Introduction to B- and T-cell Clonality Testing & Targets

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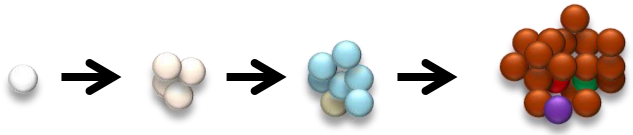
What is Clonality?

Clonality

- A proliferation of cells originating from a single progenitor cell, producing a **pool of identical clonal cells.**



- Clonality testing using molecular techniques is used to **confirm the presence of leukemia or lymphoma.**

Highly indicative of B- or T-cell malignancy

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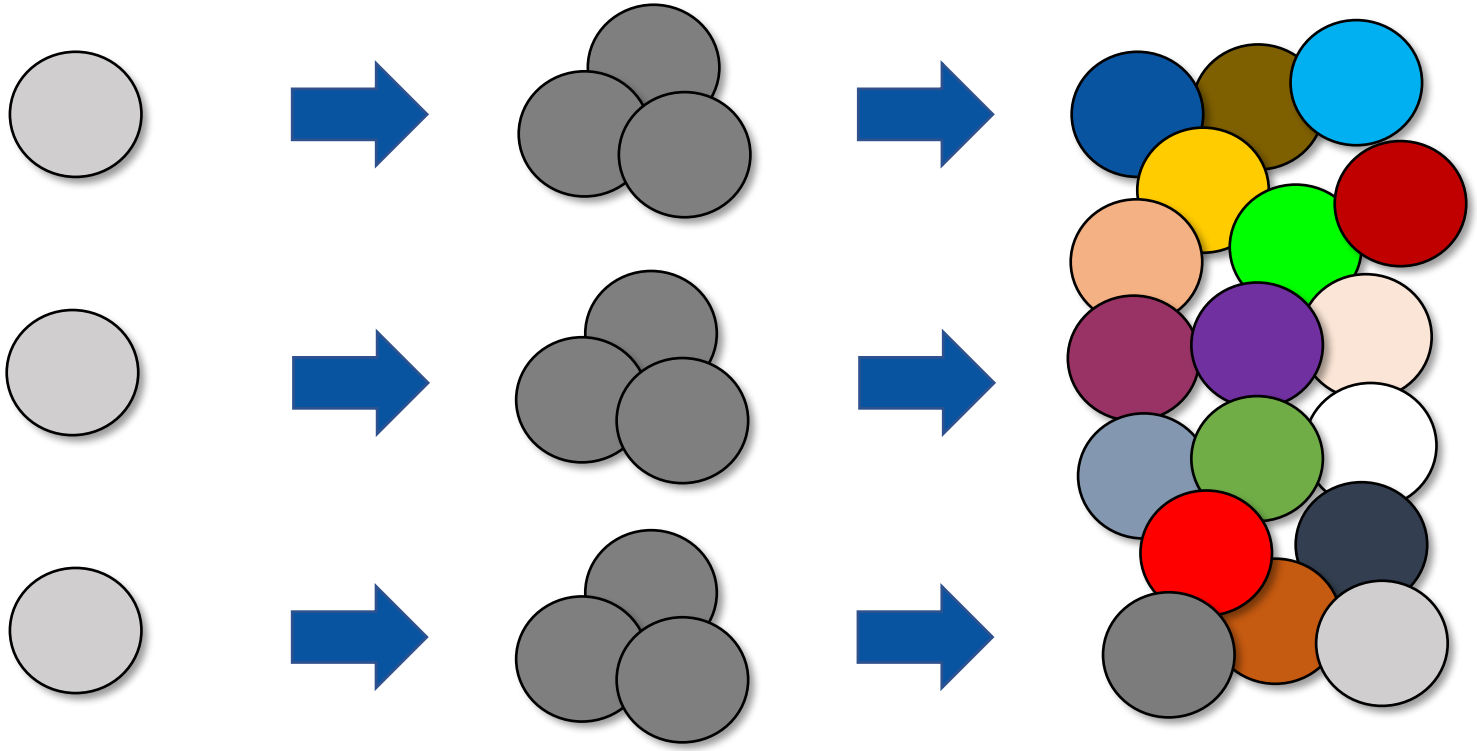
What is Clonality?

Polyclonal Progression



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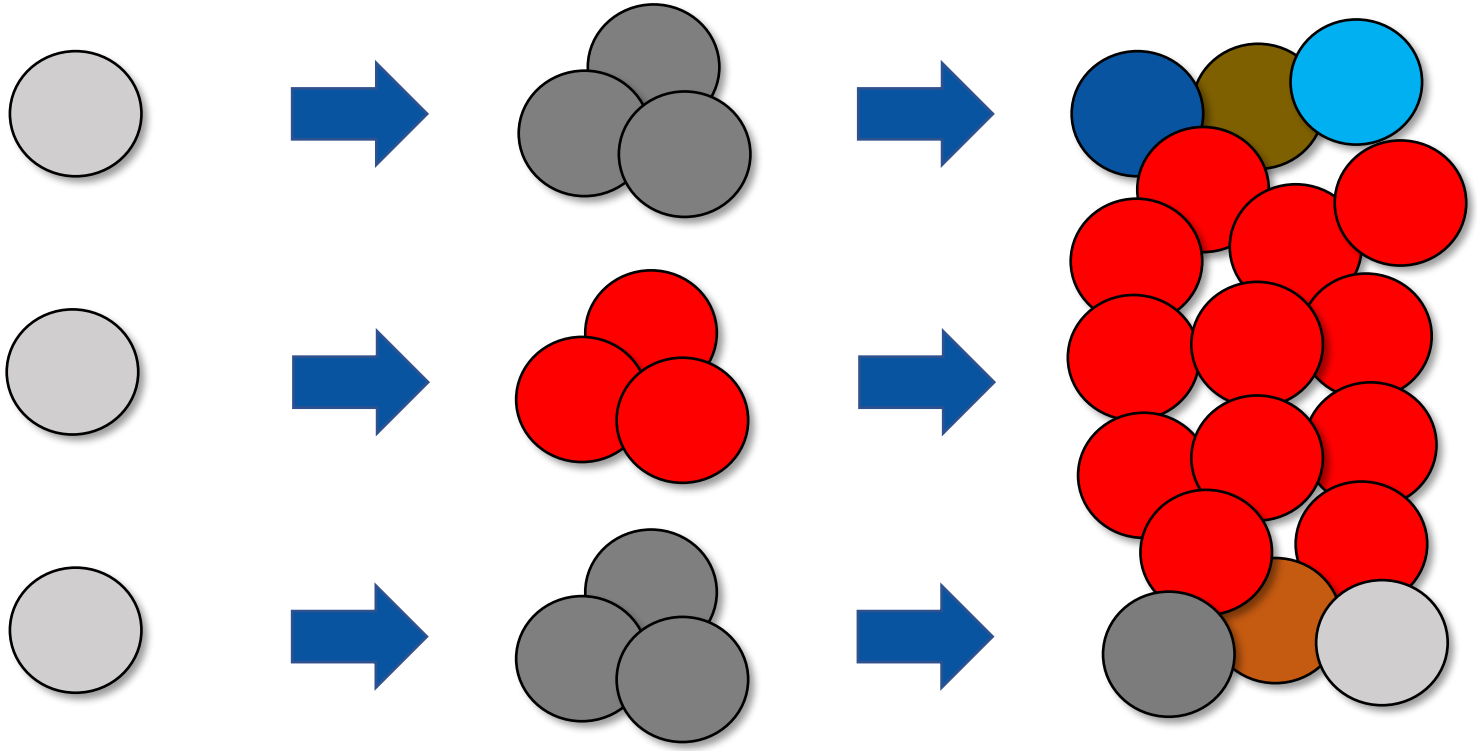
What is Clonality?

