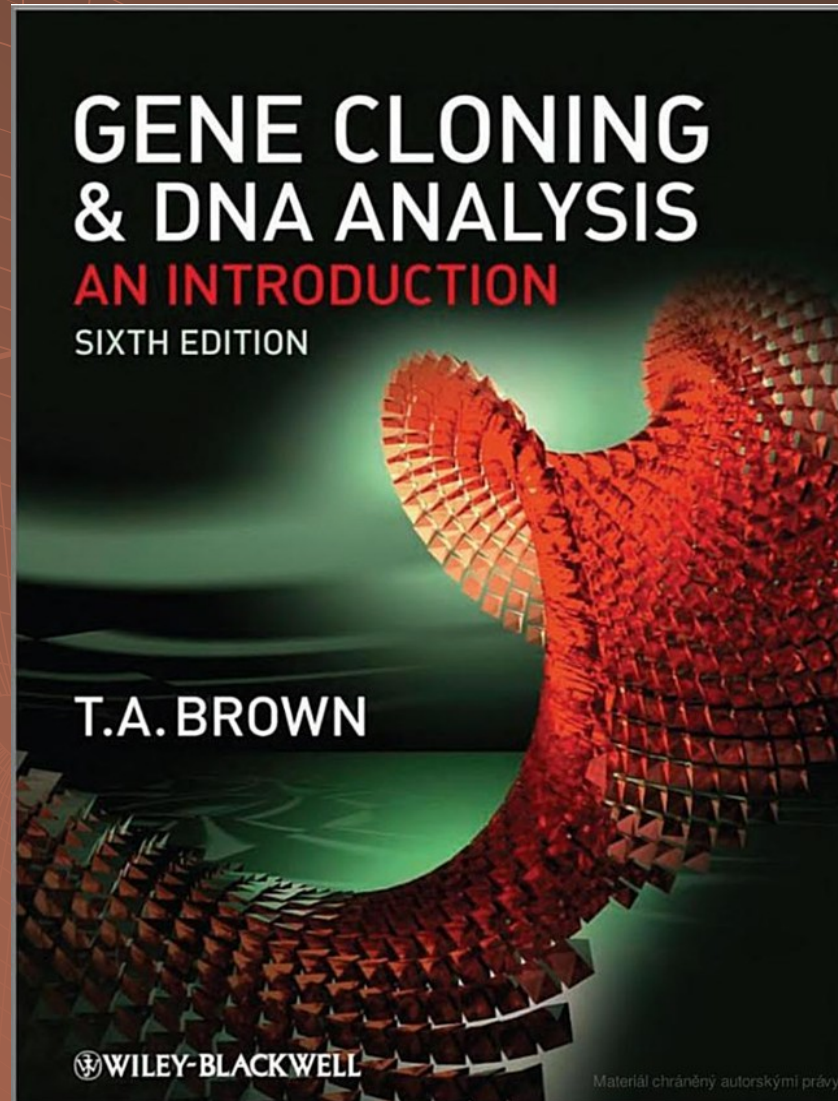


# Analýza a separace nukleových kyselin

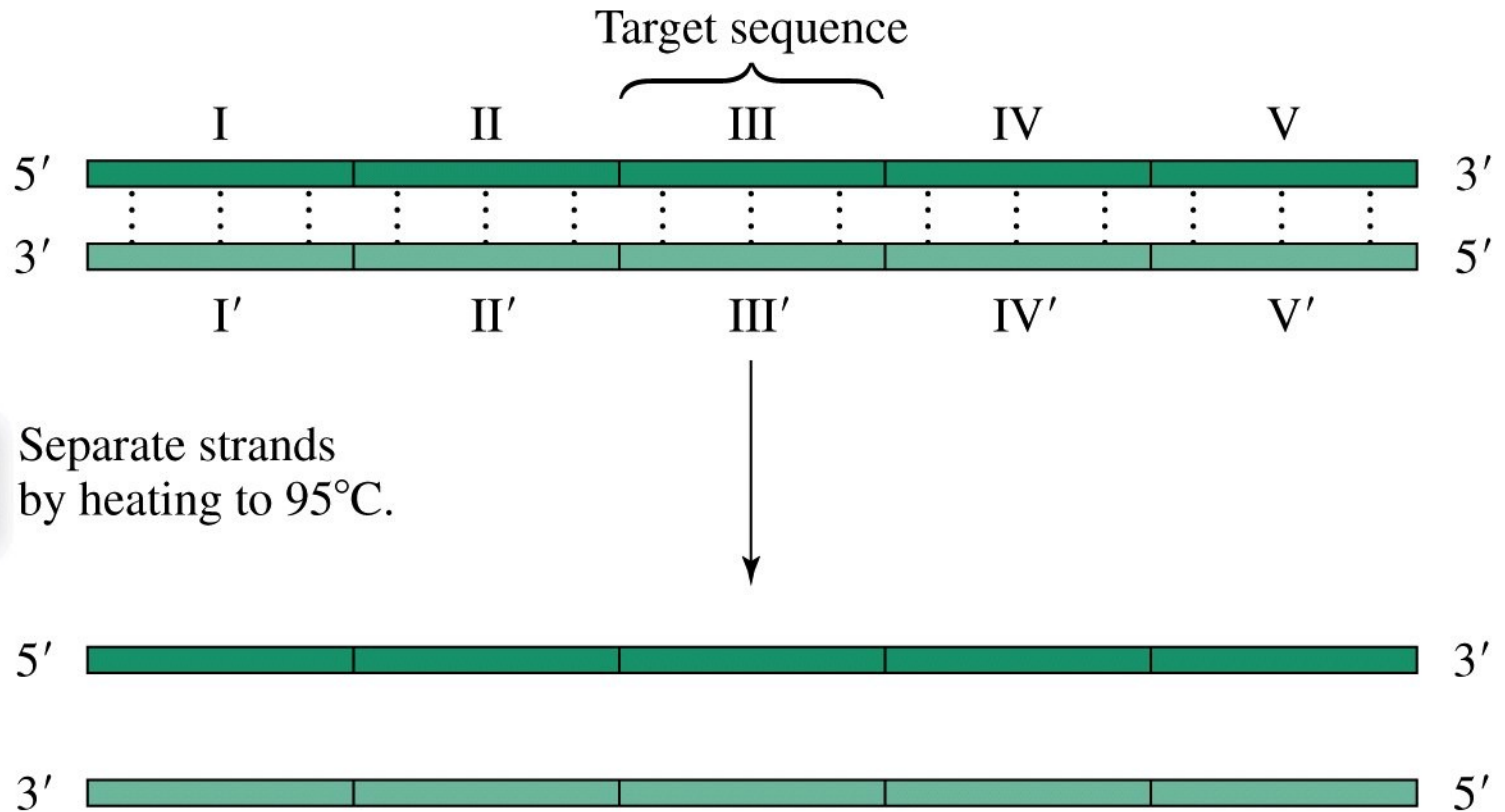


INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# Literatura



# PCR Mullis



Step 1

Separate strands  
by heating to 95°C.

Step 2

Hybridize primers by cooling to 50°C.

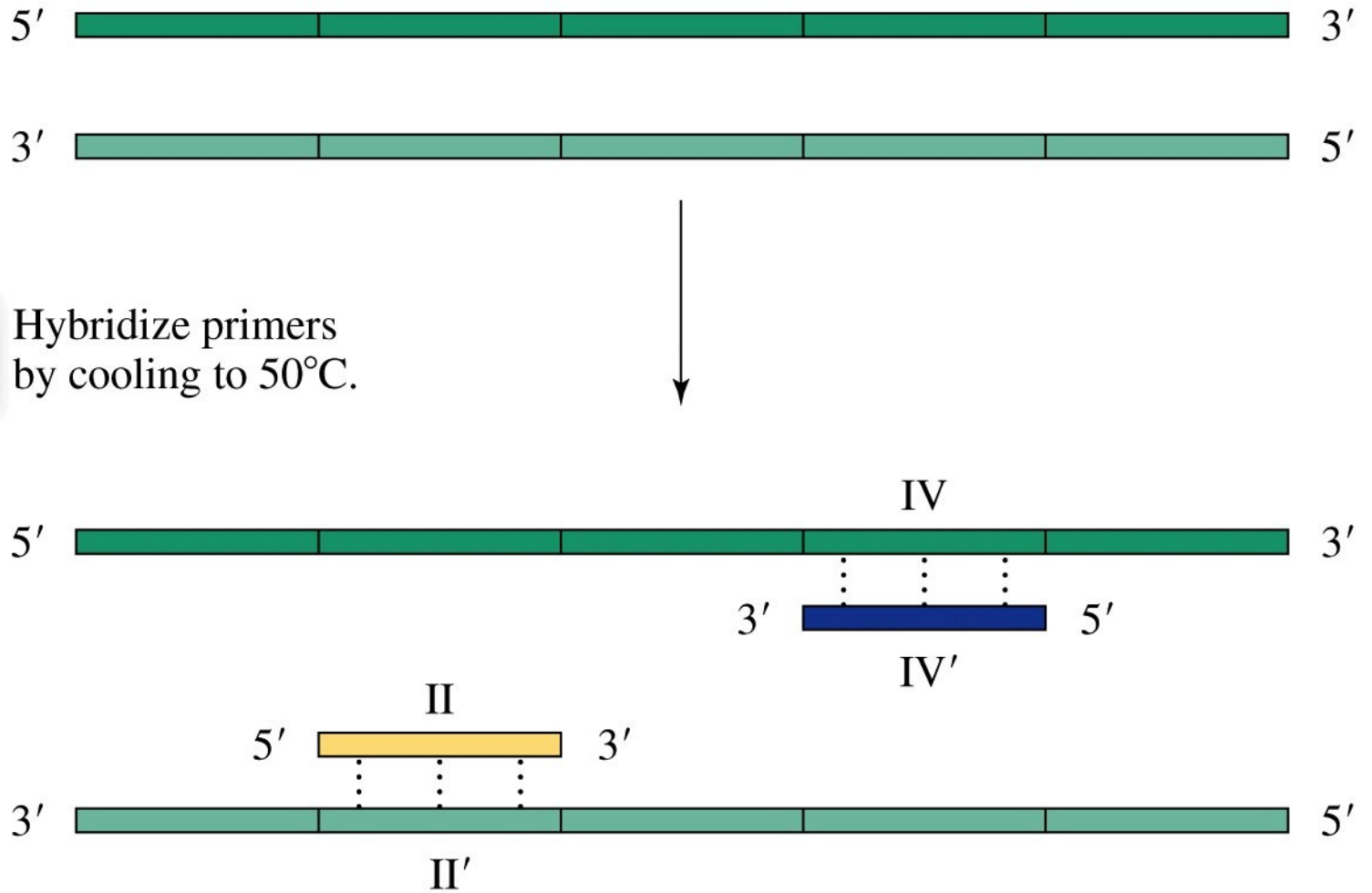
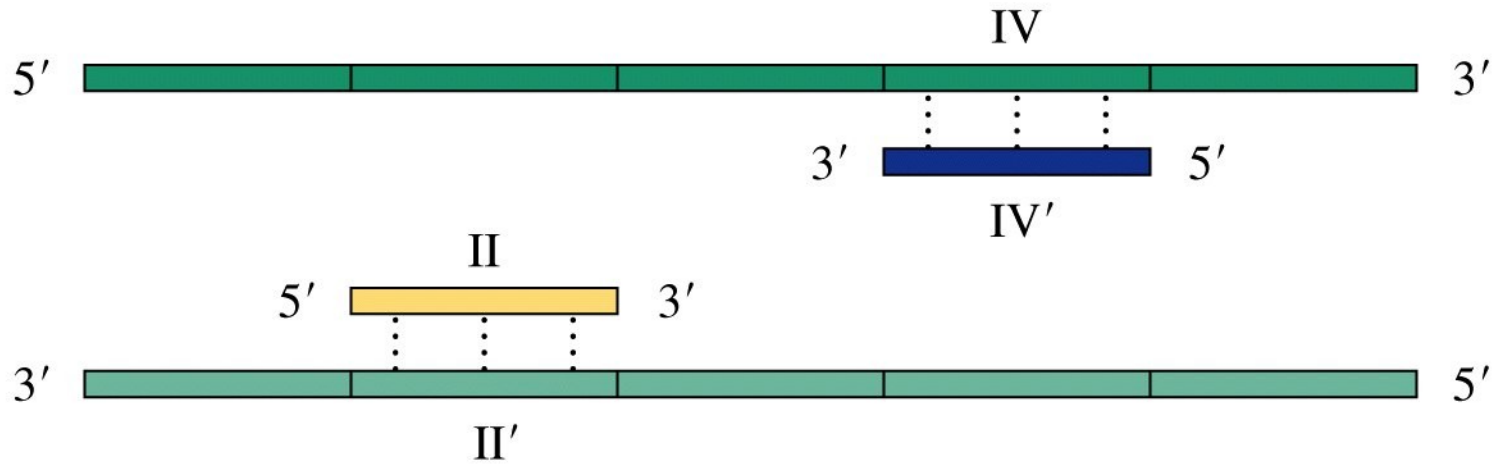


Figure 13-11 part 2 Concepts in Biochemistry, 3/e  
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Step 3

DNA is synthesized by extending the primers at 72°C.

