Acquired alterations of IG and TCR loci in lymphoproliferative disorders

Sabine Franke, PhD
Sabine.Franke@chuliege.be
CHU Ulg Liège, Centre for Human Genetics

Interuniversity course BeSHG 2021/22 29/04/22

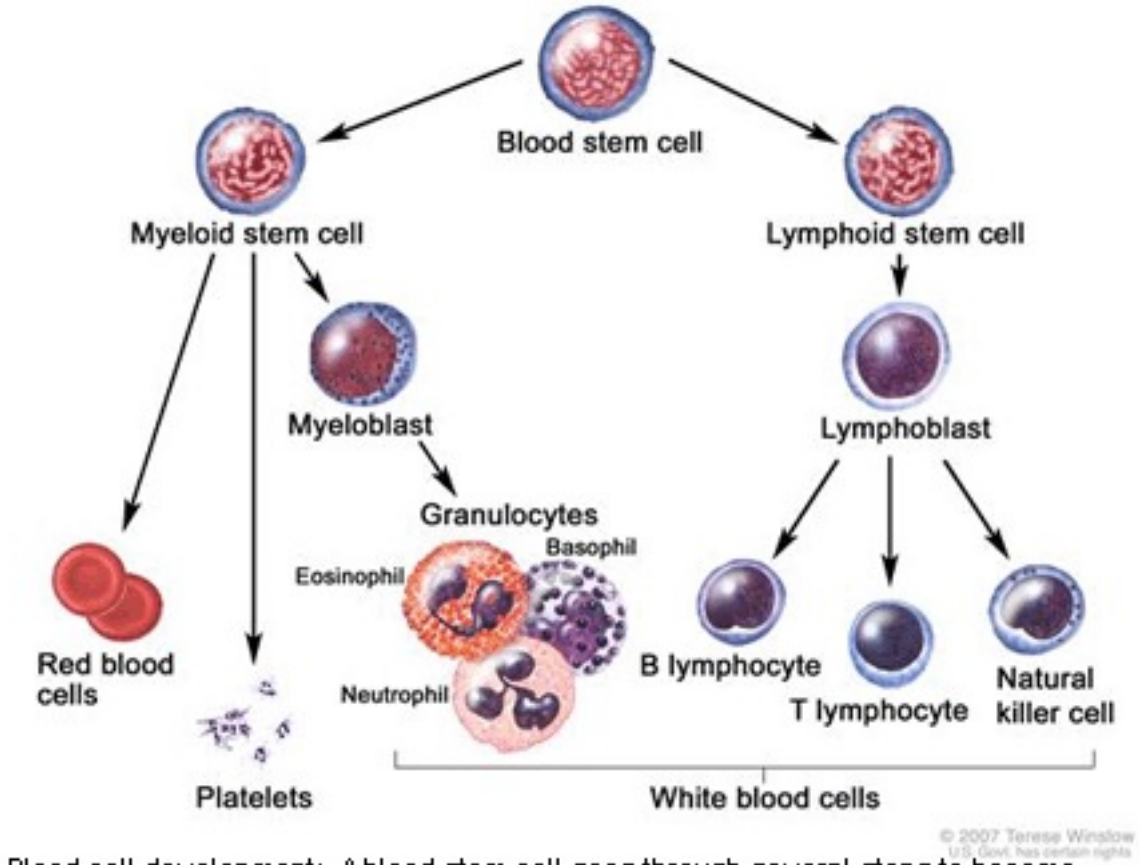
Overview

- What are lymphoproliferative disorders?
- What are ‘IG’ and ‘TCR’?
- What are IG/TCR alterations?
- How to detect IG/TCR alterations?

Overview

- What are lymphoproliferative disorders?
- What are ‘IG’ and ‘TCR’?
- What are IG/TCR alterations?
- How to detect IG/TCR alterations?

Blood cell development



Blood cell development: A blood stem cell goes through several steps to become a red blood cell, platelet, or white blood cell.

major types of lymphocytes

Three major types of lymphocytes

- B cells
- T cells
- Natural killer (NK) cells

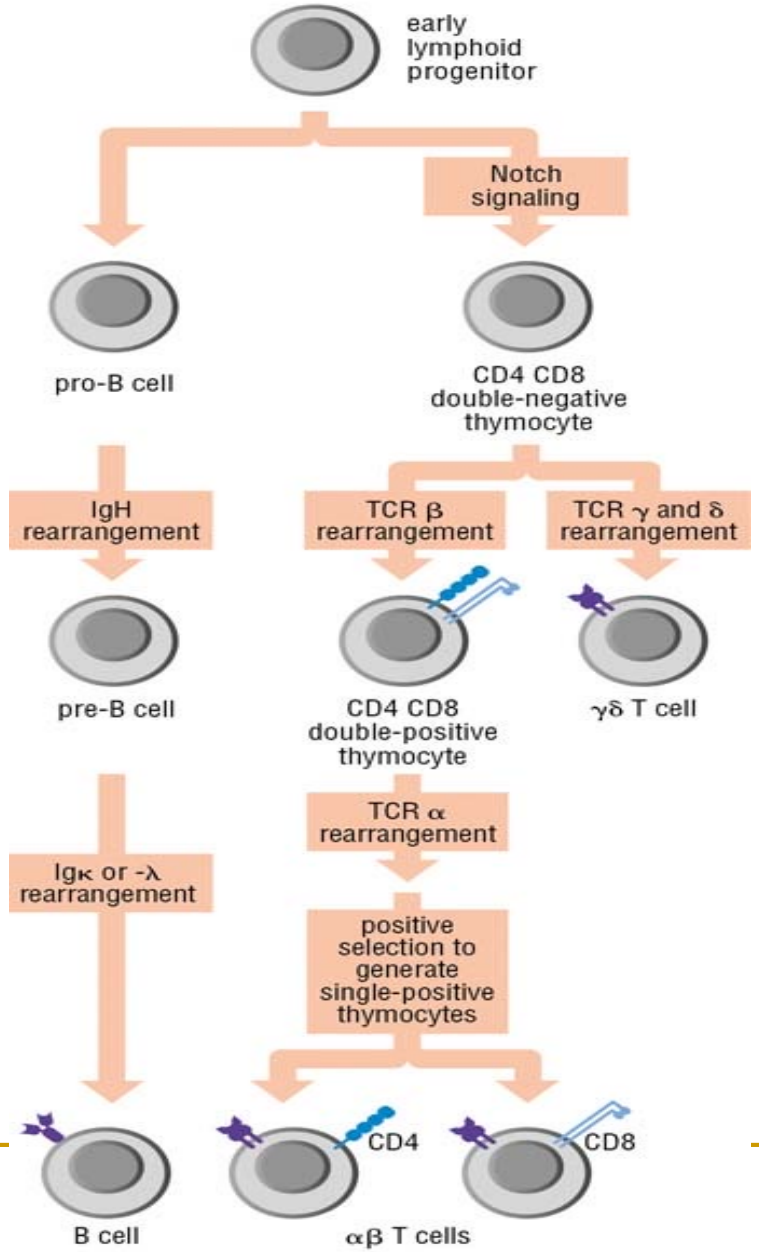
Function of B and T cells

- B cells are primary responsible for humoral immunity (relating to antibodies)
- T cells are involved in cell-mediated immunity.

B-cells

- Millions of different types of B cells each day
- In the blood and lymphatic system role of immune surveillance
- No production of antibodies until fully activated

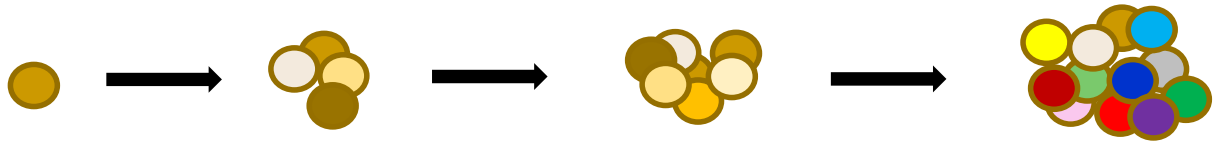
From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson



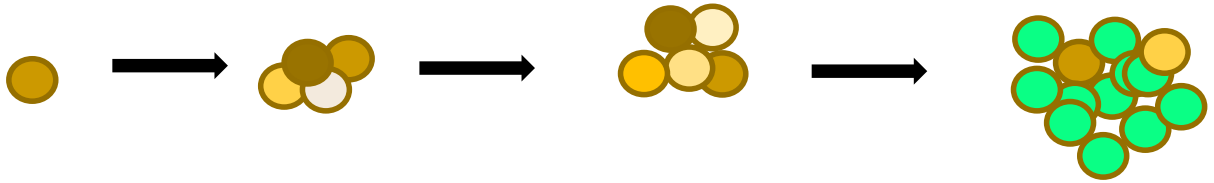
Gene rearrangements of the antigen receptor genes occur during the lymphoid proliferation

Progression of the cell

Polyclonal progression



Clonal progression



Lymphoproliferative disorders (LPDs)

LPDs refer to several conditions in which lymphocytes are produced in excessive quantities.

- **Chronic lymphocytic leukemia** (most frequent) 25%
- Acute lymphoblastic leukemia
- Hairy cell leukemia
- lymphomas
- Multiple myeloma
- Waldenstrom's macroglobulinemia
- Wiskott-Aldrich syndrome
- Post-transplant lymphoproliferative disorder
- Autoimmune lymphoproliferative syndrome (ALPS)
- 'Lymphoid interstitial pneumonia'

B, T, and NK lineage of lymphoid malignancies

Lymphoid malignancy	B lineage	T lineage	NK lineage
Acute lymphoblastic leukemia			
– children	**82 – 86%**	14 – 18%	< 1%
– adults	**75 – 80%**	20 – 25%	< 1%
Chronic lymphocytic leukemias	**95 – 97%**	3 – 5%	1 – 2%
Non-Hodgkin lymphomas			
– nodal NHL	**95 – 97%**	3 – 5%	< 2%
– extranodal NHL	**90 – 95%**	5 – 10%	< 2%
– cutaneous NHL	30 – 40%	**60 – 70%**	< 2%
Multiple myeloma	**100%**	0%	0%

1.Take home message

- Three major types of lymphocytes
- In LPDs lymphocytes are produced in excessive quantities
- Different lymphoid malignancies of different lineage origin

Overview

- What are lymphoproliferative disorders?
- What are ‘IG’ and ‘TCR’?
- What are IG/TCR alterations?
- How to detect IG/TCR alterations?