Clonal Progression



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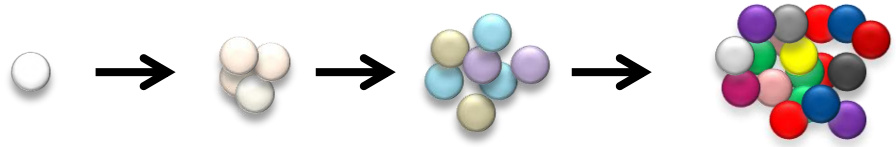
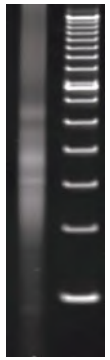
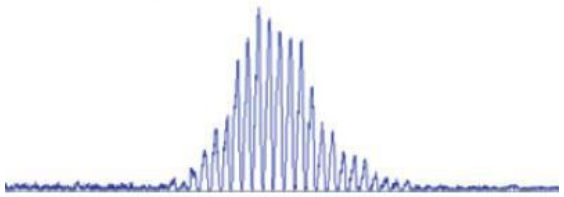
What is Clonality?

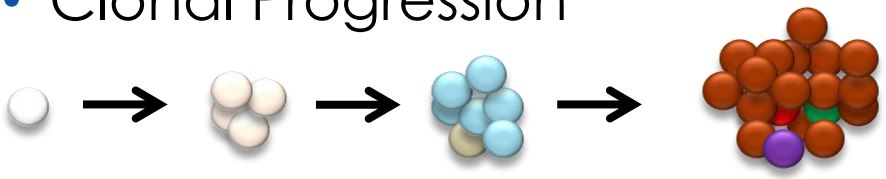
- Polyclonal Progression

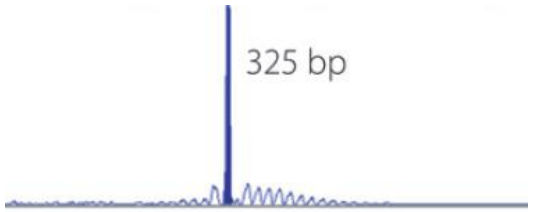






- Clonal Progression





Highly Indicative of B- or T-Cell Malignancy

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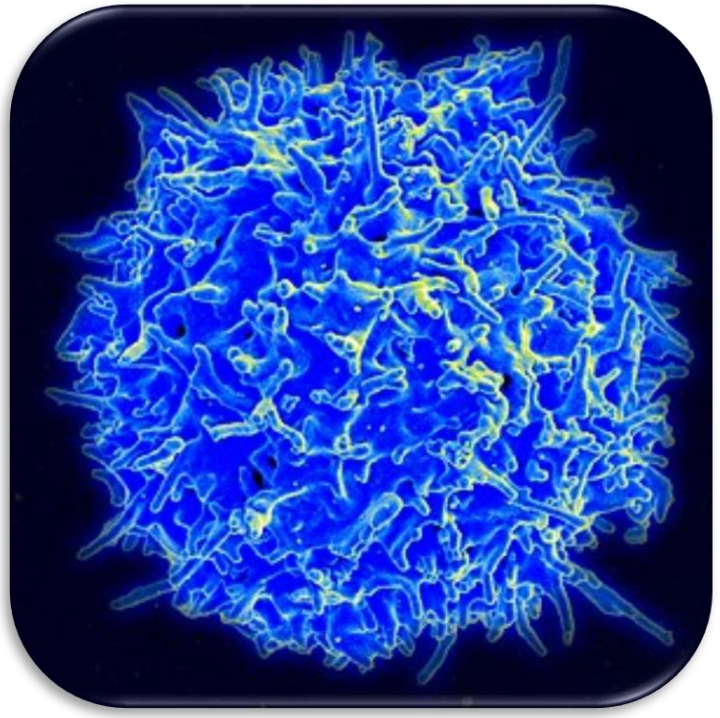
Why test for B- and T-cell clonality?



