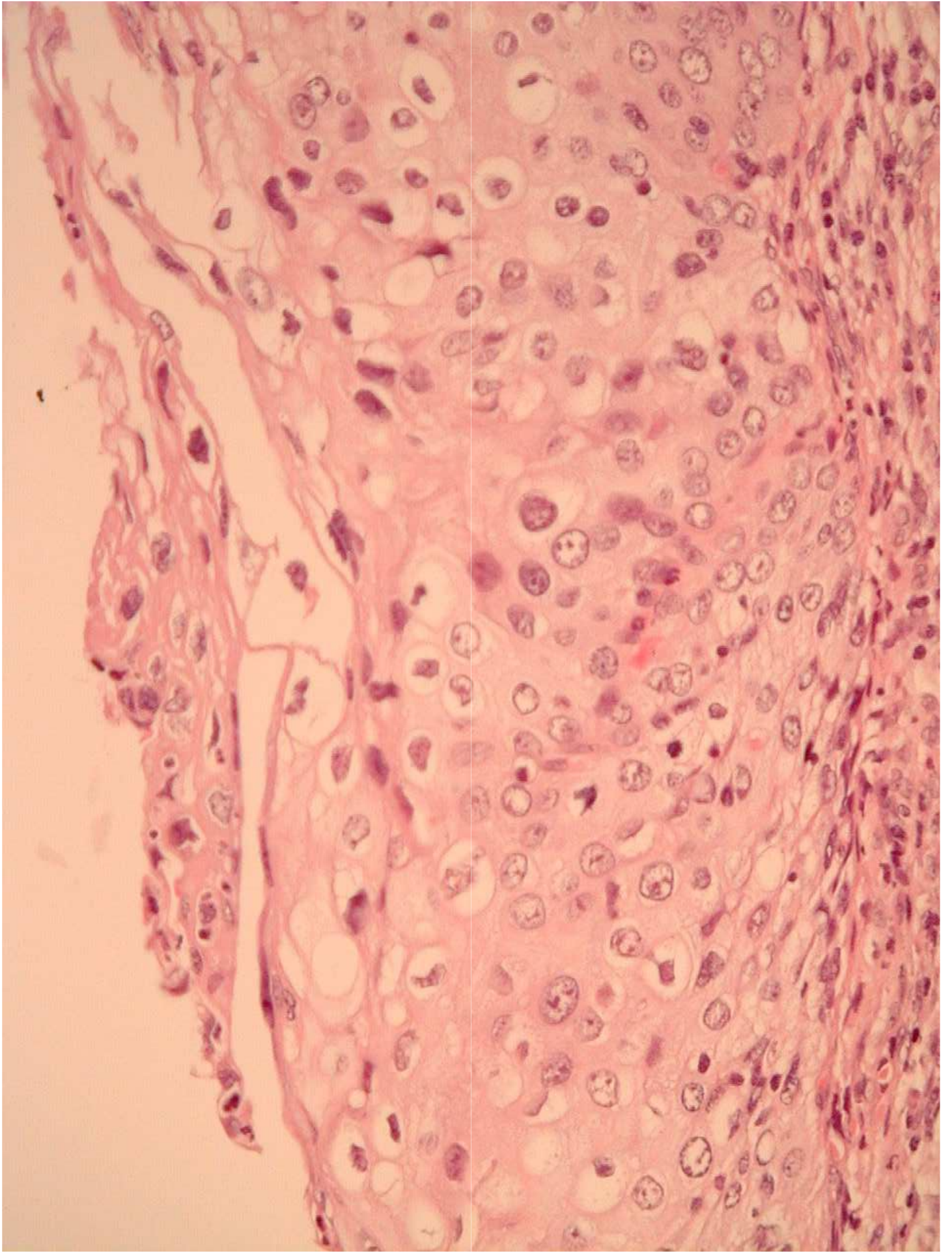


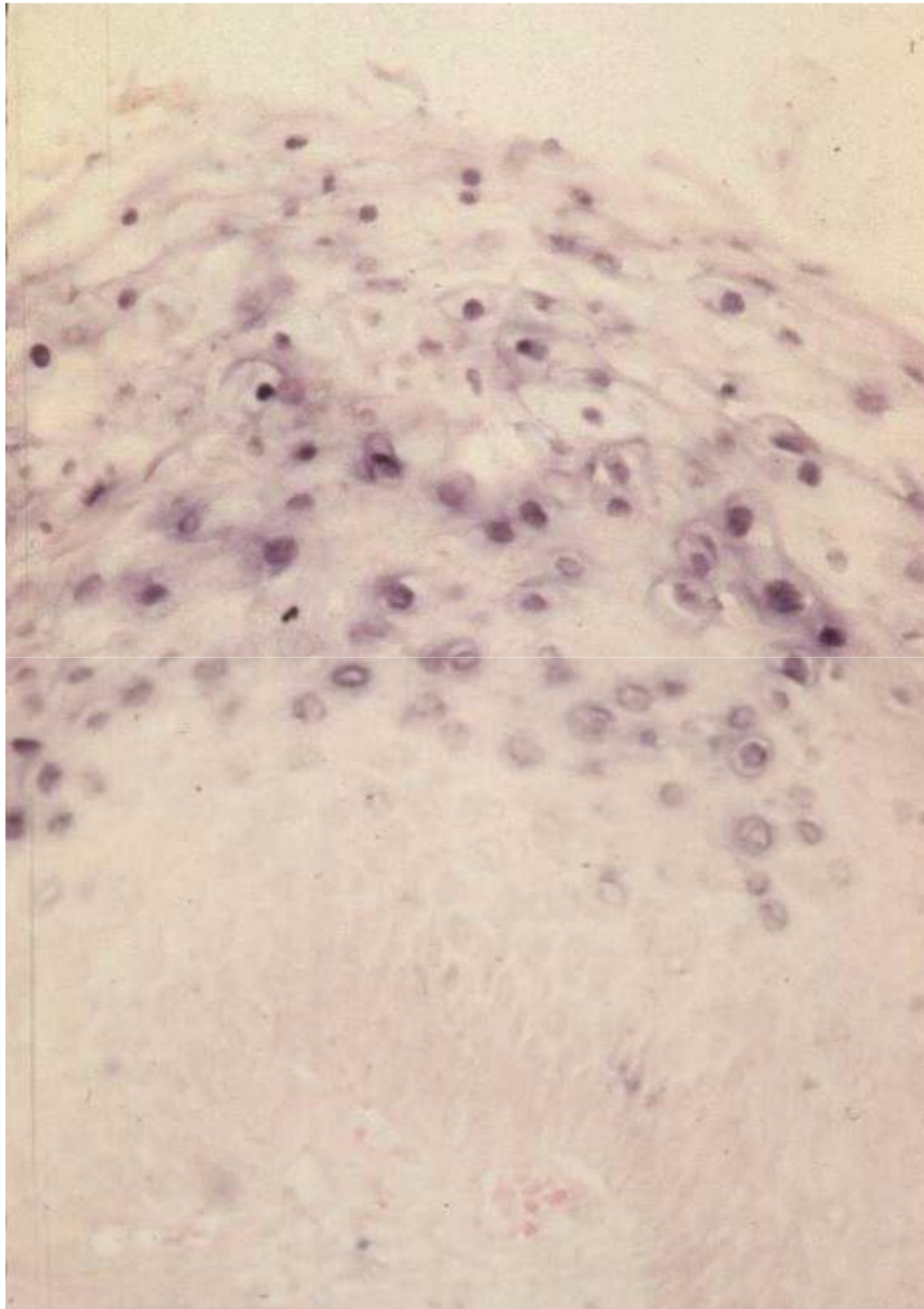
# Molekularbiologie

- Proteinnachweis, -quantifizierung
  - Western Blot, Immunhistochemie
- DNA Nachweis / Quantifizierung
  - Southern Blot, *in-situ* Hybridisierung, (quantitative) PCR
- RNA Quantifizierung
  - Northern Blot, Quantitative RT-PCR, DNA-Chip
- Mutationsanalyse
  - RFLP, Sequenzierung, Schmelzpunktanalyse, Hybridisierung
- Deletionsanalyse
  - PCR, Sequenzierung
- Translokationsanalyse
  - Southern Blot, (RT-)PCR, FISH

# Detektion von DNA / RNA

- Keine Aussage über Lebens- oder Vermehrungsfähigkeit
- Keine Screeningmethode
- Hohe Sensitivität
- Viren (ev. integriert)
- Bakterien
- Parasiten





*In-situ* Hybridisierung  
HPV 16/18

# Durchführung - PCR

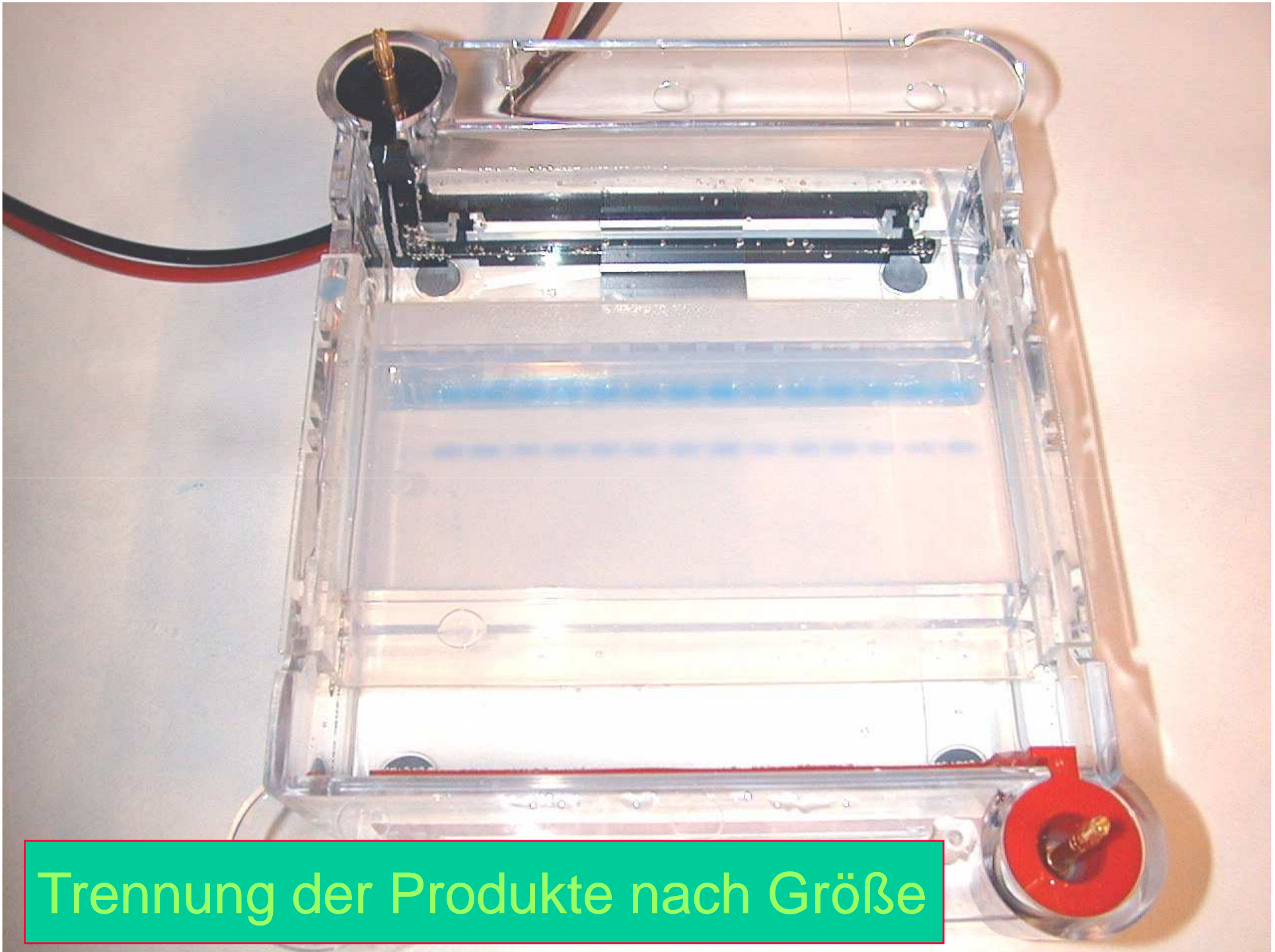
- Amplifikation
- Trennung der Produkte nach Größe
- Identifizierung und Analyse



# Amplifikation

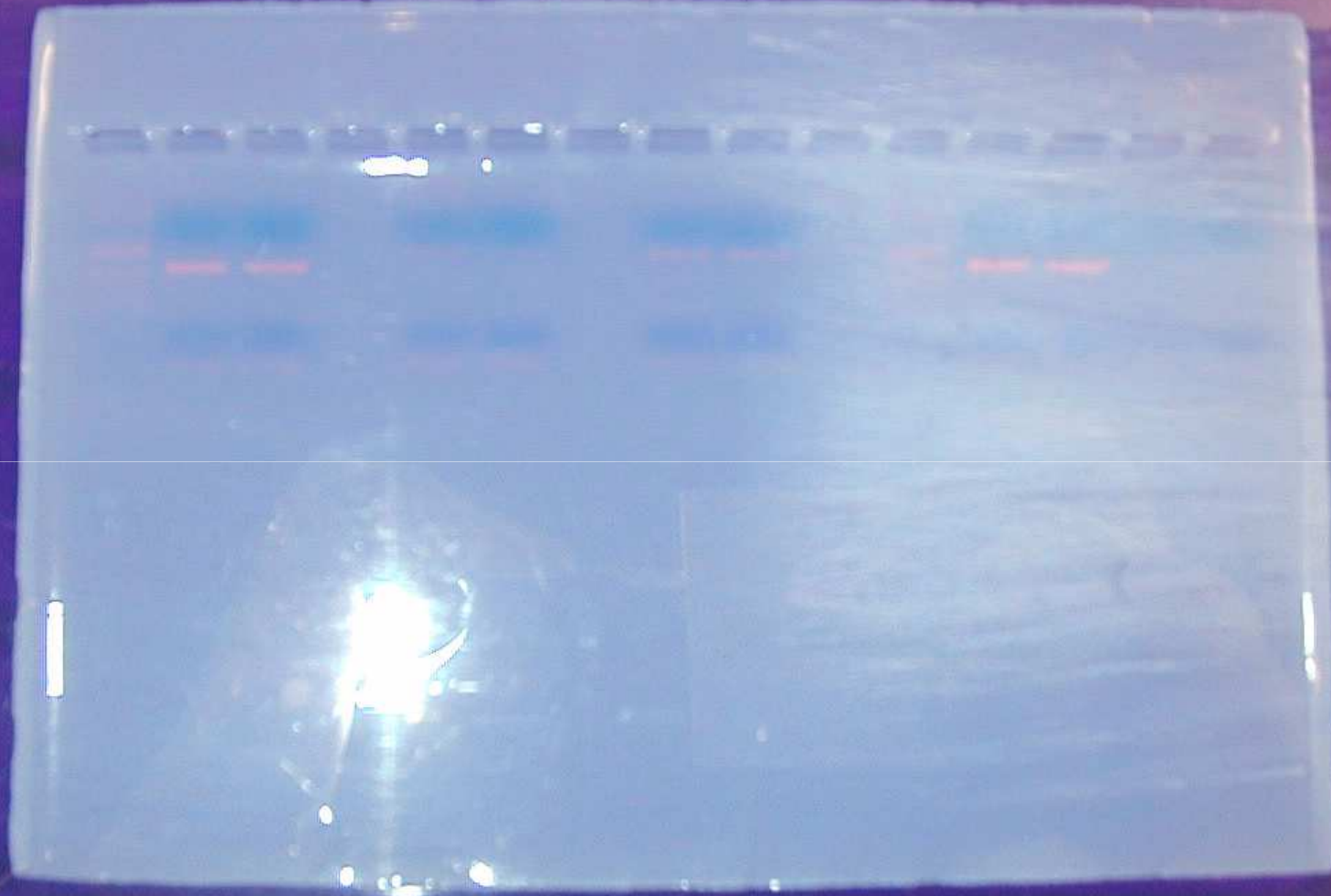






Trennung der Produkte nach Größe

# Identifizierung und Analyse





# HPV 18 - PCR



# Nachweis von Erreger-DNA mittels PCR

## Vorteile

- Unkultivierbare Organismen
- Zeitfaktor
- Hohe Sensitivität

## Nachteile

- Aufwand
- Kontaminationsgefahr (falsch positive Ergebnisse)
- Keine Screeningmethode

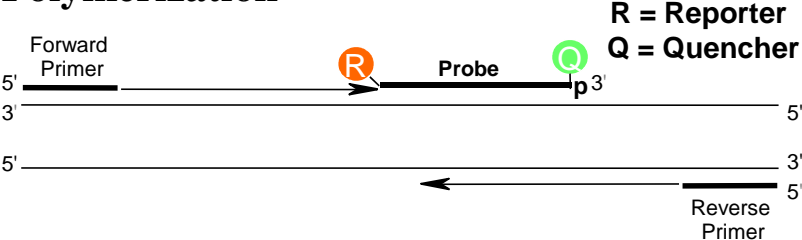
# Quantifizierung von DNA/RNA

- Blot: Southern/Northern
- (RT)PCR: (Semi-)quantitative
- „in situ“-Methoden (z.B.: FISH)
- Chip-Verfahren (high throughput)
- Seltener verwendet:
  - Nuclease protection assay, Ligase chain reaction etc.

