

# NUKLEOVÉ KYSELINY

1. H. Braconnot (30. léta 19. století)

- Strassburg – vinné kvasinky – izolace „**matière animale**“.

2. J.F. Meischer (1868-1869)

- experimenty z hnísem a mlíčem rýnského lososa – **NUKLEIN**

3. Altman (1899)

pokračoval v experimentech svého předchůdce ( živočišné tkáně – thymus a kvasinky) – nazývá izolovanou látku – **kyselina nukleová - DNA**

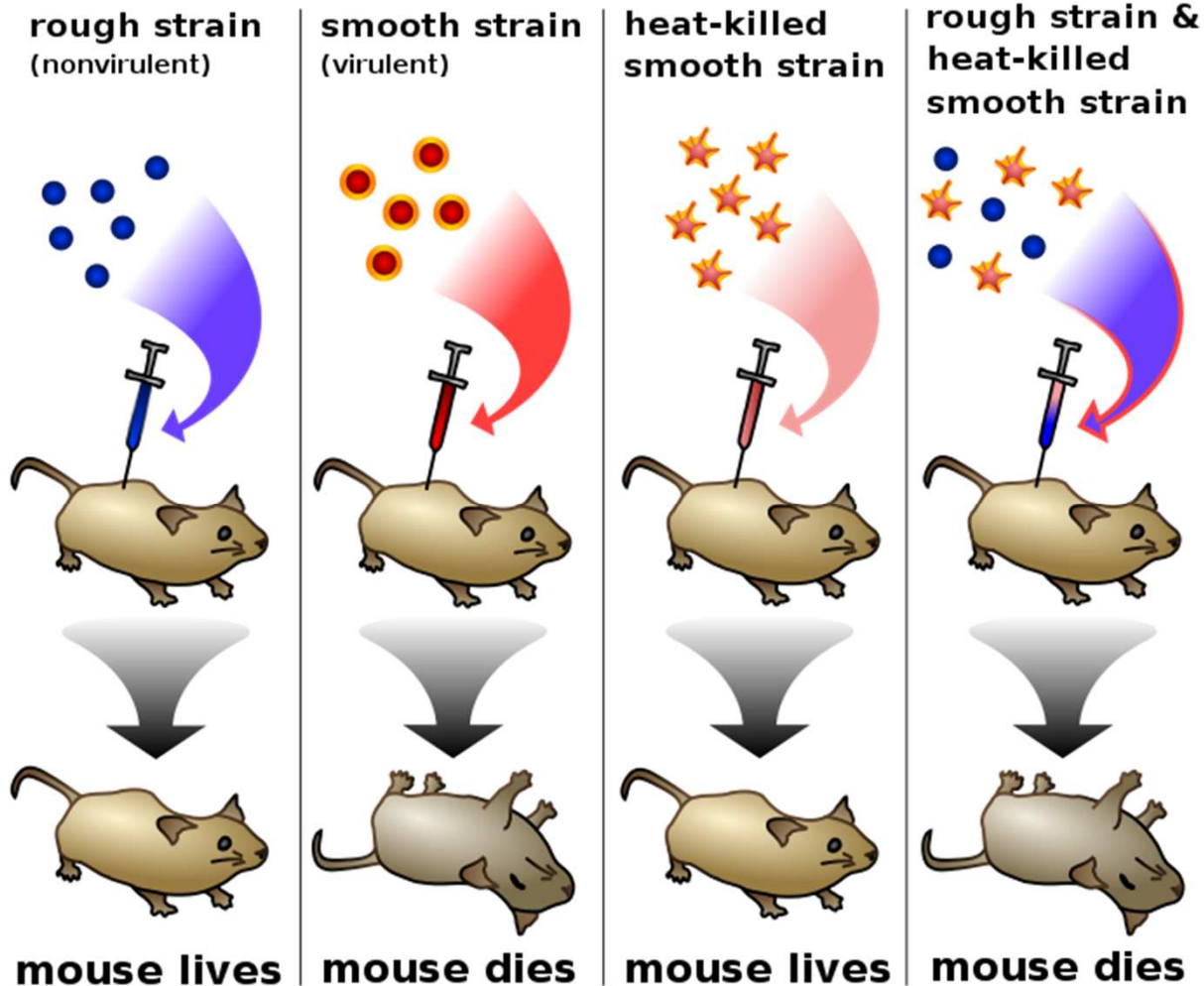
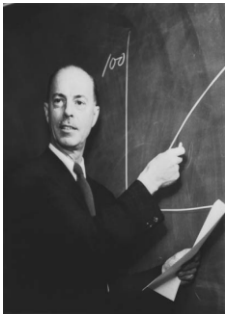
4. Leven (1909)