B-cell and T-cell receptors

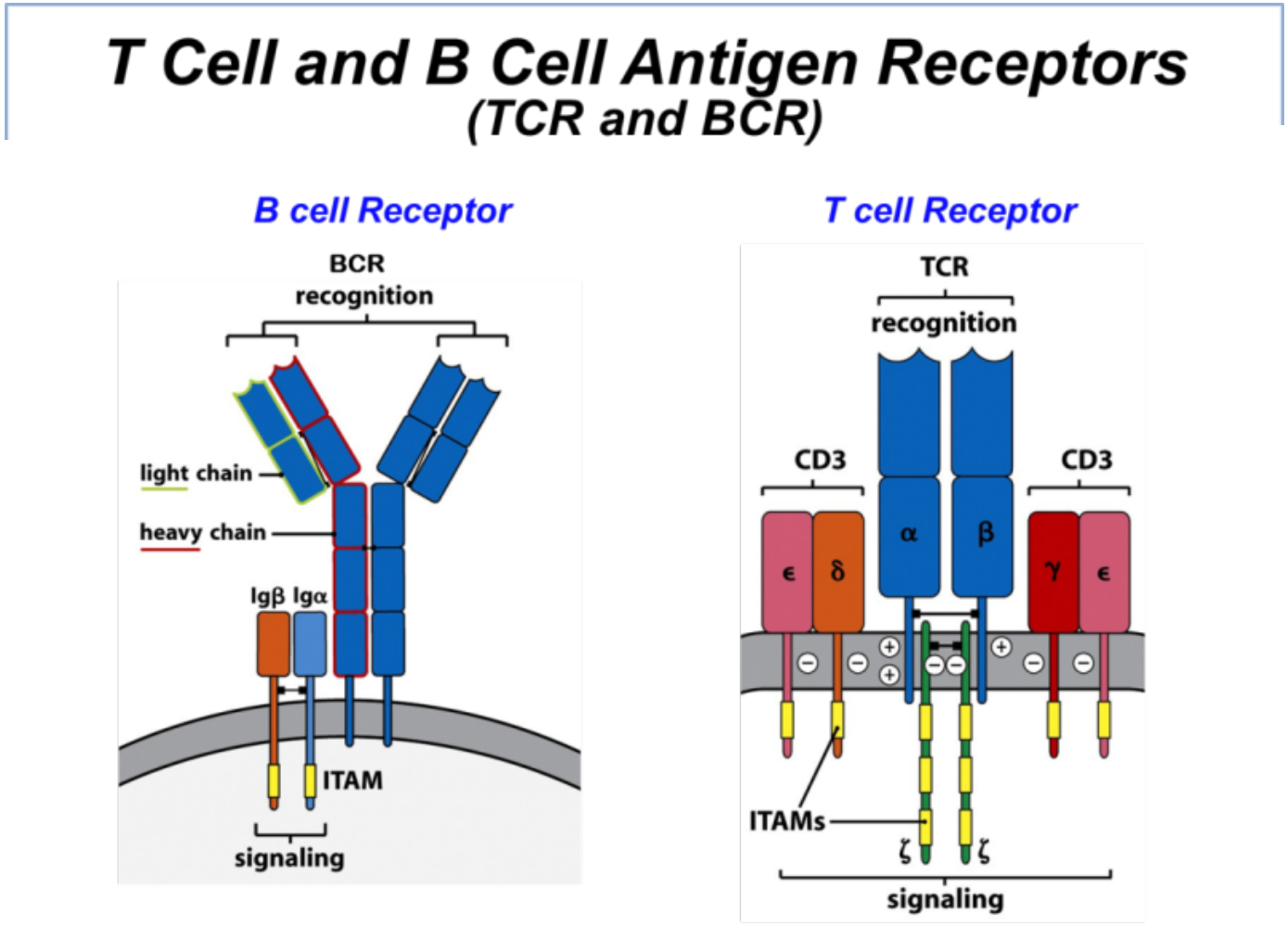
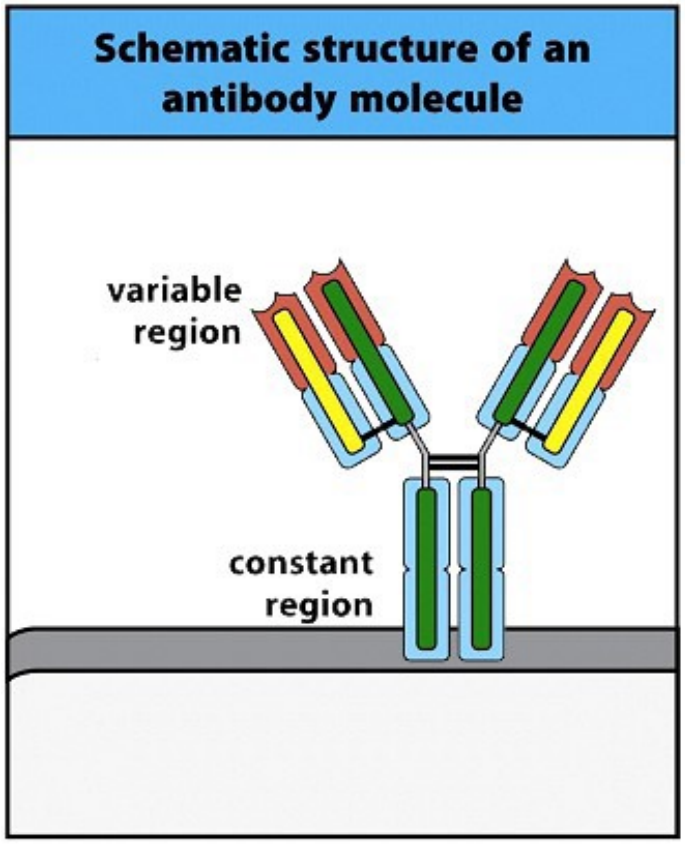


Image source: Immunobiology, 5th edition Janeway et al

Structure of the B-cell receptors

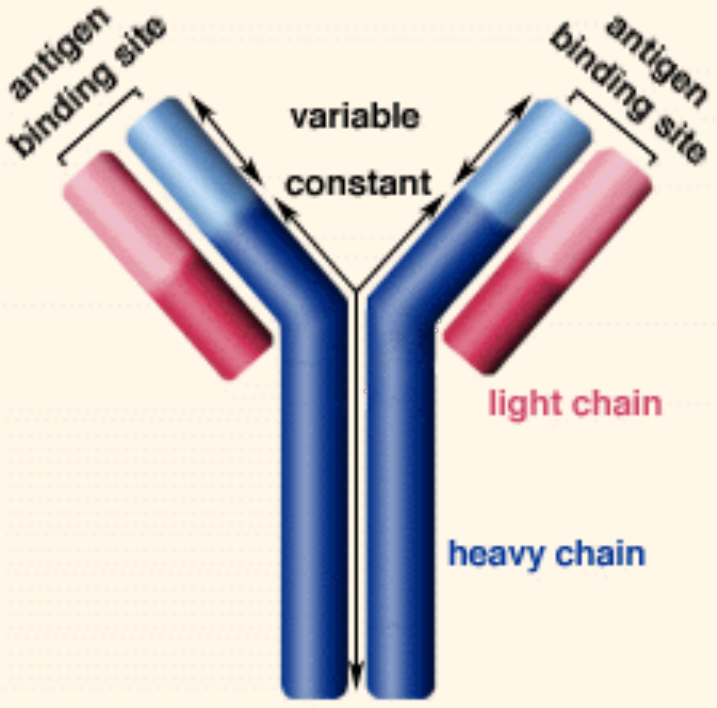


Light chain

Heavy chain

The BCR is a membrane-bound immunoglobulin, and allows the distinction of B cells from other types of lymphocytes and is the main protein involved in B cell activation.

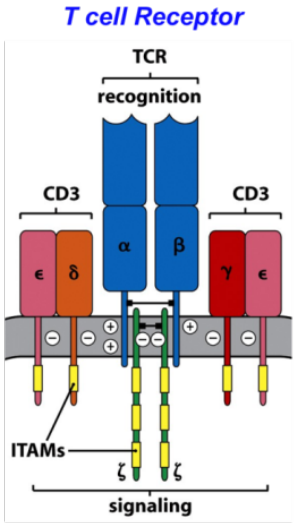
The ability to produce billions of different antibodies in humans results from the production of variable regions of light and heavy antibody genes by DNA rearrangement.



http://www.biology.arizona.edu

Heavy chain (IGH)
Light chain (IGL)

Structur of the T-cell receptor



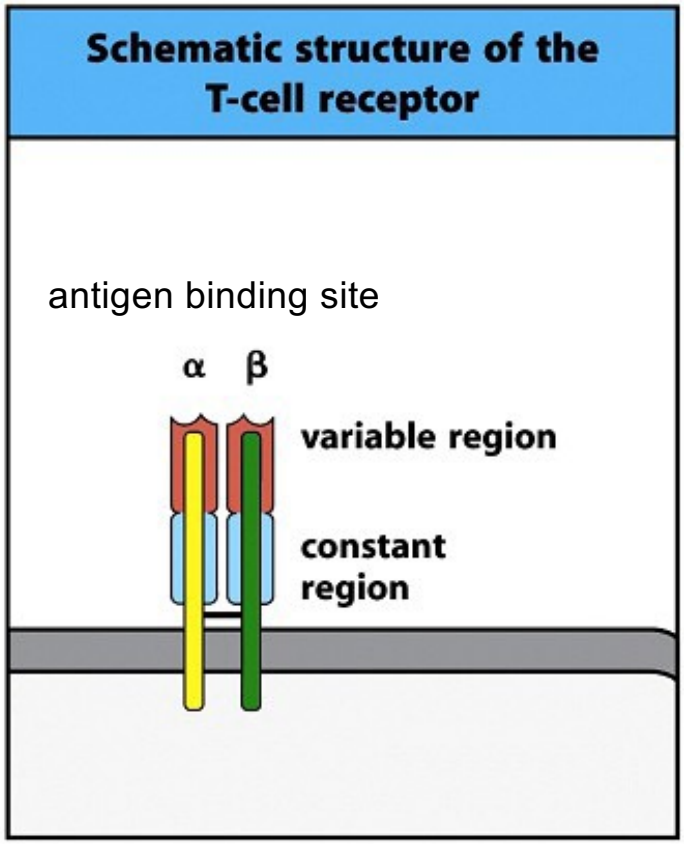


Image source: Immunobiology, 5th edition Janeway et al

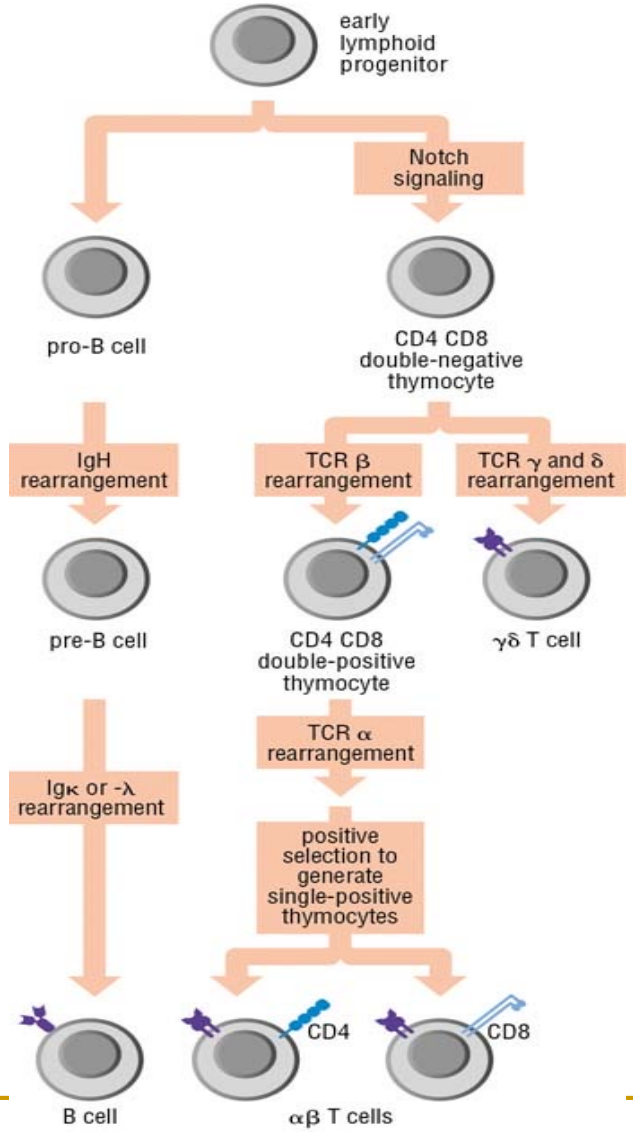
2. Take home message

- Structure of the B and T cell receptor is important for the antibodies
- Ig consists of heavy and light chains
- Ig and TCR consists of a variable and a constant region