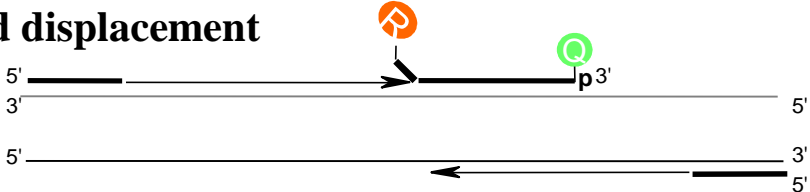


# Quantitative PCR: Exonuclease probe

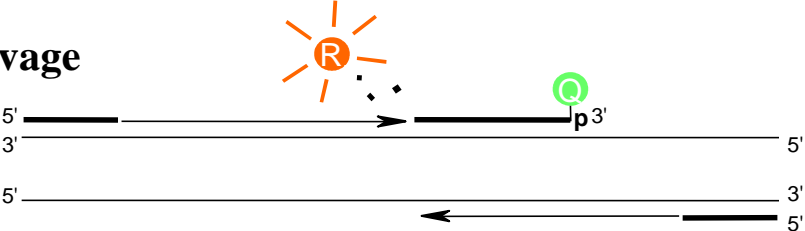
## Polymerization



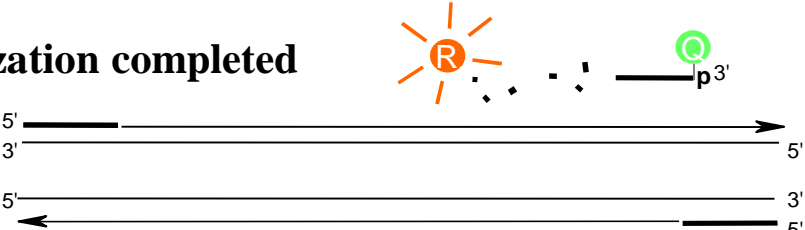
## Strand displacement



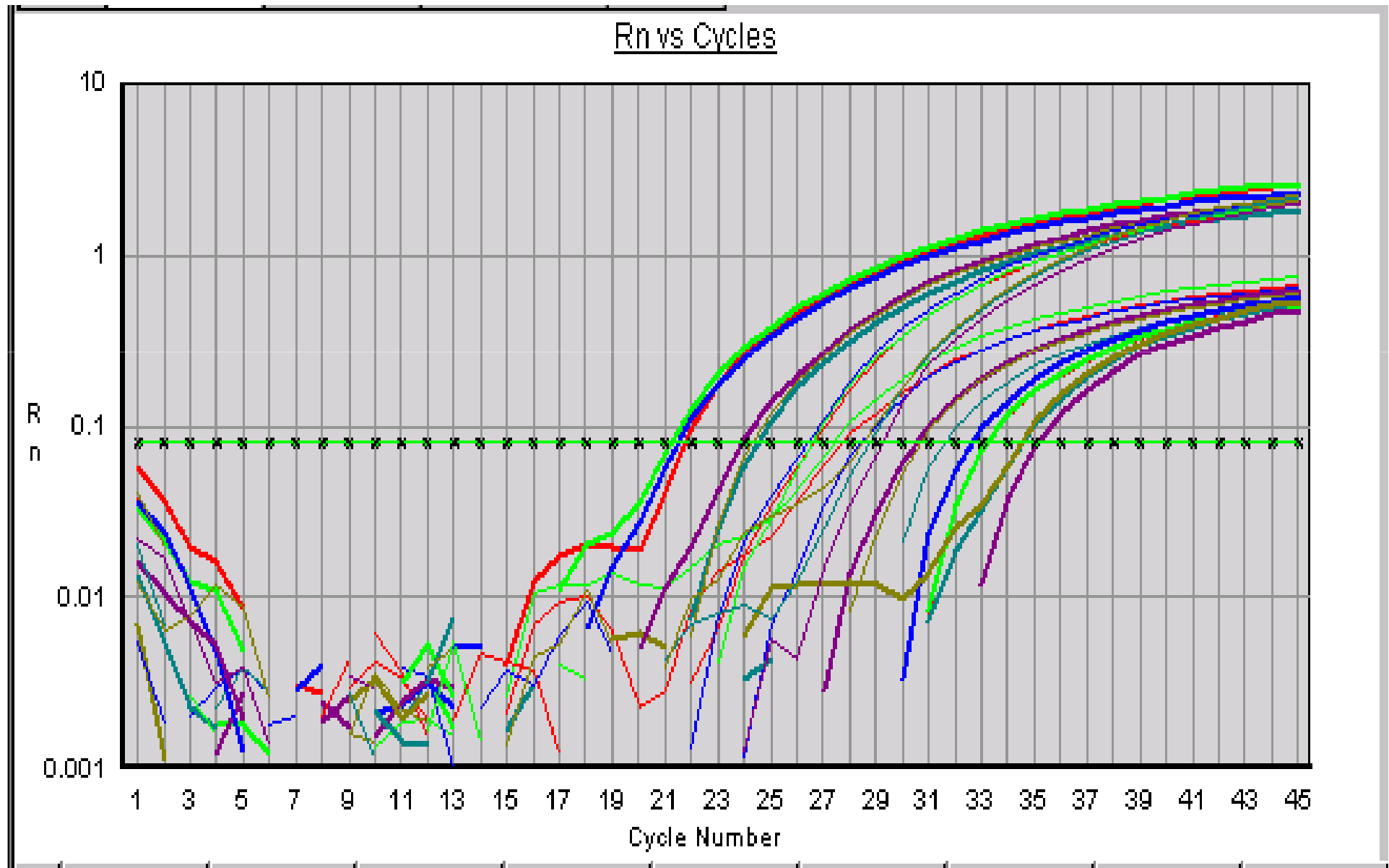
## Cleavage



## Polymerization completed

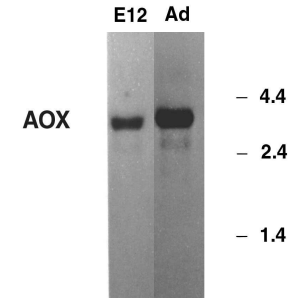


# Quantifizierung von N-myc

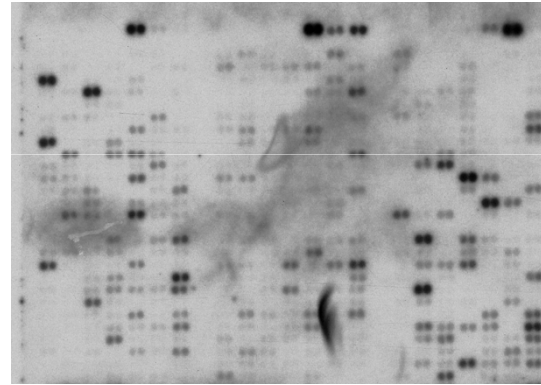


# Expressionsanalyse

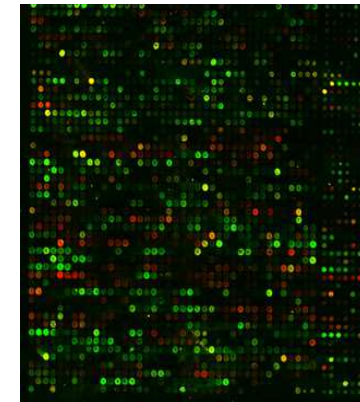
**I. Northern Blot - One Gene at a time**