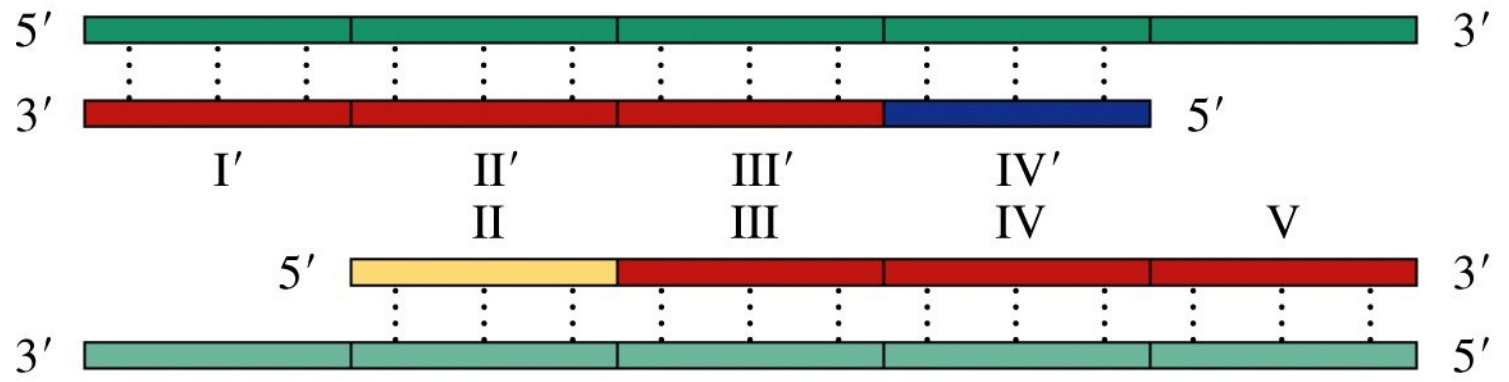
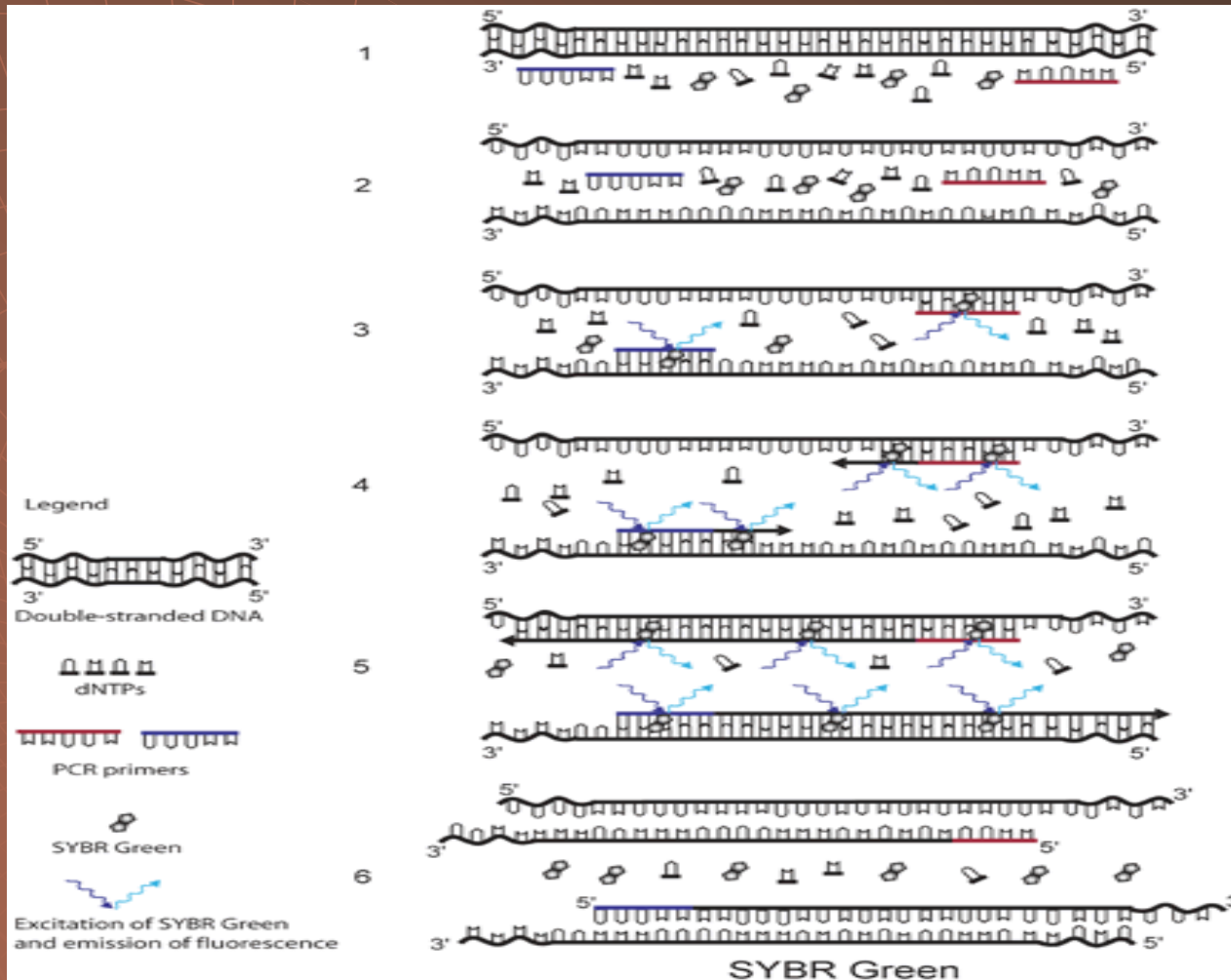
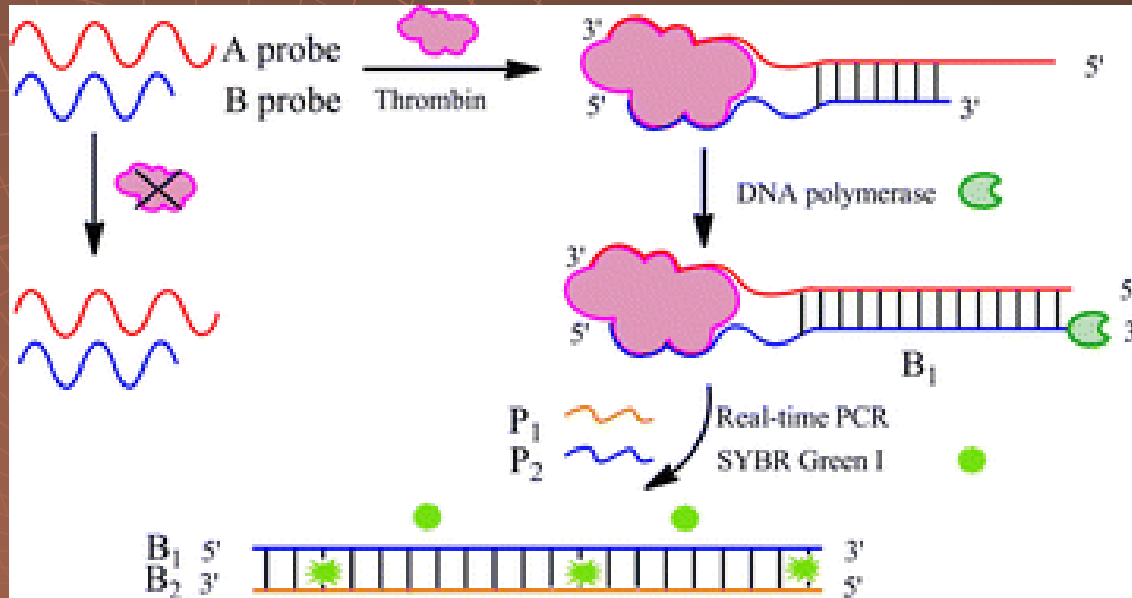