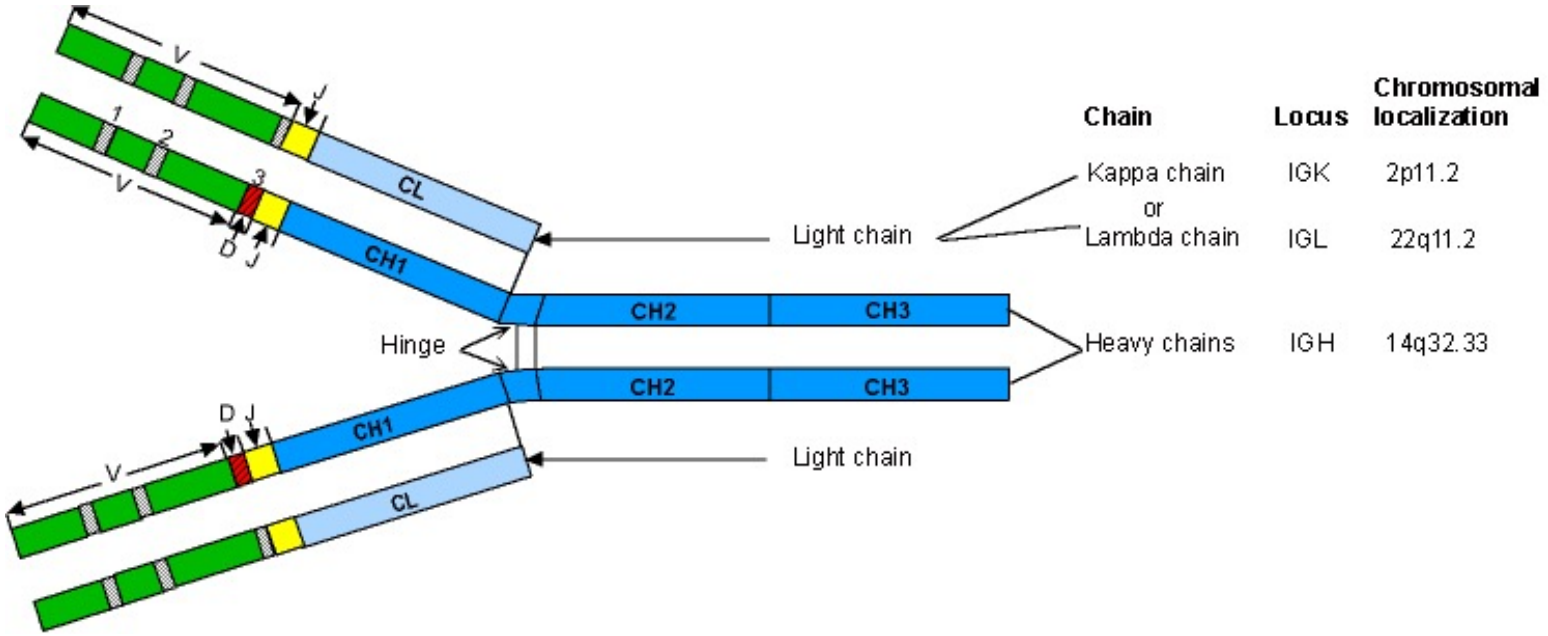
Overview

- What are lymphoproliferative disorders?
- What are ‘IG’ and ‘TCR’?
- What are IG/TCR alterations?
- How to detect IG/TCR alterations?

From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson



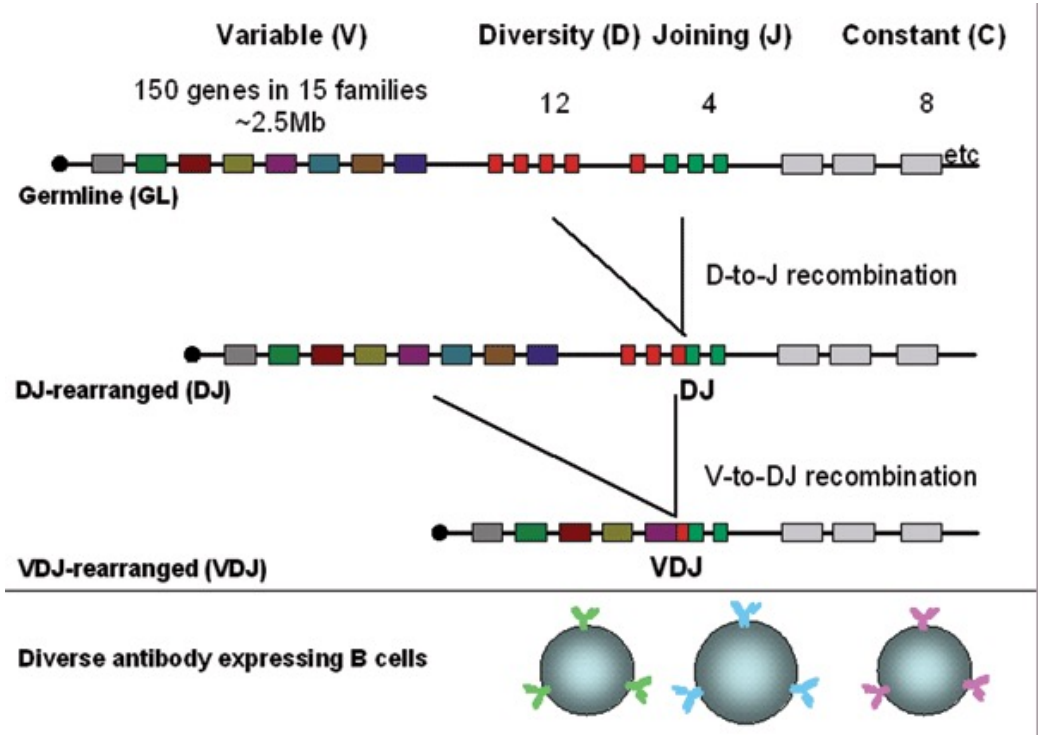
Schematic representation of an Immunoglobulin (IG)



Chain	Locus	Chromosomal localization
Kappa chain	IGK	2p11.2
or		
Lambda chain	IGL	22q11.2
Heavy chains	IGH	14q32.33

The production of variable regions of heavy antibody genes by DNA rearrangement.

stepwise rearrangement of V, D, and J gene segments



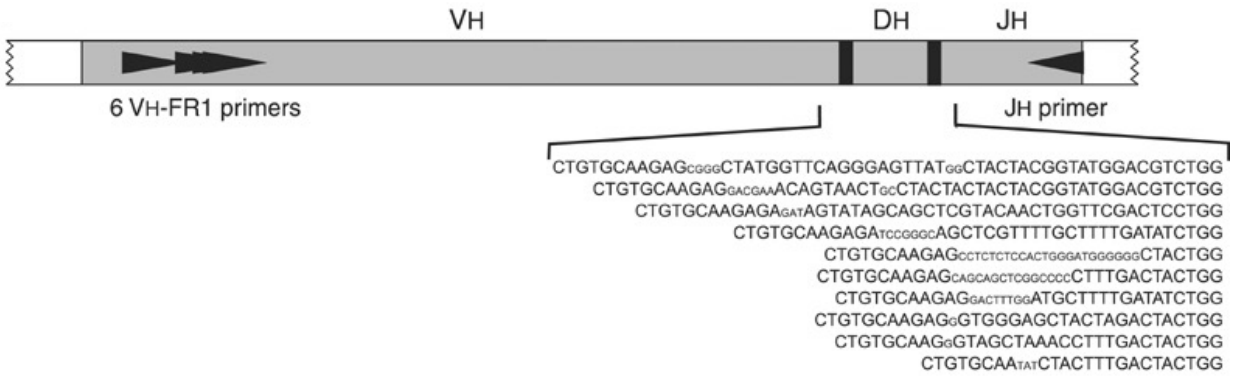
Genes encoding antigen receptors are unique:
-high diversity
-developing lymphocytes through V(D)J rearrangement.

Genetic recombination process of the DNA Level. Each of th V, D and J gene segments are randomly recombined

Gene rearrangements are lymphoid specific, developmentally-regulated, sequencial

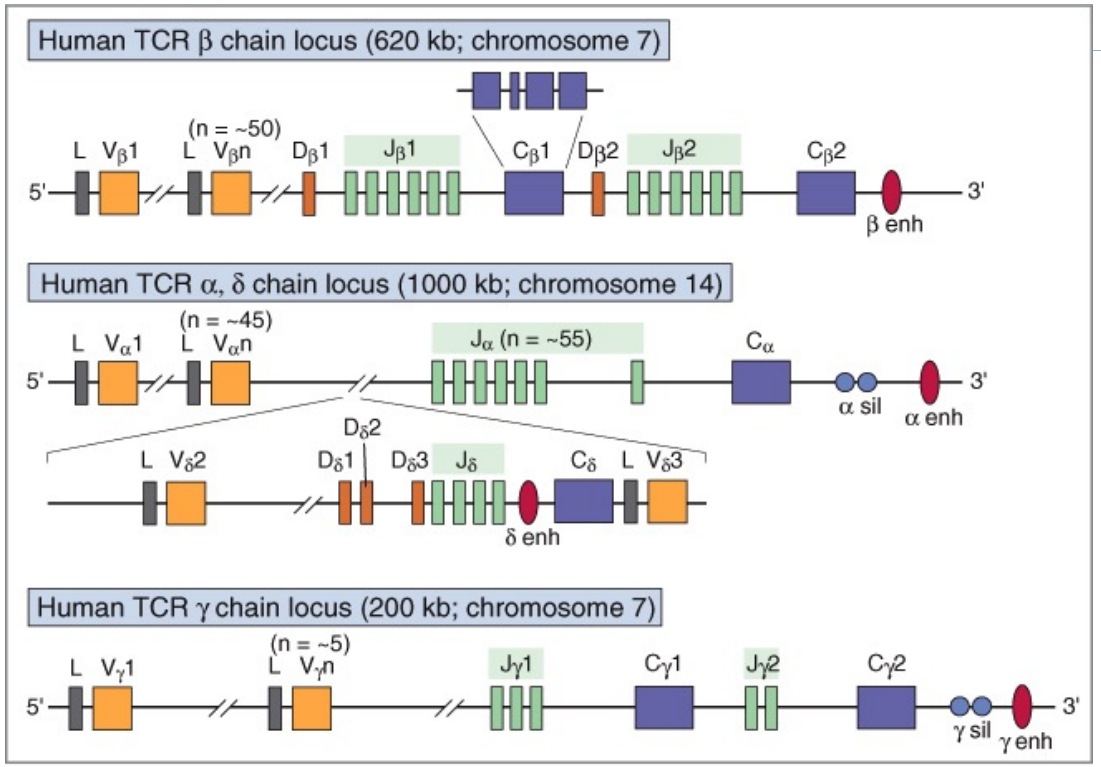
Cell development

Stage	Heavy chain	Light chain
Progenitor (or pre-pro) B cells	germline	germline
Early Pro (or pre-pre)-B cells	undergoes D-J rearrangement	germline
Late Pro (or pre-pre)-B cells	undergoes V-DJ rearrangement	germline
Large Pre-B-cells	is VDJ rearranged	germline
Small Pre-B cells	is VDJ rearranged	undergoes V-J rearrangement
Immature B cells	is VDJ rearranged	VJ rearranged
Mature B cells	is VDJ rearranged	VJ rearranged

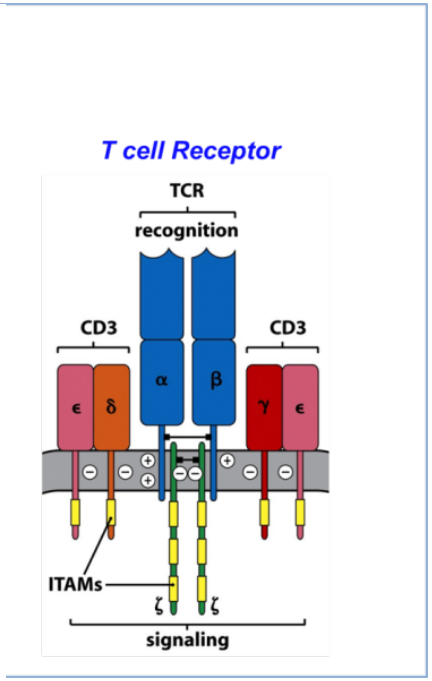


These V-J gene rearrangements generate products that are unique in length and sequence in each cell resulting in diverse antigen expressing B cells.