**II. cDNA Macroarray -**



**III. cDNA Microarray (DNA Chip) -**





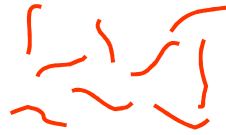
**Normale  
Mucosa**



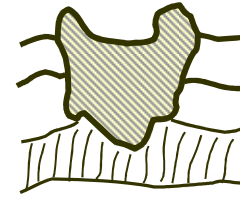
**mRNA**



**Cy 5 - markierte  
cDNA**



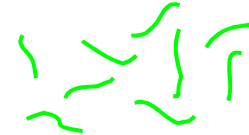
**Karzinom**



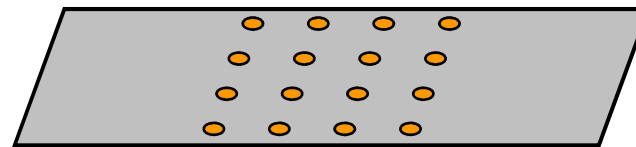
**mRNA**



**Cy 3 - markierte  
cDNA**

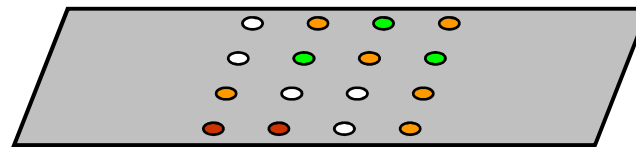


**Hybridisierung**

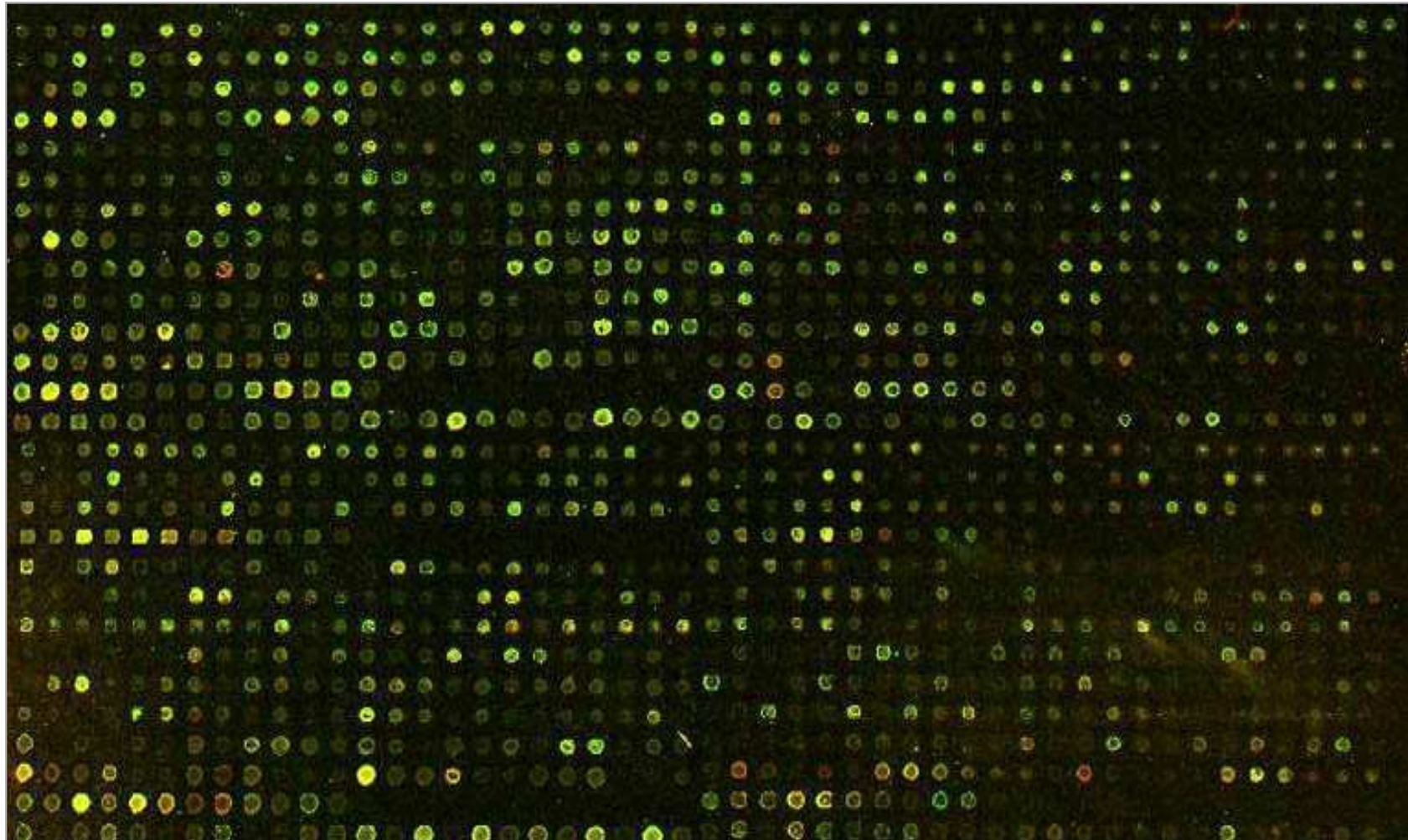


**cDNA array**

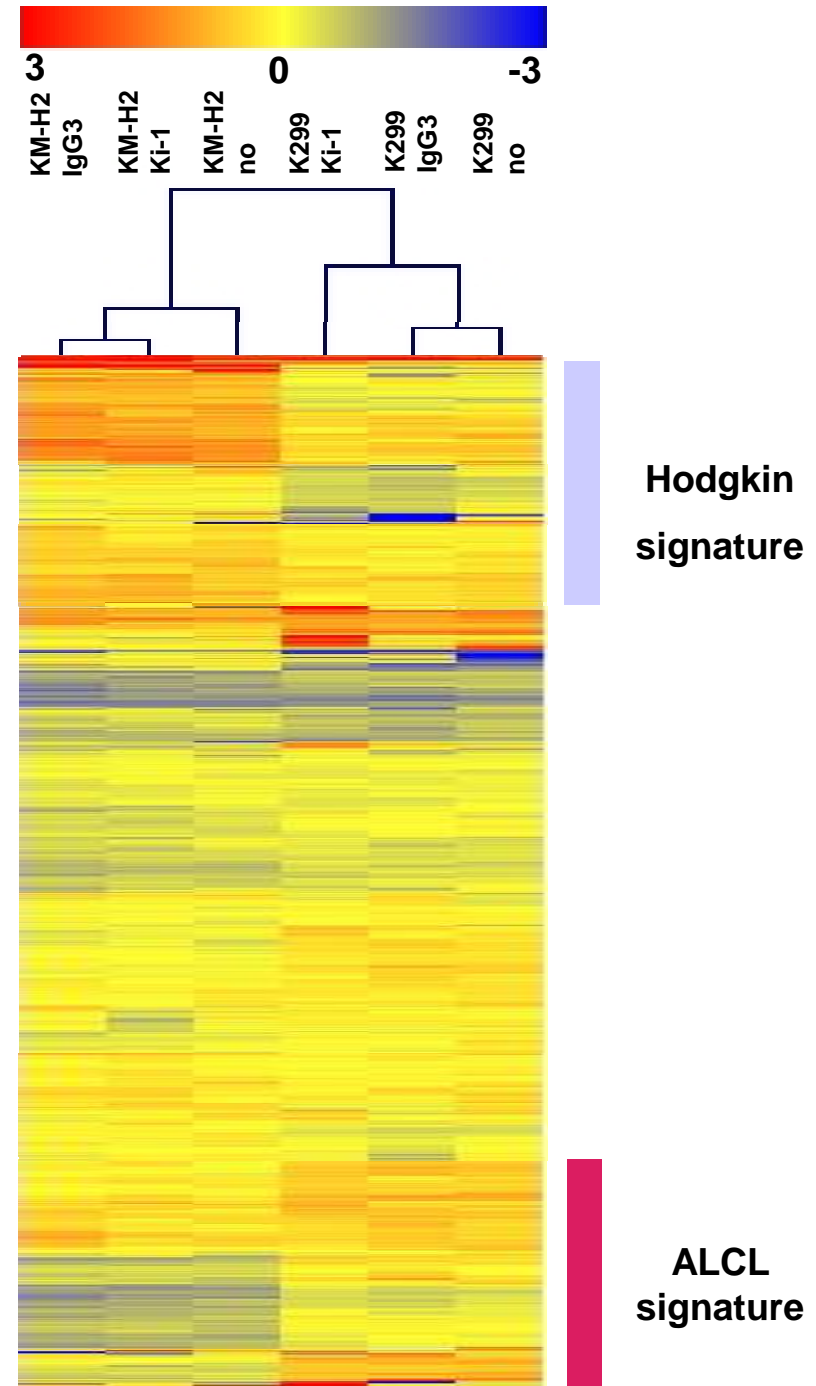
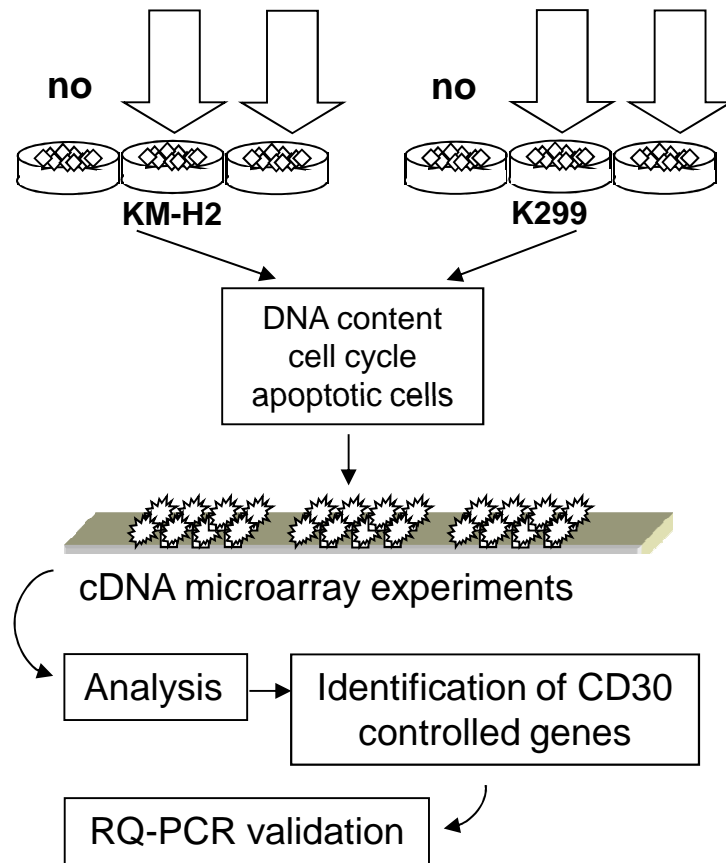
**Scanner**



# Hybridisierter cDNA-array



# RNA expression analysis





1.12.14

# Analyse von Punktmutationen

## ● Methoden:

- Restriktionsenzymanalyse
- Hybridisierung
- DGGE
- SSCP
- Allelspezifische PCR
- Sequenzierung

# Molekulare Diagnostik – Gerinnungsstörungen, Stoffwechselerkrankungen

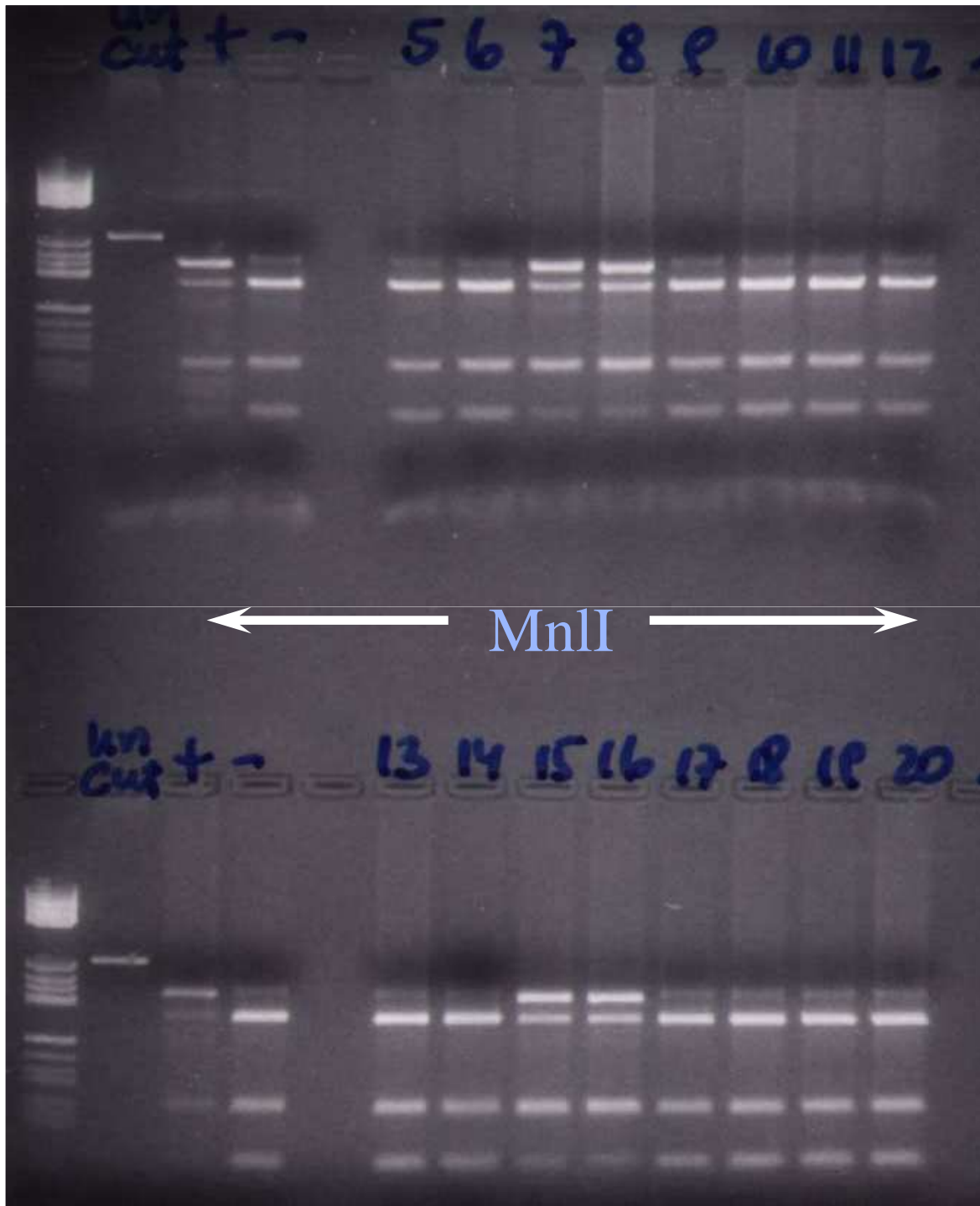
- Faktor V „Leiden“ Mutation
- Hämochromatose: HFE-Gen – Mutationen
- Mb. Wilson: ATP7B - Mutationen

# Mutation Faktor V “Leiden”

- Häufigste genetisch bedingte Ursache für venöse Thrombosen
- Prävalenz: 5-7% in der Normalbevölkerung
- Mutation: G1691A = Arg561Gln



# Faktor V „Leiden“

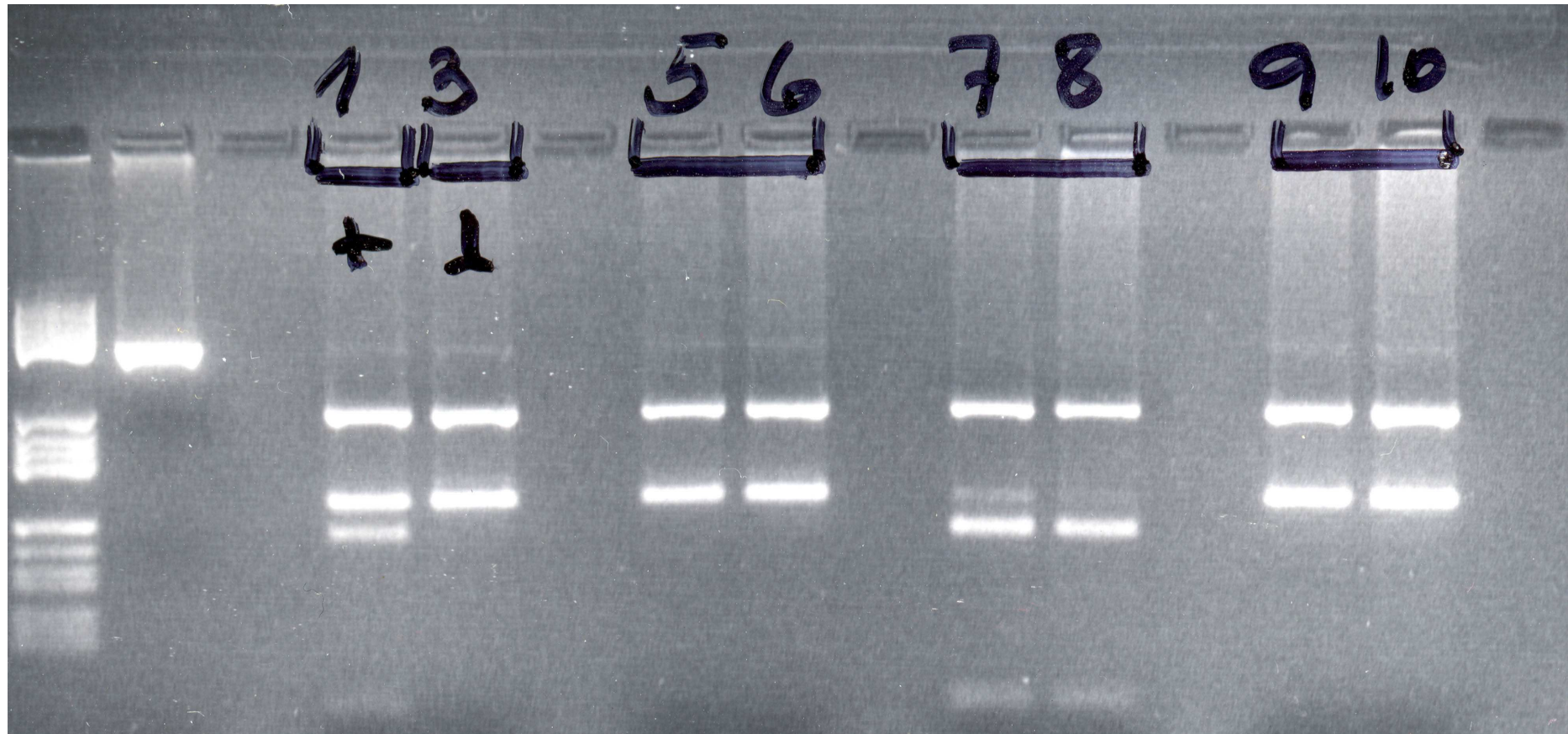


# Mutationsanalyse

◆ DNA-Polymorphismen: z.B.  
Hämochromatose

- Verwendung unterschiedlicher Analysetechniken
  - Restriktionsenzymanalyse
  - Schmelzpunktanalyse (Real-time PCR)
  - Hybridisierung (Strip-Assay)

# Mutation C282Y des HFE-Gens: RFLP-Analyse

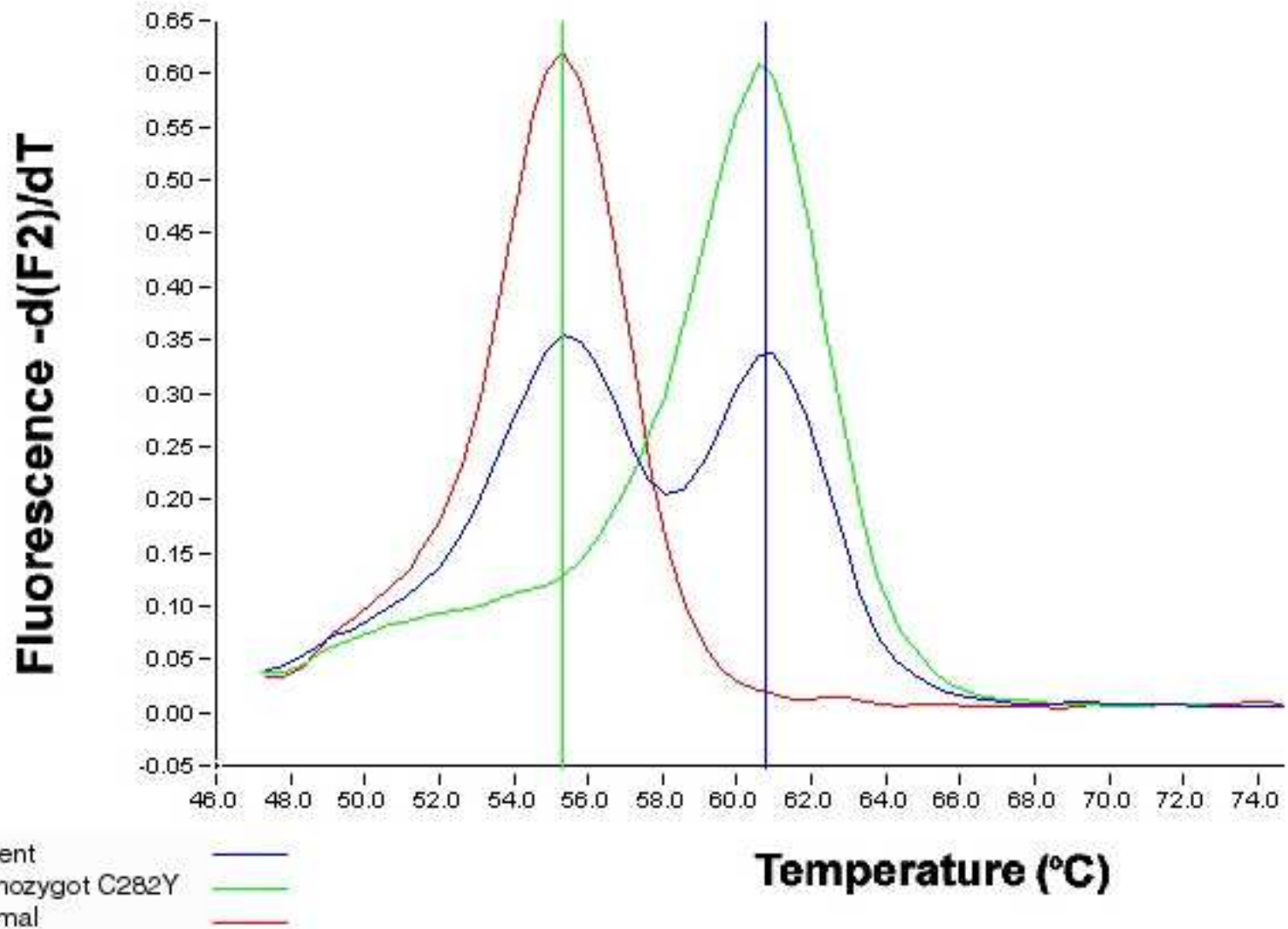


St

+ -  
Kontr.