– **izoloval z NK kyselinu fosforečnou , cukr a báze – pojmenoval další typ NK – RNA**

5. Avery, MacLeod ,McCarty (1943)

**DNA přenáší genetickou informaci – infekční princip**

# Avery-MacLeod-McCarty experiment – virulence DNA u *Pneumococcus*



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5. Avery, MacLeod ,McCarty (1943) **DNA** přenáší genetickou informaci – infekční princip

6. Watson, Crick, Wilkins, (1953) – **model sekundární struktury DNA** – **Nobelova cena 1962**

# NUKLEOVÉ KYSELINY

## Složení :

- Dusíkaté báze – purinové, pyrimidinové
- Sacharid – ribosa, deoxyribosa
- $\text{H}_3\text{PO}_4$

## Funkce :

DNA – nositel genetické informace

- Viry
- Prokaryonta –cytoplazma
- Eukaryonta – jádro, mitochondrie, chloroplasty

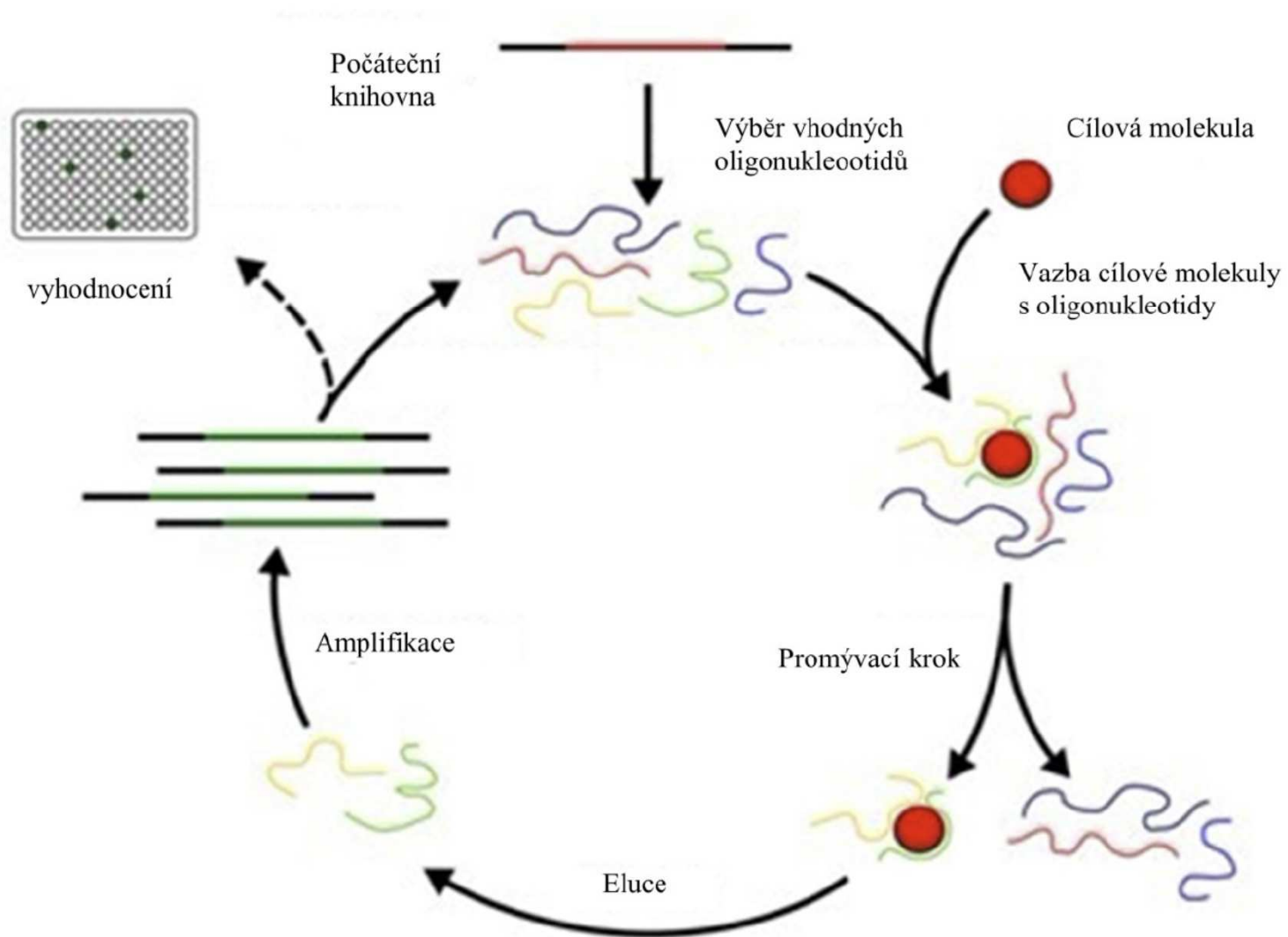
- DNAzymy

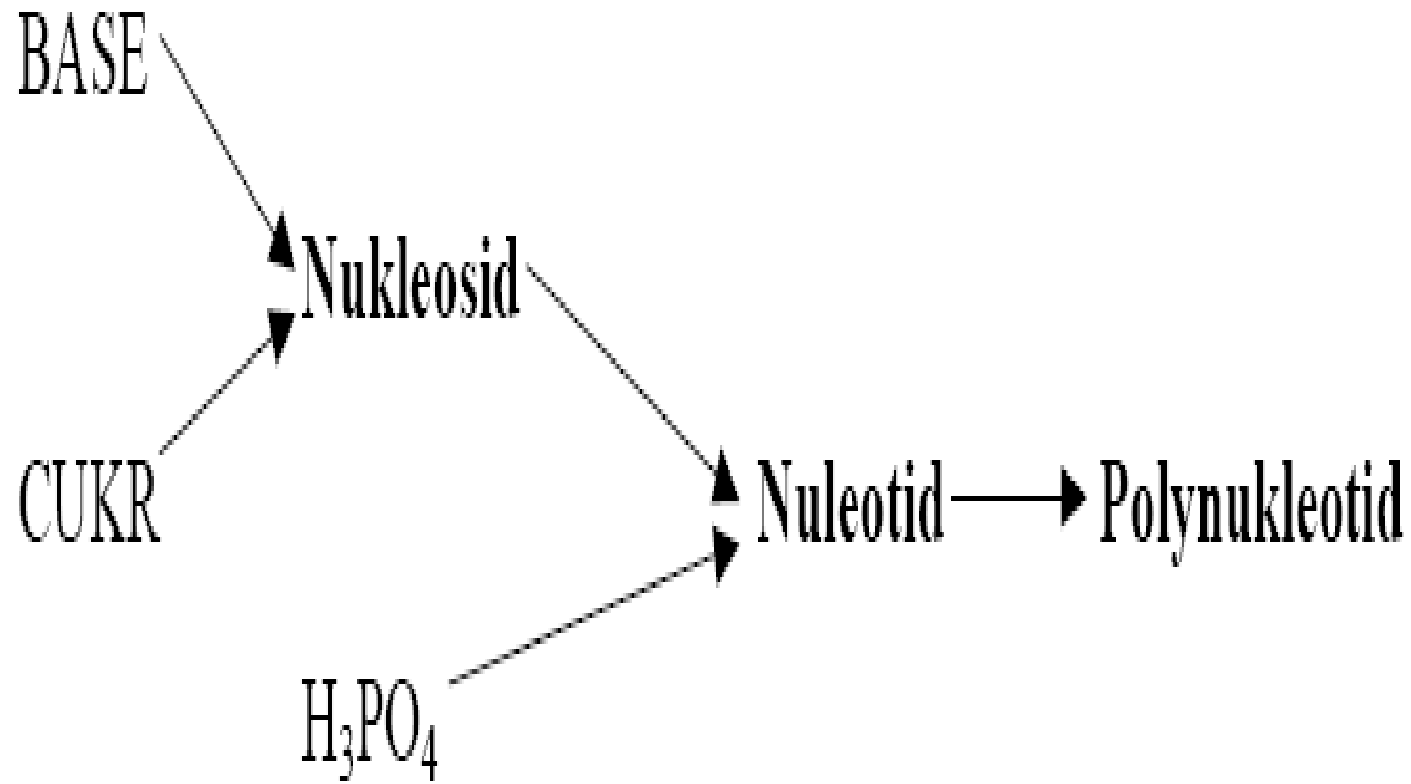
RNA – realizace genetické informace (u RNA virů i nositel genetické informace)