T cell rearrangement



© Elsevier 2005. Abbas & Lichtman: Cellular and Molecular Immunology 5e www.studentconsult.com



Estimated diversity of human Ig and TCR molecules

	IgH	Igα	Igλ	TCR α	β	γ	δ
		molecules			molecules		
Number gene segments							
V gene segments	~44	~43	~38	~46	~47	~6	~6
D gene segments	27				2		3
J gene segments	6	5	4	53	13	5	4
Combination diversity	>2x10 ^{6}			2x10 ^{6}		<5000	
Junctional diversity	++	±	±	+	++	++	++++
Total diversity	**>10 ^{14}**			**>10 ^{12}**		**>10 ^{12}**	

Unique antigen receptors are encoded -> up to 10^{14}

Belgian Society of Human Genetics

Unilab Lg.

CHU de Liège

Possible variation through recombining gene fragments?

Over 10^{14} combinations of variable, diversity and joining gene segments are possible.

Imprecise recombination and mutation increase the variability into billions of possible combinations.

3. Take home message

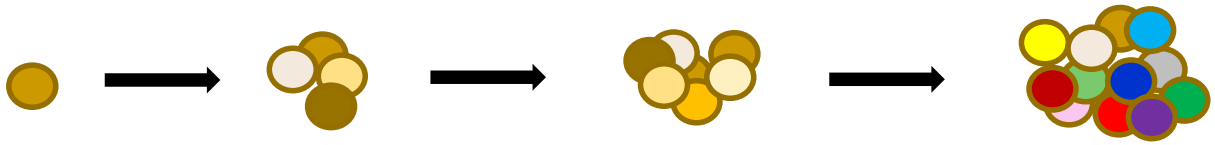
- Gene rearrangements of the antigen receptor genes occur during the lymphoid proliferation
- B-cells: Stepwise rearrangement of V, D, and J gene segments (randomly recombined)
- Unique products of V(D)J rearrangements resulting in diverse antigen expressing B-cells

Overview

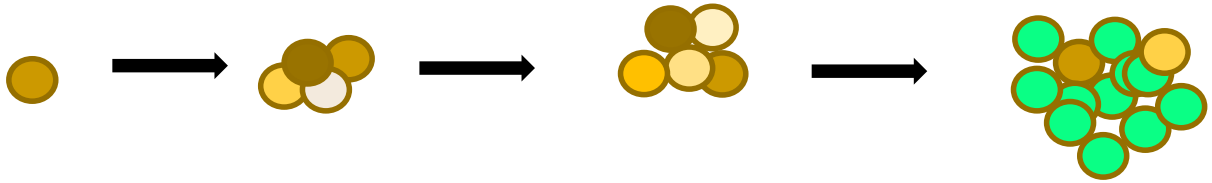
- What are lymphoproliferative disorders?
- What are ‘IG’ and ‘TCR’?
- What are IG/TCR alterations?
- How to detect IG/TCR alterations?

Progression of the cell

Polyclonal progression



Clonal progression



Clinically relevant testing

- Diagnosis
- B-cell versus T-cell malignancy (origine)
- Reactive versus malignant
- New lymphoma versus recurrence
- Assessment of remission and relapse
- Bone marrow involvement
- Evaluation of treatment effectiveness (MRD)

Analysis techniques

- Detecting gene rearrangements
  of the antigen receptor genes
- Discrimination between clonal and polyclonal Ig/TCR gene PCR products
- NGS: identification of the sequence

Design of primer sets for detection of Ig/TCR rearrangements

BIOMED-2 study (multiplex PCR)

Ig genes:	*IGH*:	VH-JH and DH-JH
	IGK:	Vκ-Jκ and Kde rearrangements
	IGL:	Vλ-Jλ
TCR genes:	*TCRB*:	Vβ-Jβ and Dβ-Jβ
	TCRG:	Vγ-Jγ
	TCRD:	Vδ-Jδ, Dδ-Dδ, Dδ-Jδ, and Vδ-Dδ

Analysis of *TCRB* gene rearrangements

Vβ family primers

32 27

Jβ primers

15

TCRB tubes A and B

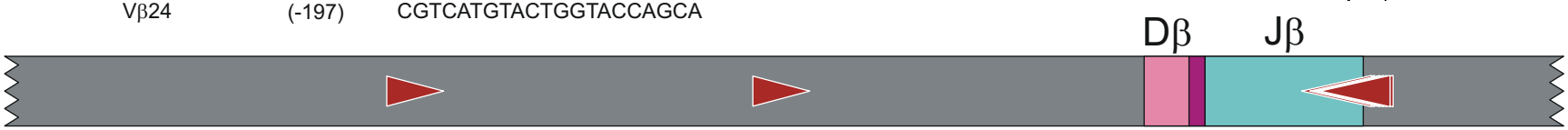
		5' 3'
Vβ2	(-204)	AACTATGTTTTGGTATCGTCA
Vβ4	(-201)	CACGATGTTCTGGTACCGTCAGCA
Vβ5/1	(-197)	CAGTGTGTCCTGGTACCAACAG
Vβ6a/11	(-201)	AACCCTTTATTGGTACCGACA
Vβ6b/25	(-201)	ATCCCTTTTTTGGTACCAACAG
Vβ6c	(-201)	AACCCTTTATTGGTATCAACAG
Vβ7	(-198)	CGCTATGTATTGGTACAAGCA
Vβ8a	(-201)	CTCCCGTTTTCTGGTACAGACAGAC
Vβ9	(-198)	CGCTATGTATTGGTATAAACAG
Vβ10	(-201)	TTATGTTTACTGGTATCGTAAGAAGC
Vβ11	(-198)	CAAAATGTACTGGTATCAACAA
Vβ12a/3/13a/15	(-198)	ATACATGTACTGGTATCGACAAGAC
Vβ13b	(-198)	GGCCATGTACTGGTATAGACAAG
Vβ13c/12b/14	(-198)	GTATATGTCCTGGTATCGACAAGA
Vβ16	(-201)	TAACCTTTATTGGTATCGACGTGT
Vβ17	(-198)	GGCCATGTACTGGTACCGACA
Vβ18	(-201)	TCATGTTTACTGGTATCGGCAG
Vβ19	(-201)	TTATGTTTATTGGTATCAACAGAATCA
Vβ20	(-193, inv)	CAACCTATACTGGTACCGACA
Vβ21	(-201)	TACCCTTTACTGGTACCGGCAG
Vβ22	(-201)	ATACTTCTATTGGTACAGACAAATCT
Vβ23/8b	(-201)	CACGGTCTACTGGTACCAGCA
Vβ24	(-197)	CGTCATGTACTGGTACCAGCA

TCRB tubes A and C: Jβ A primers

3' 5'		
GTGGTCTAAGTGTCAACATCCATTC	(+53)	Jβ1.1
CTGGTCCAATTGGCAACATCCATTC	(+53)	Jβ1.2
TTCAACCGAGTGACAACATCCATTC	(+55)	Jβ1.3
CTTGGGTCGAGAGACAGAACCCATAC	(+56)	Jβ1.4
CTGAGCTGAGAGGTAGGATCCATTC	(+55)	Jβ1.5
GTCCGAGTGACACTGTCCATAC	(+58)	Jβ1.6
TCCGACTGGCATGACCCATTC	(+56)	Jβ2.2
TCCGACTGGCACGACCCGCTC	(+58)	Jβ2.6
GTCCGAGTGCCAATGTCCATTC	(+52)	Jβ2.7

TCRB tubes B and C: Jβ B primers

3' 5'		
AGTGGCACGATCCATTCTTCC	(+59)	Jβ2.1
ACTGTCACGAGCCATTCGCCC	(+58)	Jβ2.3
AGAGTCACGACCCATTCGACC	(+59)	Jβ2.4
CACGAGCCACACGCGC	(+57)	Jβ2.5



