PRACTICAL APPLICATIONS OF MOLECULAR BIOLOGY IN SURGICAL PATHOLOGY

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Tumor types

- Lymphoma
- G.I. tract

I. Lymphoma

- **B and T cell monoclonalities**

  - Rearrangement of immunoglobin and TCR genes.

- **Identification of non-random chromosomal abnormalities detectable by PCR**

  - t(14;18) or t(11;14) translocations in FL and MCL respectively.

B and T cell monoclonality

- Genotype does not correspond to phenotype !

Lineage infidelity of Ig and TCR gene rearrangements ("Illegitimate rearrangements"):

- **50-60 % of lymphoblastic B cell malignancies.**
- **20-30% of lymphoblastic T cell malignancies.**
- **~10% of mature B and T cell malignancies.**

Therefore, Ig and TCR gene rearrangements cannot be used as markers for B and T cell lineages, respectively.

B and T cell monoclonality

- Monoclonality is not always equivalent to malignancy !

  - Clinically benign lymphoproliferations may consist of clonal cell populations.

  - Although this pitfall is encountered in B cells, it is mainly observed in T cell monoclonality (cf limited combinatorial diversity of TCR-γ and -δ genes)

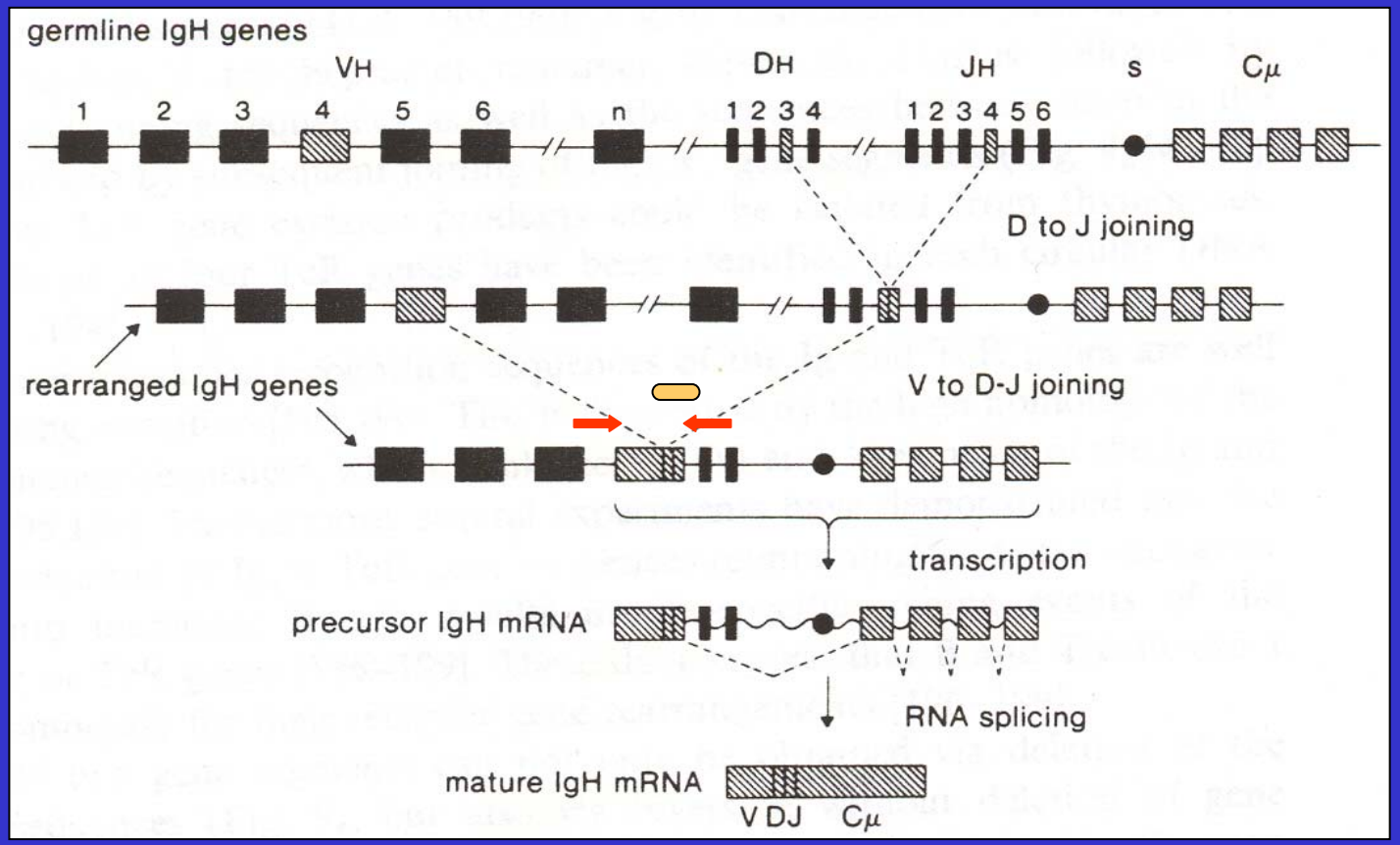
B and T cell monoclonality

Molecular tools

- Southern Blot

- PCR

Schematic diagram of IgH gene rearrangements

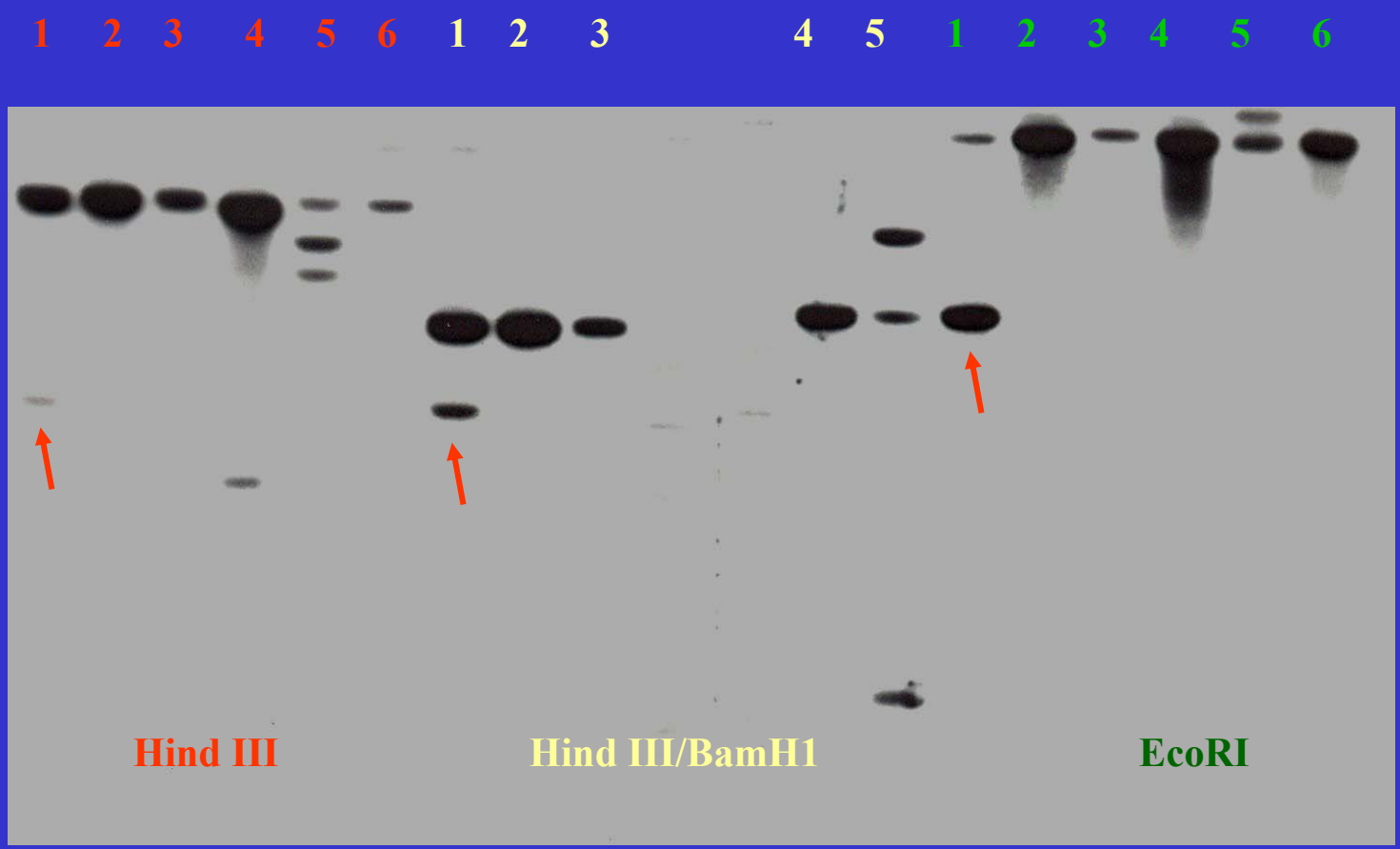


: indicate the JH probe location for Southern Blot method

: indicate the primers location for PCR method

B cell monoclonality - Southern Blot

Illustration



Southern Blot

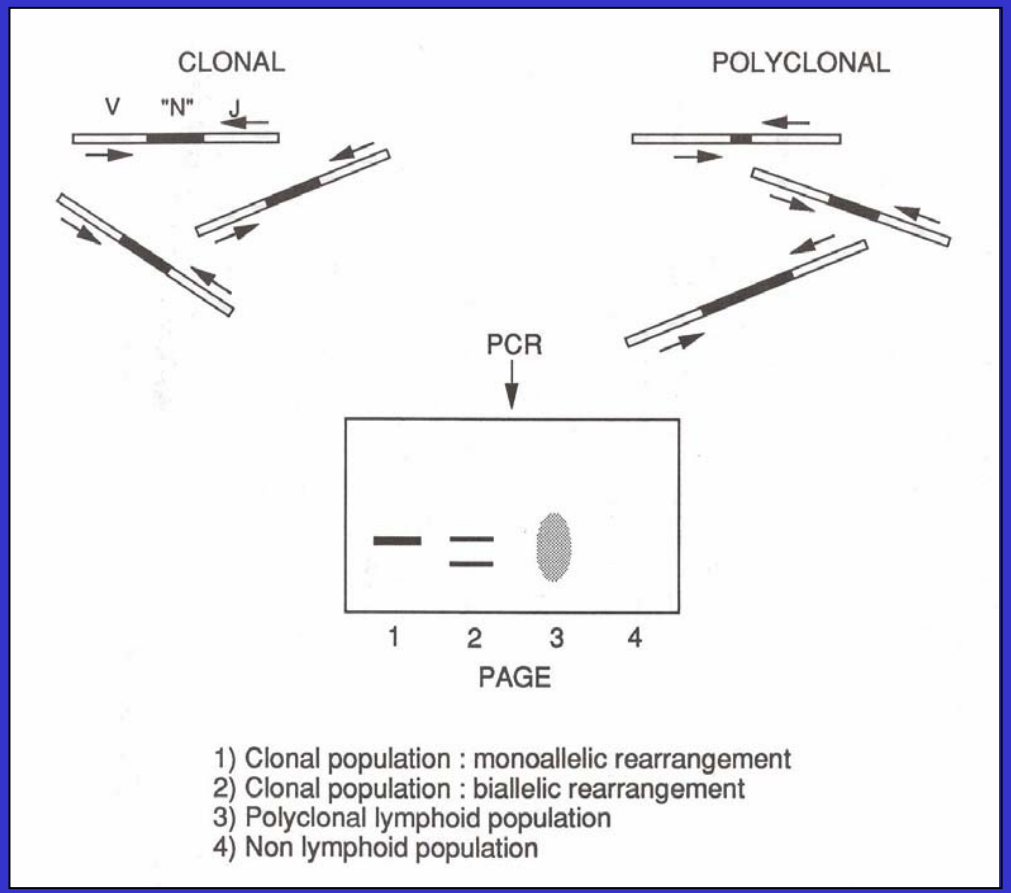
Advantages:

- Very good qualitative sensitivity since ~100% of B and T cell malignancies are detectable by Southern Blot

Disadvantages:

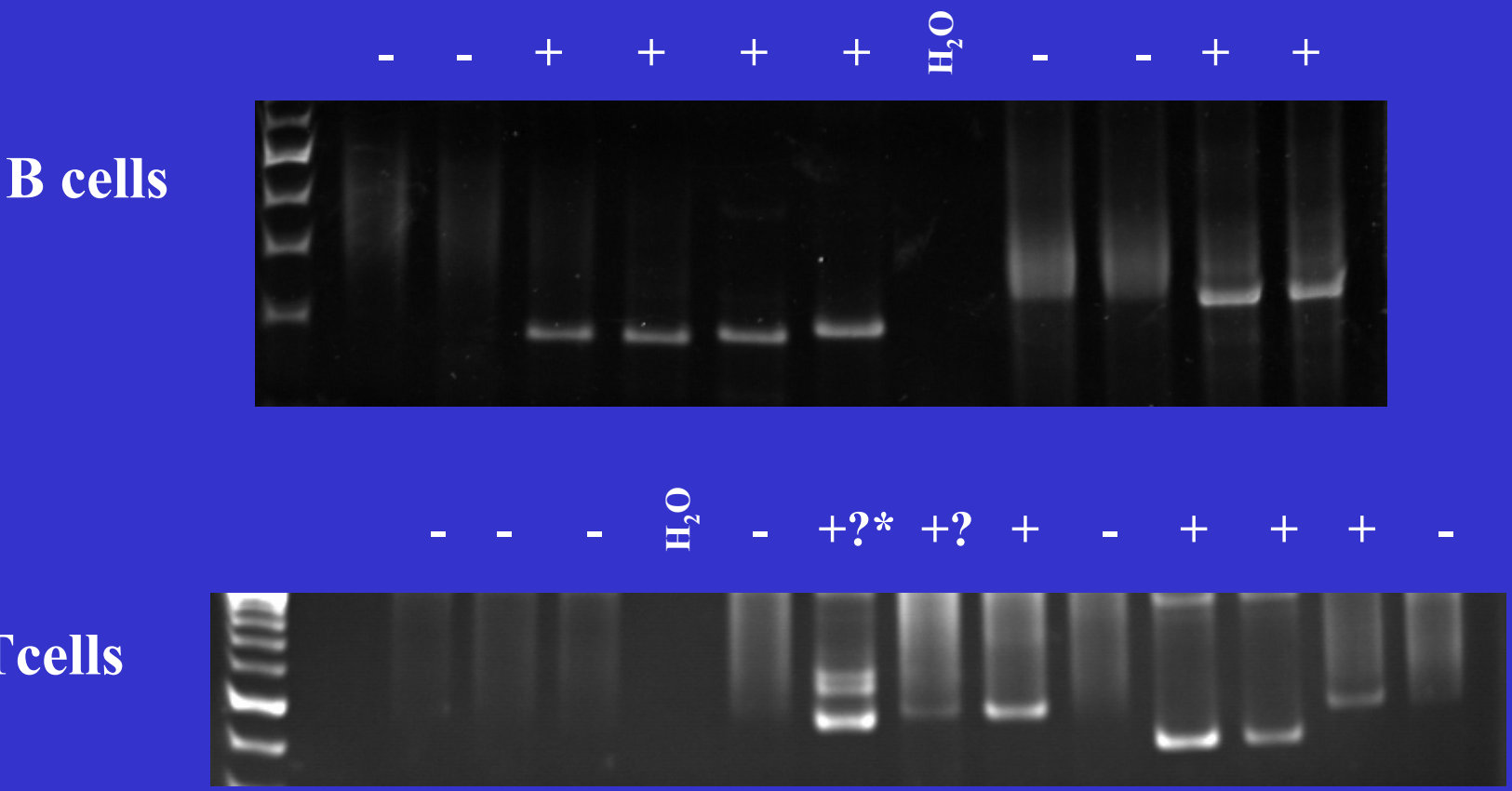
- time-consuming
- requires relatively large amounts of pathological material
- low quantitative sensitivity (~ 5 %)

Schematic representation of mono and polyclonal populations detected by PCR.