- Ribozymy - biokatalyzátory

- RNA a ssDNA aptamery – SELEX Systematic Evolution of Ligands by EXponential enrichment

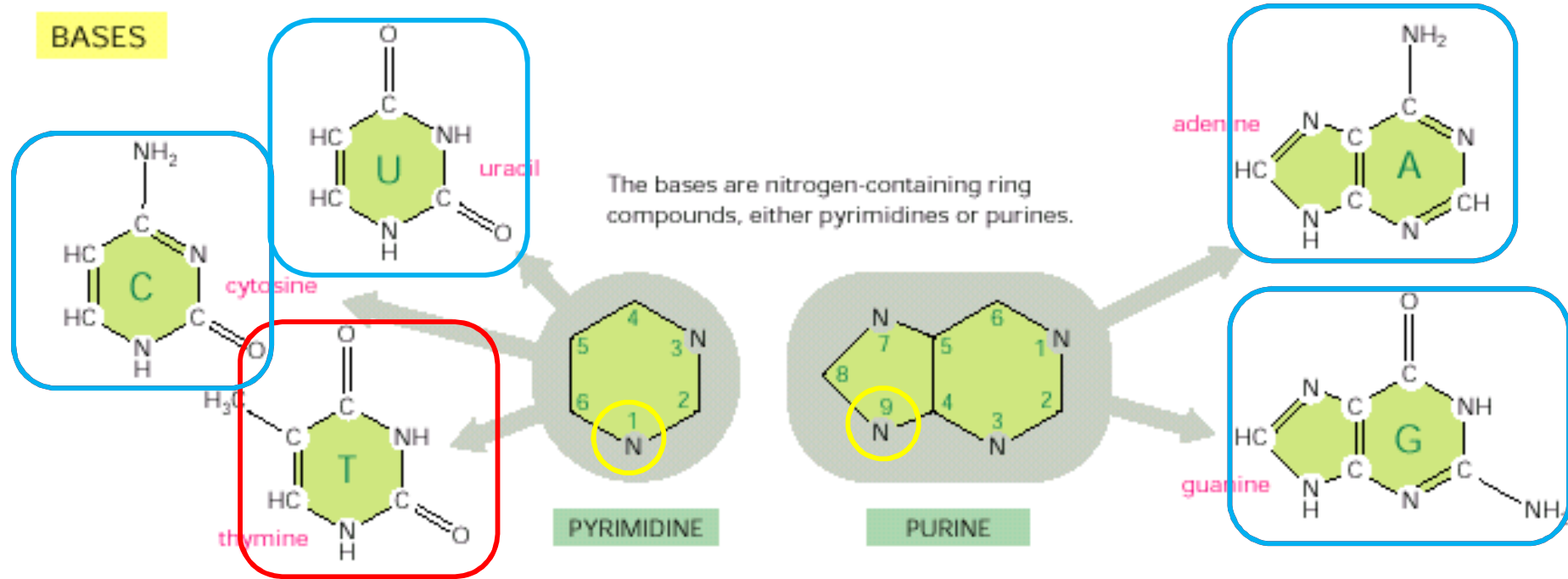
# SELEX





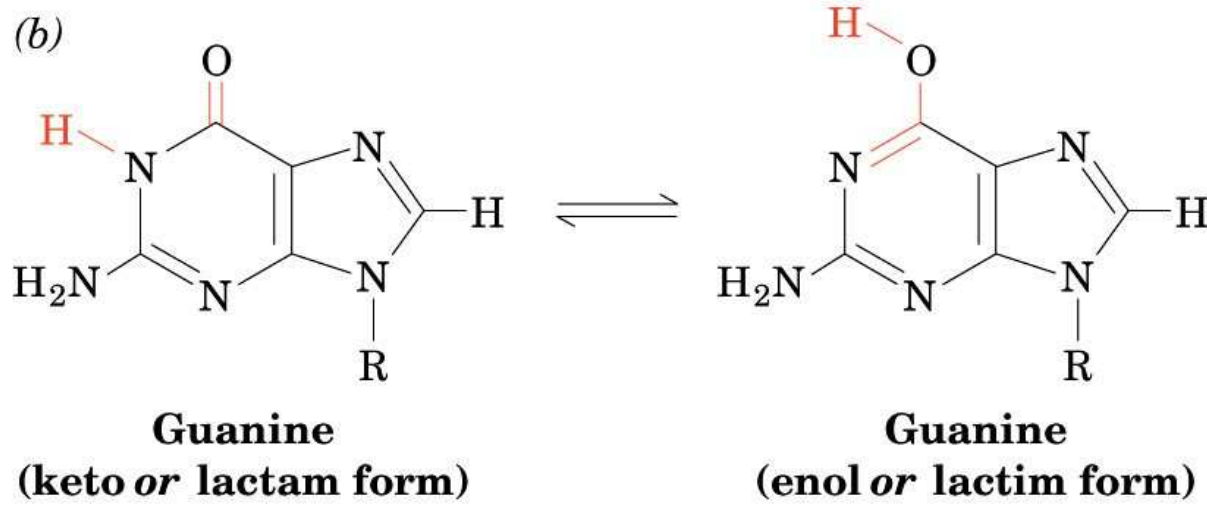
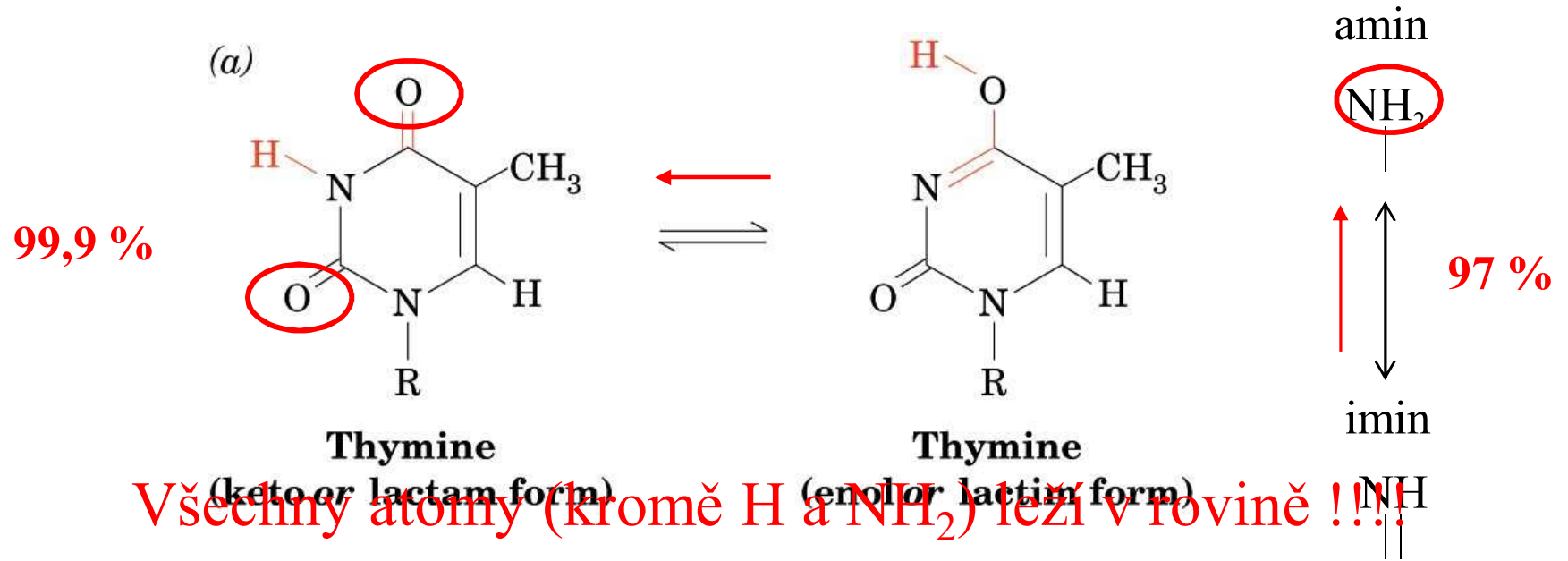
# Báze