Dβ1 primer

Dβ2 primer

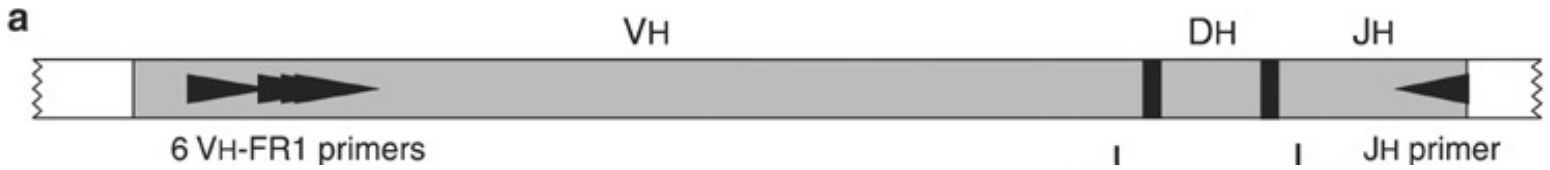
Jβ primers

TCRB tube C

		5' 3'		5' 3'
Dβ1	(-252)	GCCAAACAGCCTTACAAAGAC	Dβ2 (-137)	TTTCCAAGCCCCACACAGTC

BIOMED-2 report: Leukemia 2003; 17: 2257-2317

Analysis of *IG* gene rearrangements



BIOMED-2 clonality strategy

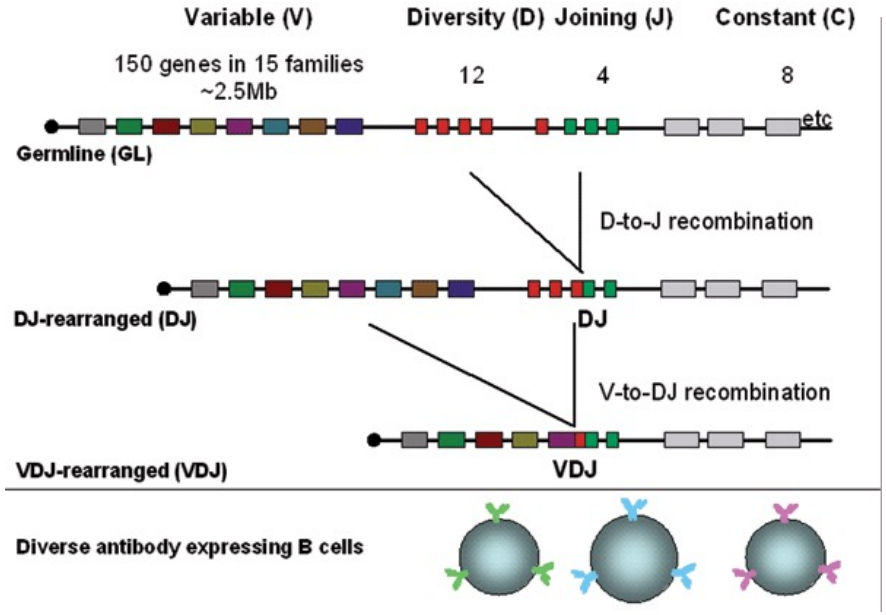
Suspected
B-cell lymphoma

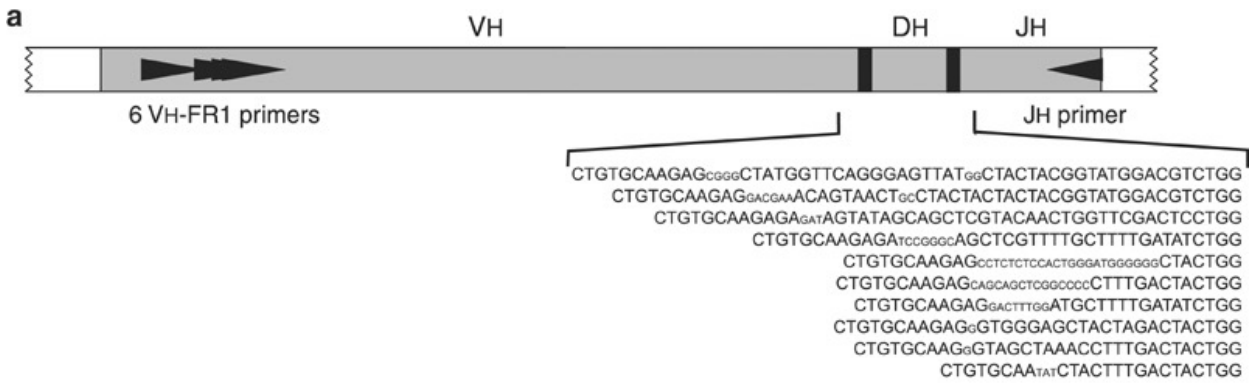
IGH V(D)J FR1, FR2, FR3
IGH DJ(A)
IGK-VJ and DE

Suspected lymphoma
of unknown origin

Suspected
T-cell lymphoma

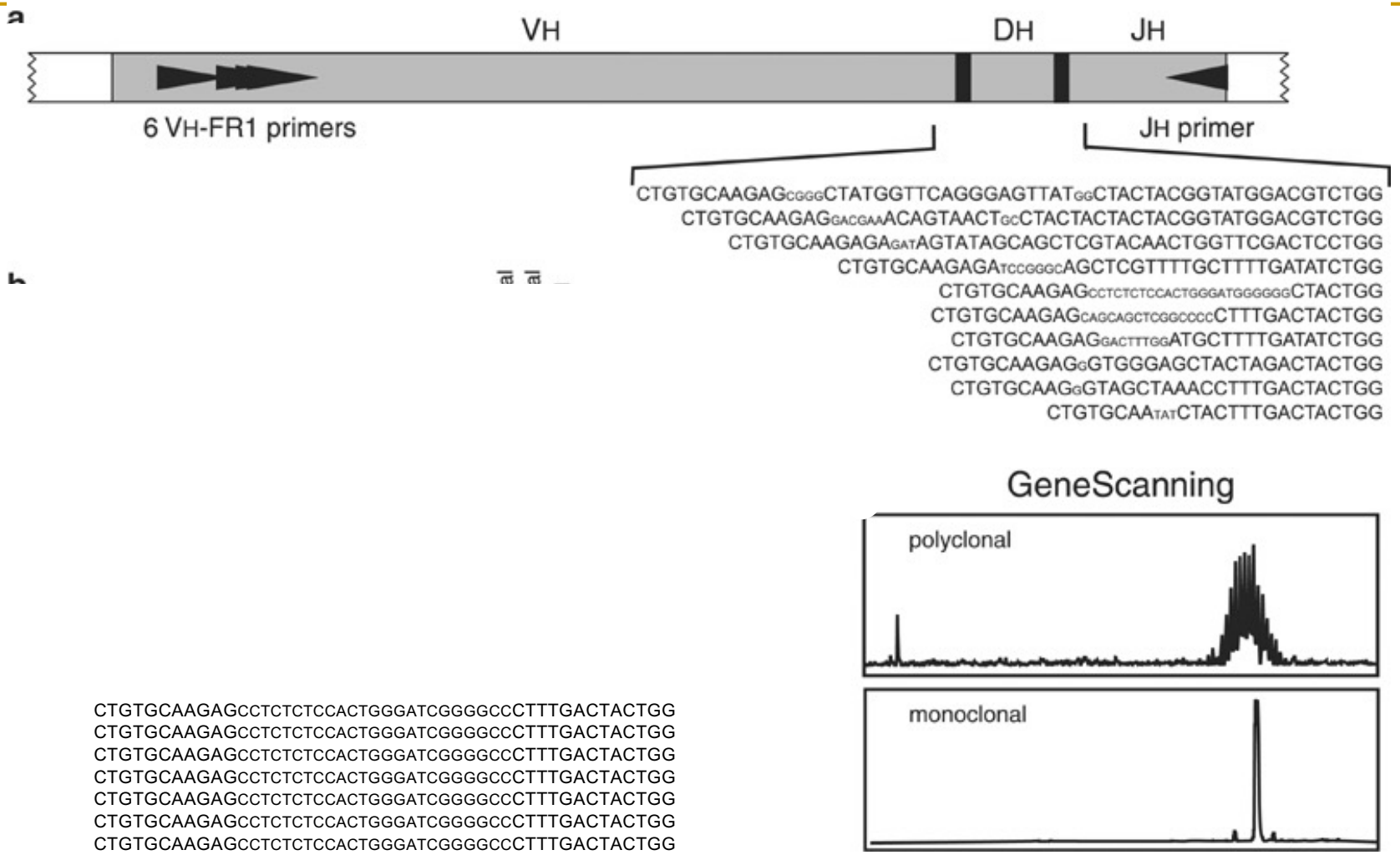
TCRGVJ (A and B)
TCRB V(D)J (A and B)
TCRB DJ



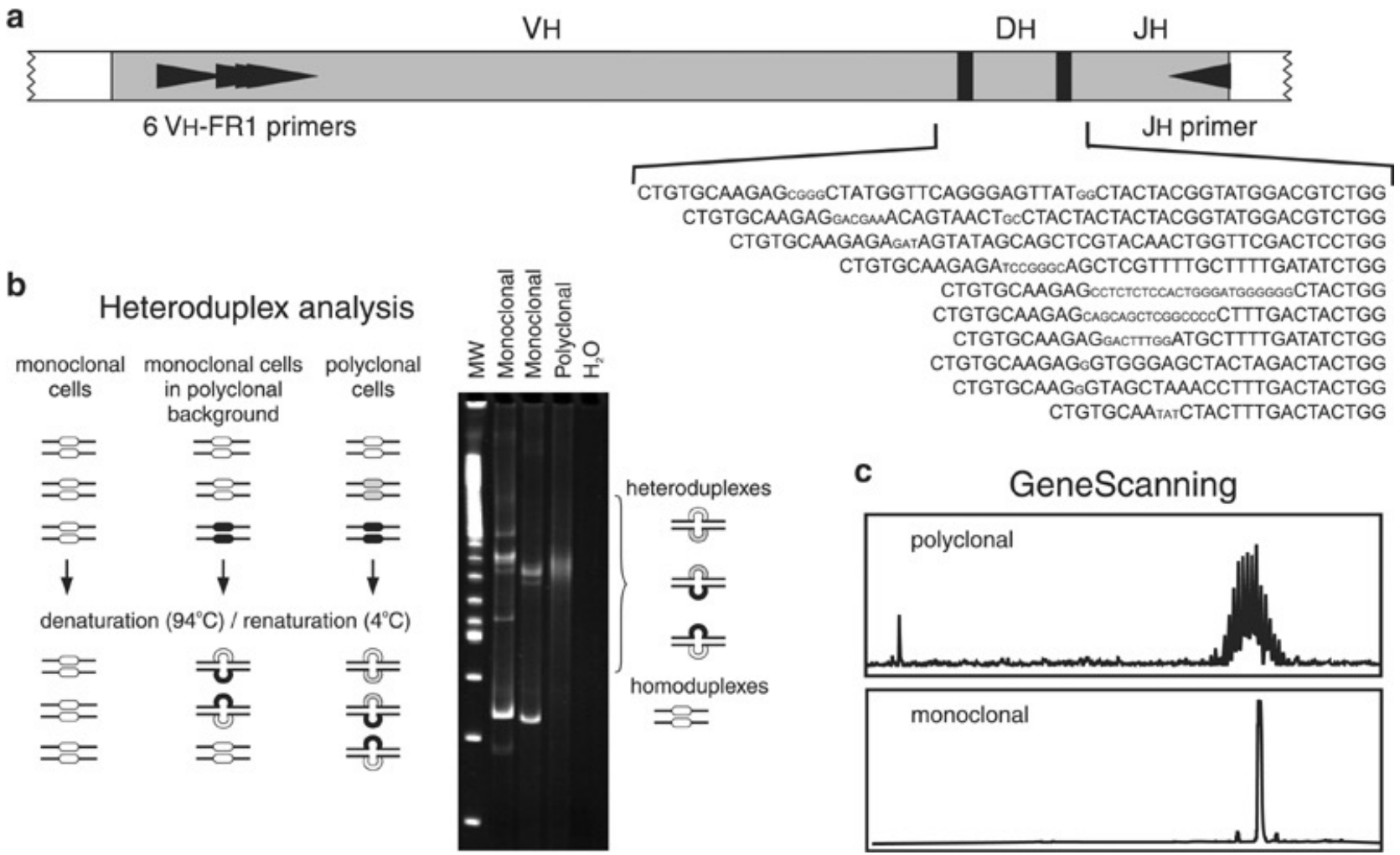


CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
CTGTGCAAGAGCCTCTCTCCACTGGGATCGGGGCCCTTTGACTACTGG
.....