Patient

# Mutation C282Y des HFE-Gens: Schmelzpunktanalyse





# Translokationsnachweis - Techniken

- Zytogenetik

- Molekularbiologie

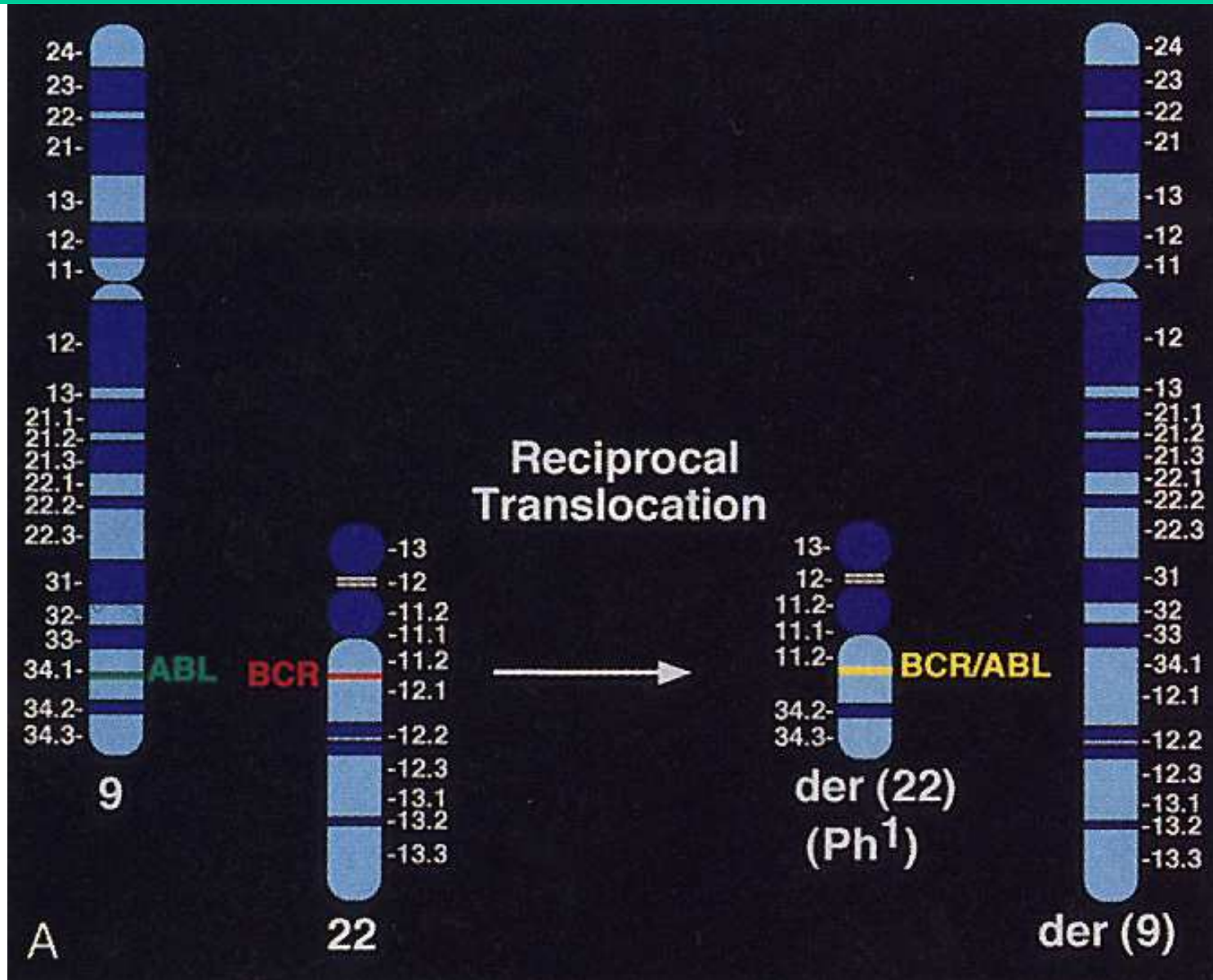
- Southern Blot

- PCR

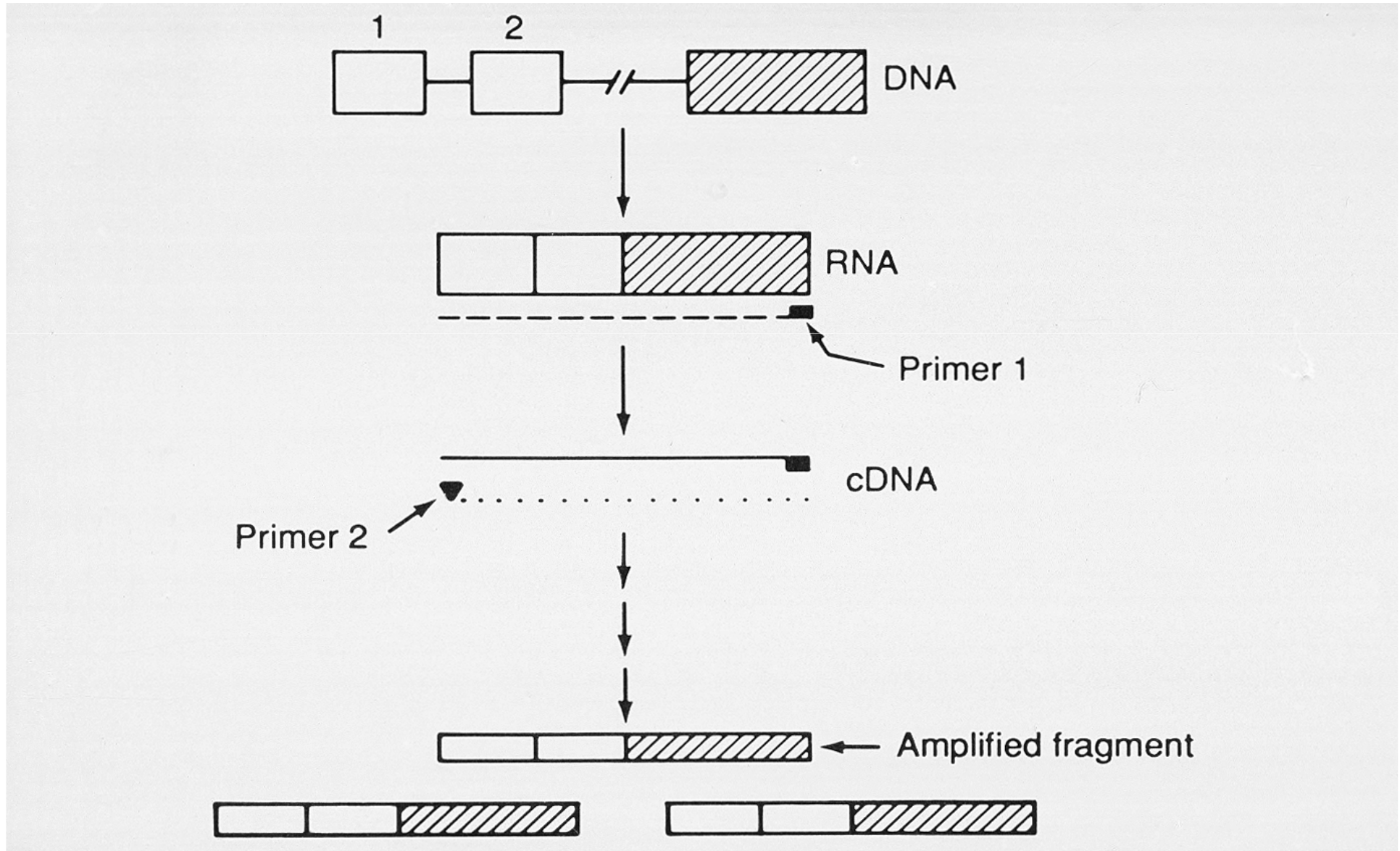
- RT-PCR

- FISH (Fluoreszenz *in situ* Hybridisierung)

# Translokation BCR-ABL



# Nachweis BCR-ABL mittels RT-PCR

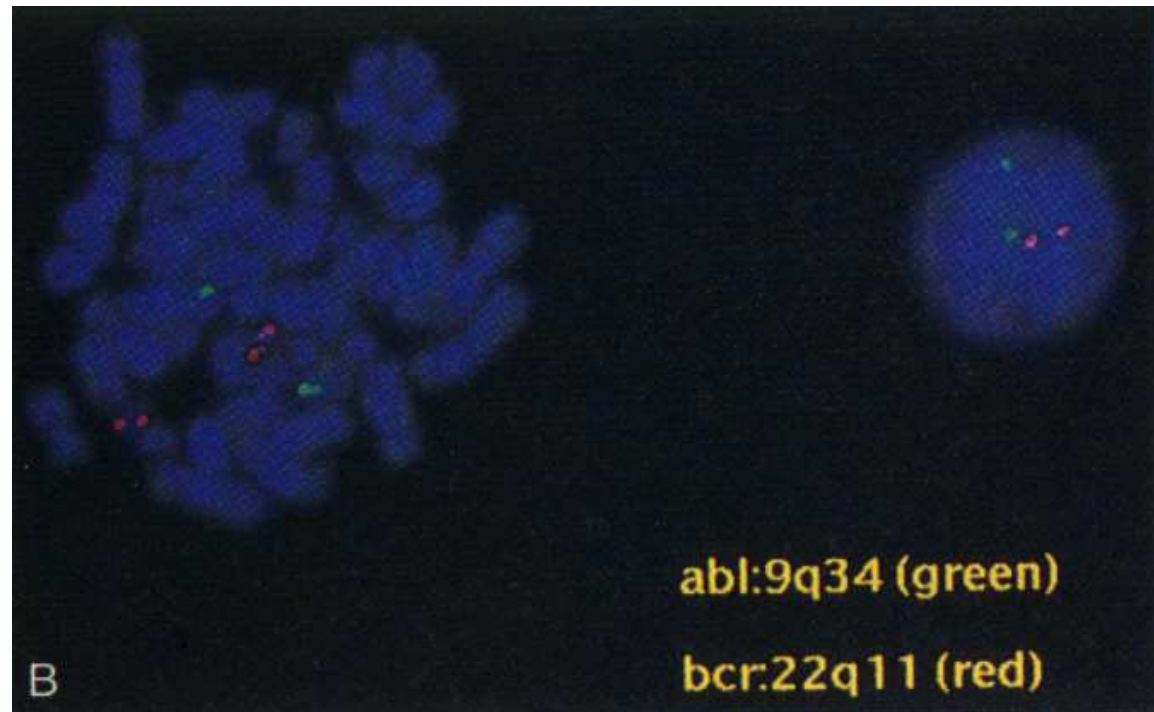


+ 1 2 3 4 5 6 7 8 9 10 11 12

RT-PCR BCR/Ab1 MBr



# FISH BCR- ABL



# Anwendungen

- Diagnostik:

- CML

- Individuelle Risikoabschätzung:

- AML, ALL

- Therapiekontrolle:

- KMT, (Chemotherapie, Interferon)

- Früherfassung eines Relaps

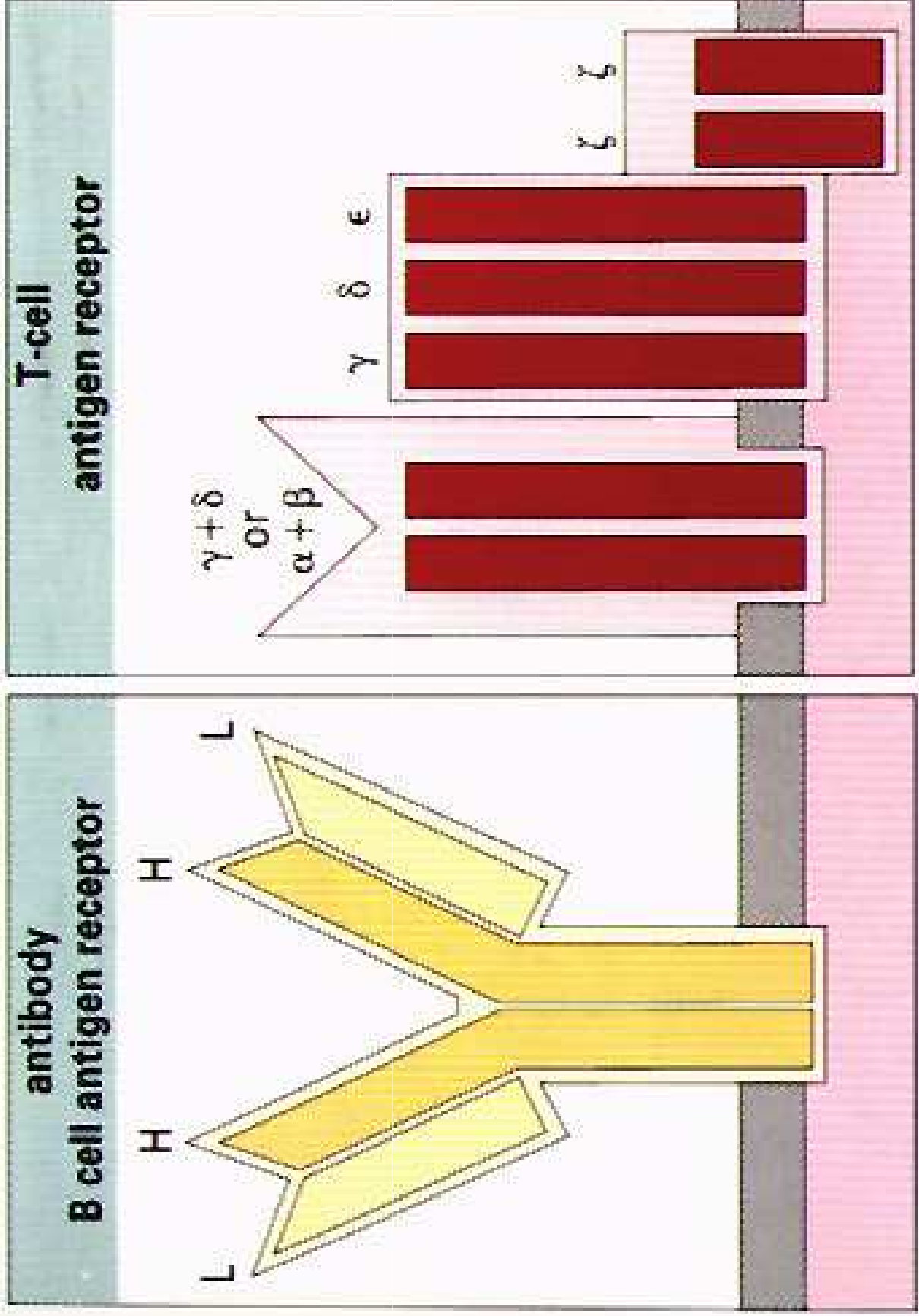
# B-Lymphozyten

- B-Zell Rezeptor
- Effektorzellen: Plasmazellen -  
Antikörperproduktion
- Unterstützung des Makrophagensystems