## RNA





# Báze -tautomerie

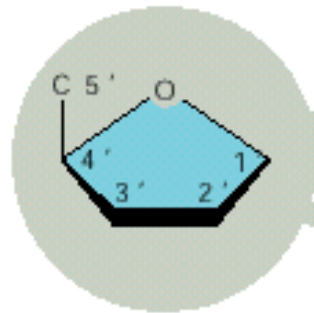


# Monosacharidy

## SUGARS

### PENTOSE

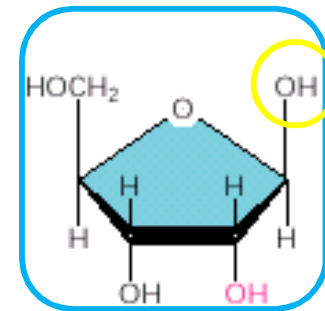
a five-carbon sugar



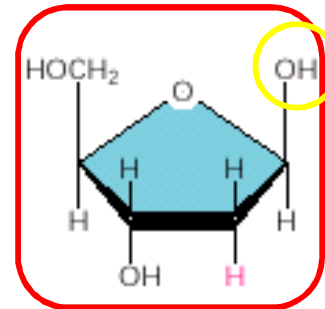
two kinds are used

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.

## RNA



$\beta$ -D-ribose  
used in ribonucleic acid

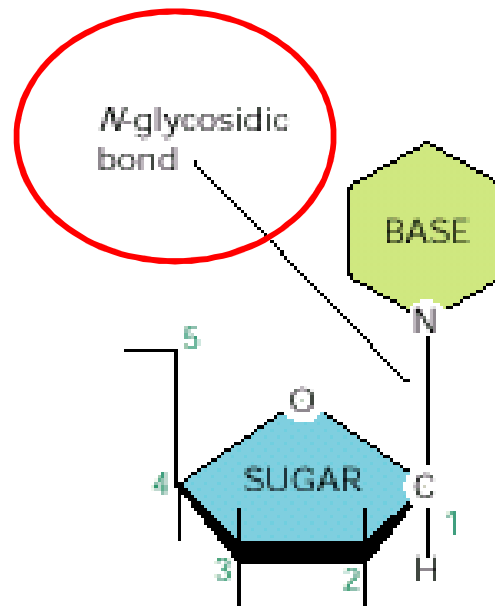


$\beta$ -D-2-deoxyribose  
used in deoxyribonucleic acid

## DNA

# Nukleosid

## BASIC SUGAR LINKAGE

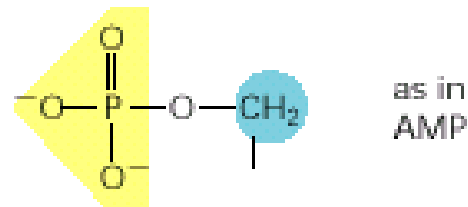