Van Dongen et al Leucemia 2003



Van Dongen et al Leucemia 2003



Van Dongen et al Leucemia 2003

Analysis of *TCRB* gene rearrangements

Vβ family primers

Jβ primers

TCRB tubes A and B

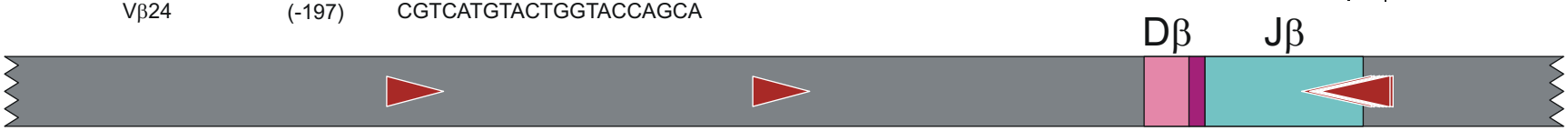
		5'	3'
Vβ2	(-204)	AACTATGTTTTGGTATCGTCA	
Vβ4	(-201)	CACGATGTTCTGGTACCGTCAGCA	
Vβ5/1	(-197)	CAGTGTGTCCTGGTACCAACAG	
Vβ6a/11	(-201)	AACCCTTTATTGGTACCGACA	
Vβ6b/25	(-201)	ATCCCTTTTTTGGTACCAACAG	
Vβ6c	(-201)	AACCCTTTATTGGTATCAACAG	
Vβ7	(-198)	CGCTATGTATTGGTACAAGCA	
Vβ8a	(-201)	CTCCCGTTTTCTGGTACAGACAGAC	
Vβ9	(-198)	CGCTATGTATTGGTATAAACAG	
Vβ10	(-201)	TTATGTTTACTGGTATCGTAAGAAGC	
Vβ11	(-198)	CAAAATGTACTGGTATCAACAA	
Vβ12a/3/13a/15	(-198)	ATACATGTACTGGTATCGACAAGAC	
Vβ13b	(-198)	GGCCATGTACTGGTATAGACAAG	
Vβ13c/12b/14	(-198)	GTATATGTCCTGGTATCGACAAGA	
Vβ16	(-201)	TAACCTTTATTGGTATCGACGTGT	
Vβ17	(-198)	GGCCATGTACTGGTACCGACA	
Vβ18	(-201)	TCATGTTTACTGGTATCGGCAG	
Vβ19	(-201)	TTATGTTTATTGGTATCAACAGAATCA	
Vβ20	(-193, inv)	CAACCTATACTGGTACCGACA	
Vβ21	(-201)	TACCCTTTACTGGTACCGGCAG	
Vβ22	(-201)	ATACTTCTATTGGTACAGACAAATCT	
Vβ23/8b	(-201)	CACGGTCTACTGGTACCAGCA	
Vβ24	(-197)	CGTCATGTACTGGTACCAGCA	

TCRB tubes A and C: Jβ A primers

3'	5'	
GTGGTCTAAGTGTCAACATCCATTC	(+53)	Jβ1.1
CTGGTCCAATTGGCAACATCCATTC	(+53)	Jβ1.2
TTCAACCGAGTGACAACATCCATTC	(+55)	Jβ1.3
CTTGGGTCGAGAGACAGAACCCATAC	(+56)	Jβ1.4
CTGAGCTGAGAGGTAGGATCCATTC	(+55)	Jβ1.5
GTCCGAGTGACACTGTCCATAC	(+58)	Jβ1.6
TCCGACTGGCATGACCCATTC	(+56)	Jβ2.2
TCCGACTGGCACGACCCGCTC	(+58)	Jβ2.6
GTCCGAGTGCCAATGTCCATTC	(+52)	Jβ2.7

TCRB tubes B and C: Jβ B primers

3'	5'	
AGTGGCACGATCCATTCTTCC	(+59)	Jβ2.1
ACTGTCACGAGCCATTCGCCC	(+58)	Jβ2.3
AGAGTCACGACCCATTCGACC	(+59)	Jβ2.4
CACGAGCCACACGCGC	(+57)	Jβ2.5



Dβ1 primer

Dβ2 primer

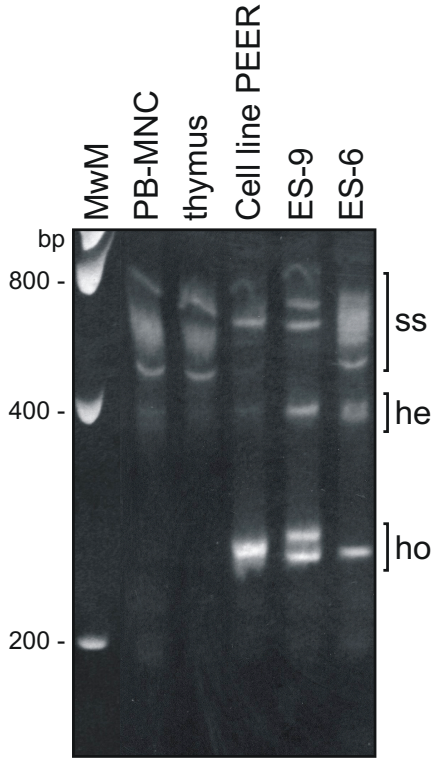
Jβ primers

TCRB tube C

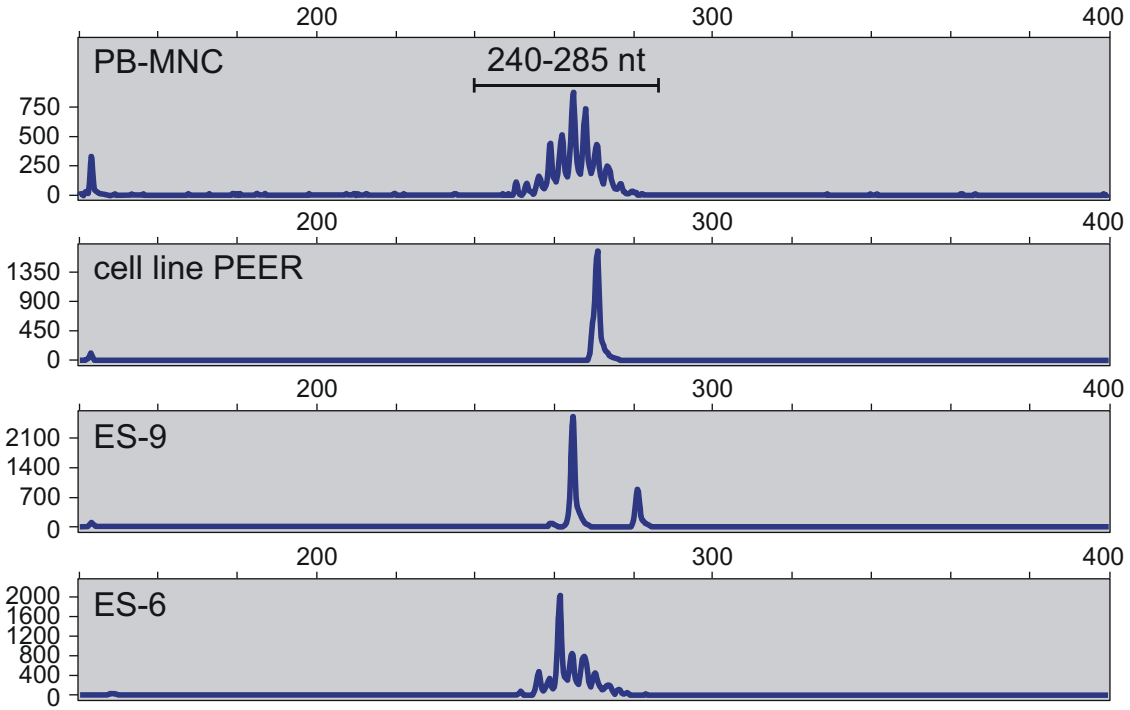
		5'	3'		5'	3'
Dβ1	(-252)	GCCAAACAGCCTTACAAAGAC		Dβ2	(-137)	TTTCCAAGCCCCACACAGTC

BIOMED-2 report: Leukemia 2003; 17: 2257-2317

Analysis of *TCRB* gene rearrangements



TCRB tube B Vβ-Jβ



From patient to analysis to patient

- Selection of material
- Protocol
- Check DNA quality
- Clonality analysis
- Control check
- Interpretation

Examples of cases

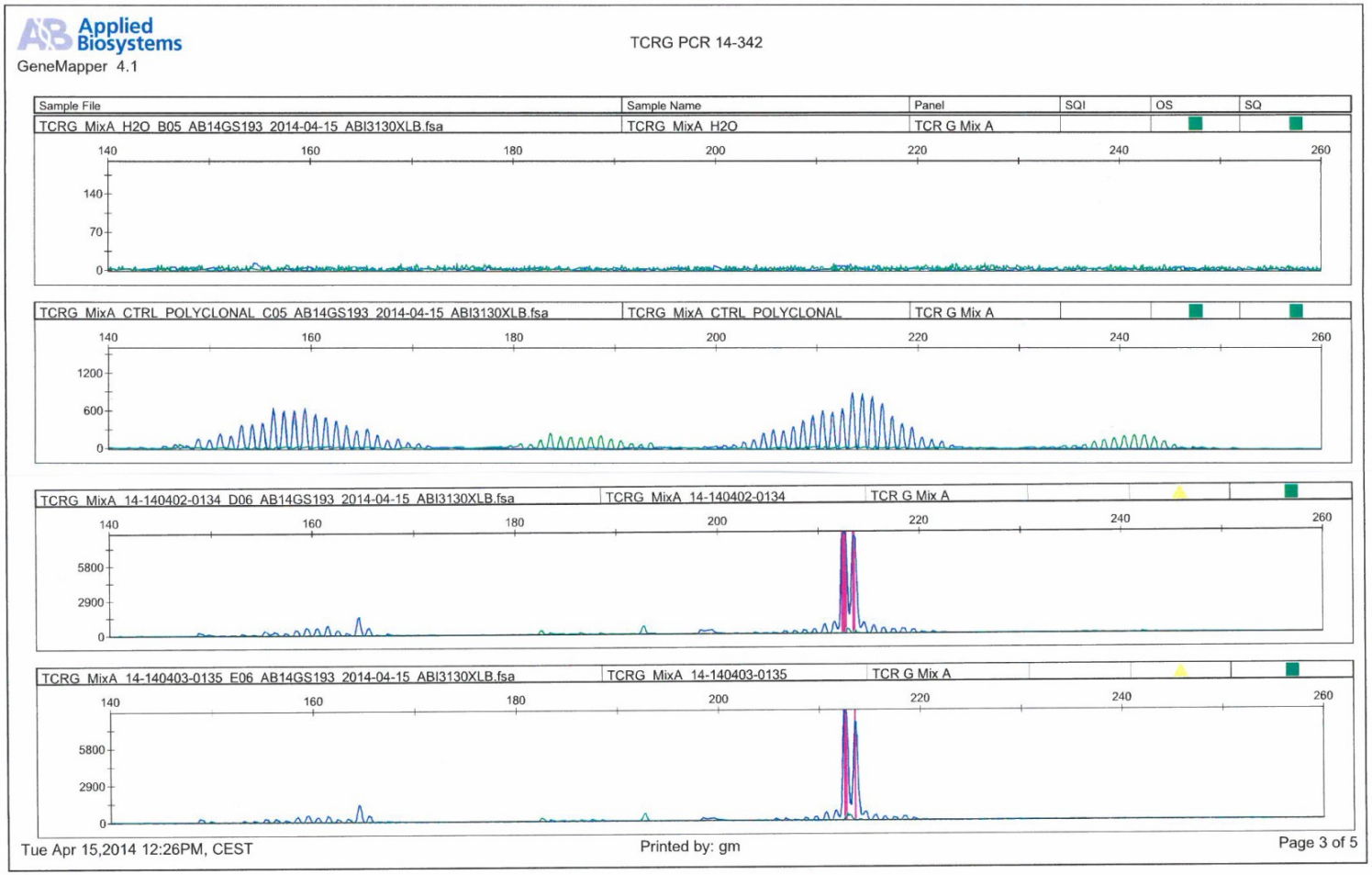
- Case 1: Lymphoma. Origine?
- Case 2: Relapse?
- Case 3: Lymphoma? Reactive?