Leukemias and lymphomas can be challenging to diagnose by

- morphology
- immunohistochemistry
- flow cytometry

5-15% of above cases result in inconclusive diagnoses

Diagnosis of lymphoid malignancies is greatly supported and facilitated by **clonality testing**

Adopted in routine diagnostics for further MRD testing

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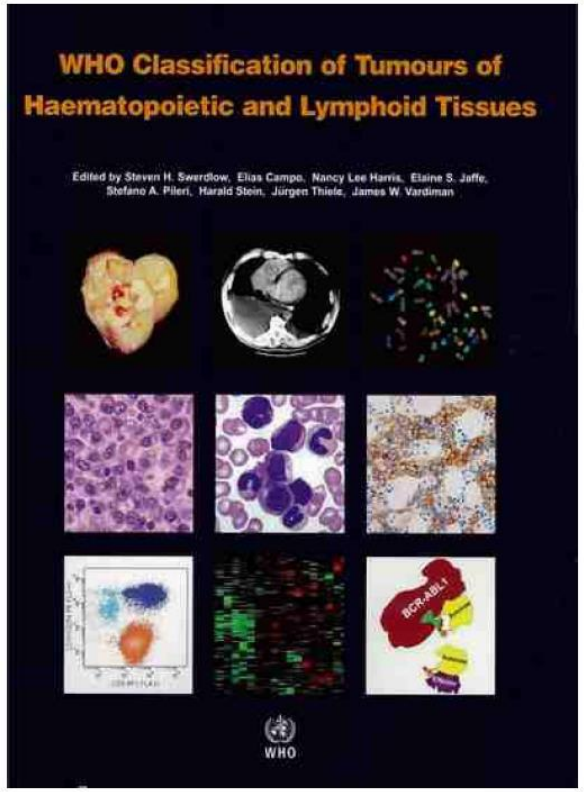
Why test for B- and T-cell clonality?



We are facing a growing number of hematopoietic tumors with specific or characteristic molecular changes.

Diagnosis of lymphoid malignancies is greatly supported and facilitated by **clonality testing**

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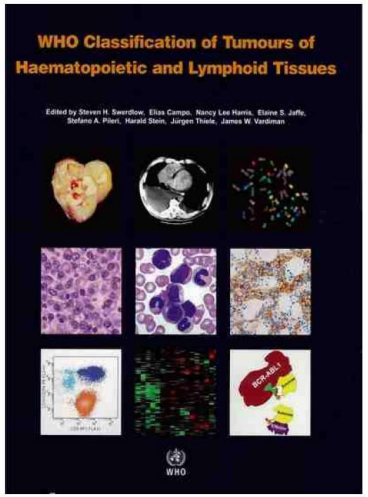
Why test for B- and T-cell clonality?

Monoclonality is a dominant characteristic of cancer

Allows for discrimination between :

- Reactive lesions **(polyclonal)** - generally considered **benign**
- Hematologic malignancies **(clonal)** - generally considered **neoplastic**



Aids in diagnosis:

- of Minimal tumor infiltration
- on limited diagnostic tissue when the architecture is not evaluable
- of neoplastic proliferations without specific cytological, histological or immunohistochemical criteria

Helps to identify tumor-specific markers for post-treatment monitoring

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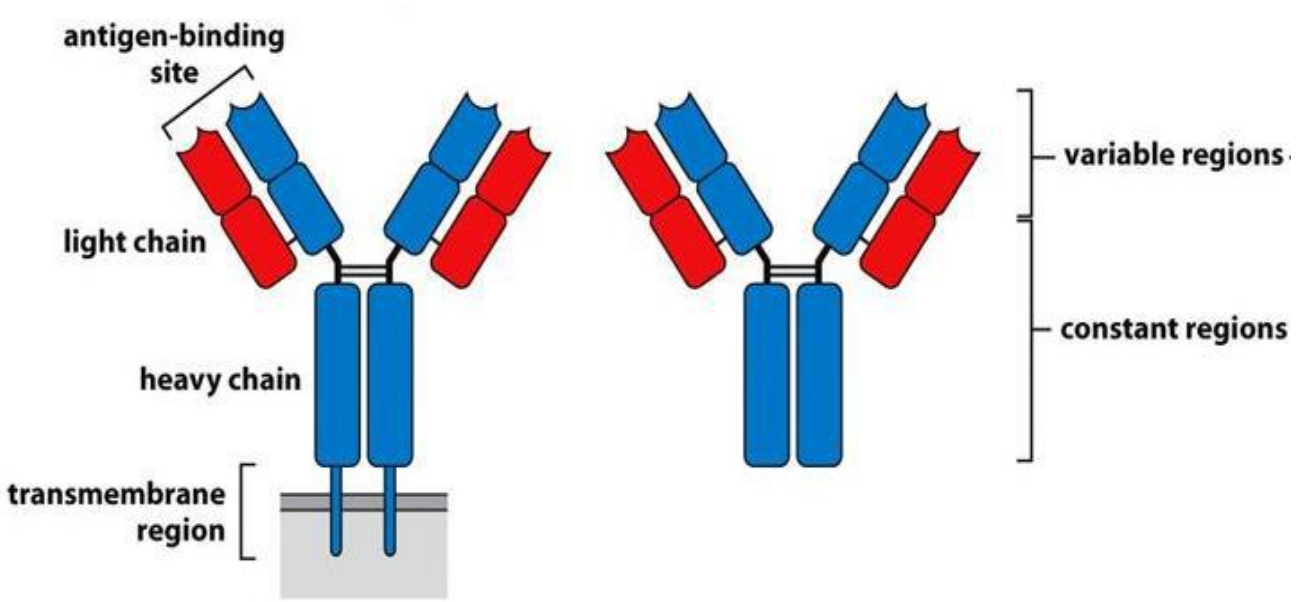
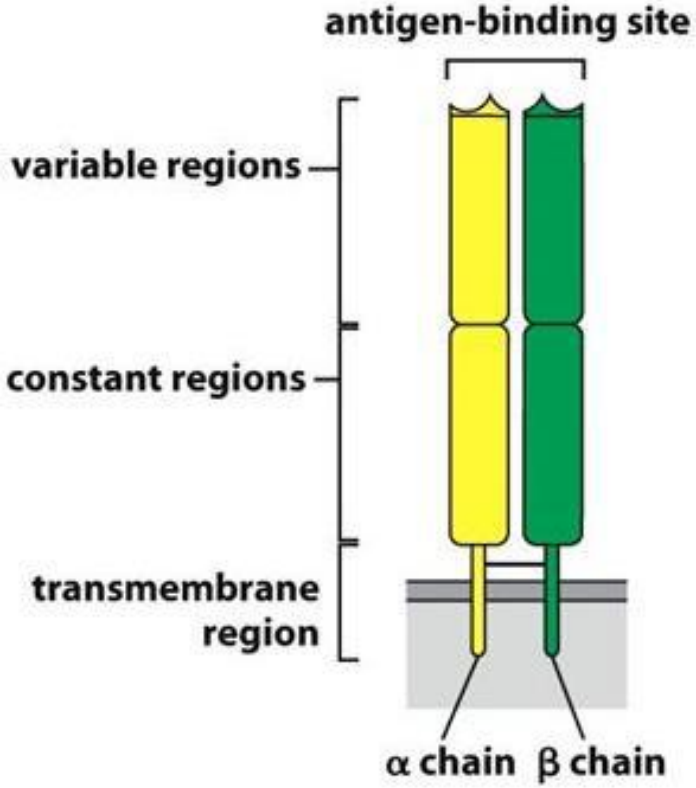
Clonality Targets