B and T cell monoclonalities - PCR

Illustration on paraffin embedded tissue



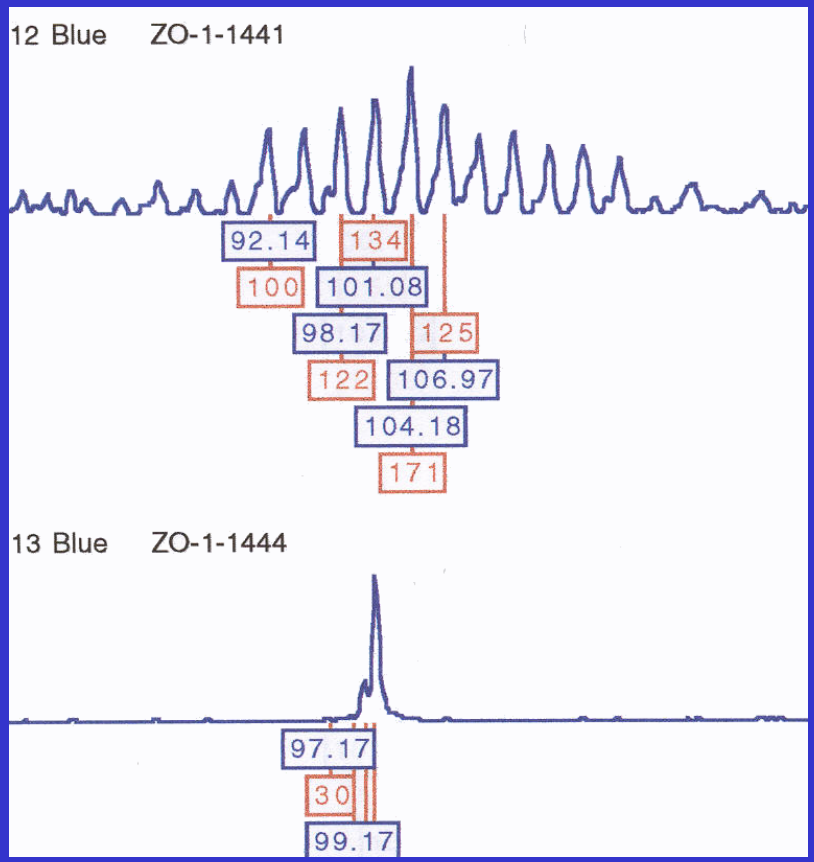
*? oligoclonality

B cell monoclonality - PCR

Illustration (Genescan)

polyclonality

monoclonality



PCR

Advantages: (vs Southern Blot)

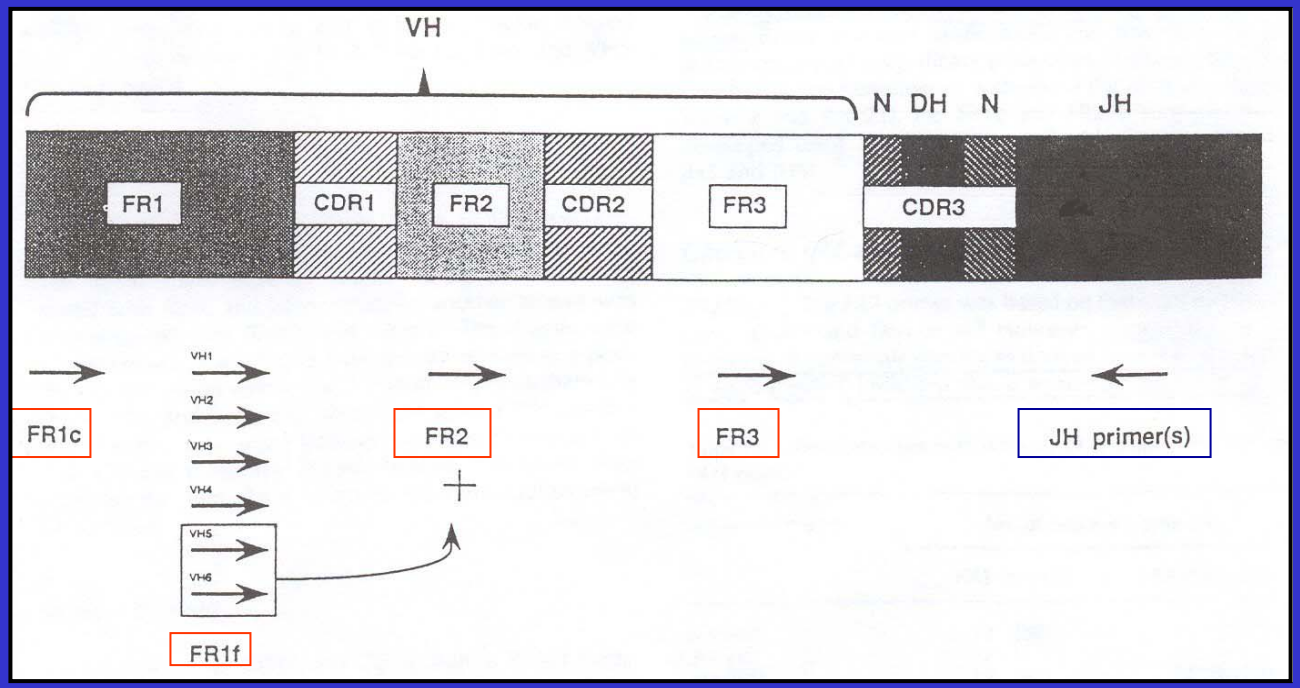
- simple and faster
- requires much less amounts of pathological material
- greater quantitative sensitivity
- can be applied on DNA paraffin-embedded tissue

Disadvantages: (vs Southern Blot)

- lower qualitative sensitivity
- need to use several different PCR strategies in order to increase the overall detection rate.