Case 1:

Male
75 years
Lymphoma origine?
2 biopsies

First results:
FR1, 2, 3 kappa polyclonal

Case 1: TCRG

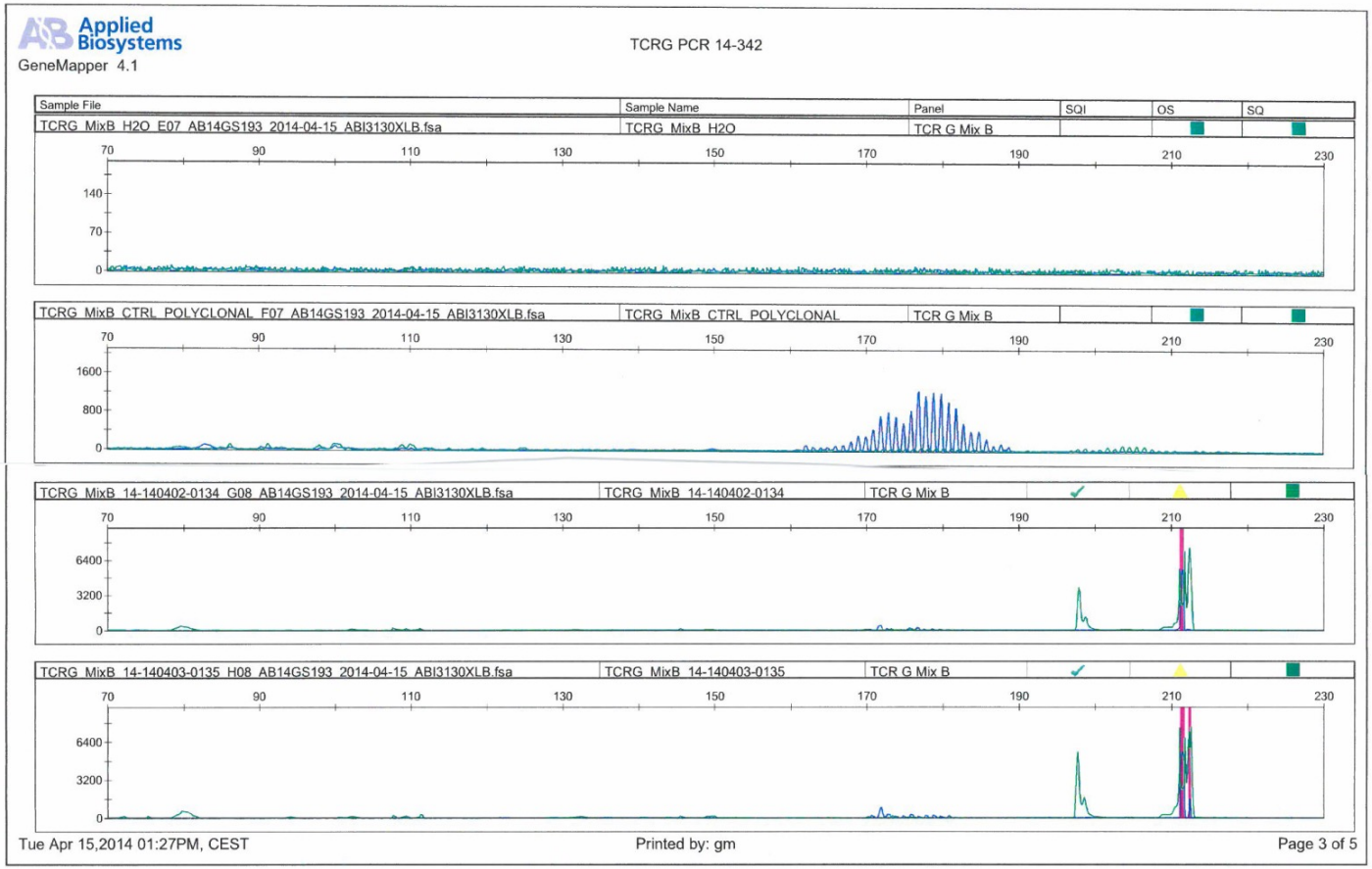


Belgian Society of Human Genetics

Unilab Lg.

CHU de Liège

Case 1: TCRG



Belgian Society of Human Genetics

Unilab Lg.

CHU de Liège

Case 1: Results

IgH et kappa polyclonal
TCR gamma clonal

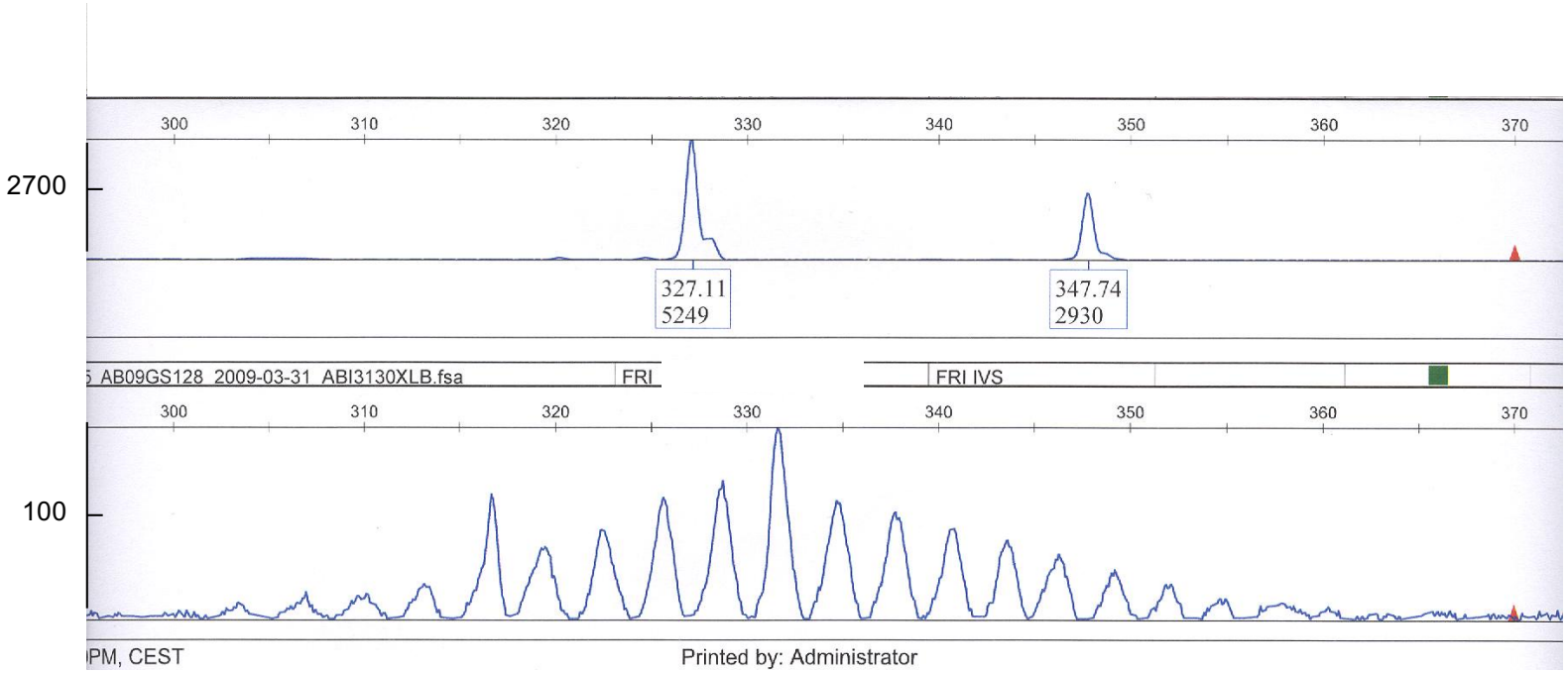
Conclusion of the molecular results:

Clonal rearrangements of the TCRgamma gene were detected in this specimen.
This gene rearrangement profile fits to the presence of a clonal T-cell population.

Case 2

- Female
- 81 years
- Biopsie
- Lymphoma?

Case 2



3/2009
biopsie
FR1
327,11 bp

control

Case 2: Results

Controls ok
B-cell targets:
IGH(VDJ) FR1 clonal
FR2 clonal

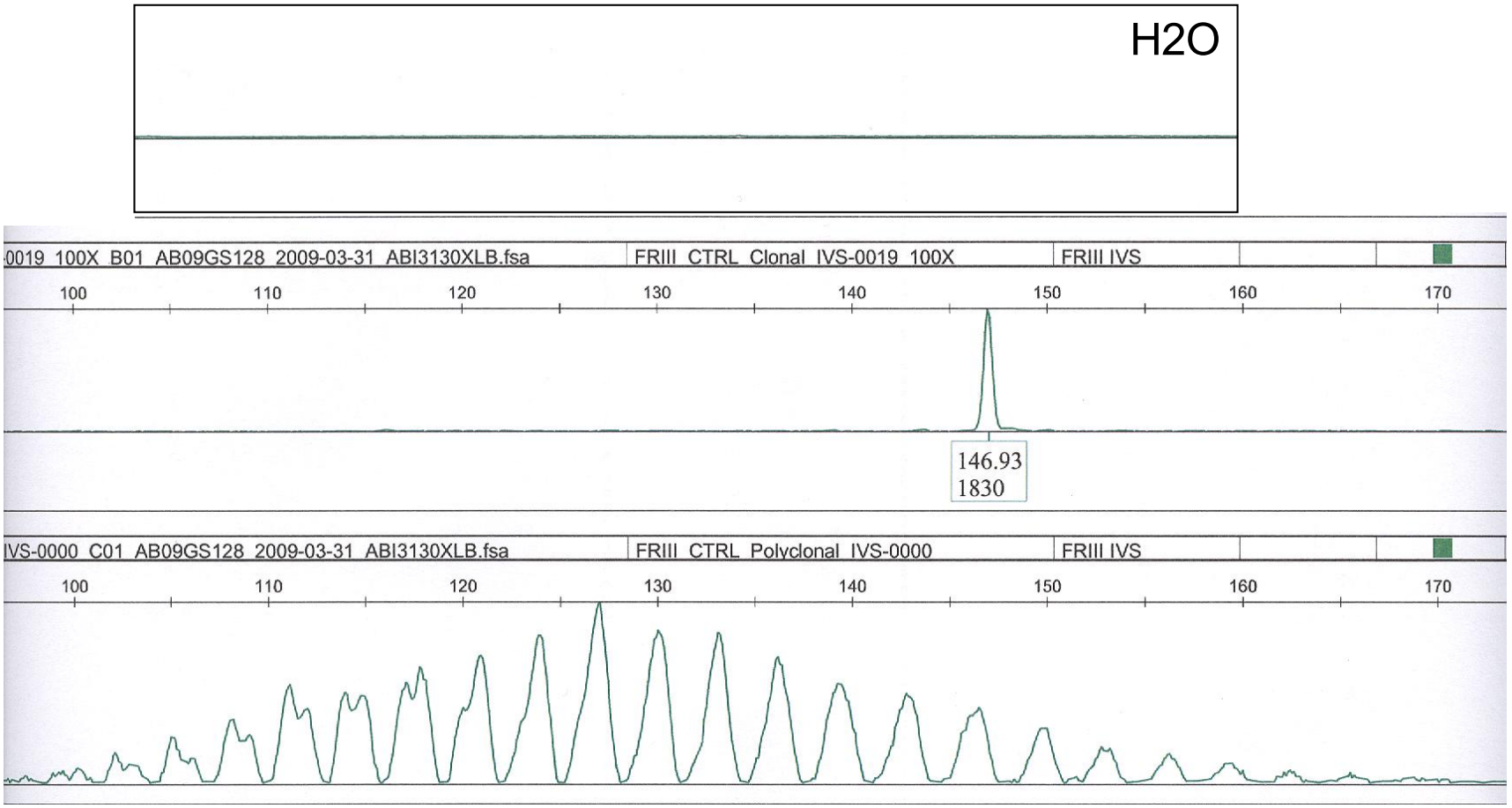
Conclusion of the molecular results:

Clonal rearrangements of the IG gene
were detected in this specimen.
This gene rearrangement profile fits to the presence of a clonal B-cell population/B-NHL.

Case 3

- Female
- 63 years
- Lymphoma in dec. 2004
- FR1 polyclonal, FR3 clonal
- Feb. 2009 biopsy
- Relapse?