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



T-Cell Receptor (TCR)



Each Lymphocyte has a Unique Antigen Receptor

Magnitude ~10^{12} (*i.e.*, 1,000,000,000,000) different Ig or TCR molecules

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Clonality Targets

Antigen Receptor Molecules (AgRs) are the Molecular Targets

- **B- and T- Cell Receptors**
  - *IGH, IGK, IGL* (B-Cell Receptors)
  - *TRG, TRB, TRD* (T-Cell Receptors)

- **Each lymphocyte has a unique AgR** (single specificity)

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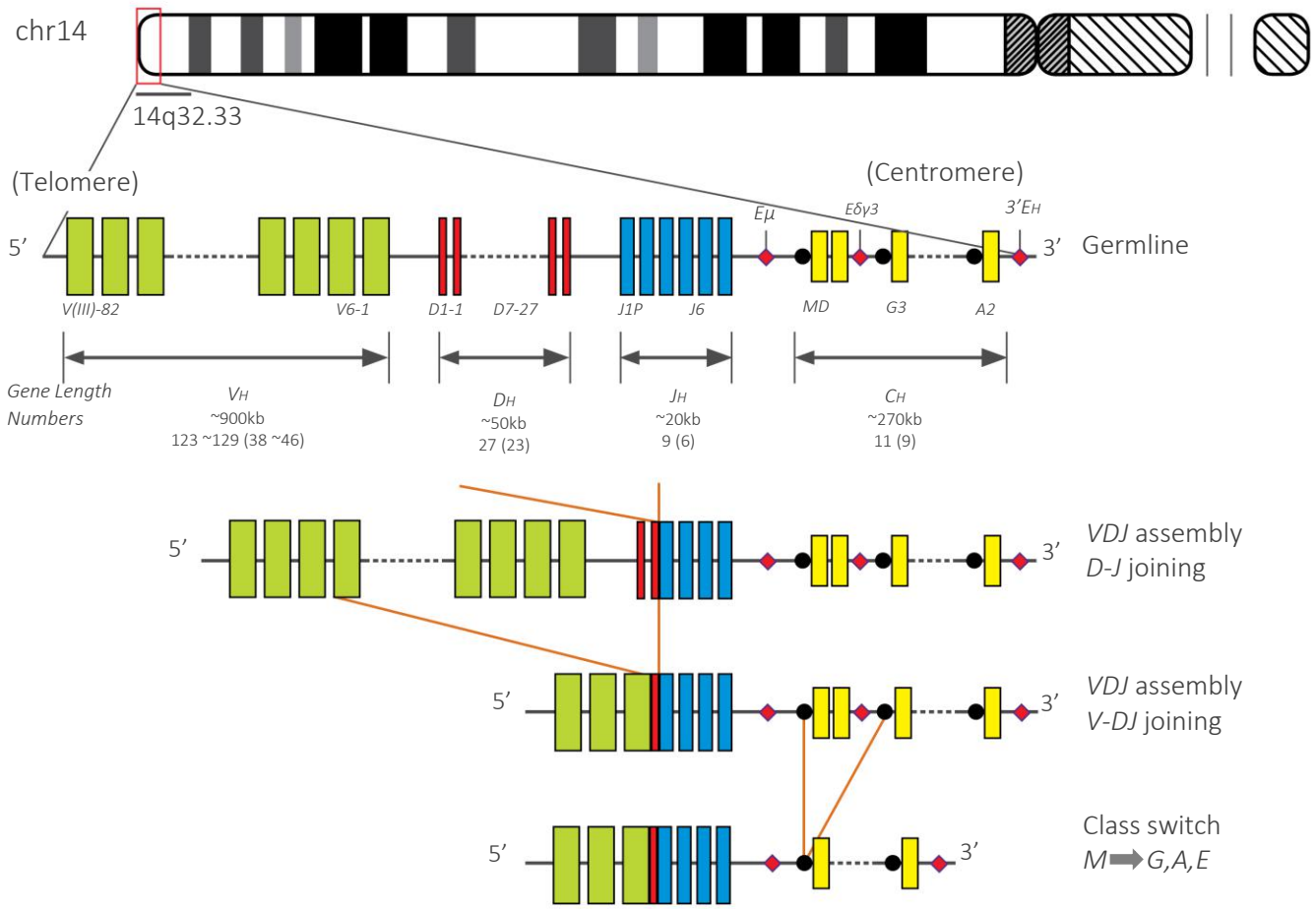
Human *IGH* Gene Locus



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

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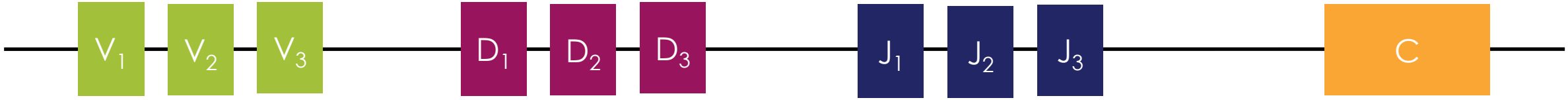
Generation of Diversity

Rearranged V-D-J gene :



Adapted from Dr. Yury Monczak, PhD. Jewish General Hospital & McGill University
"Utility for NGS Assays for the Evaluation of Lymphoid Malignancies." 2020 Feb. 27. AMP Webinar
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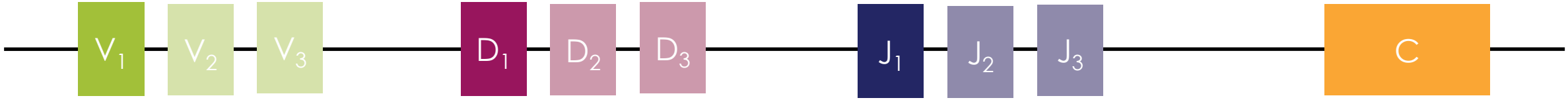

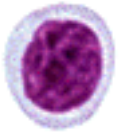

B-cell “X”

