1	NOT DETECTED	> 99% at 1E-5
2	Seq2	NOT DETECTED	> 99% at 1E-5

clone 1 (69%)

clone 2 (17%)

Both clones "negative", but
with 99% confidence at 10E-5



But why negative at 10E-5, when the negative dilution is 10E-7?

Because not enough reads for a % confidence at 10E-6 or 10E-7.

Remember: up to 8 µg of DNA for MRD5, 18 µg for MRD6 …

… but we had only **4.8 ug** of DNA

Specimen #1 with a twist – NextSeq 2000:

MDL #	Replicate	Primer set	Total reads
24.3118	1 (10E-3)	FR1	4,131,240
	2 (10E-3)	FR1	3,206,312
	3 (10E-3)	FR1	3,163,014
	4 (10E-3)	FR1	2,976,909
			13,477,475
	1 (10E-4)	FR1	3,122,775
	2 (10E-4)	FR1	3,049,050
	3 (10E-4)	FR1	3,005,897
	4 (10E-4)	FR1	2,664,700
			11,842,422
	1 (10E-5)	FR1	1,941,895
	2 (10E-5)	FR1	3,238,936
	3 (10E-5)	FR1	3,522,034
	4 (10E-5)	FR1	4,539,105
			13,241,970
	1 (10E-6)	FR1	3,492,428
	2 (10E-6)	FR1	5,265,399
	3 (10E-6)	FR1	4,930,720
	4 (10E-6)	FR1	4,850,051
			18,538,598
	1 (10E-7)	FR1	5,589,559
	2 (10E-7)	FR1	4,607,548
	3 (10E-7)	FR1	4,842,572
	4 (10E-7)	FR1	5,173,188
			20,212,867

- 5 dilutions in tonsil DNA:
  - 10E-3, 10E-4, 10E-5, 10E-6, 10E-7

  Each dilution represents an MRD timepoint after treatment
- 4 replicates / dilution
- 1,200 ng DNA / replicate = 4.8 µg total DNA
- 3-5 million reads per replicate (>4x greater)
  > 11 million total per dilution
- Illumina NextSeq 2000 with P2 XLEAP

Specimen #1 with a twist – NextSeq 2000:

MRD Results for Collection/Timepoint: 2025/03/15			
Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
1	Seq1 ← clone 1 (69%)	DETECTED	5.59E-6
2	Seq2 ← clone 2 (17%)	NOT DETECTED	> 99% at 1E-5

One clone still positive (5.59E-6), while the other one is “negative” with 99% confidence at 10E-5

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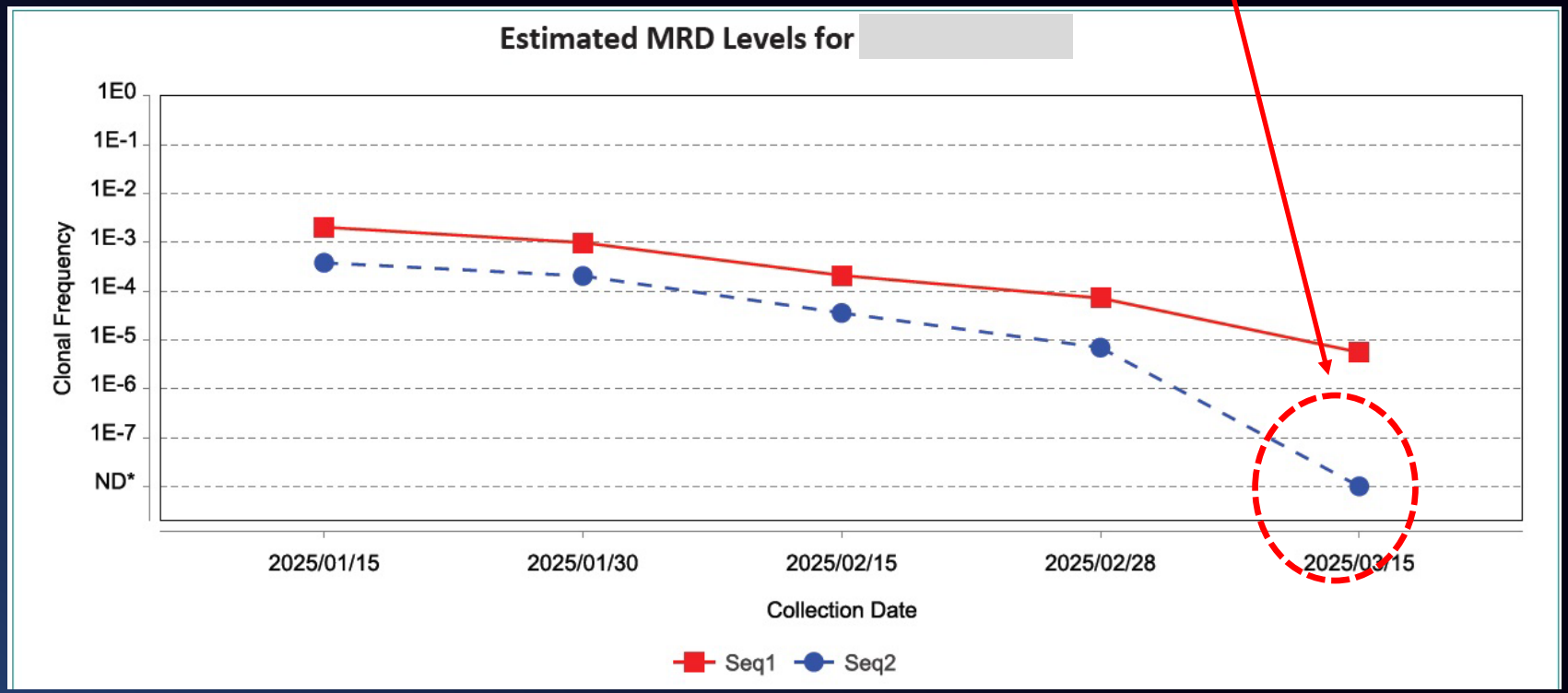