# Immunglobuline

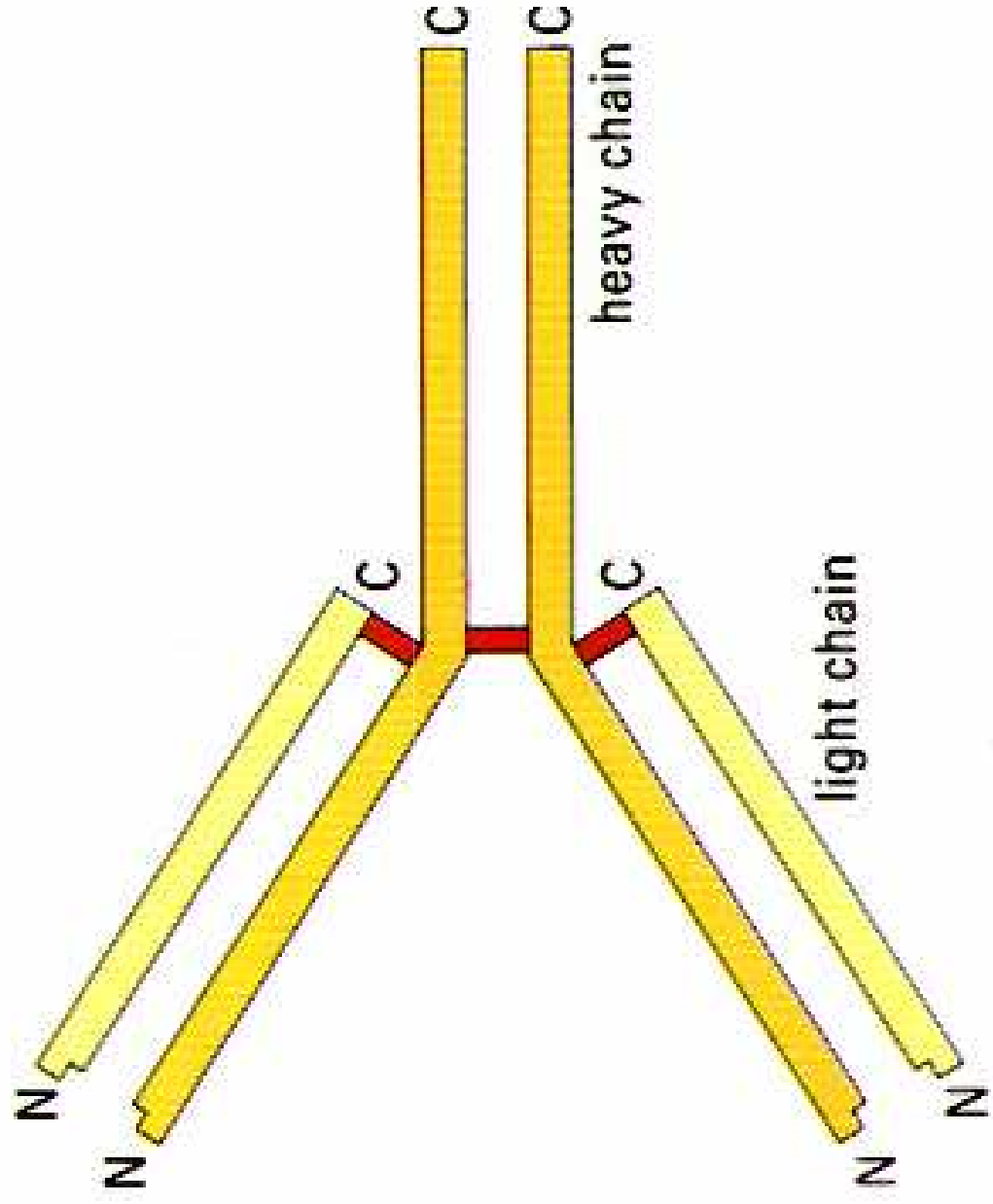
- **Glykoproteine**, im Serum u. Gewebsflüssigkeiten aller Säugetiere
- **AG-Rezeptoren**: an Zelloberfläche
- **AK**: frei im Blut oder Lymphflüssigkeit
- **5 Klassen**: IgG, IgA, IgM, IgD, IgE
- **Unterschiede**: Größe, Ladung, AA-Zusammensetzung, Kohlehydratgehalt

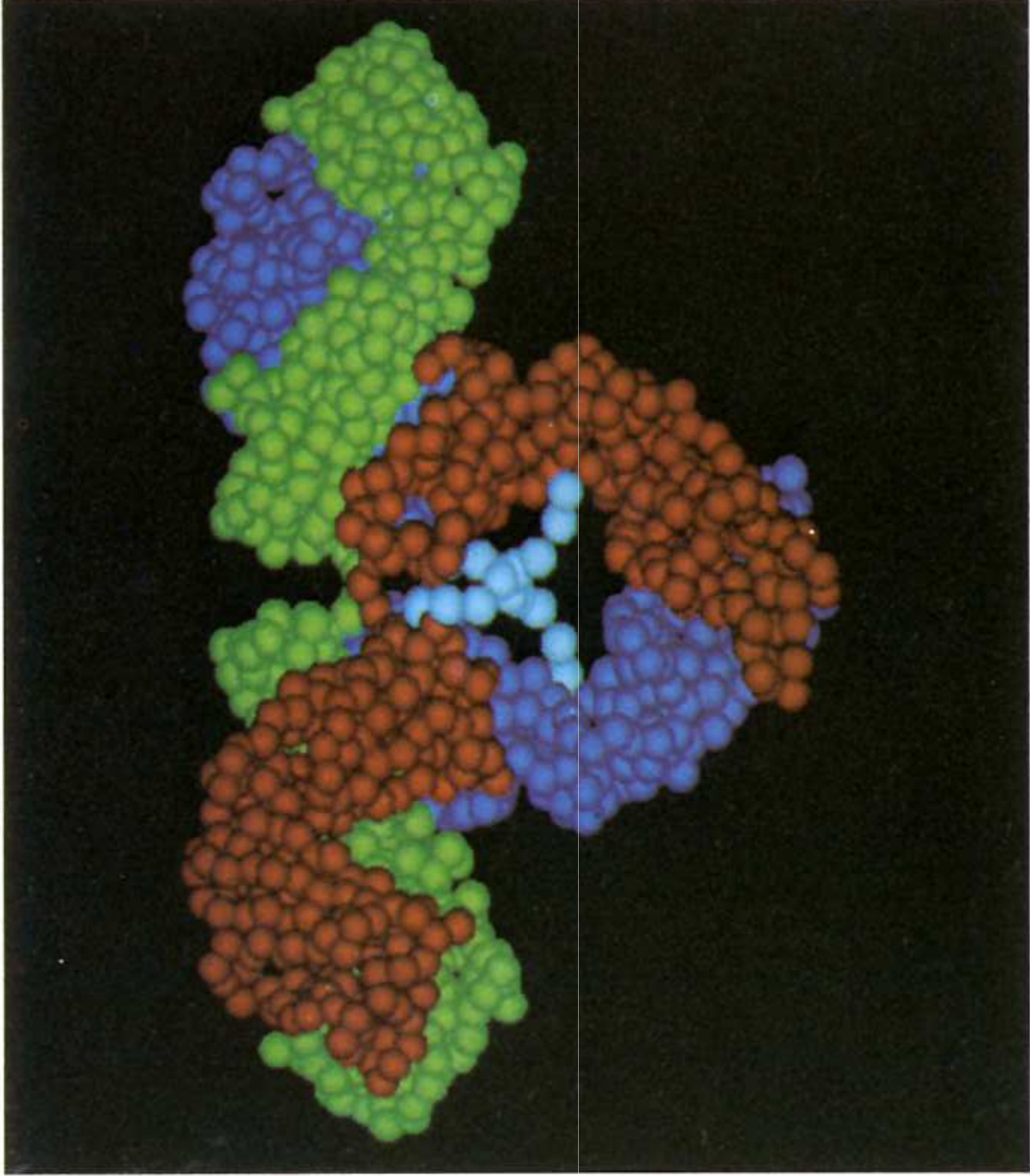
# Antigen recognition molecules





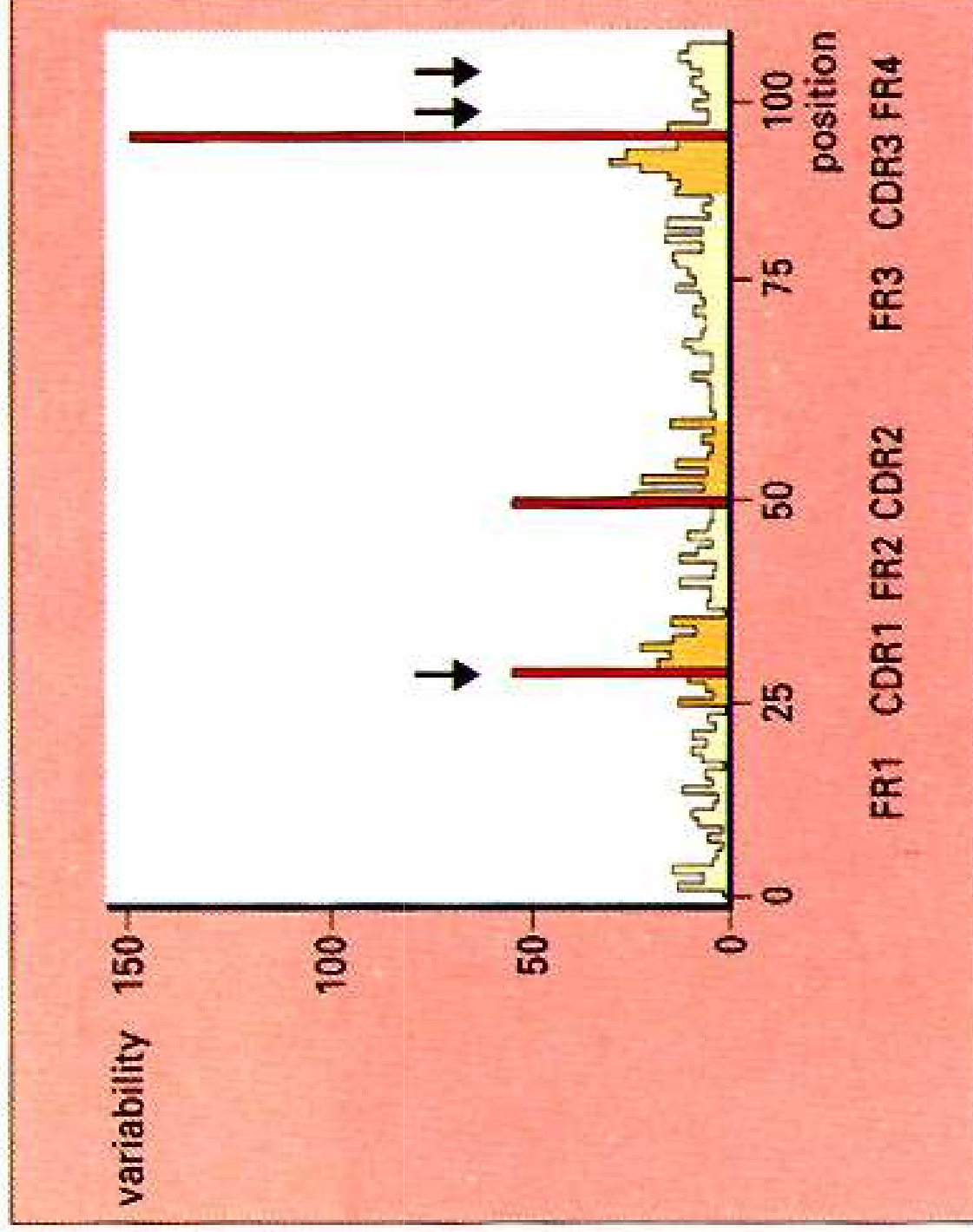
## The basic chain structure of immunoglobulins





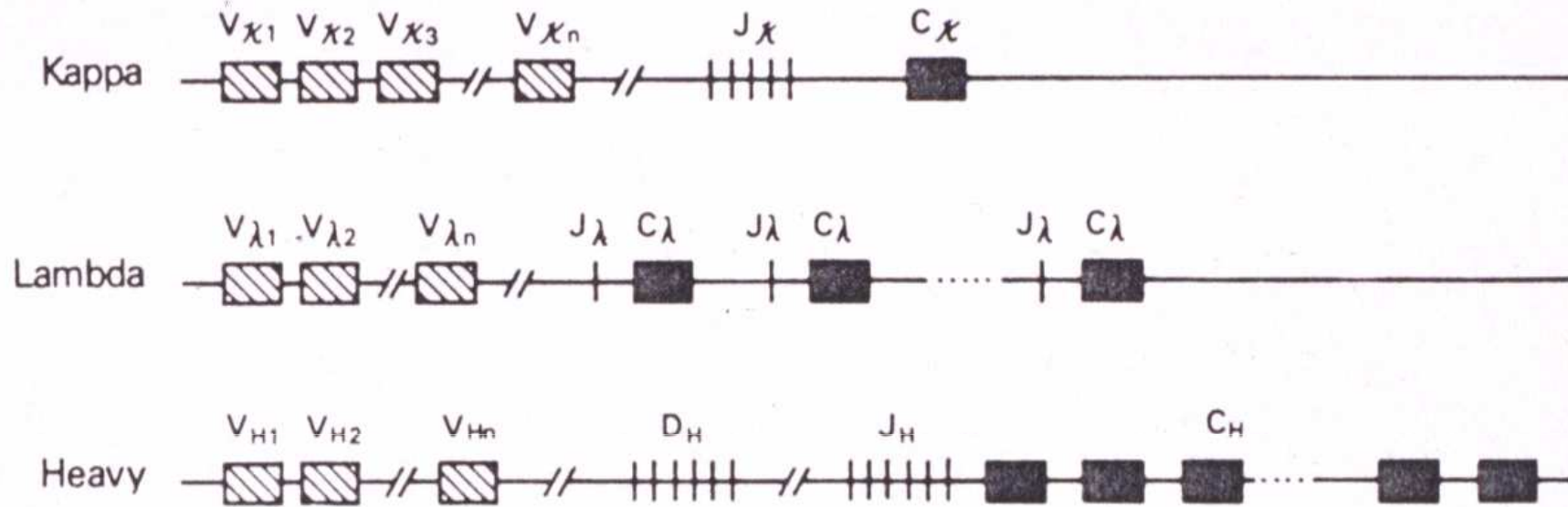
**Computer-generated model of the hinge-deleted human IgG1 protein Dob.**