Figure 13-11 part 3 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

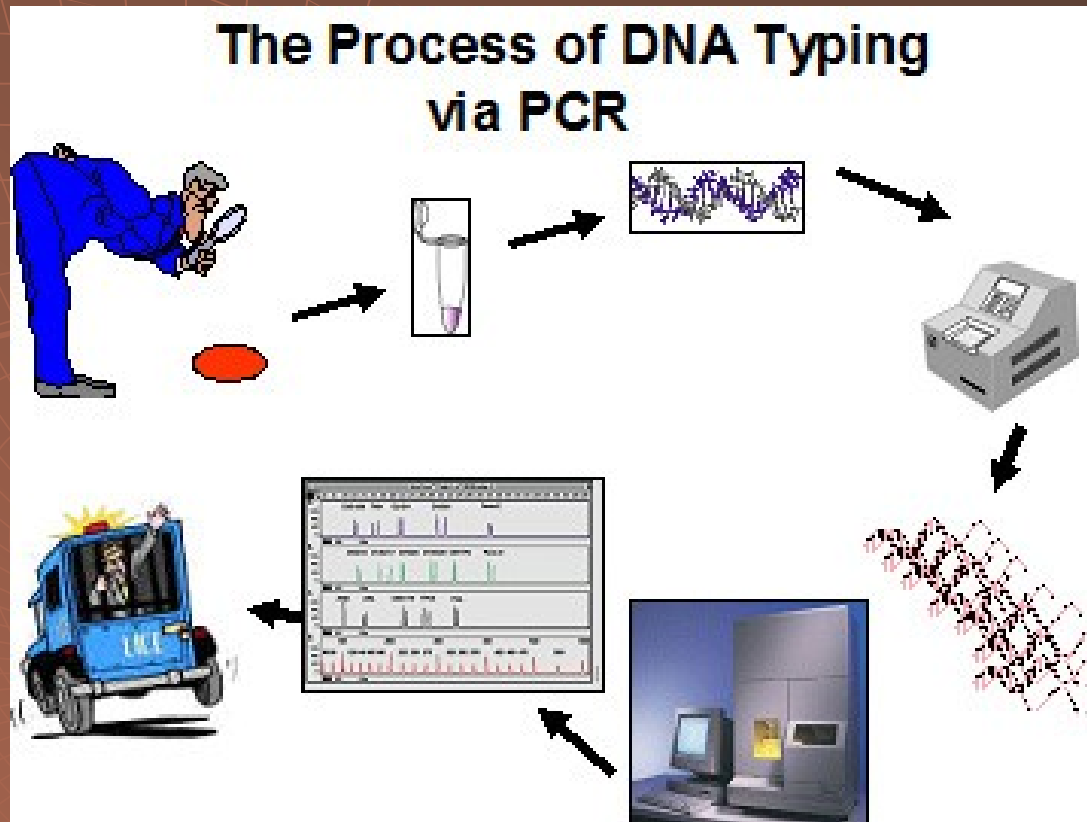
# Real time PCR



# Real time PCR proteinů



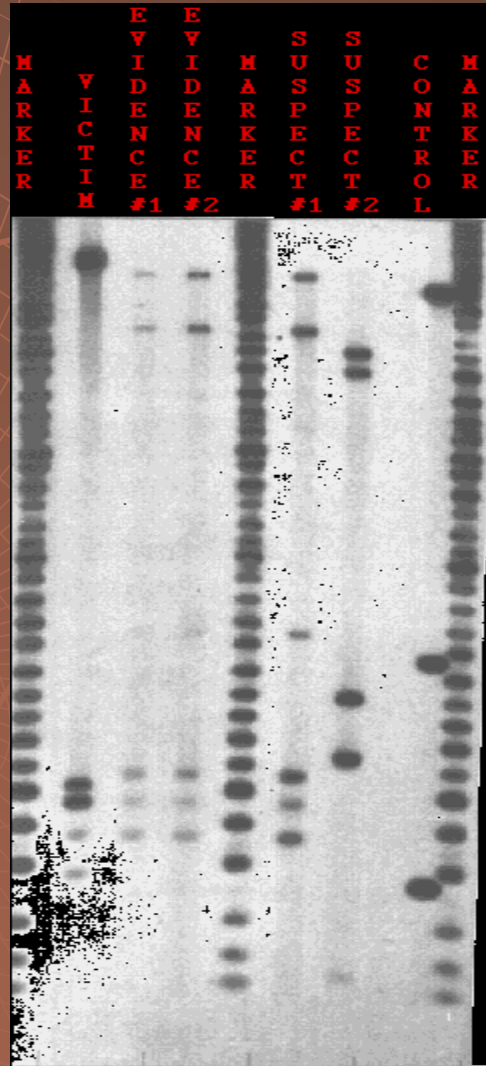
# Genetická daktyloskopie



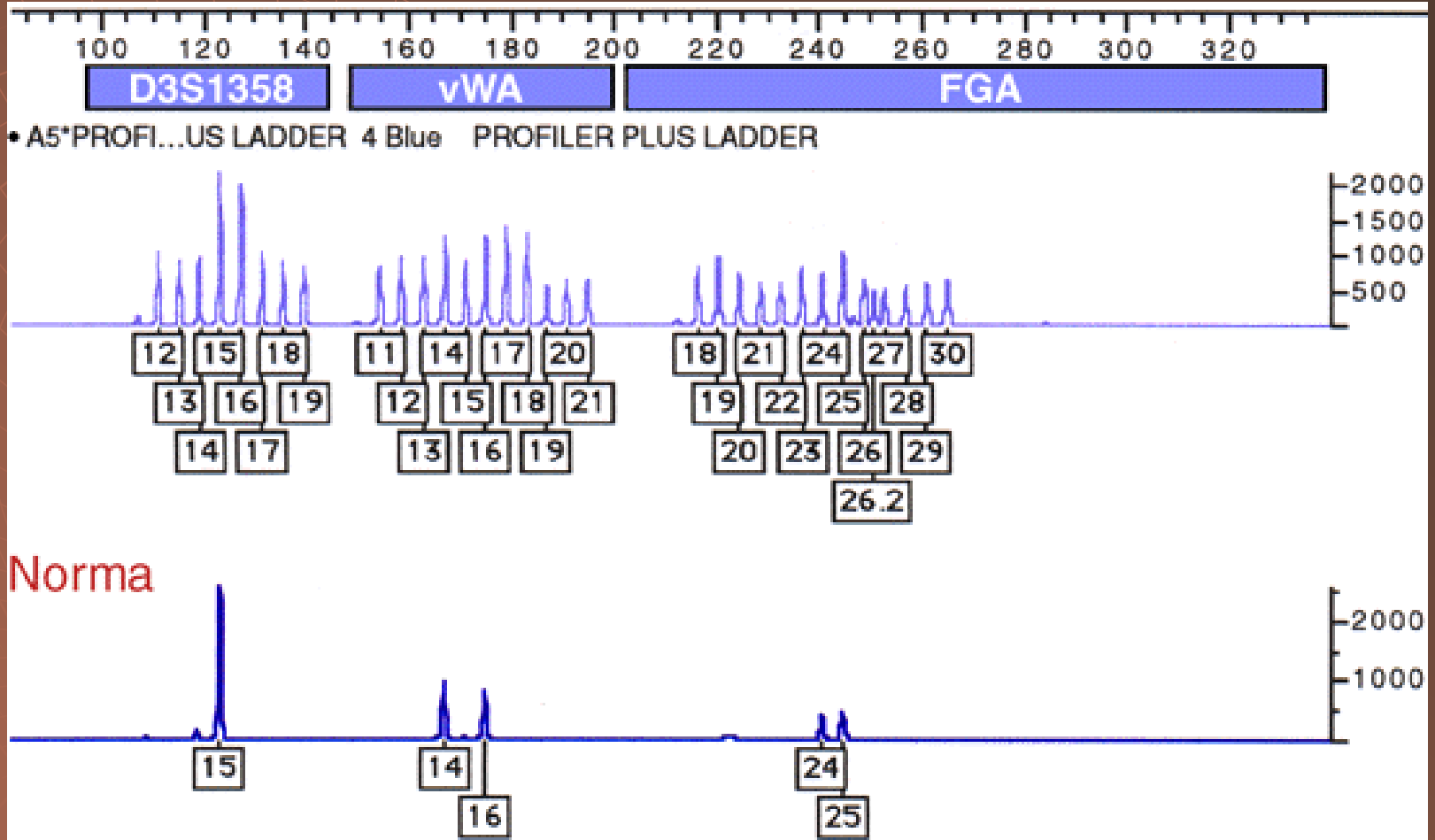


# Použití restrikčních enzymů - RFLP

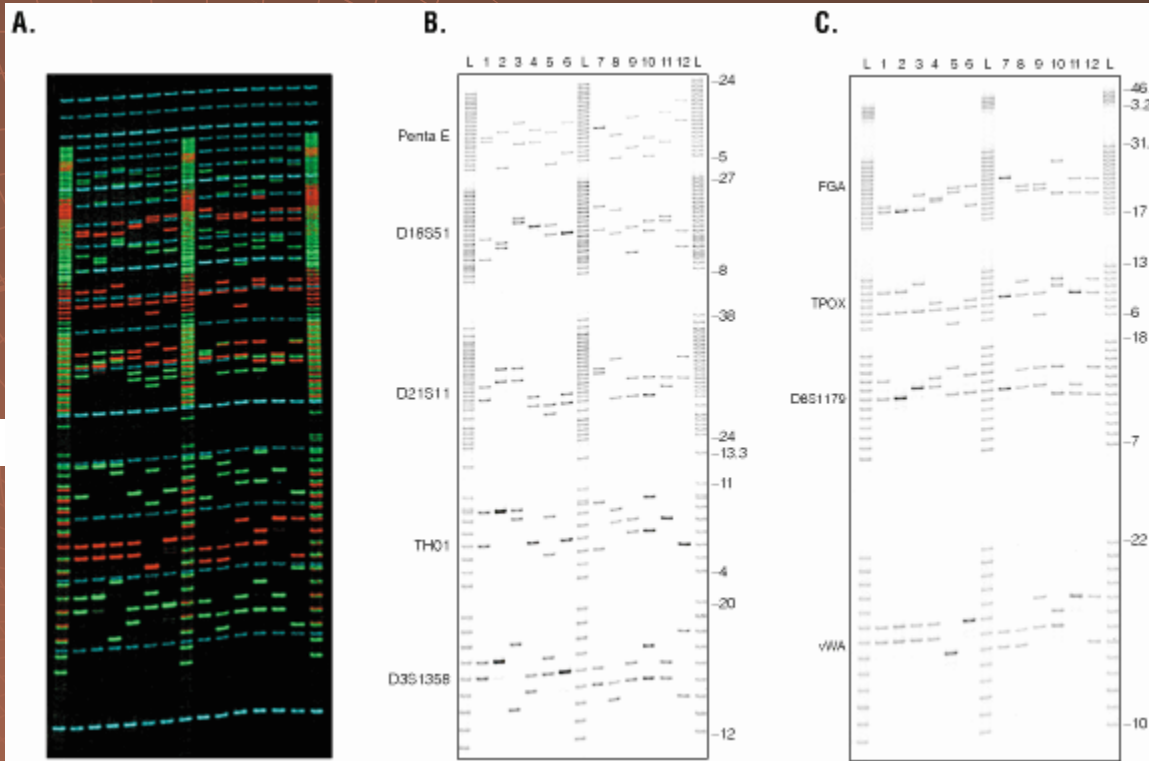
## Restriction Fragment Length Polymorphism



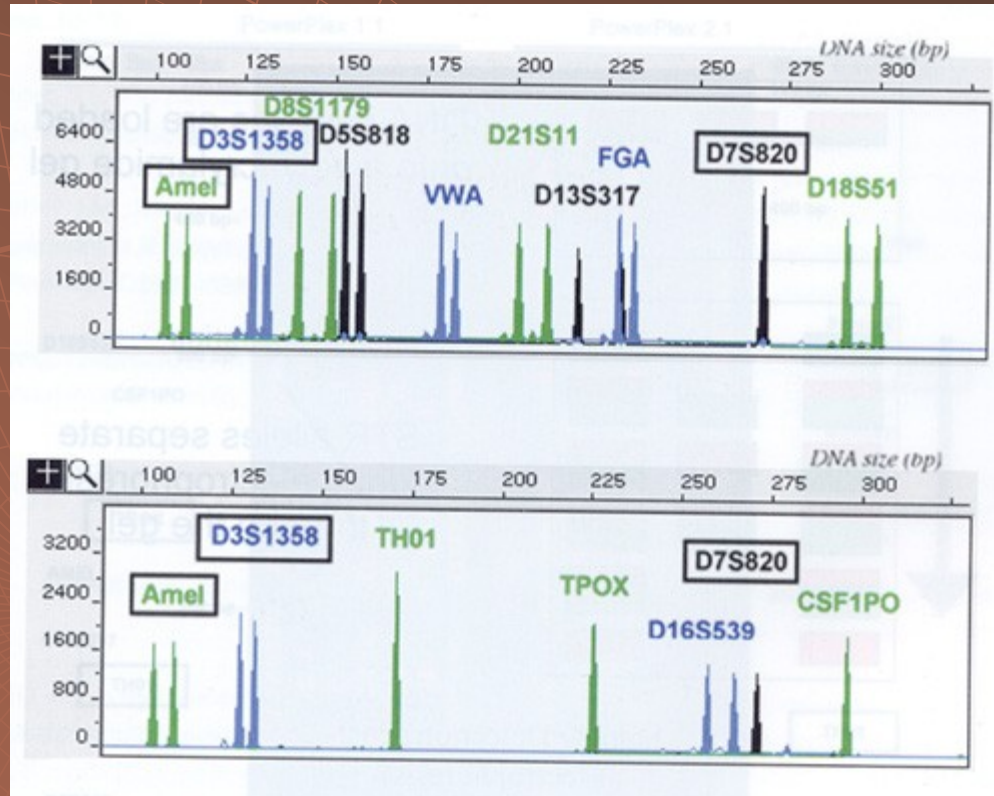
# Short tandem repeats



# Short tandem repeats



# Short tandem repeats



# Testy paternity

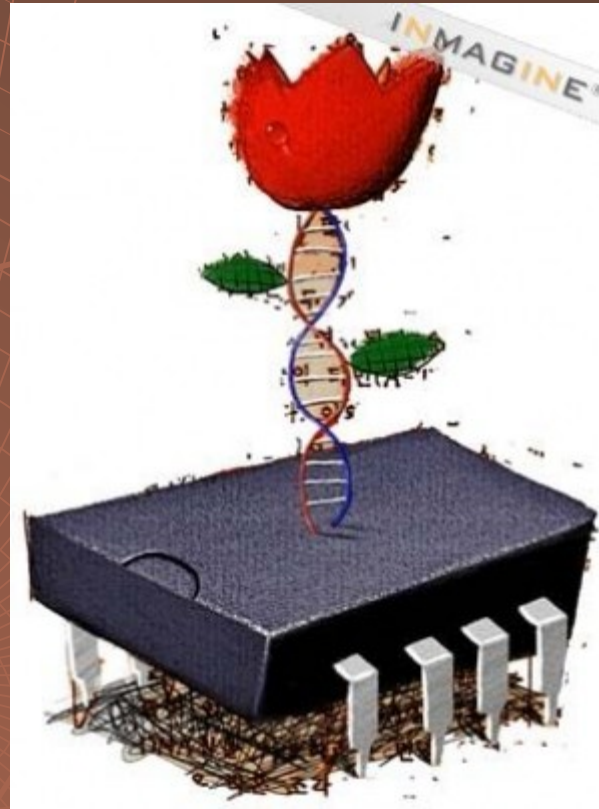
## Zjednodušené testy

- ◆ STR na Y chromosomu – mužských potomků srovnání s otcem
- ◆ Mitochondriální DNA – dědí se po matce – matroklinní dedičnost