PCR strategies

- Necessity to use several sets of primers in order to increase the overall detection rate (~90 %) of the PCR method: FR3 -JH
  FR1c-JH
  FR1f-JH,...



- This detection rate varies according to the underlying disorders

Detection rates by PCR according to pathological subtypes

- SLL	**~100 %**
- MCL	**~100 %**
- SNCL	**~80 %**
- PCN	**~70 %**
- DLCL	**~60 %**
- LC (IBL)	**~50-60 %**
- LF	**~50 %**

PCR - Pitfalls

False negative:

- chromosomal translocations into the IgH locus (in FL or DLCL)
- Somatic mutation (in FL and DLCL)
- partial D-J rearrangements (in immature malignancies)
- no VDJ rearrangement produced (in immature malignancies)
- failure of the IgH primers to recognize the VH segment involved

False positive:

- very weak amount of DNA
- reactive lymphoid populations

B and T cell monoclonalities

- Some cases of unequivocal B-cells lymphoma do not generate a clonal signal by PCR despite a demonstrated clonality by Southern Blot.

- Any result must be interpreted in view of other findings and clinical informations

Recurrent molecular abnormalities in lymphoma

t(14;18) / Bcl2 - J_H	in follicular lymphoma
t(11;14) / Bcl1 - J_H	in Mantle Zone lymphoma
t(3;14) / Bcl6 - J_H	in Diffuse Large Cell lymphoma
t(8;14) / cMyc - J_H	in Burkitt lymphoma
t(2,5) / ALK-NPM	in Anaplastic Large Cell Lymphoma

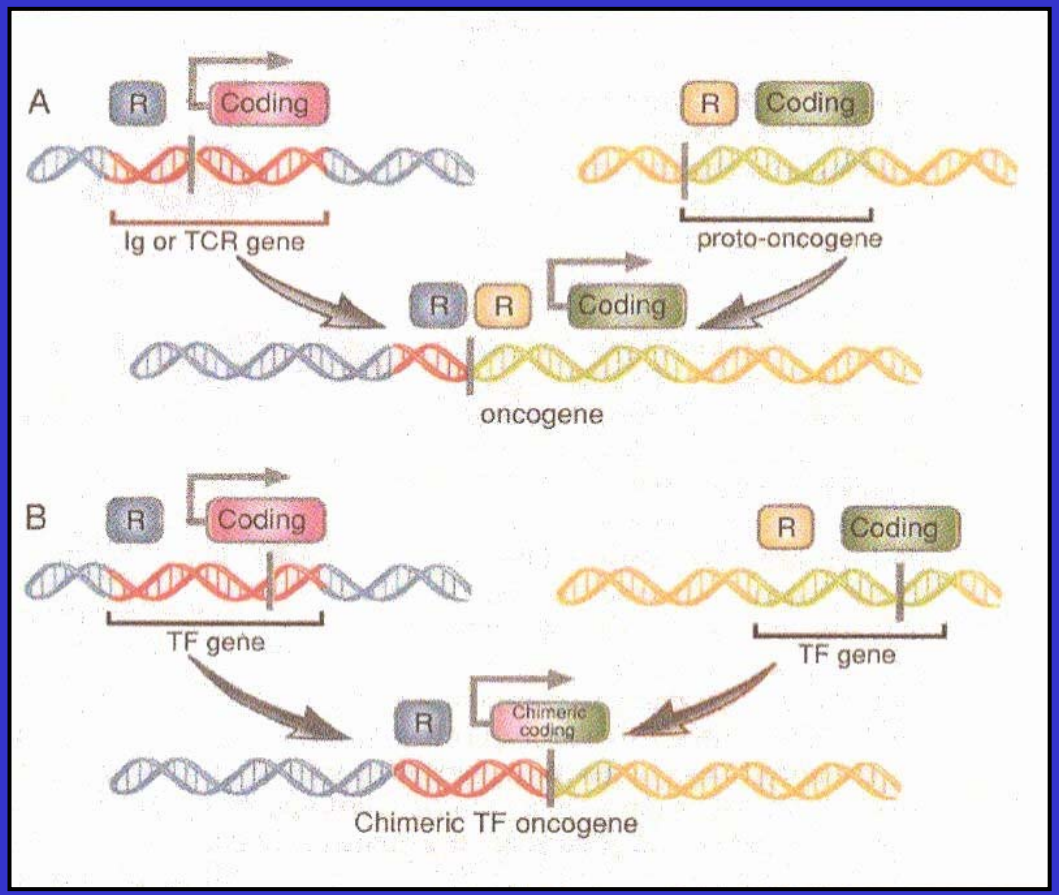
Two distinct types of chromosomal translocations at molecular level