## Amino acid variability in the variable region of immunoglobulin light chains

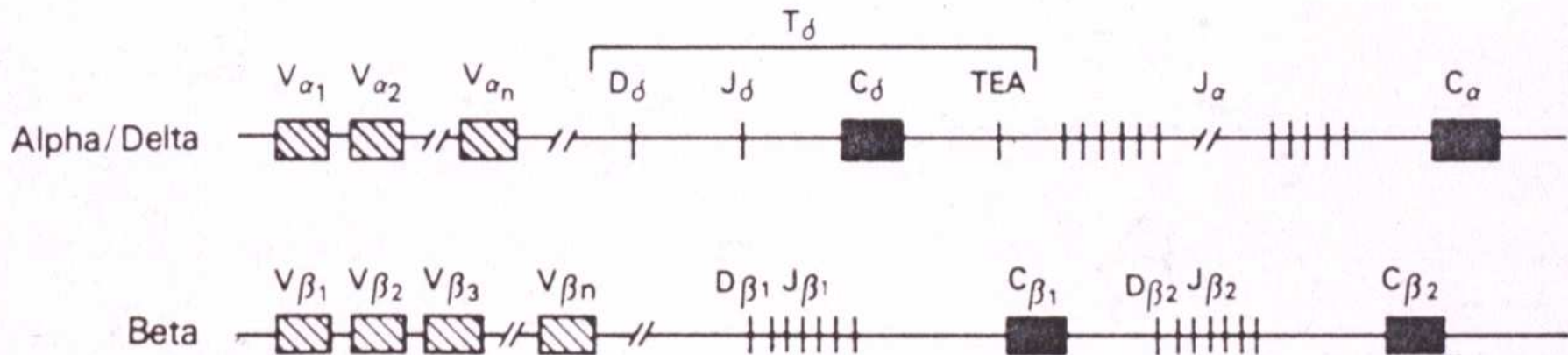


# Genstruktur

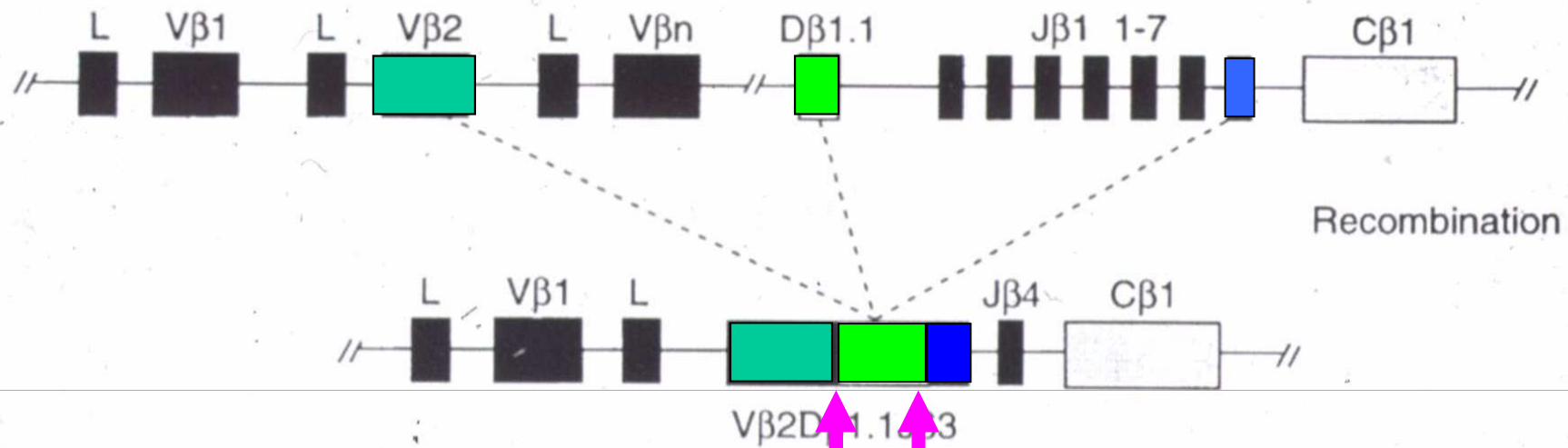
## B CELL



## T CELL



# TCR- $\beta$ Gen-rearrangement



Recombination

Transcription

Splicing

Translation

Addition of a variable number of nucleotides in a template independant fashion

**N - region**

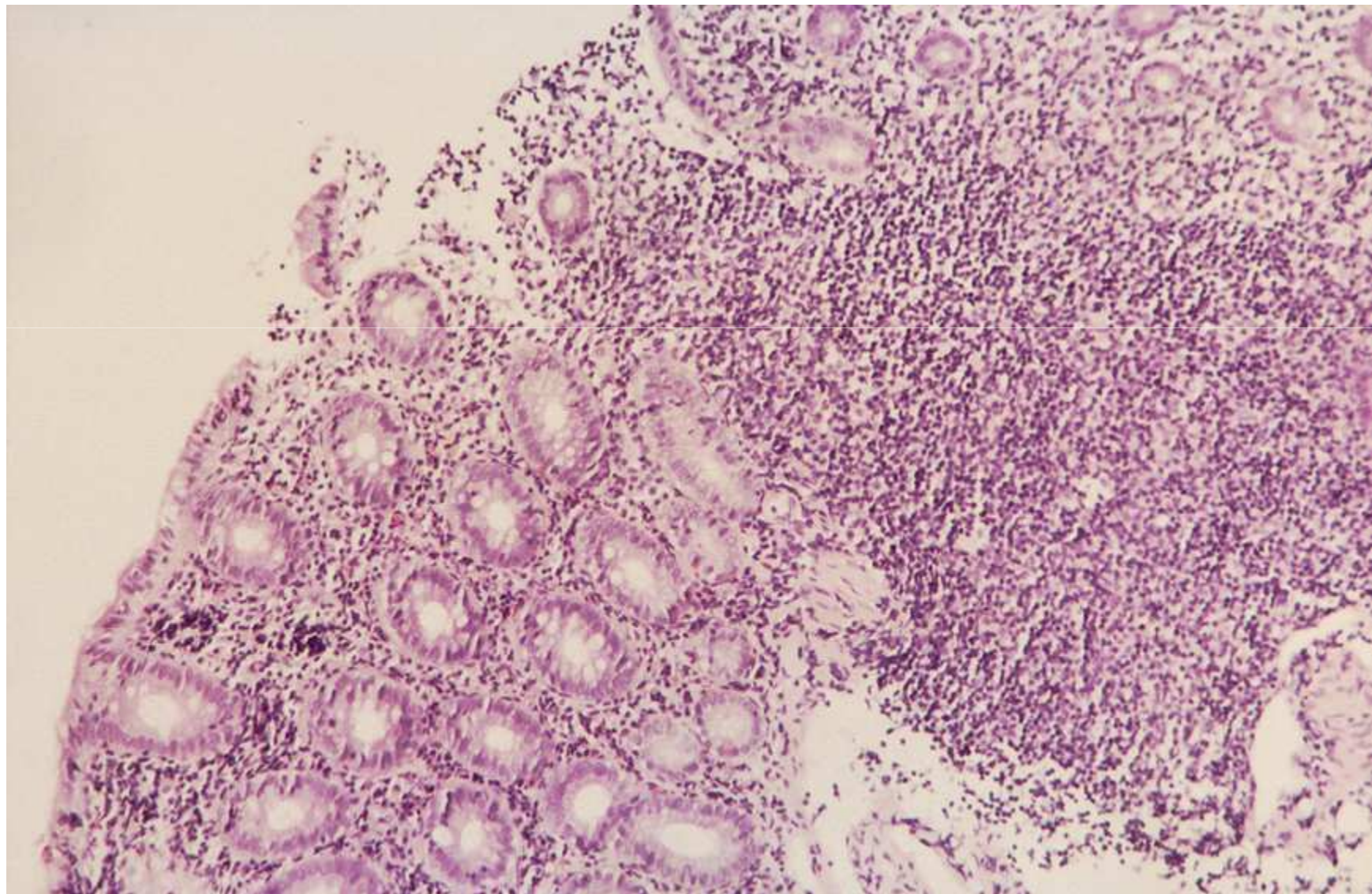
NH<sub>2</sub>  COOH



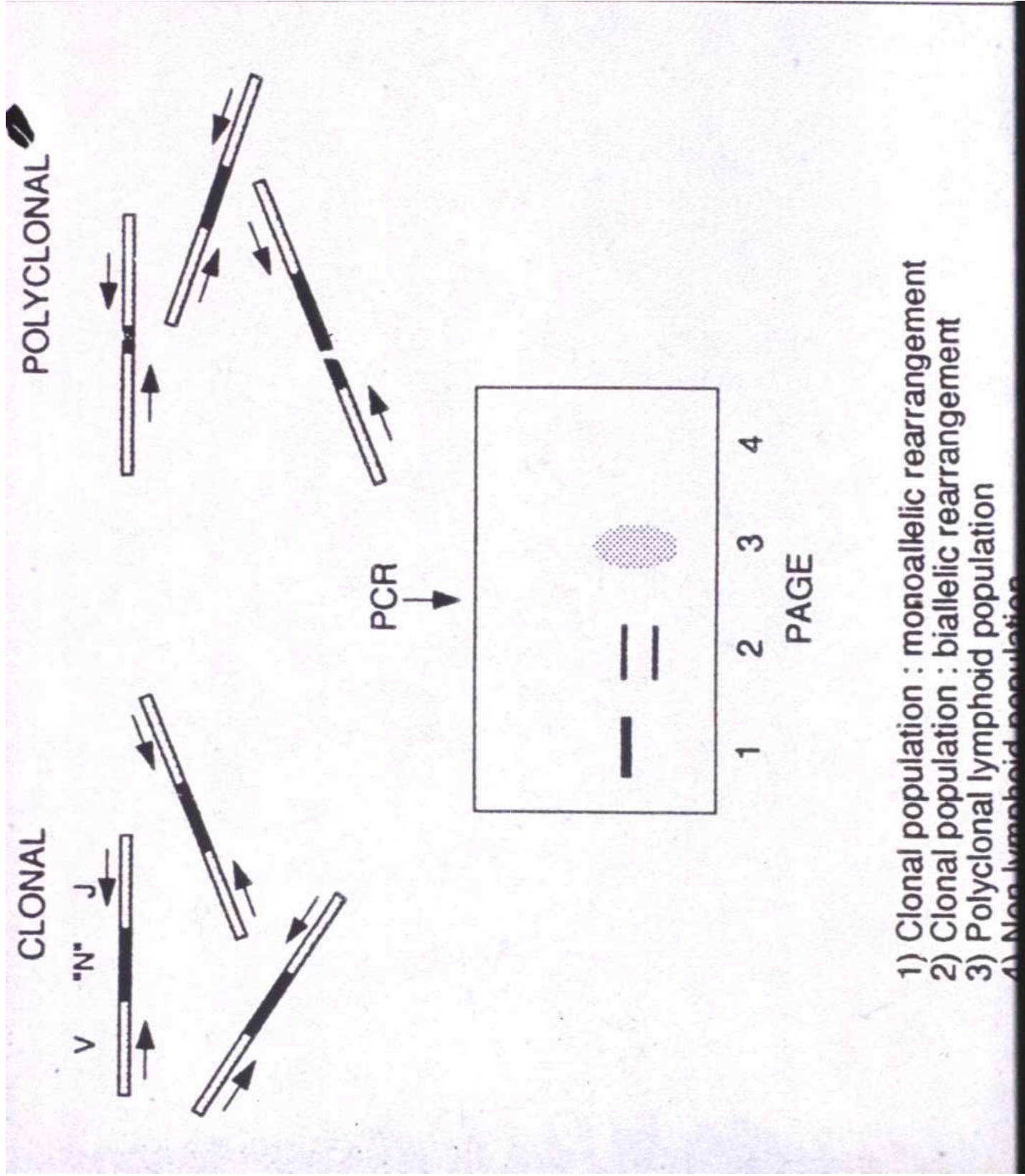
# Hypervariable Regionen

- CDR (complementarity determining regions)
- Jede Leicht- und Schwereketten: 3, CDR1-CDR3
- Umgeben von 4 FR (framework regions)
- CDR's aufgrund von Faltung nahe aneinander

Reaktiv ? CLL ?

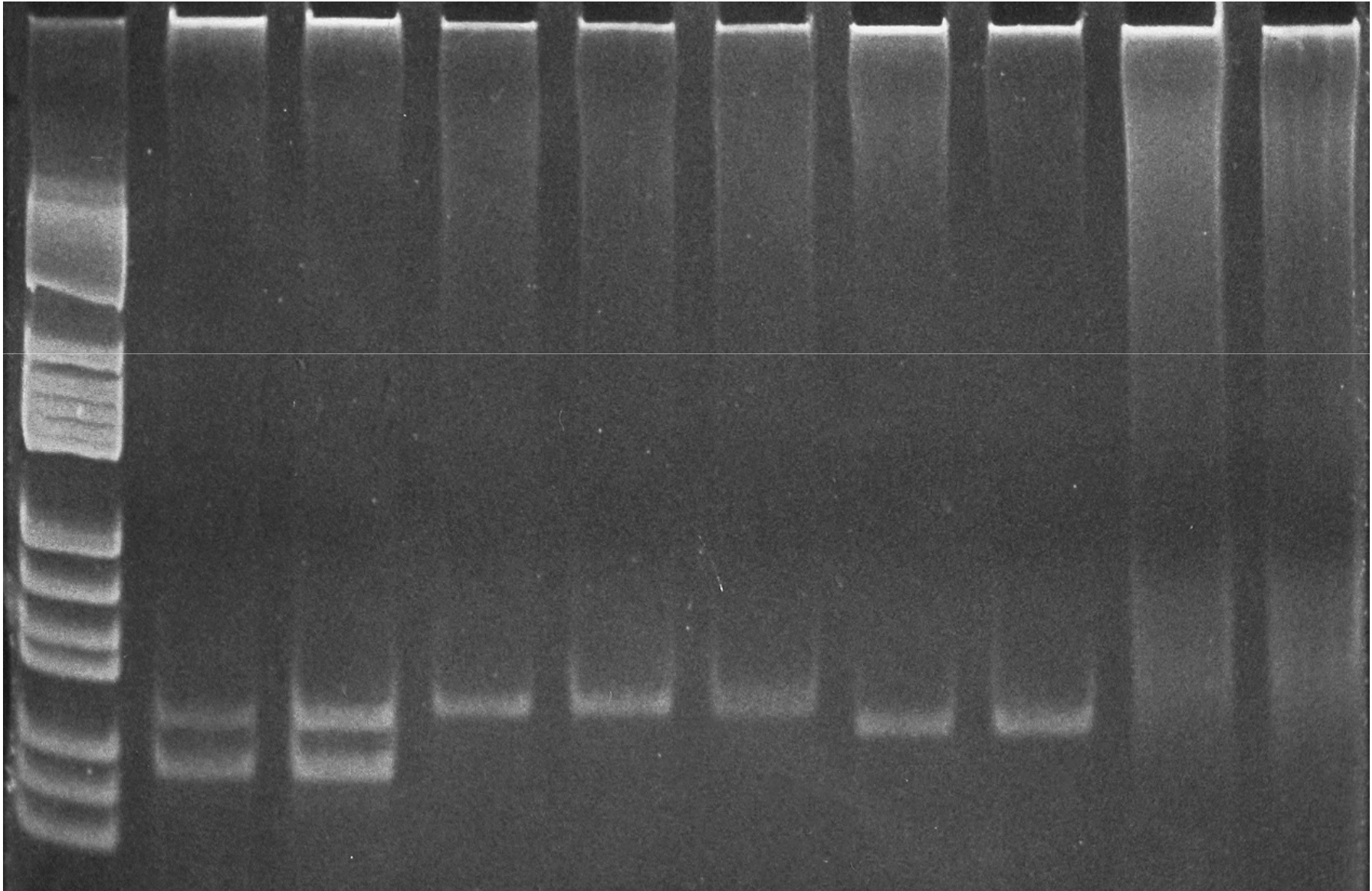






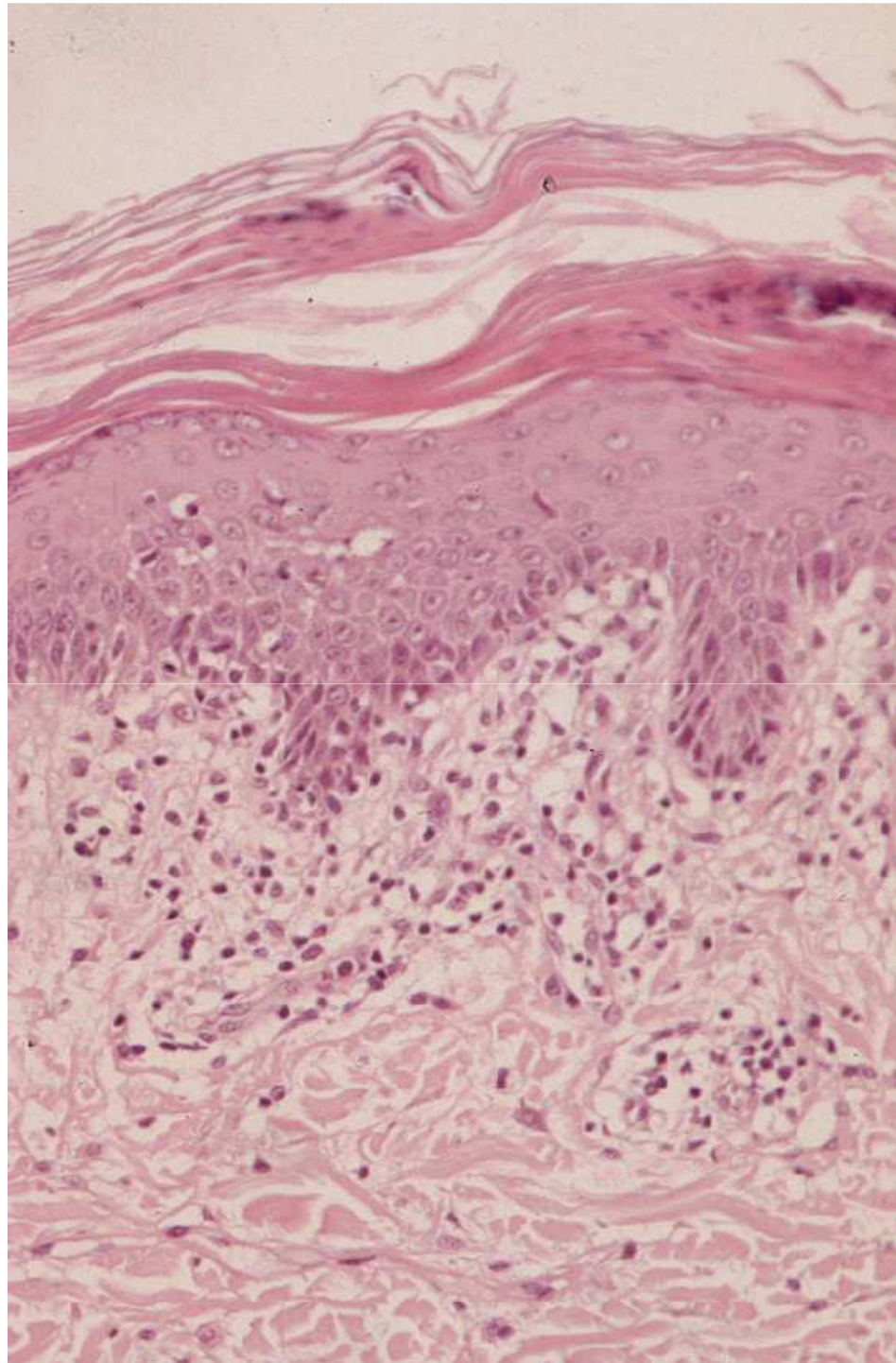
- 1) Clonal population : monoallelic rearrangement
- 2) Clonal population : biallelic rearrangement
- 3) Polyclonal lymphoid population
- 4) Non-lymphoid population