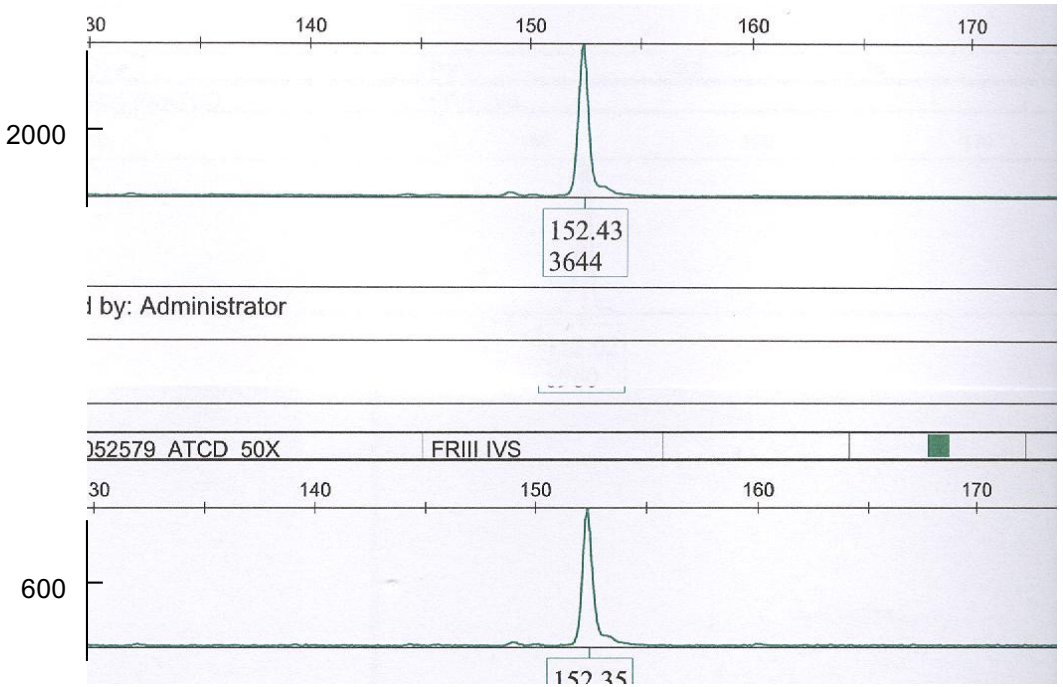
Case 3: GeneScan analysis of controls



Case 3: Relapse?

2/2009
Biopsie
FR3: 152 bp

12/2004
Biopsie
FR3: 152 bp



Case 3: Results

Controls ok
B-cell targets:
IGH(VDJ) FR3 clonal

Conclusion of the molecular results:

Clonal rearrangement of the IG gene
was detected in this specimen.
This gene rearrangement profile **could be** identical to the one detected in the biopsie of 12/2004 and **seems to** confirm the relapse of the disease.

2022

Case would be done by NGS

Advantages:

- Identification of the DNA sequence of clonal rearrangement
- Information if the clone is identical

Case 1 and 2 for clonal identification at diagnosis NGS is not necessaire

NGS Lymphotrack workflow (lymphotrack)

DNA

Amplification

Purification PCR products

Library quantification

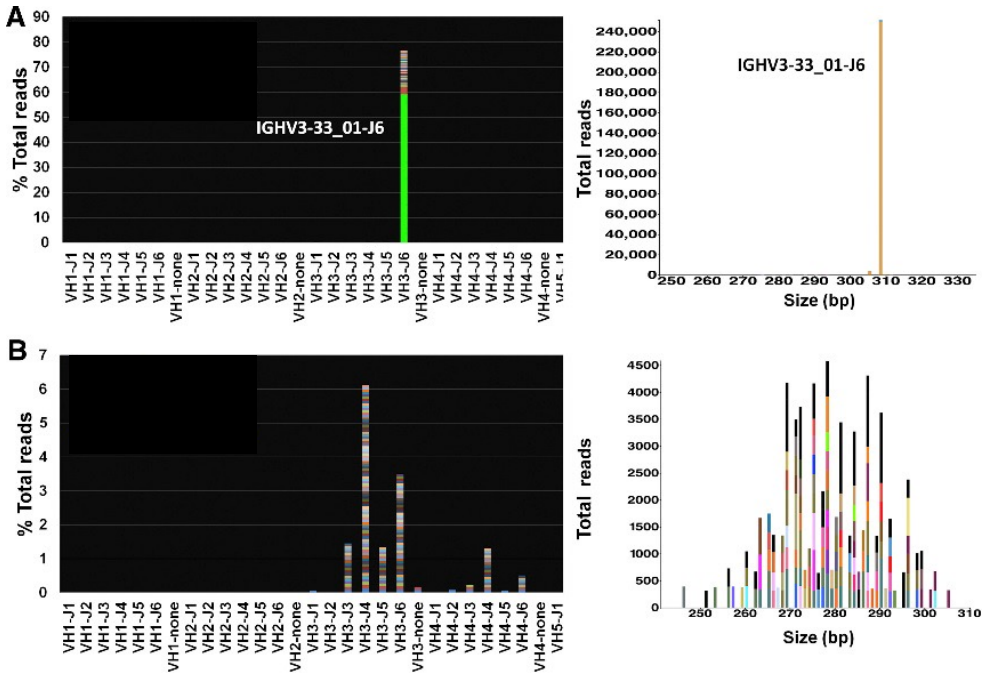
Run prep

FASTQ files

Analysis

NGS results with the lymphotrack IVS

Sample	Reads total	Rank	Sequence	Length	V-gene	J-gene	% total reads	in-frame	v-coverage
Control pos	183754	1	CATCTGGAT…	295	IGHV1-46_03	IGHJ4_02	2,54	Y	100
		2	CATCTGGAT…	295	IGHV1-46_03	IGHJ4_02	0,08	Y	
		3	GCCTCTGGG…	146	IGHV3-73_02	IGHJ2_01	0,04	N	
		4	GCCTCTGGG…	174	IGHV3-64_01	IGHJ5_02	0,04	N	
Sample D0108	148898	1	GCCTCTGGA…	272	IGHV3-23_04	IGHJ1_01	8,72	Y	100
		2	GCCTCTGCA…	291	IGHV3-48_01	IGHJ5_02	1,30	Y	
		3	GCCTCTGGA…	272	IGHV3-23_04	IGHJ1_01	1,15	Y	
		4	GCCTCTGCA…	291	IGHV3-48_01	IGHJ5_02	0,83	Y	
Sample D0082	63025	1	CGCTGTCTA…	273	IGHV4-34_02	IGHJ2_01	0,34	Y	100
		2	GCGTCTGGA…	278	IGHV3-30_02	IGHJ4_02	0,31	Y	
		3	CGCTGTCTA…	273	IGHV4-34_02	IGHJ2_01	0,28	Y	
		4	GCCTCTGGA…	150	IGHV3-64_05	IGHJ4_02	0,24	Y	



clonal

polyclonal

Adapted from M.E. Arcile et al. The Journal of Molecular Diagnostics

Case 3

- Female
- 63 years
- Lymphoma in dec. 2004
- FR1 polyclonal, FR3 clonal
- Feb. 2009 biopsy
- Relapse?

This gene rearrangement profile **could be** identical to the one detected in the biopsie of 12/2004 and **seems to** confirm the relapse of the disease.

By NGS: result!

Advances of NGS clonality testing:

- Determines the sequence of clonal rearrangements
- Reduce subjectivity in interpretation
- Identifies several clonal populations, if present
- Allows Ig and TCR analyses in the same run
- MRD possible

MRD (Minimal Residual Disease) analysis by NGS

Need of the sequence of the diagnostic sample

Spiking control: MRD quantification

LymphoTrack
MRD Report

Sequence #1	Sequence Name:	Sample			
Replicate:	9	MRD Status	Sequence Detected (1)	Reads in Replicate	915981 (2)

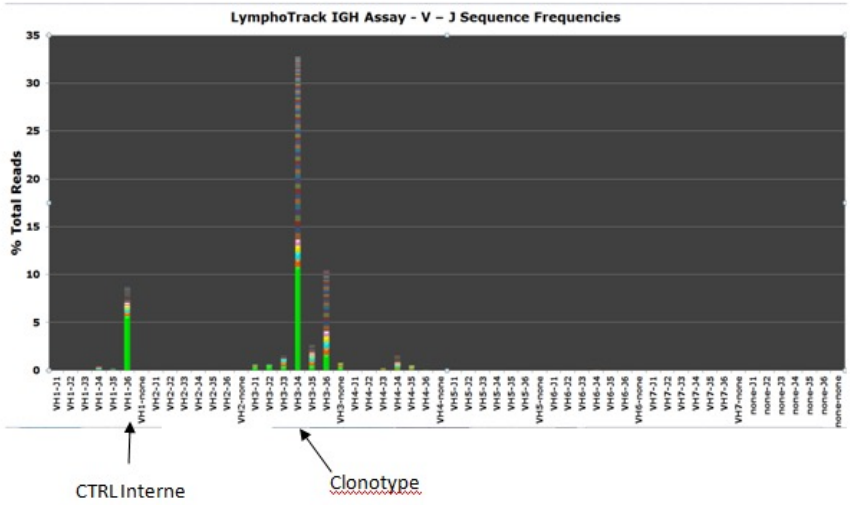
CATCTGGATACACCTTCACCAGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGAATAATCAACCCTAGTGGTGGTAGCACAAGCTACGCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGGGA
CACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCTA
GAGATCTCACAGGTTGTATTAGTACCAGCTGCTATCCTCCGAACTACTTTGACTACTGGGGCCAGGGAACCCT

Replicate Details:	Read Count	Cumulative Read Count:	Cumulative Read Frequency
Exact Match	(3) 71	71	7.75E-5
1 Mismatch	40	111	1.21E-4
2 Mismatch	10	121	1.32E-4 (4)

Detection Limit:	% Confidence
1e-3	NA
1e-4	NA
1e-5	NA
1e-6	NA

Image of detected sequences

170322-0041



MRD

Same kit as for clonality detection
Internal control
Sensitivity only limited by DNA input

Search for sequence of diagnosis

Follow-up patient during treatment: efficancy of the treatment
Follow up in remission (monitor patient)
Detect recurrence

Good sensitivity and specificity
Kits available
Sequence identity
Track multiple clones

Discrimination between clonal and polyclonal Ig/TCR gene PCR products

GeneScanning analysis

- ☝ Fast, accurate, sensitive, monitoring of clonal proliferations
- 👎 Need sequence equipment

Heteroduplex analysis

- ☝ Available to most laboratories
- 👎 Sensitivity ~5-10%

NGS (Next Generation Sequencing) analysis

- ☝ Accurate, easy interpretation, very sensitive, determination of the DNA sequence of clonal rearrangement, quantitative monitoring of clonal proliferations possible
- 👎 Need NGS equipment, expensive

Use of clonality analysis

1. Making the diagnosis

   Normal ↔ reactive ↔ malignant
2. Assessment of remission and relapse

   Normal ↔ reactive ↔ malignant
3. Involvement (staging)
4. Evaluation of treatment effectiveness

   Detection of minimal residual disease (MRD)

   MRD-based risk-group stratification (treatment reduction or treatment intensification)

4. Take home message

- Gene rearrangements of the antigen receptor genes occur during the lymphoid proliferation
- These gene rearrangements generate products that are unique in length and sequence in each cell.
- In a clonal population (from one cell) all cells have the identical gene rearrangement.
- Unique length allows by PCR discrimination of clonality and polyclonality.
- Standardized protocol.
- NGS has advandages in the follow up of patients (MRD)