A. Quantitative changes

Bcl2-J_H, Bcl1-J_H,…

B. Qualitative changes

ALK-NPM,...



Bcl2 in Follicular lymphoma

- t(14;18) detectable by cytogenetic in ~ 90 % of cases

- this translocation gives rise to an overexpression of the antiapoptotic Bcl2 protein

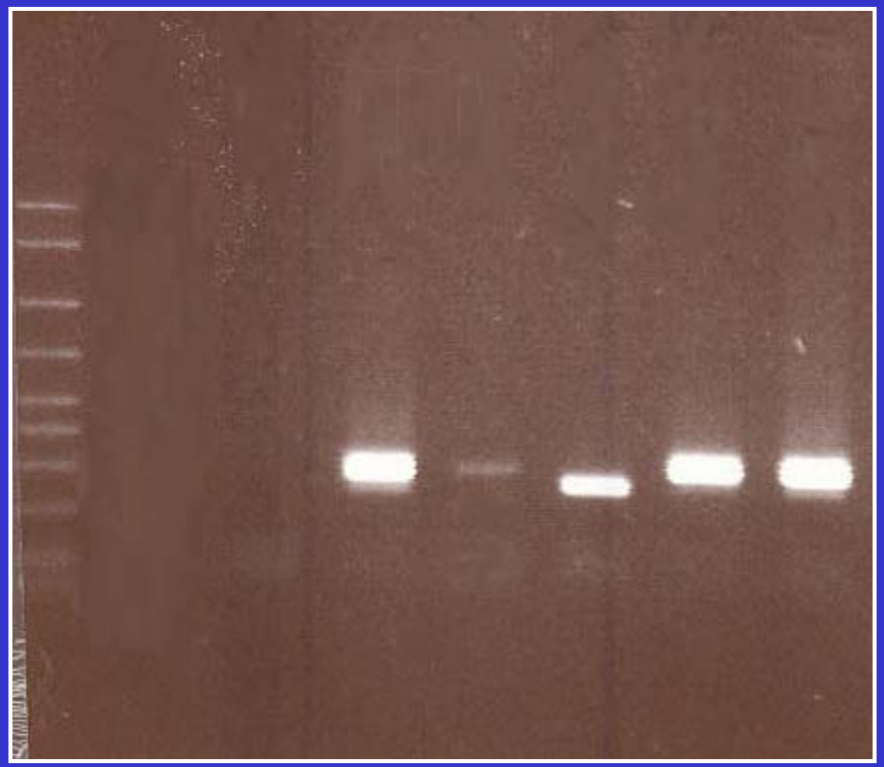
- four different breakpoints on Bcl2 gene

mbr	in ~ 45 % of cases
mcr	in ~ 7 % of cases
3'UTR	in ~ 10 % of cases
icr	in ~ 10% of cases

PCR Bcl2-JH in follicular lymphoma

Illustration

M H_2O - + + + + +



mbr breakpoint

Bcl2 in Follicular lymphoma

Method	Detection rate
FISH	**>95%**
Cytogenetic	~80-90%
S.B.	65-80%
PCR Bcl2 / mbr-JH	**40-50%**
PCR Bcl2 / mcr-JH	**~10%**

Bcl2 in Follicular lymphoma

False positivity in normal patients (~ 23 %) and in benign follicular hyperplasia where a very low percentage of positive cells (10^{-3} - 10^{-4}) are detectable by nested PCR ⟶ need to use a less sensitive method (standard PCR) to avoid false positive cases

Bcl2 in Diffuse Large Cell Lymphoma

Bcl2-JH rearrangement is detectable in ~35 % of DLCL and seems to be associated with a better prognosis