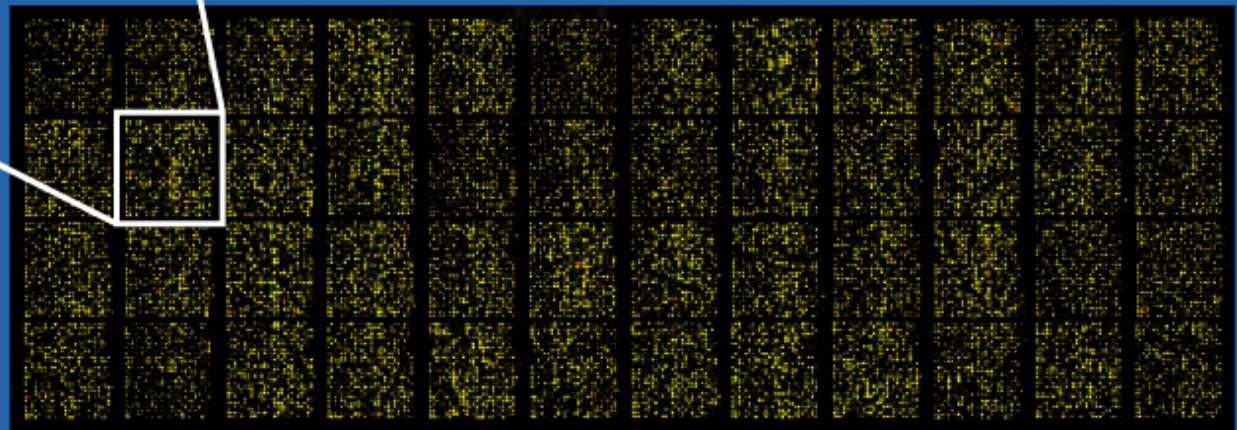
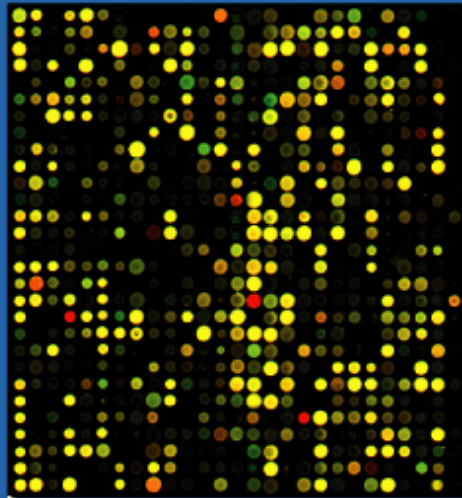
# Mitochondriální Eva