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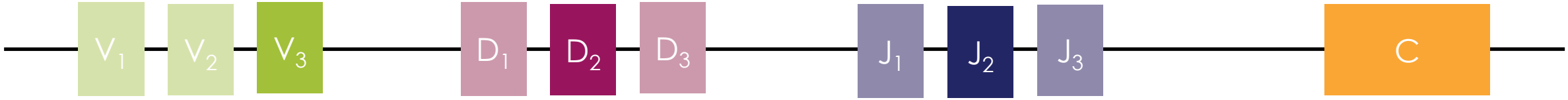
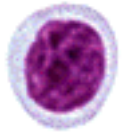
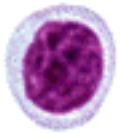




B-cell “X”



B-cell “Y”

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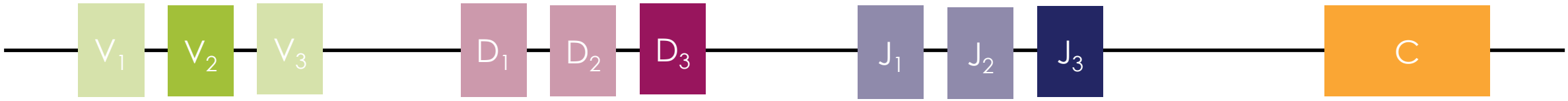

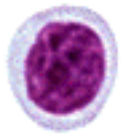

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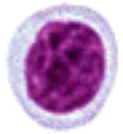

B-cell “Y”

B-cell “Z”

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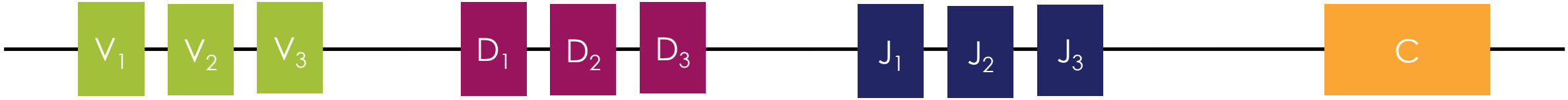

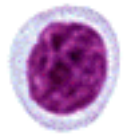

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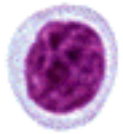

B-cell “Y”

B-cell “Z”

Unique sequences

=

Unique fingerprints

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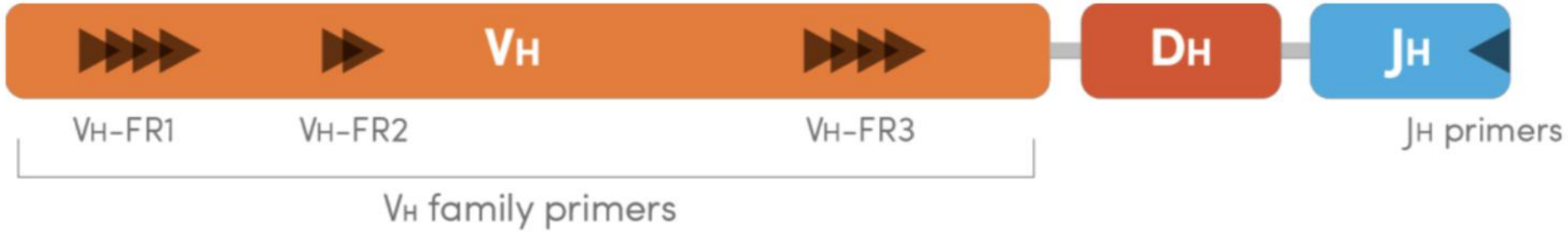
B- and T-cell Clonality Simplified

IGH Locus (14q32.33)



TRG Locus (7p14)

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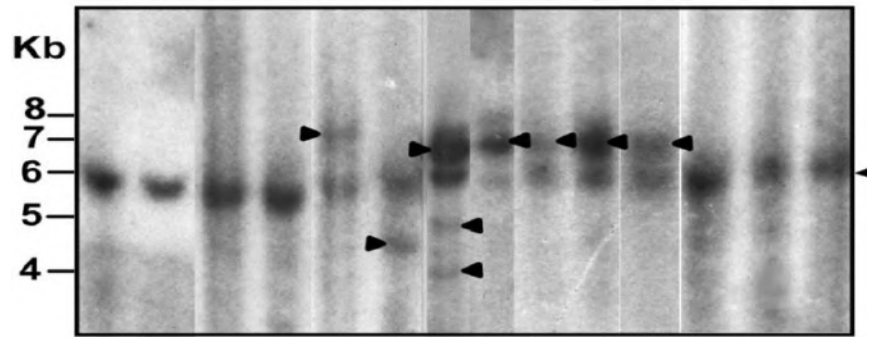
Evolution of Clonality Testing



Clonality Testing was originally performed by Southern Blots

- Labor intensive
- Restricted repertoire
- High DNA quality and quantity required
- Time consuming
- Moderate limits of detection
- Subjective interpretation

Southern Blot

Fragment Analysis / CE

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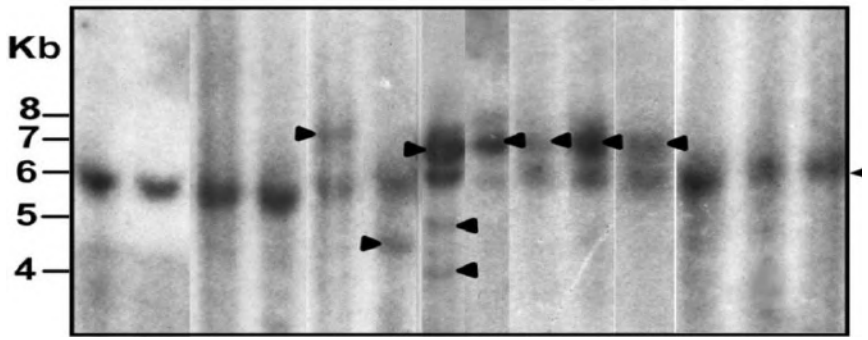
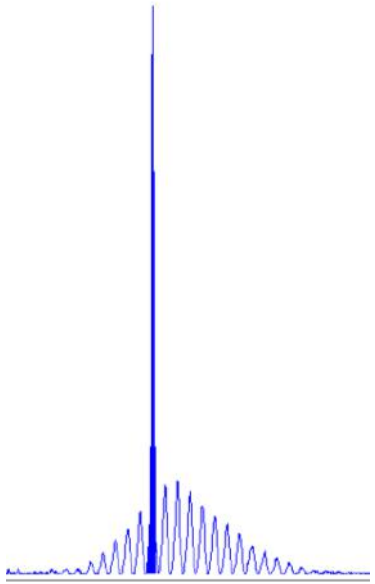
Evolution of Clonality Testing