Bcl1 in Mantle Cell lymphoma

- t(11;14) detectable in ~ 100 % of MCL
  observed also in MM (~ 20 %)
  in SLVL (~ 20 %)

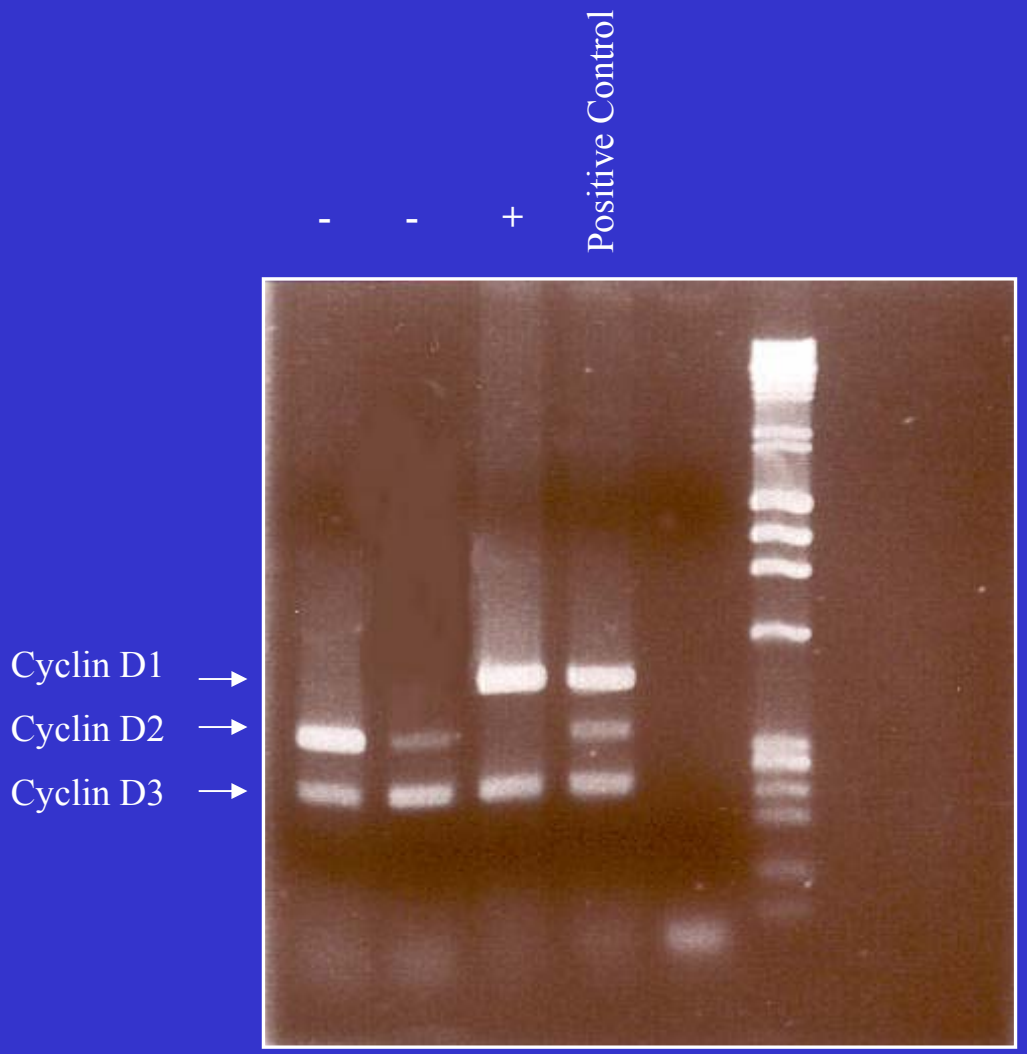
- this translocation gives rise to an overexpression of the Bcl1 gene encoding the cyclin D1 protein(positive cell cycle regulatory protein)

- the majority of the Bcl1 breakpoints are clustered in the MTC region

Bcl1 in Mantle Cell lymphoma

Method	Detection rate
FISH	**≥ 95 %**
Cytogenetic	50-80 %
S.B.	60-70 %
PCR Bcl1/MTC - J_H	40 %
Northern Blot	**100 %**
RT-PCR (cyclinD1 overexpression)	**100 %**

BCL1 Overexpression in MCL



<u>Competitive RT-PCR</u>:
Comparison of the different expression profiles of the three cyclins D1, D2 and D3

This method avoids any false positive results