# IgH PCR - Klonalitätsbestimmung



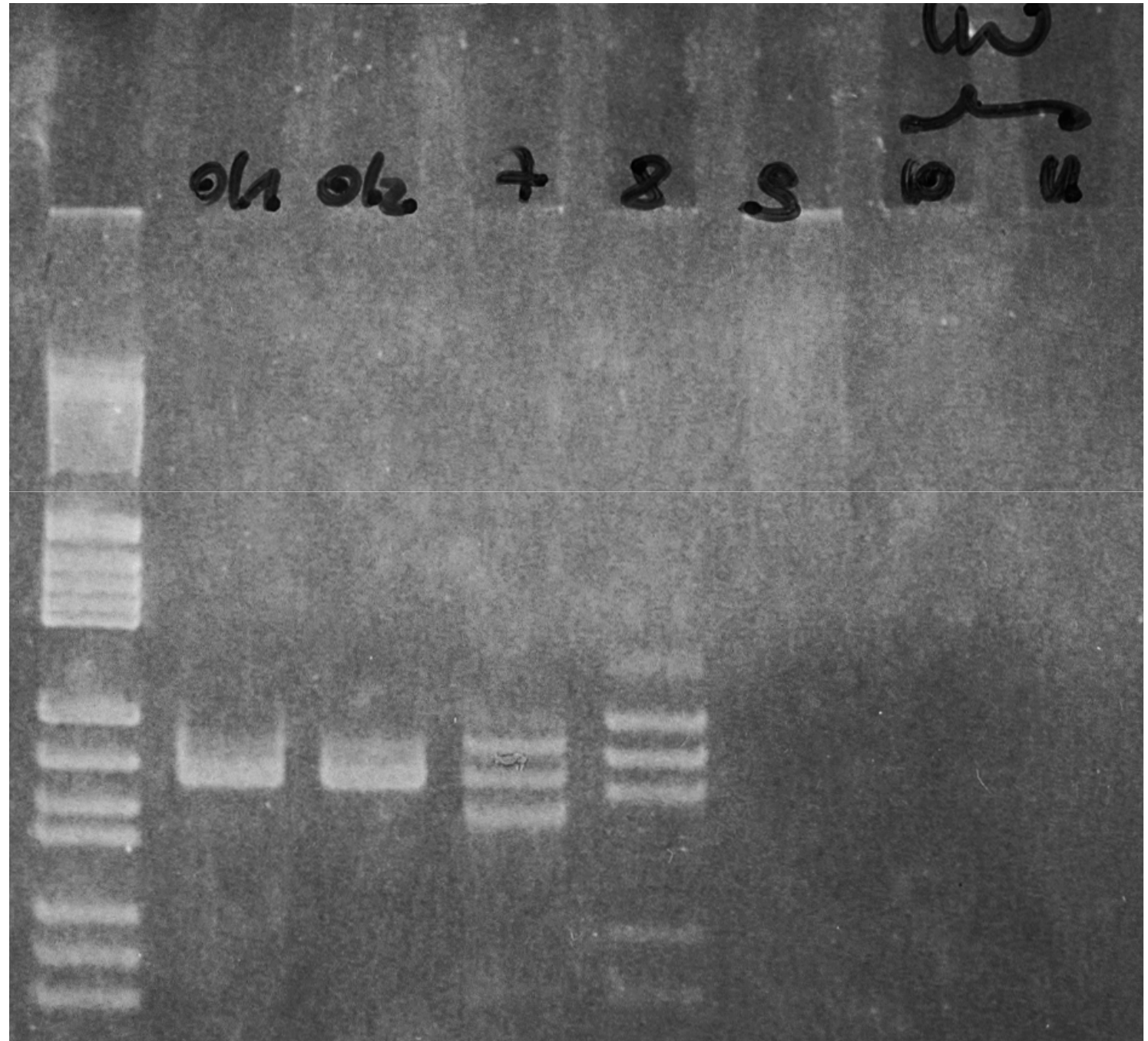


Lymphom ?



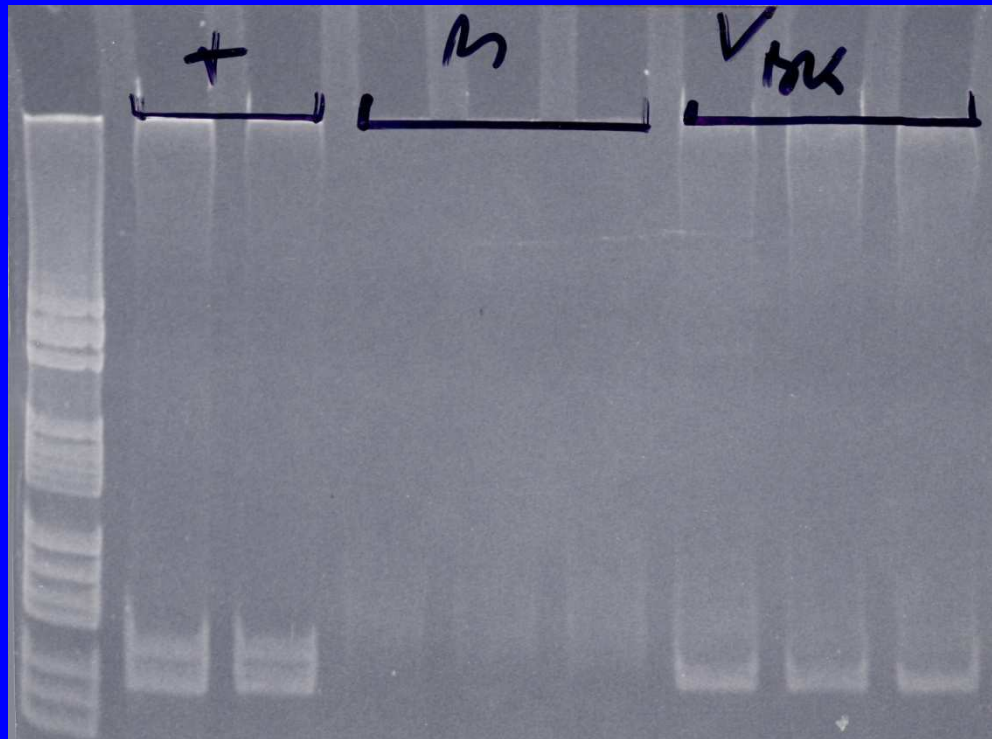


TCR $\gamma$

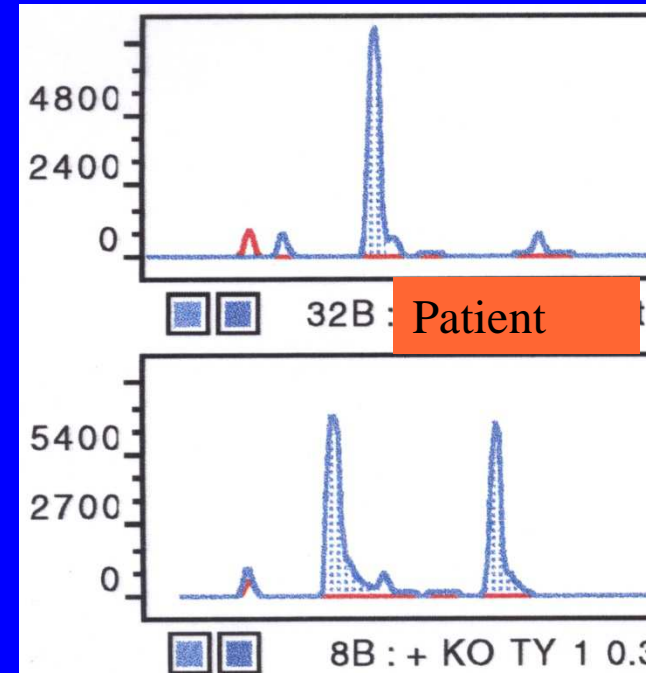


# Analysis of PCR products – Method Comparison

## TCR $\gamma$ - PCR



PAGE analysis



Capillary electrophoresis

# Verlaufskontrolle nach KMT

- Analyse des X-Chromosoms
- RFLP
- VNTR
  - Minisatelliten
  - Mikrosatelliten – STR
    - Short Tandem Repeats

# STR-Analyse

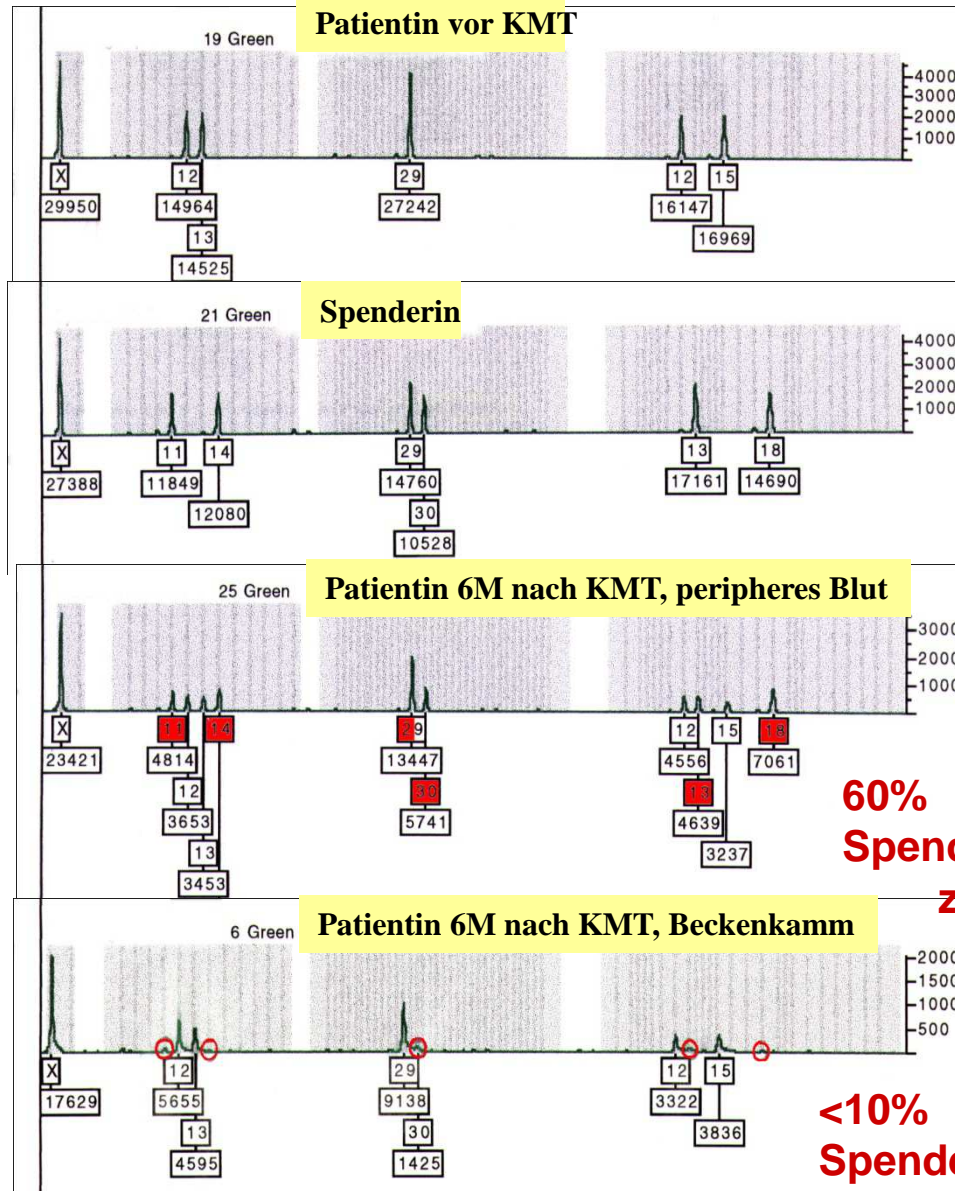
Farbstoff: JOE

Loci:  
Größe (bp):