# DNA chipy

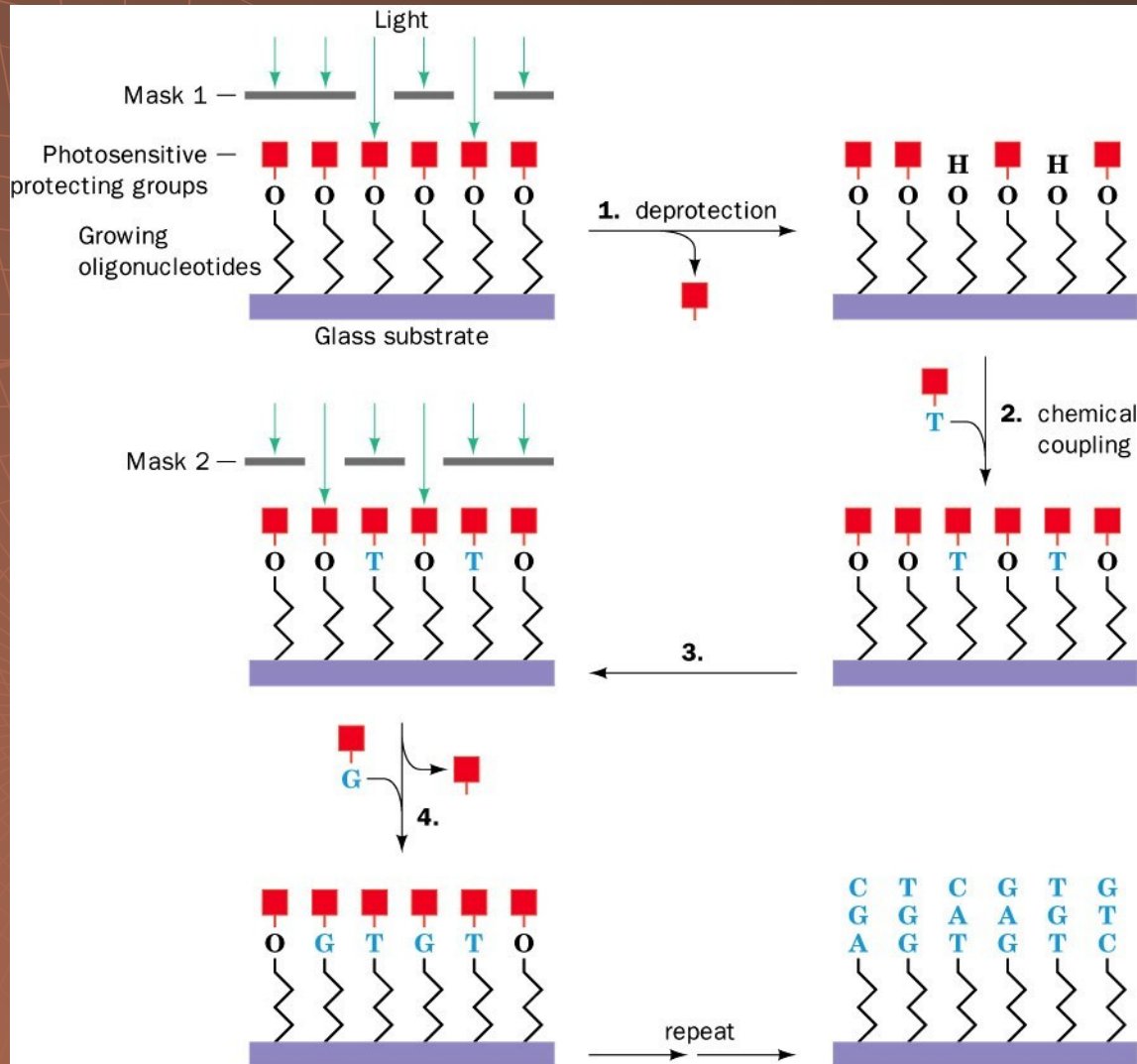


# DNA chipy

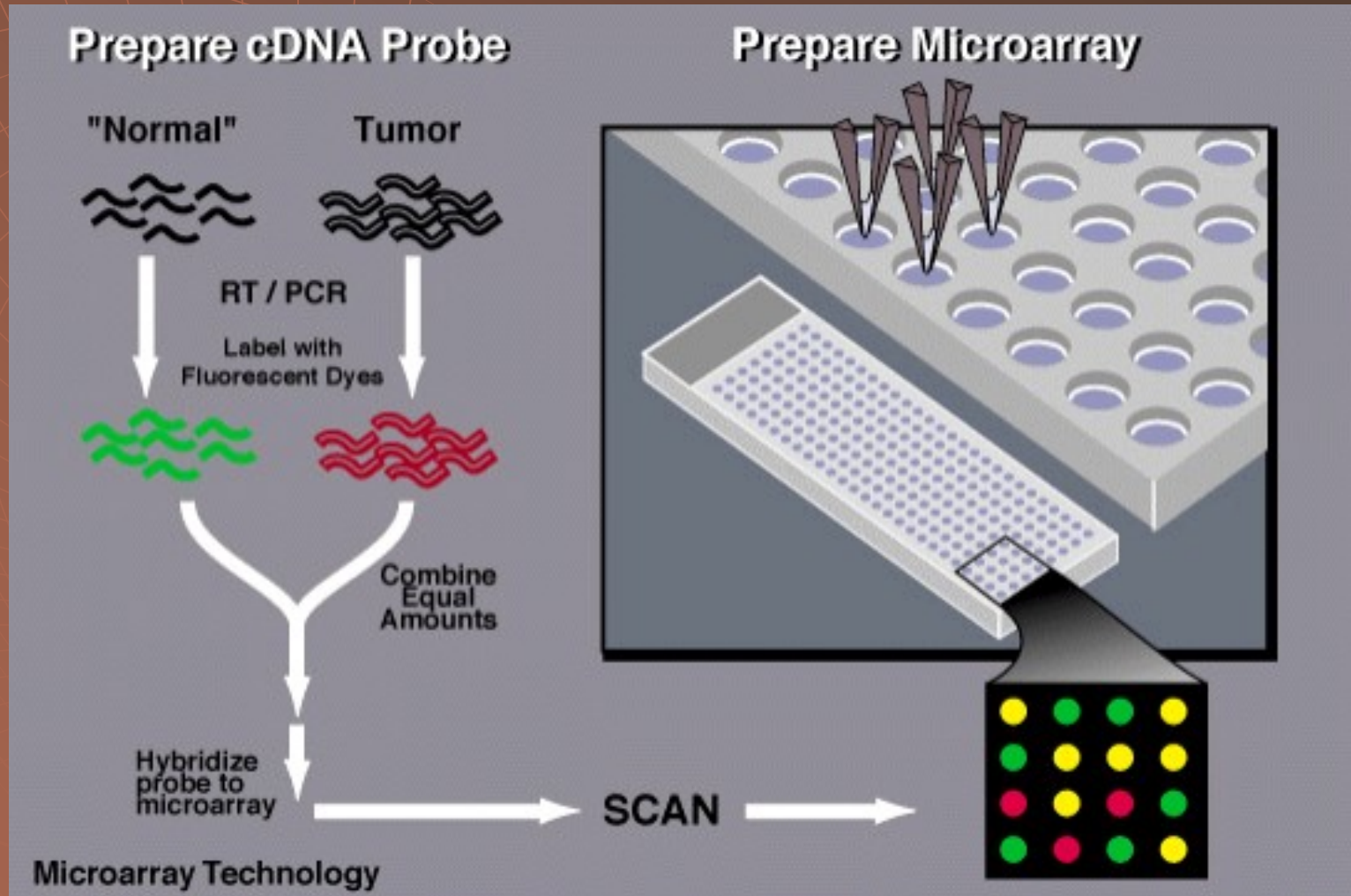




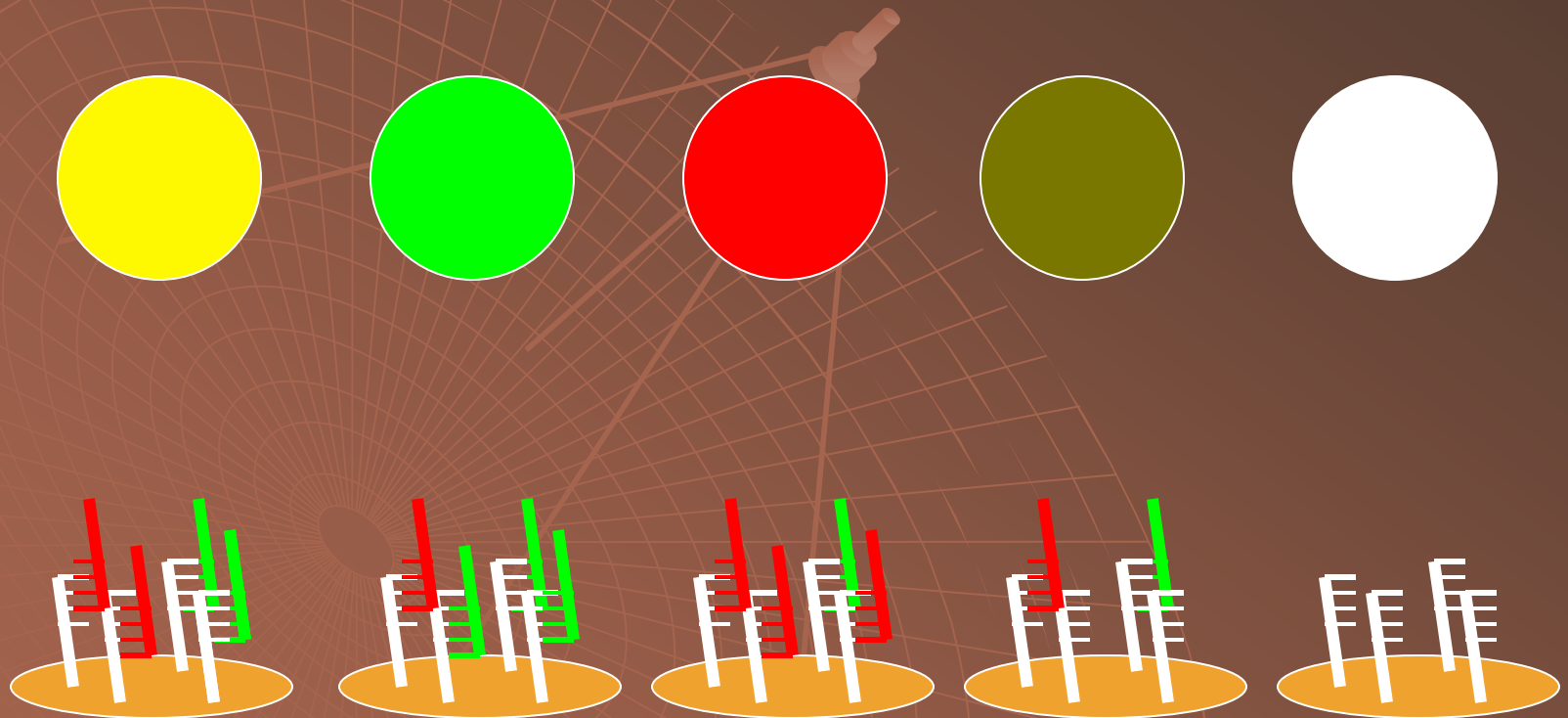
# DNA chipy



# DNA chipy



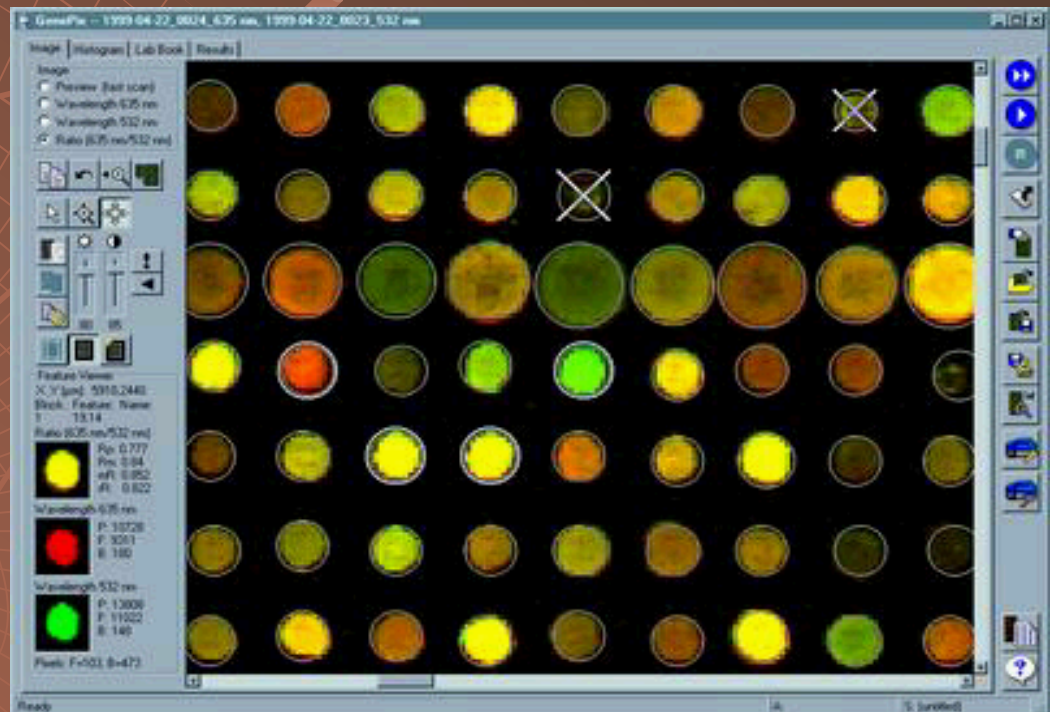
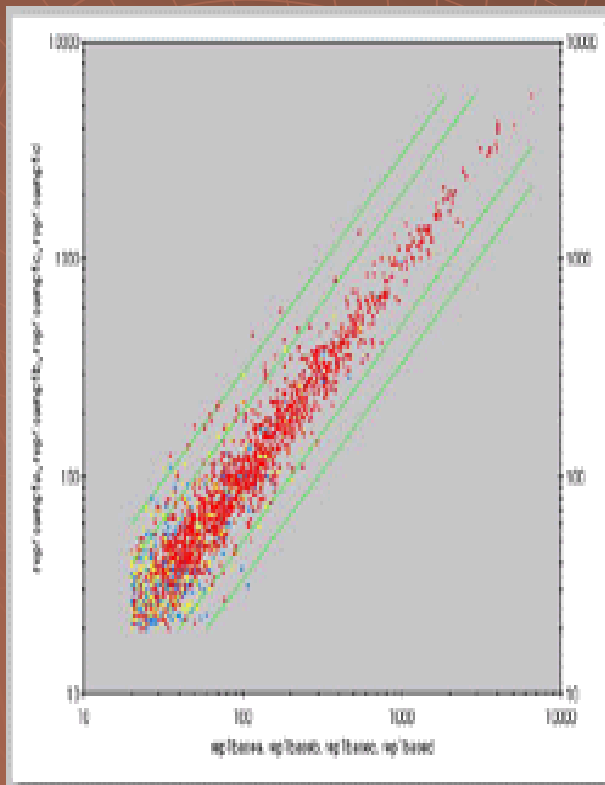
# DNA chipy - barva skvrn