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

- Sensitivity
- Testing time
- Coverage
- Limit of detection
- Sample type suitability

Southern Blot → **Fragment Analysis / CE** → **NGS**

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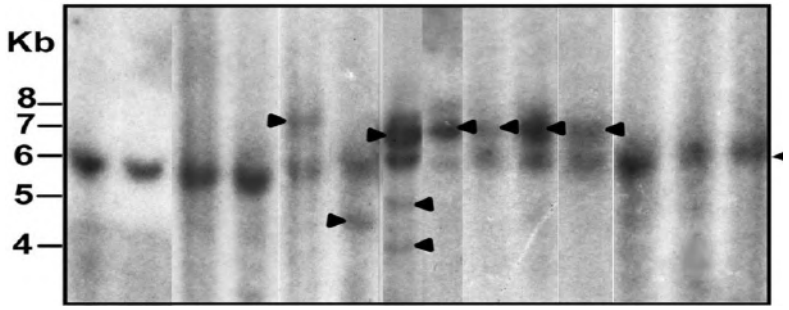
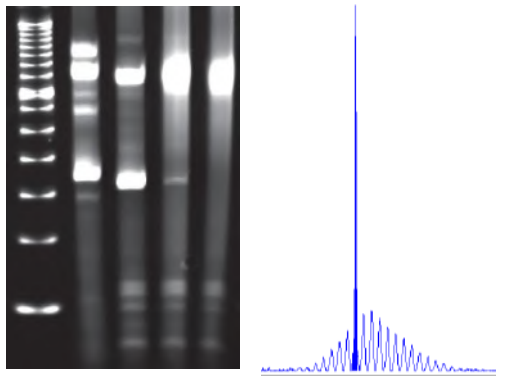
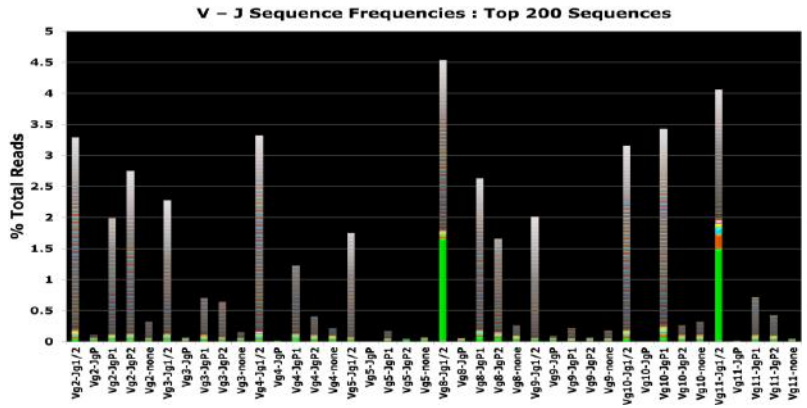
Evolution of Clonality Testing



- Labor intensive
- Time consuming
- Restricted repertoire
- Requires high DNA quantity & quality



- Increased sensitivity
- Reduced testing time
- Better coverage
- Lower limits of detection
- More sample types



- DNA sequence of clones
- Highest sensitivity
- Ability to track clones

Southern Blot → **Fragment Analysis / CE** → **NGS**

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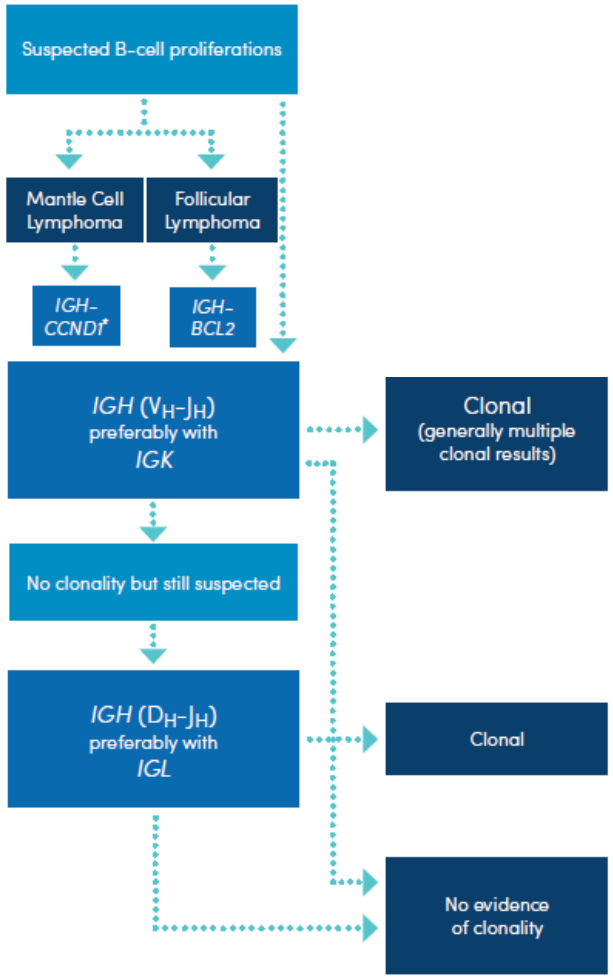
Hematologic Malignancy Research & Testing Guide