D8 S1179  
128-168

D21 S11  
189-243

D18 S51  
273-341



60%  
Spender-  
zellen

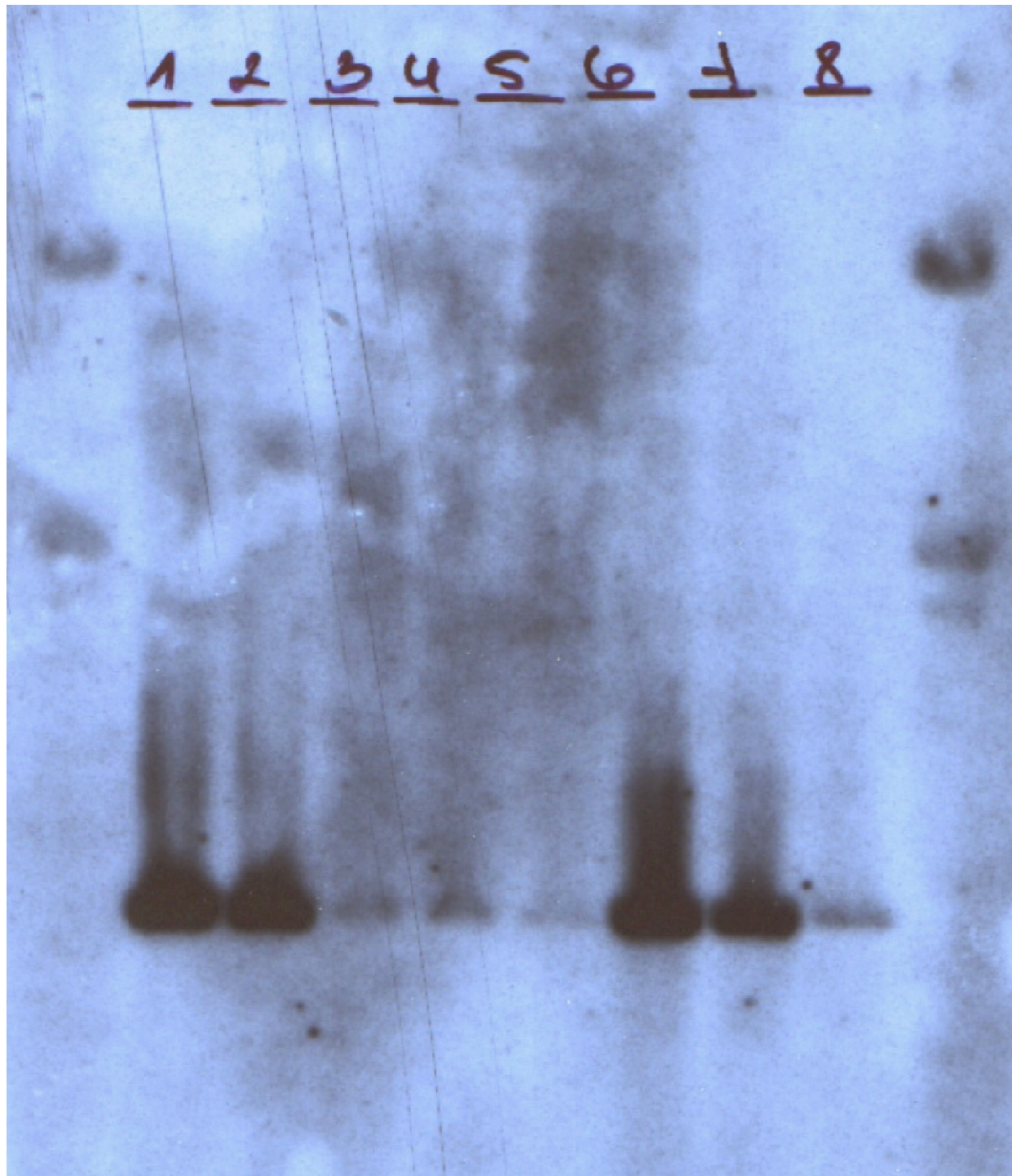
<10%  
Spender-  
zellen

# Molekulare Diagnostik - Onkologie

## Nachweis der:

- Amplifikation von Onkogenen
- Mikrosatelliteninstabilität
- Punktmutationen (Onkogene,  
Tumorsuppressorgene)
- Klonalität



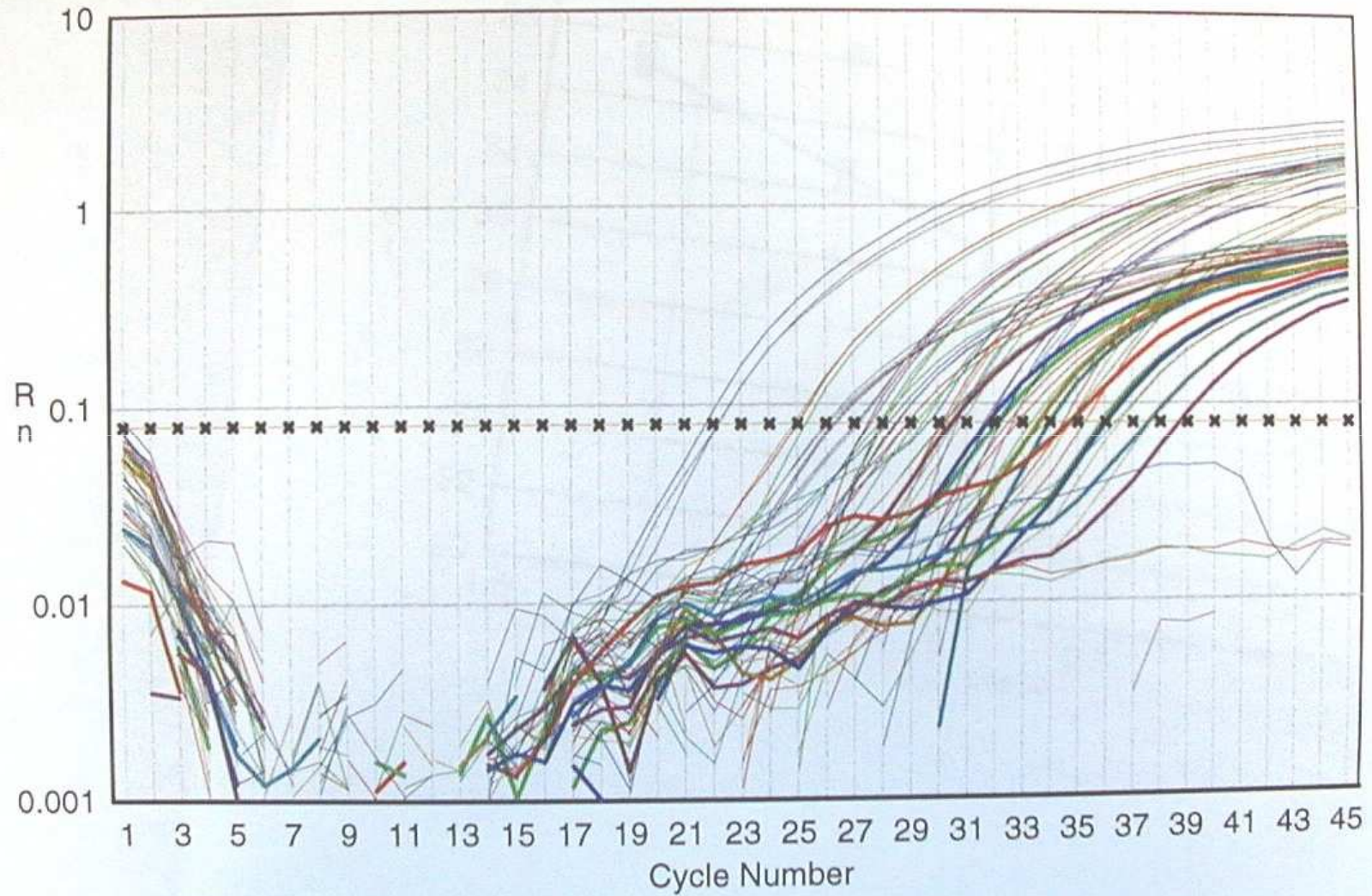


*N-myc*  
Southern-blot

- 1,2: pos. Ko. (IMR)
- 3,4: normale DNA
- 5: Patient 1
- 6: Patient 2, 10µg
- 7: Patient 2, 5µg
- 8: Patient 2, 2µg DNA



# Quantitative PCR – N-*myc*

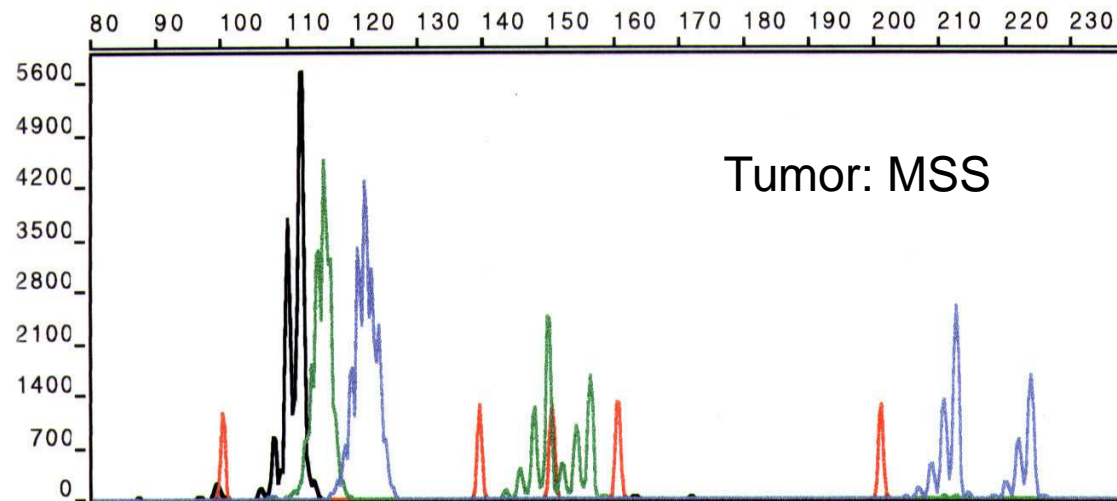
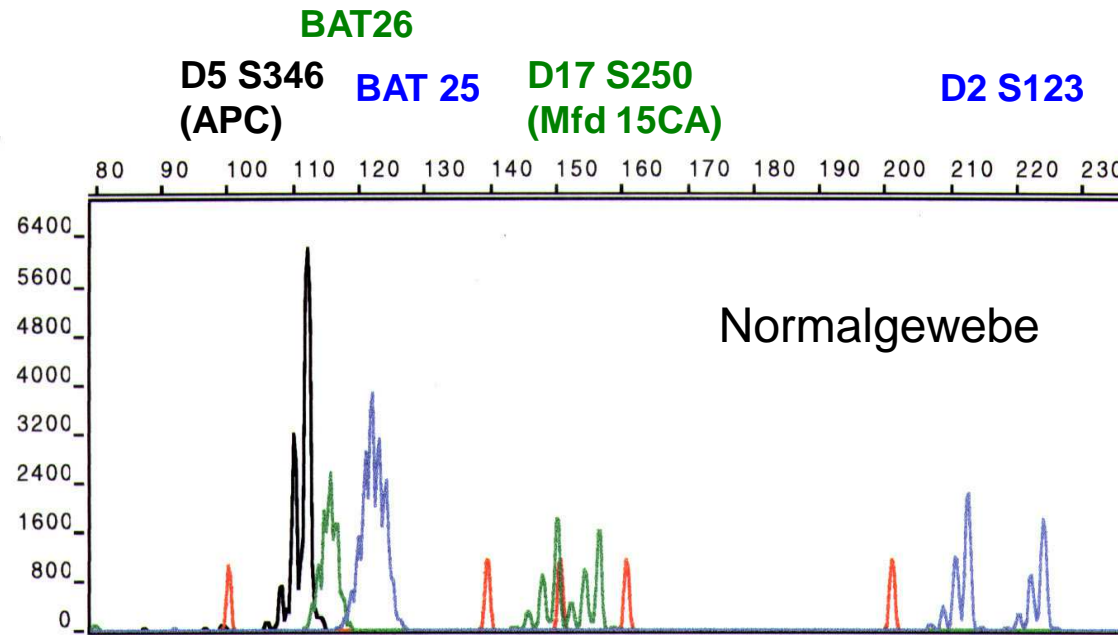


# HNPPC – Hereditary Non-Polyposis Colon Carcinoma

- Junge Patienten
- Colon ascendens
- Muzinöses Adenokarzinom
- Mutation in DNA-Reparaturgen (*hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*)
- Hohe Mutationsrate
- Mikrosatelliteninstabilität

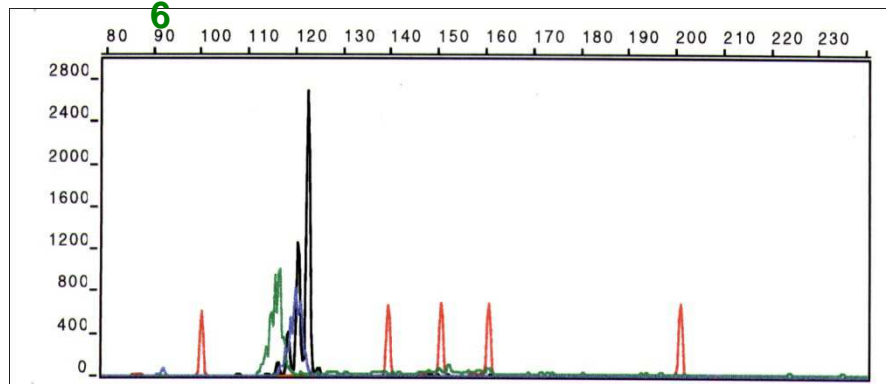
# HNPCC MSI- Analyse

- 6-FAM**
- BAT 25: 110-130bp**
- D2 S123: 200-230bp**
- TET**
- BAT 26: 100-120bp**
- D17 S250: 140-170bp**
- HEX**
- D5 S346: 100-130bp**
- TAMRA**
- Interner Standard**



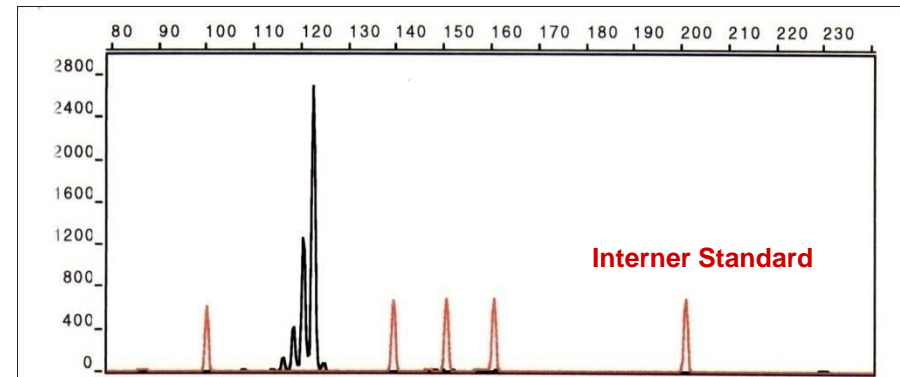
# MSI-Analyse

BAT 25  
BAT2 D5 S346 (APC)

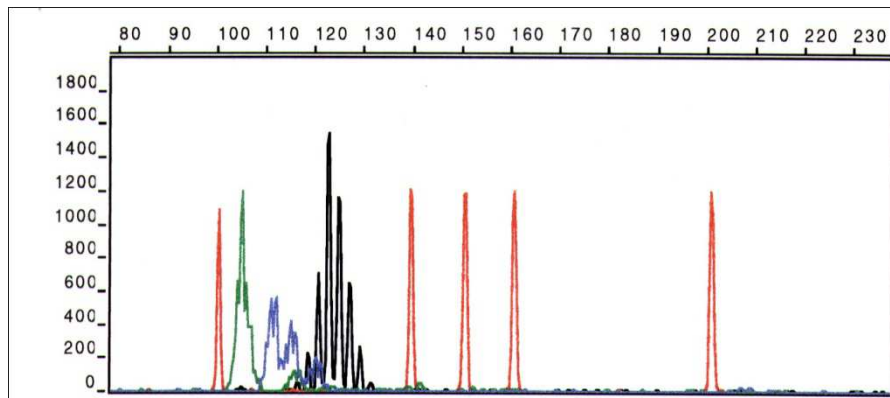


**NORMALGEWEBE**

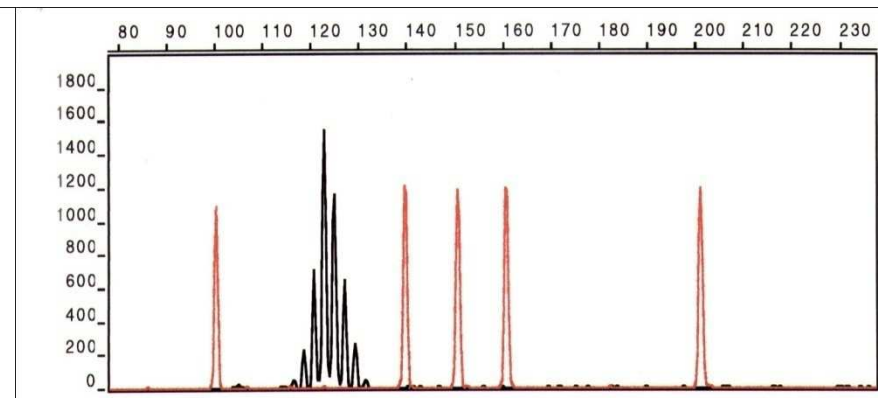
D5 S346 (APC)



**NORMALGEWEBE**



**TUMORGEWEBE: MSI-H**



**TUMORGEWEBE: MSI-H**