Specimen #2:

MDL #	Replicate	Primer set	Total reads
24.3633	1 (10E-3)	FR1	2,564,084
	2 (10E-3)	FR1	4,903,685
	3 (10E-3)	FR1	3,388,958
	4 (10E-3)	FR1	5,991,333
			16,848,060
	1 (10E-4)	FR1	8,599,957
	2 (10E-4)	FR1	3,606,248
	3 (10E-4)	FR1	3,305,999
	4 (10E-4)	FR1	3,124,012
			18,636,216
	1 (10E-5)	FR1	2,406,009
	2 (10E-5)	FR1	3,360,993
	3 (10E-5)	FR1	4,455,488
	4 (10E-5)	FR1	2,594,690
			12,817,180
	1 (10E-6)	FR1	2,533,631
	2 (10E-6)	FR1	2,912,782
	3 (10E-6)	FR1	4,618,804
	4 (10E-6)	FR1	7,505,885
			17,571,102
	1 (10E-7)	FR1	9,896,854
	2 (10E-7)	FR1	4,803,691
	3 (10E-7)	FR1	48,110
	4 (10E-7)	FR1	2,323,174
			17,071,829

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Sequence #	Sequence Name	MRD Result	% Confidence[†] OR Clonal Frequency
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Specimen is “negative”, but with 99% confidence at 10E-5