Disease	Gene Rearrangement								Translocation				Mutations	
Recommended Primary Test / Recommended Secondary Test	IGH (V_H-J_H)	IGH (D_H-J_H)	IGK	IGL	IGHV SHM	TRB	TRD	TRG	IGH-BCL1 (CCND1)	IGH-BCL2	BCR-ABL1*	PML RARa*	FLT3	NPM1
Lymphoid/Lymphoma														
Marginal Zone Lymphoma (MZL), extranodal[12,13,27]	88%	58%	84%	29%		23%	10%	16%						
Marginal Zone Lymphoma (MZL), nodal[13]	100%	30%	80%	30%		10%	20%	10%						
Mantle Cell Lymphoma (MCL)[2,6,7,12,13,27,37]	100%	11%	100%	44%	*	9%	4%	11%	75%					
Follicular Lymphoma (FL)[3,7,12,13,27,28]	84%	19%	84%	21%		6%	5%	2%		90%				
Diffuse Large B-cell Lymphoma (DLBCL)[3,12,13,27]	80%	30%	80%	28%		21%	14%	15%		30%				
Multiple Myeloma (MM) and other Plasma Cell Neoplasms (PCN)[2,9,10,20,25]	84%	60%	57%	97%					20%					
Chronic Lymphocytic Lymphoma (CLL)[11,12,13,15,23,27,35]	100%	43%	100%	30%	*	25%	12%	18%						
B-cell Acute Lymphoblastic Leukemia (B-ALL)[4,12,14,19,21,22,27,29,30,31,32,33,34]	96%	57%	95%	20%		81%	86%	75%			30%			
Suspect B-cell Proliferations[12,26,27,33]	93%	93%	90%	40%		20%		20%						
Peripheral T-cell Lymphoma (PTCL)[12,13,14,24]	35%	4%		2%		98%		94%						
T-cell Acute Lymphoblastic Leukemia (T-ALL)[12,14,21,22,29,31]	24%	25%	4%			92%	68%	95%						
Angioimmunoblastic T-cell Lymphoma (AILT)[12,13,14]	19%	11%	30%	5%		99%	35%	92%						
Adult T-cell Leukemia/Lymphoma[39]						97%		96%						
Anaplastic Large-Cell Lymphoma (ALCL)[12,13,14]						74%	12%	74%						
T-cell Prolymphocytic Leukemia (T-PLL)[12,13,14]	3%	3%	3%	3%		100%	6%	94%						
T-cell Large Granular Lymphocytic Leukemia (T-LGL Leukemia)[12,13,14]			4%	4%		97%	29%	96%						
Suspect T-Cell Proliferations[12,26,40]	10%		10%			90%	11%	90%						
Myeloid														
Acute Myeloid Leukemia (AML)[8,16]													33%	64%
Acute Promyelocytic Leukemia (APL)[1,5,16,17]												90%		
Chronic Myeloid Leukemia (CML)[7,18,19,21,38]											87%			
Myeloproliferative Neoplasms (MPNs)[38]											10%			

Note: The percentage of samples within a given disease category were detected using each gene target. Percentages indicate the highest referenced value.

Clonality Testing Guide

- A test algorithm for suspect B-cell lymphoproliferations
- Developed in concert with the EuroClonality/BIOMED-2 group for PCR-based clonality assessment of suspected B-cell lymphoproliferative disorders



J.J.M. van Krieken *et al., Leukemia* 2007 21: 201-206.
A.W. Langerak *et al., Leukemia* 2012 26: 2159-71.

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Clonality Testing Guide

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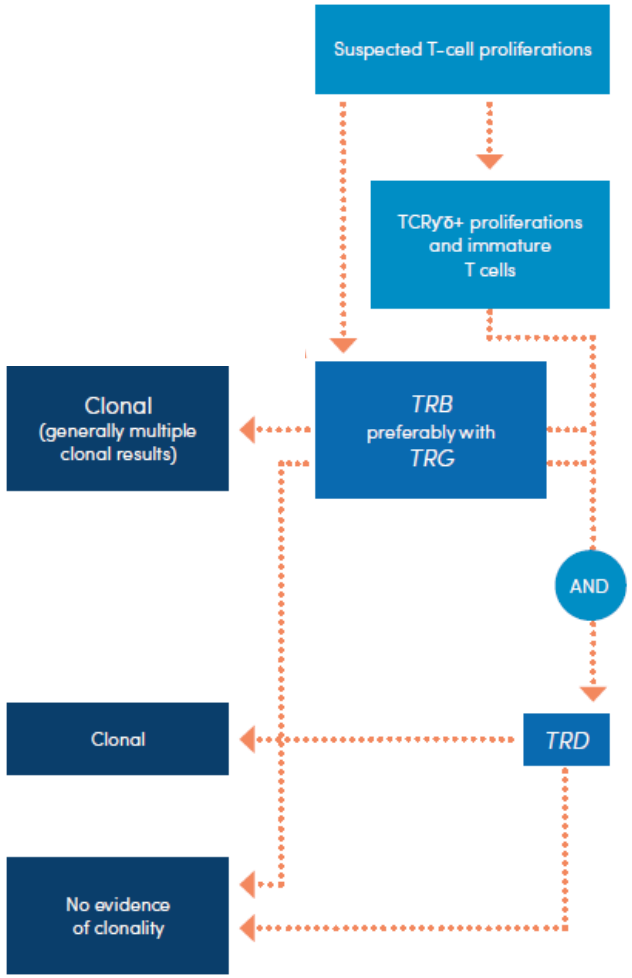