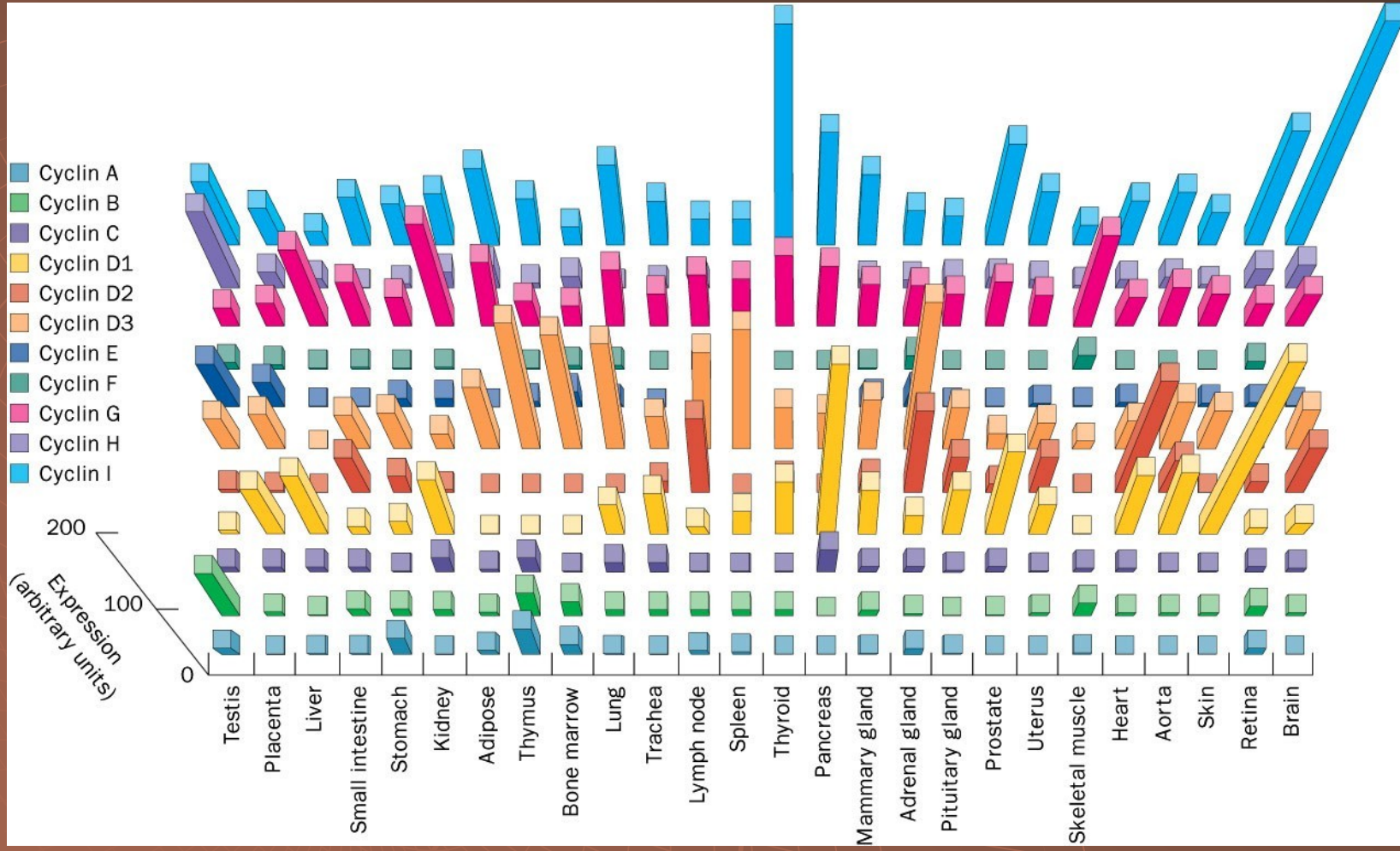


# DNA chipy - vybavení

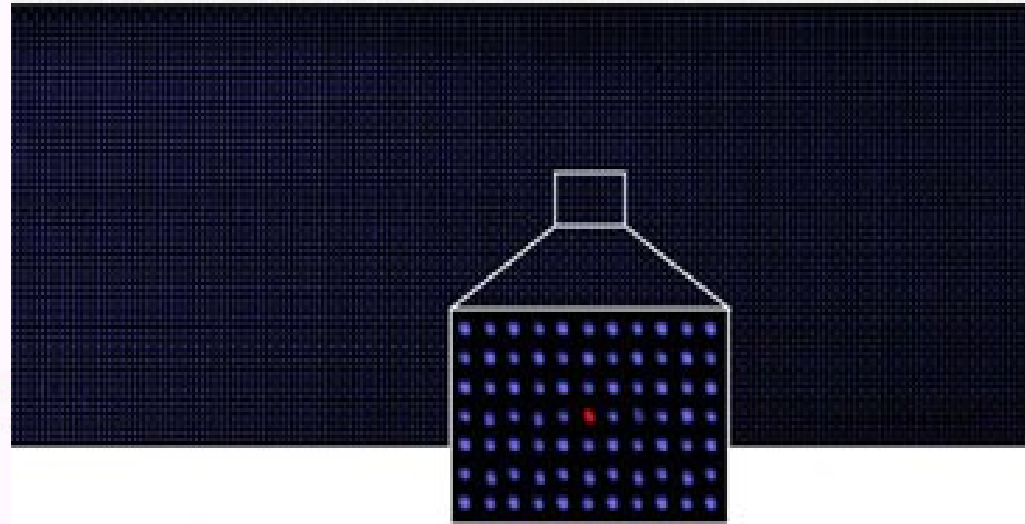


# DNA chip software





# Proteinové chipy

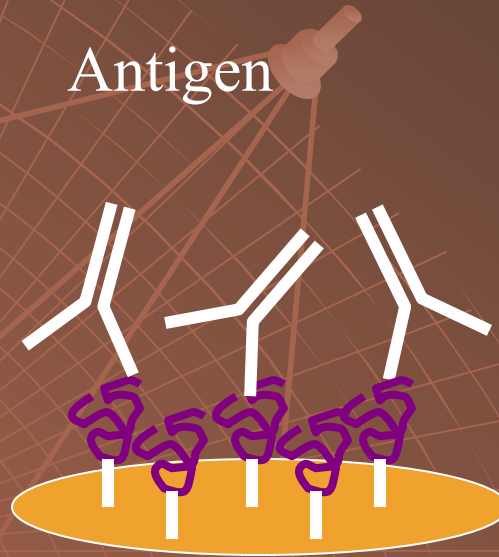


# Proteinové chipy – typy interakcí

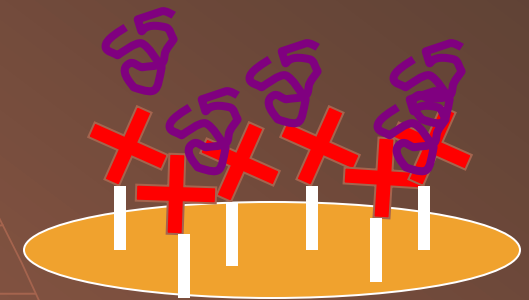
- Protilátka



- Antigen



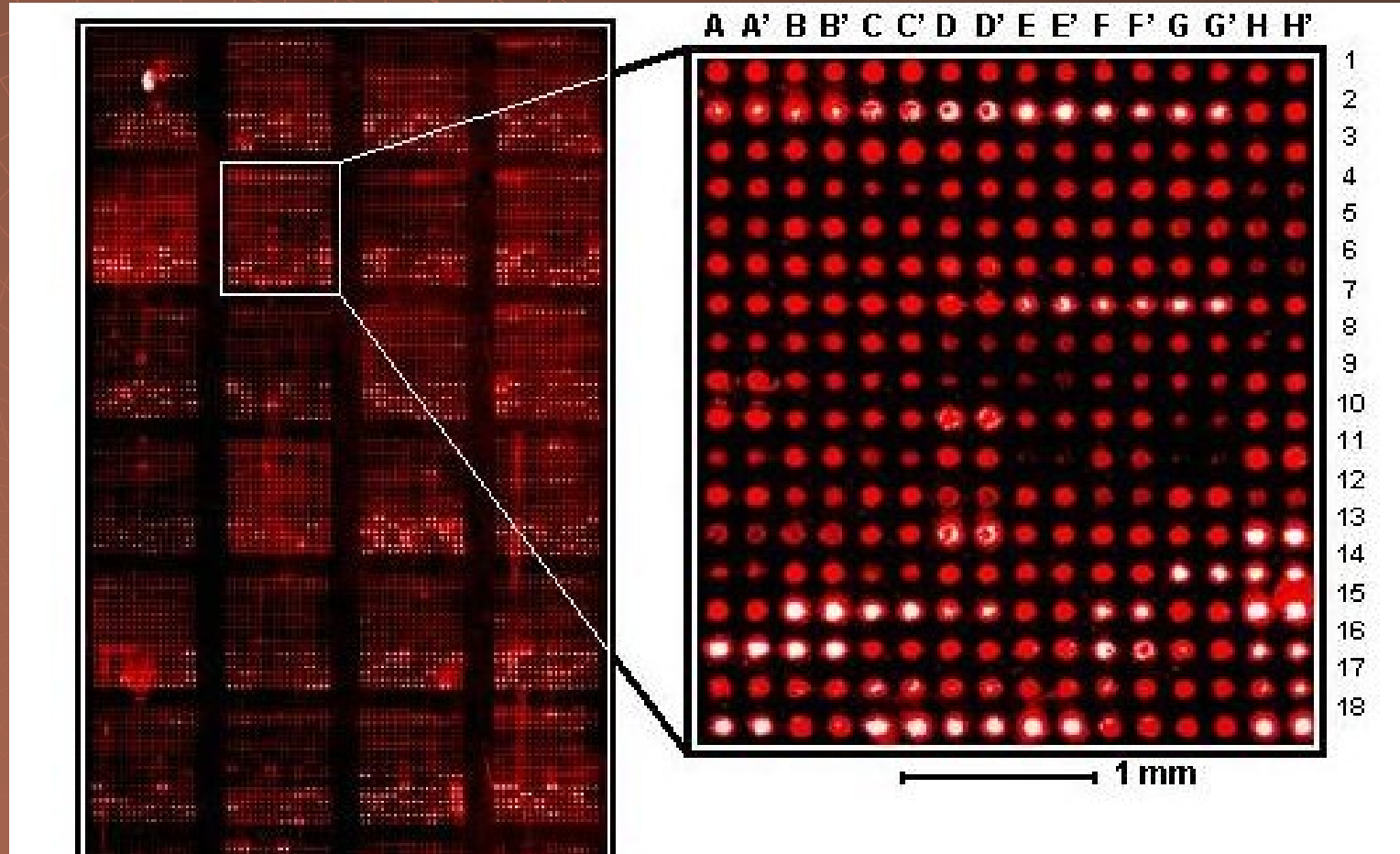
- Ligand



- Detekce: SELDI MS, fluorescence, SPR, electrochemická, radioaktivita,



# Anti-GST Probe



# Blotting

- ◆ Southern – DNA
- ◆ Northern – RNA
- ◆ Western - bílkoviny



# Izolace nukleových kyselin

# Cíl izolace

- ◆ Odstranění proteinů
- ◆ DNA vs RNA
- ◆ izolace specifického typu NK

# Typy NK

- ◆ genomická (chromosomální)
- ◆ organelová (mitochondrie, chloroplasty)
- ◆ plasmidy (extra-chromosomální)
- ◆ virová (ds nebo ss)
- ◆ komplementární (mRNA)

# Nejpoužívanější metody

- ◆ na základě rozdílné rozpustnosti – extrakce, srážení
- ◆ na základě vlastností - chromatografie – polarita- adsorpční, náboj-ionexová
- ◆ sedimentace - gradientová ultracentrifugace

# Postup

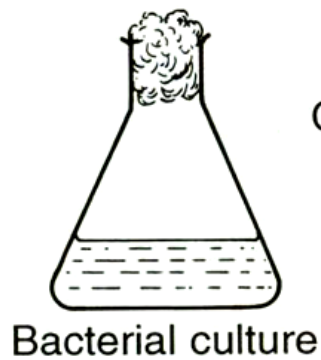
1. Rozbití buněk a membrán pro uvolnění NK
2. Inaktivace DNA- nebo RNA-degradujících enzymů (DNasy, RNasy).
3. Separace NK od dalších komponent uvolněných z buňky.
  - Extrakce/Precipitace
  - Chromatografie
  - Ultracentrifugace



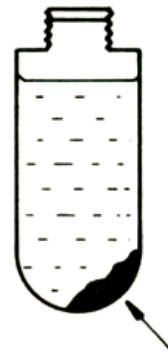
# Extrakce/Precipitace



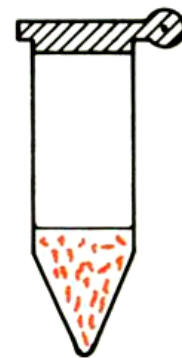
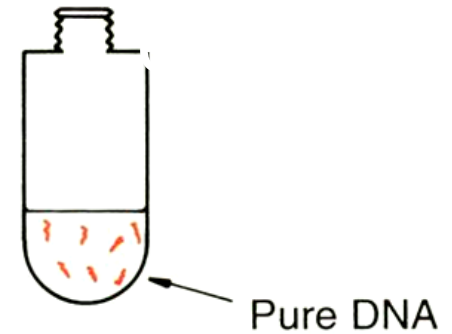
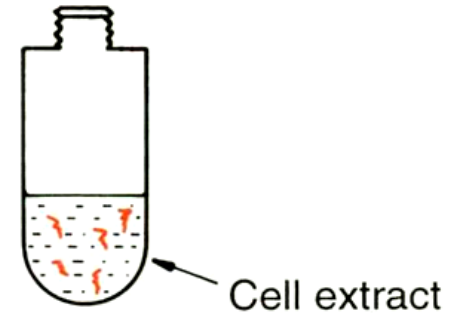
**1 A culture of bacteria is grown and then harvested**



Centrifugation



**2 The cells are removed and broken to give a cell extract**



**4 The DNA is concentrated**

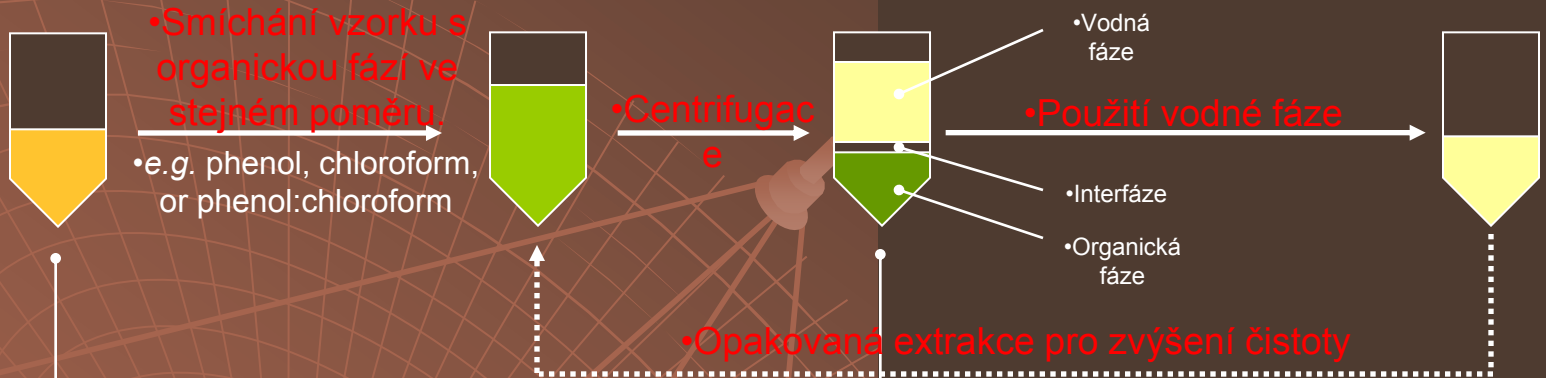
**3 The DNA is purified from the cell extract**

# • Izolace genomické DNA

- Typická procedura
  - *1. Sklizení buněk*
  - *2. Lyse buněk*
    - 0.5% SDS + proteinase K  
(55° několik hodin)
  - *3. Fenolová extrakce*
    - Jemné třepání několik hodin
  - *4. Ethanolová precipitace*
  - *5. Působení RNAsy a proteinasy  
K*
  - *6. Opakování kroku 3 a 4.*

# •Extrakce/Precipitace

## •Krok 3: Organická extrakce

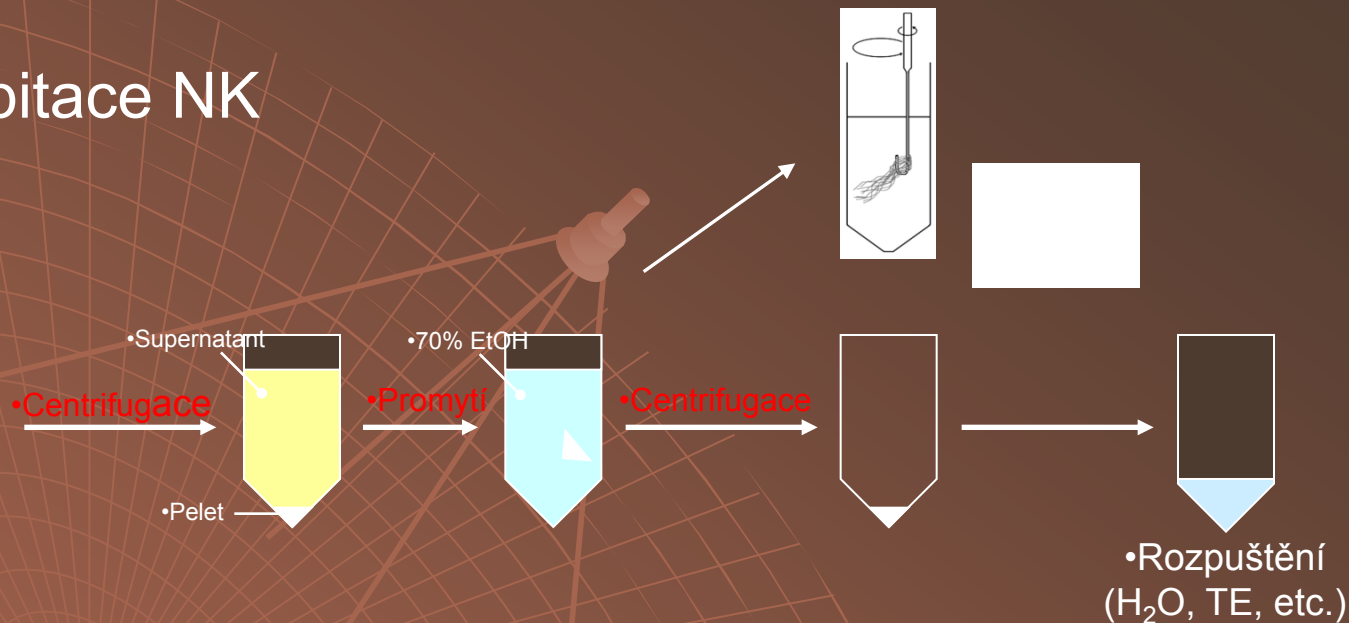
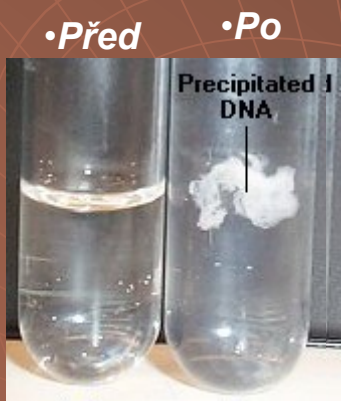


•Hrubý lyzát obsahující NK a další součásti buňky

•Vodná fáze obsahuje NK, organická fáze bílkoviny a lipidy. Nerozpustné složky přítomné v **interfázi**.