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Sequence #	Sequence Name	MRD Result	% Confidence† OR Clonal Frequency
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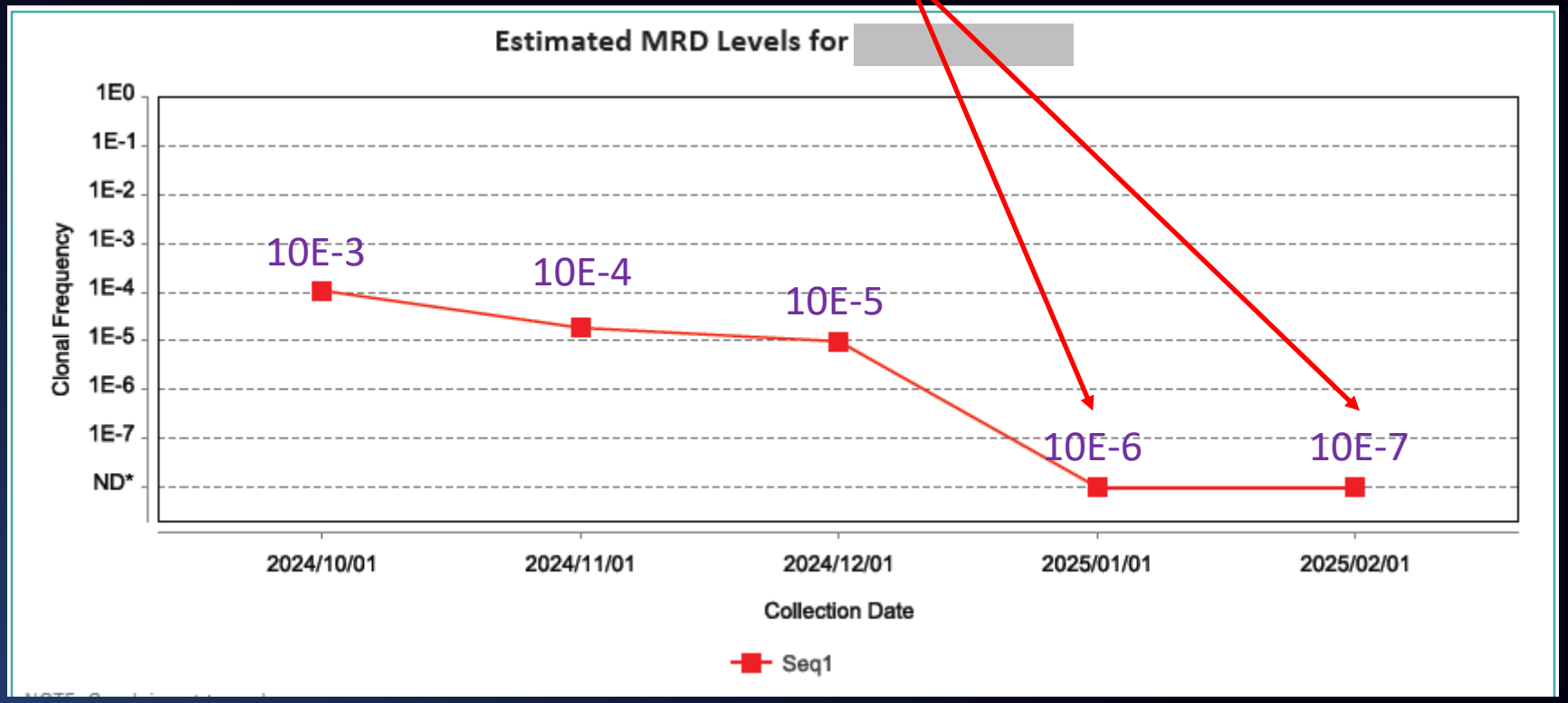

How deep do we go for MRD?

Using samples from the CLL11 trial:

uMRD4 ("undetectable at MRD4", or < $1:10^{-4}$)

- some patients reach durable remission
- most patients relapse
- PFS with chemoimmunotherapy < PFS with rituximab/venetoclax

uMRD5:

- PFS of uMRD5 (< $1:10^{-5}$) better than PFS at MRD ≥ $1:10^{-5}$
- no difference in OS between uMRD5 and ≥ MRD5

Hengeveld et al, Blood, 2023

Blood or bone marrow for MRD assessment?

- Several trials report better correlation between PFS and BM;
- Depends on type of therapy, but overall parallel conclusions;
- BM specimen remains most sensitive, but most invasive and costly;
- Suggestion: reach MRD in BM, then move to monitor relapse in blood.

Rios-Olais & Hilal, Cur Treat Options in Oncology, 2023

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   - unique “identifier” of clonal cells (DNA sequence)
   - % clonal fraction in specimen (although not truly quantitative)
   - exact number of “reads” for quality, depth and sensitivity determination
   - 24 indices allow multiplexing of specimens
   - panel-specific identifiers allow second level of multiplexing (7 cumulative panels for one flow-cell);

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3. Same chemistry can be used on MiSeq and NextSeq 2000;

4. Most FFPE specimens are acceptable for NGS analysis;

5. High tolerance for DNA concentrations (100 ng - 1,200 ng / reaction).

General conclusions:

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10. Up to 5 clonal sequences per specimen can be used to assess MRD of each sequence – potential for detecting and following multiple clones and their response to therapy

11. Seamless software analysis from clonality detection to MRD

12. % confidence level of MRD depends on number of reads – limited by lymphocyte counts in post-therapy follow-up specimens

Future of MRD testing in lymphoid malignancies:

- Data indicates NGS is robust, reproducible and convenient
- NGS analysis reaches higher sensitivities than MCF
- More precise monitoring of treatment
- Significantly aids in prognosis and therapy
- ctDNA MRD may provide further benefits – validation planned in our lab


Thank you!

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Questions?

To learn more about our comprehensive MRD products & services email sales@invivoscribe.com

Or scan the QR code

LymphoTrack Assays, LymphoQuant Internal Controls and Software are for Research Use Only (RUO); not for use in diagnostic procedures.