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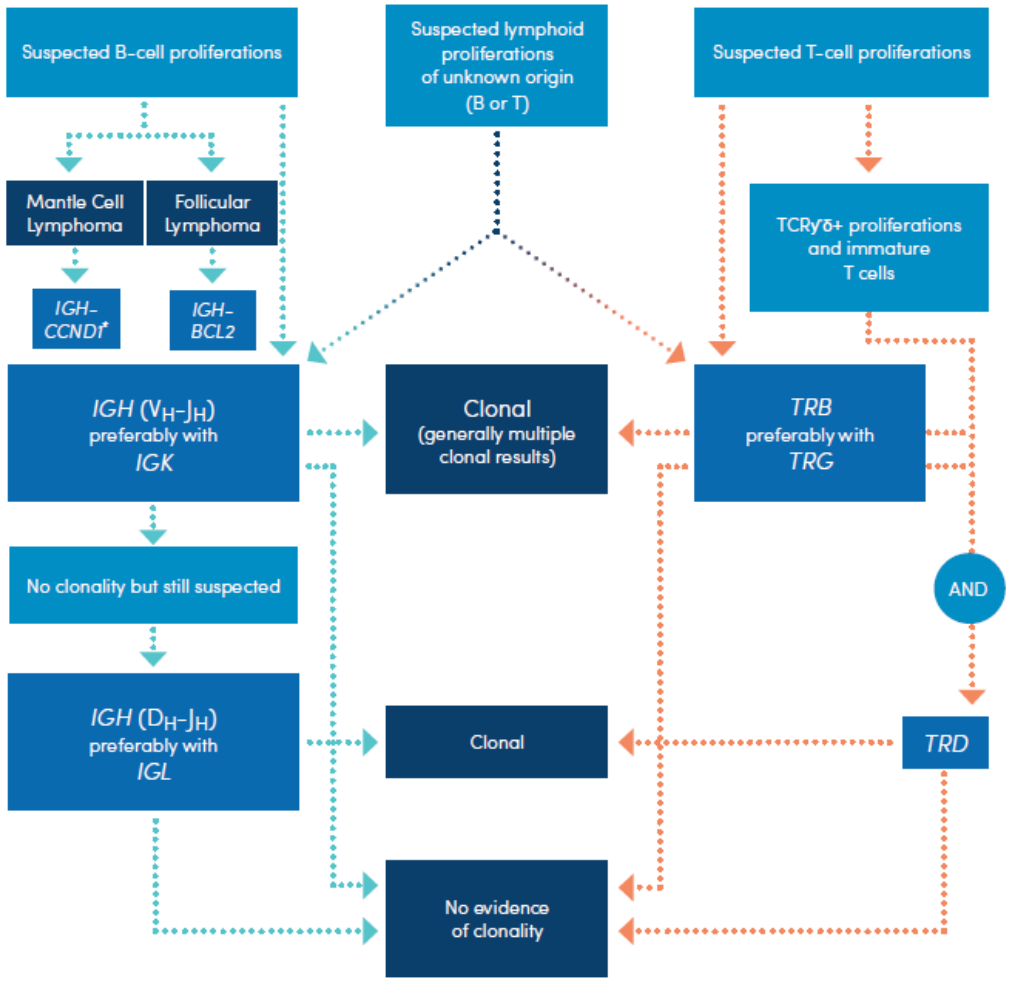
Clonality Testing Guide

- A test algorithm for suspect B- and T-cell lymphoproliferations
- Developed in concert with the EuroClonality/BIOMED-2 group for PCR-based clonality assessment of suspected B- and T-cell lymphoproliferative disorders



J.J.M. van Krieken *et al., Leukemia* 2007 21: 201-206.
A.W. Langerak *et al., Leukemia* 2012 26: 2159-71.

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Multiplexing Targets

Why combine testing of *IGH* V-J & *IGK*?

The majority of mature B-cell malignancies can be identified by targeting three *IGH* (V_H-J_H) frameworks*

	IGH FR1 (V_H - J_H)	*IGH* FR2 (V_H - J_H)	*IGH* FR3 (V_H - J_H)	*IGH* (FR 1, 2 & 3)
MCL (n=54)	100%	98%	96%	100%
B-CLL/SLL (n=56)	95%	91%	93%	100%
FL (n=109)	73%	76%	52%	84%
MZL (n=41)	73%	85%	68%	87%
DLBCL (n=109)	68%	61%	50%	79%
Total (n=369)	79%	78%	66%	88%

Abbreviations: B-CLL, B-cell chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lyn cell lymphoma; MZL, marginal zone B-cell lymphoma.

[1]PA Evans et al. Leukemia. 2007 21:207-214

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Multiplexing Targets

Why combine testing of *IGH* V-J & *IGK*?

The majority of mature B-cell malignancies can be identified by targeting three *IGH* (V_H-J_H) frameworks and two *IGK* (V_k-J_k and Kde) rearrangements*

	IGH FR1 (V_H - J_H)	*IGH* FR2 (V_H - J_H)	*IGH* FR3 (V_H - J_H)	*IGH* (FR 1, 2 & 3)	*IGK* (Vk - Jk & Kde)	Total (FR1, 2, 3 & *IGK*)
MCL (n=54)	100%	98%	96%	100%	100%	100%
B-CLL/SLL (n=56)	95%	91%	93%	100%	100%	100%
FL (n=109)	73%	76%	52%	84%	84%	100%
MZL (n=41)	73%	85%	68%	87%	83%	97%
DLBCL (n=109)	68%	61%	50%	79%	80%	96%
Total (n=369)	79%	78%	66%	88%	88%	98%

Abbreviations: B-CLL, B-cell chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone B-cell lymphoma.

[1]PA Evans et al. Leukemia. 2007 21:207-214

Clonality can be Identified in 98% of all B-Cell Malignancies

Testing Complementary Gene Targets in Parallel Improves Confidence!

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* PA Evans et al., Leukemia. 2007 21:207-214.

Multiplexing Targets

Why combine testing of *TRB* & *TRG*?

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

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

Clonality can be Identified in 94% of all T-Cell Malignancies

Testing Complementary Gene Targets in Parallel Improves Confidence!

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Why Multiplexing Targets?

Easy to combine:

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

Advantages of Combining Targets:

- Highest sensitivity
- Helps confirm diagnosis in difficult cases
- Improves Reliability

B-Cell Targets

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

T-Cell Targets

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

Testing Complementary Gene Targets in Parallel Improves Confidence!

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Improving Lives with Precision Diagnostics®

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

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Quiz

Clonality is defined as a proliferation of cells originating from a single progenitor cell, producing a pool of identical clonal cells. True or False?

- **TRUE**

Why test for clonality?

- Monoclonality is a dominant feature of cancer.
- Clonality testing facilitates the diagnosis of leukemias and lymphomas.
- Clonality testing allows for discrimination between reactive lesions and hematologic malignancies.
- **All of the above**

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Quiz

The molecule targets for clonality testing are B- and T-Cell Receptors. True or False?

- **TRUE**

Which detection method is NOT available in Invivoscribe assay kits?

- Gel Electrophoresis
- **Flow Cytometry**
- Capillary Electrophoresis (ABI)
- Next-Generation Sequencing (NGS)

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Quiz

True or False? Multiplexing targets is important because:

- It increases the detection rate of clonal rearrangements
- It increases the test sensitivity
- It improves confidence in results

- **TRUE**

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Take Home Message

- **PCR-based Clonality Testing** of B- and T- Cell Gene Rearrangements is the worldwide Gold Standard.
- **Clonality** testing should be performed **at the minimum,** in all cases where the pathological results contradict the clinical findings.
- **Combining targets** results in excellent sensitivity.
- **NGS** allows for unprecedented **detection levels** and information.

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