# •Extrakce/Precipitace

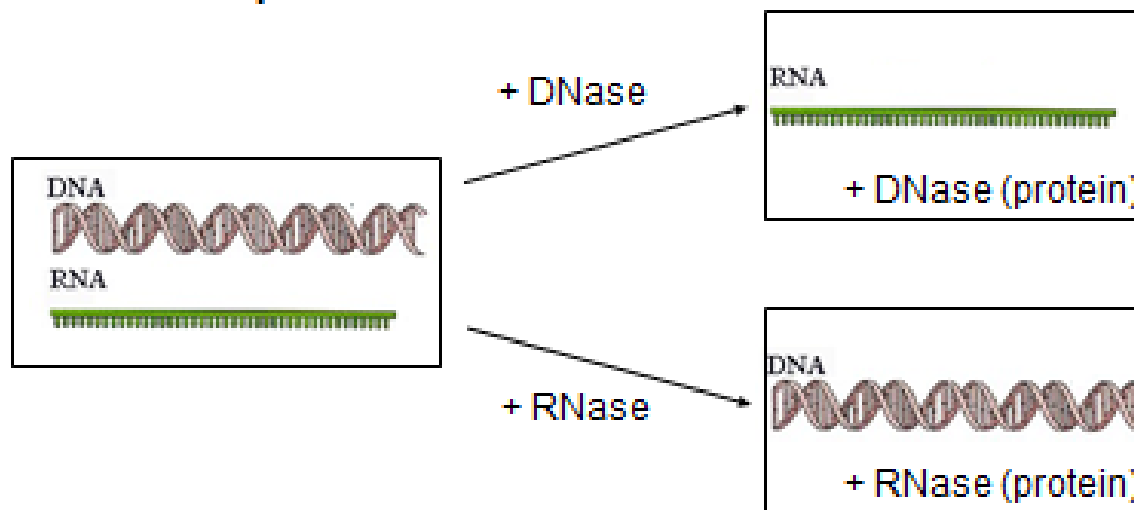
## •Krok 4: Precipitace NK



- Přidání EtOH a soli
- 2-2,5 objem EtOH
  - -20° C
  - Vysoká I
  - pH 5-5.5

## Detail kroku 5

Použití nukleas pro odstranění nechtěné DNA nebo RNA



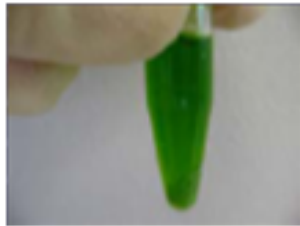
The background features a faint, light-colored grid pattern typical of a chromatogram. The grid consists of concentric circles and radial lines. A prominent red spot is visible on the right side of the grid, and a blue line extends from the center towards the right, passing through the red spot.

# Chromatografie

# Adsorpční chromatografie

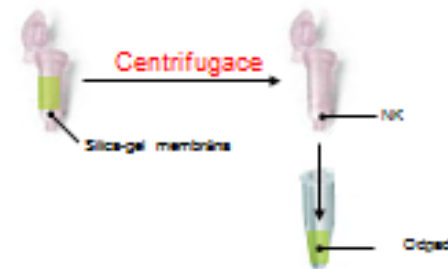
## Adsorpční chromatografie

Krok 1: Příprava lyzátu



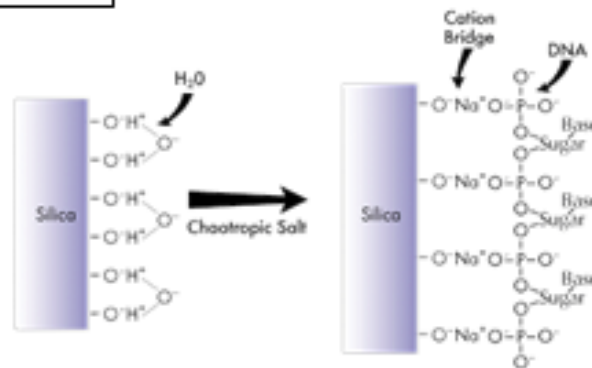
Aplikace na  
kolonku

Krok 2: Adsorpce na silikagel



Extrakční pufr pro vazbu DNA a  
RNA na silikagel:

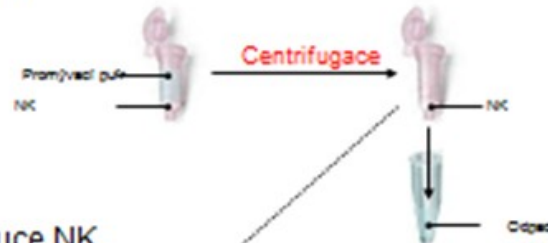
- nízké pH
- vysoká iontová síla
- chaotropní soli



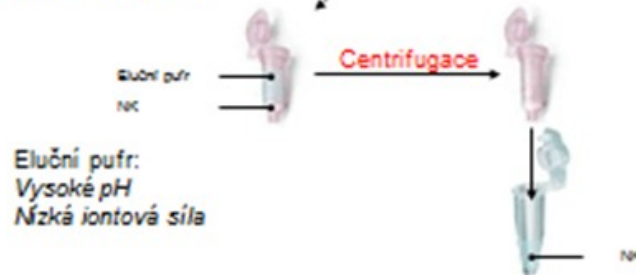
# Adsorpční chromatografie

## Adsorpční chromatografie

Krok 3: Vymytí kontaminant



Krok 4: Eluce NK

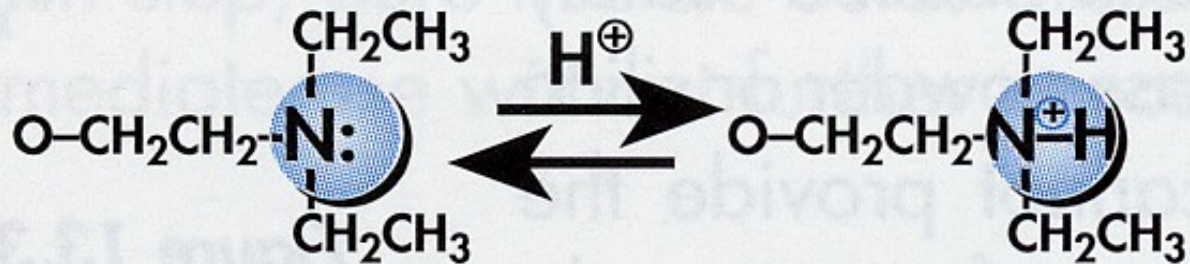


Eluční pufr:  
Vysoké pH  
Nízká iontová síla

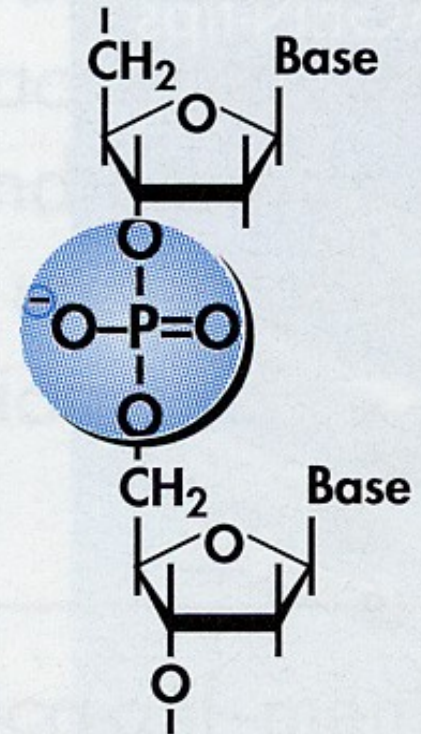


# • Ionexová chromatografie

• Vazba při nízkém pH nízké I



• Eluce zvýšením pH nebo vysokou I



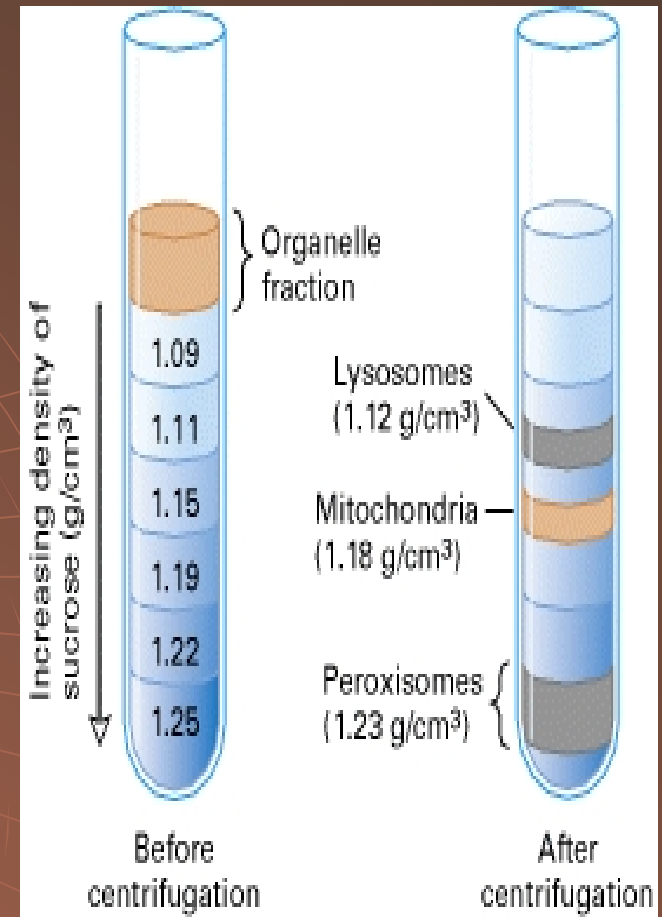
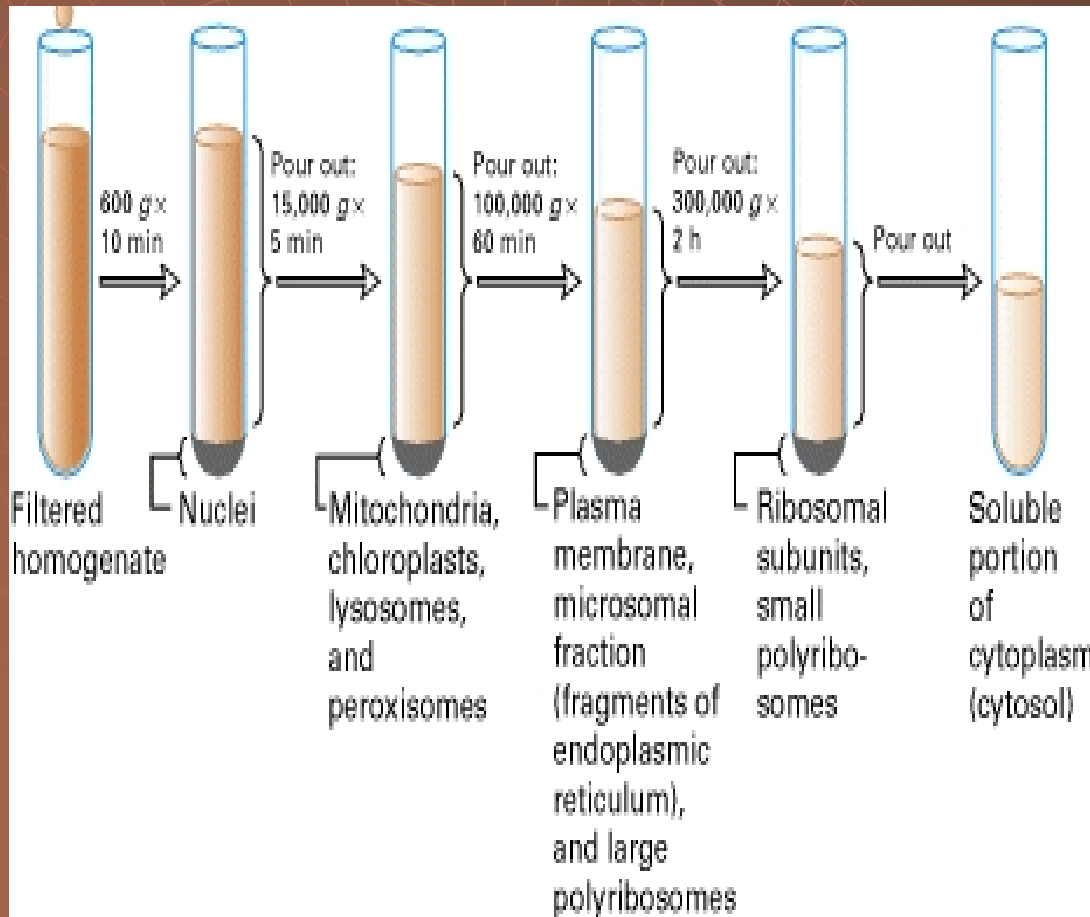
DEAE (diethylaminoethanol)

Chemical structure  
of DNA

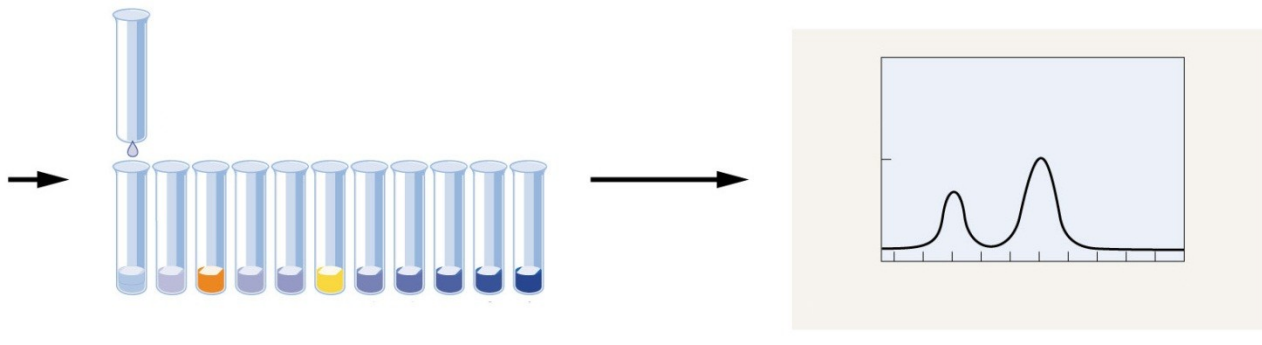
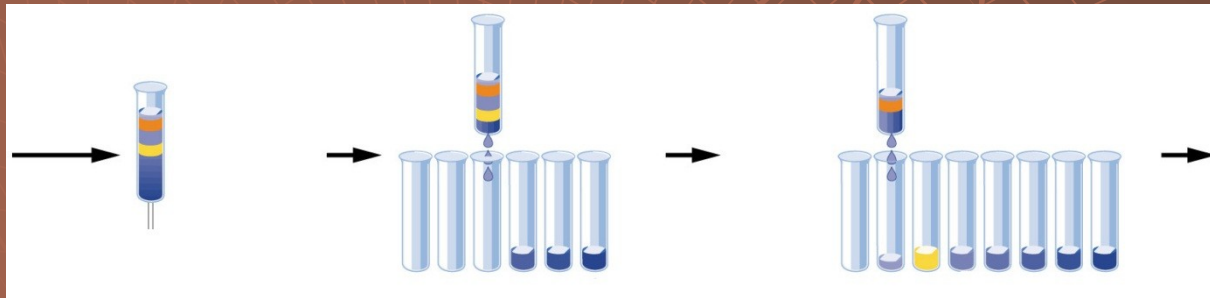
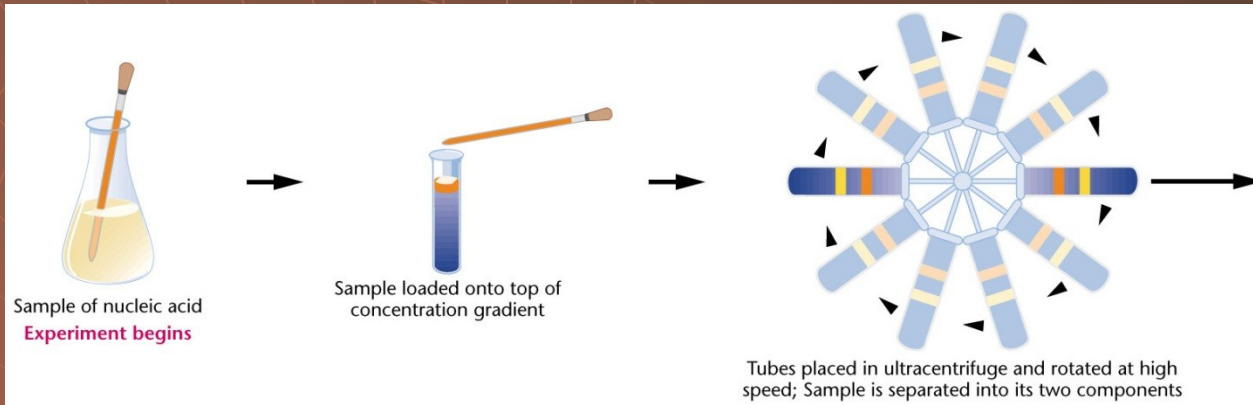


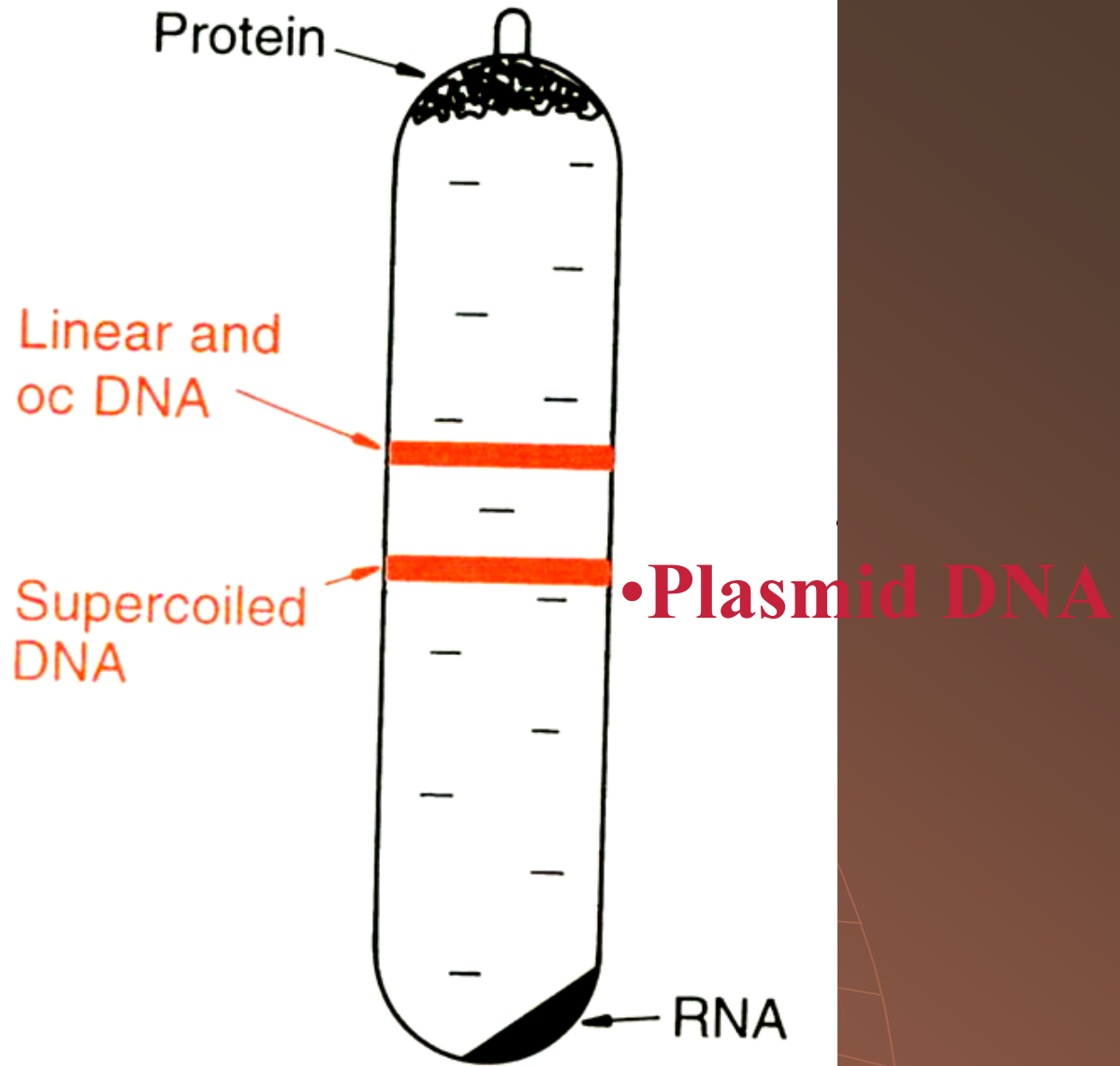
# Gradientová centrifugace

# Diferenciální versus gradientová centrifugace



# • Separation of Nucleic Acids by $\text{CeCl}_2$ Gradient Centrifugation





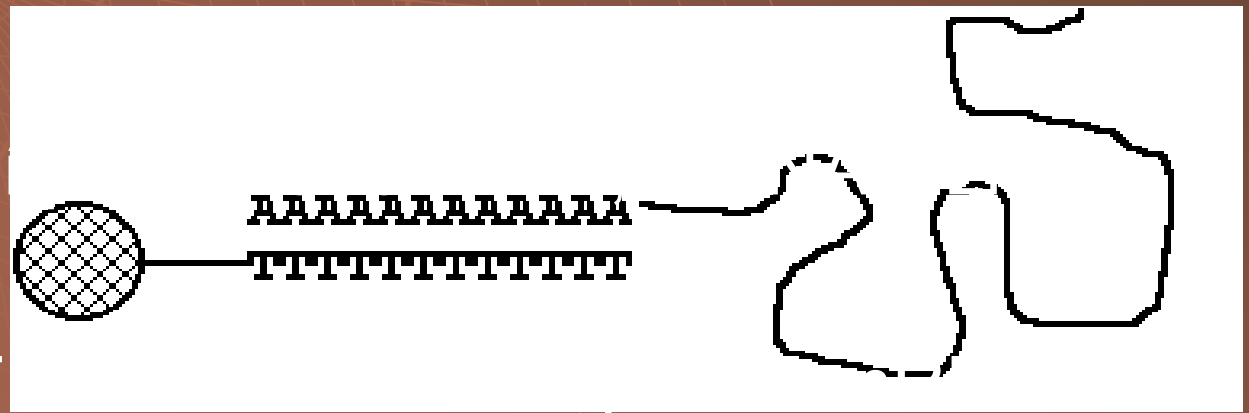
(a) An EtBr-CsCl density gradient

# • Izolace RNA - speciální přístupy

- nutno použít inhibitory RNÁsy
- extrakce guanidinium chloridem
- fenolová extrakce při pH < 4 (pH 8 pro DNA)
- působení RNase-free Dnase

• selektivní

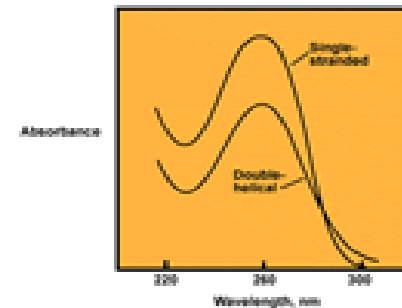
• oligo-dT



# Kontrola čistoty a kvantifikace NK

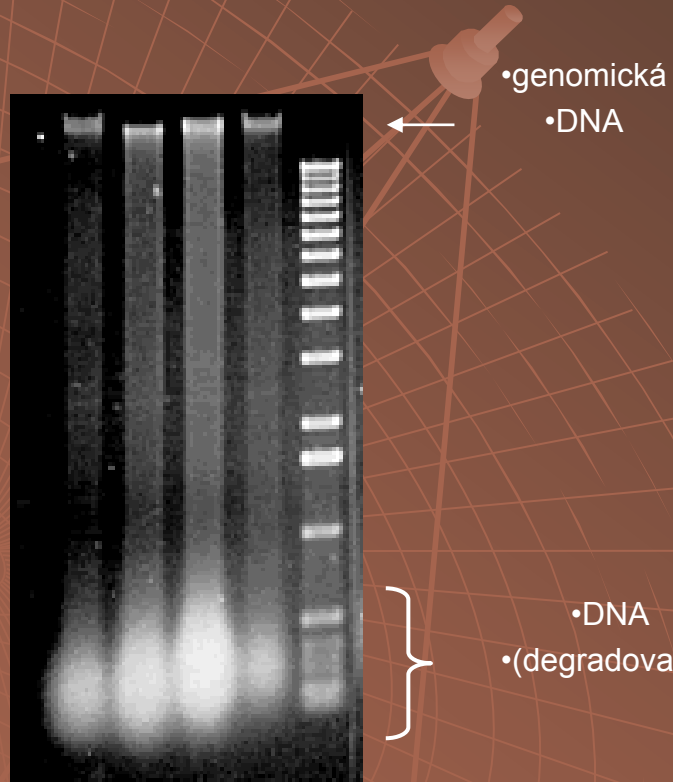
## Kontrola NK

- spektrofotometricky
  - kvalita
  - kvantita
- gelová elektroforéza
  - kvalita



DNA	$A_{260}$	$1.0 \approx 50 \mu\text{g/ml}_{ds} \approx 33 \mu\text{g/ml}_{ss}$
	$A_{260}/A_{280}$	1.6 - 1.8
RNA	$A_{260}$	$1.0 \approx 40 \mu\text{g/ml}$
	$A_{260}/A_{280}$	$\sim 2.0$

# •Kontrola degradace: DNA





- Kontrola degradace: RNA