The base is linked to the same carbon (C1) used in sugar-sugar bonds.

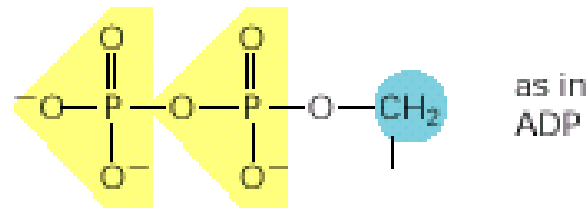
# Kyselina fosforečná

## PHOSPHATES

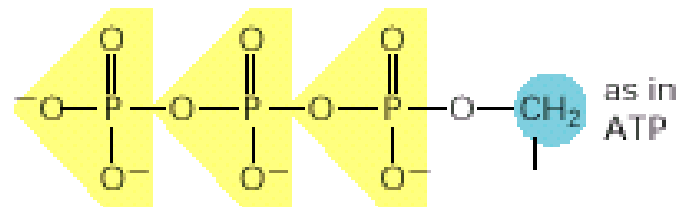
The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.



Ester



Anhydrid



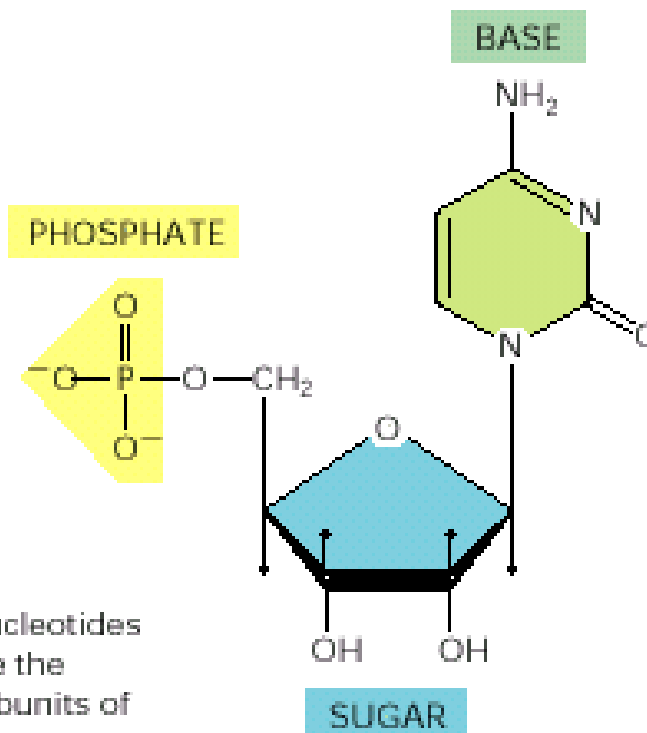
Anhydrid

The phosphate makes a nucleotide negatively charged.

# Nukleotid

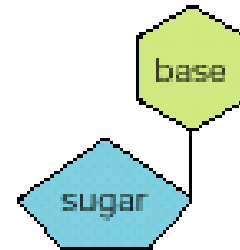
## NUCLEOTIDES

A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.

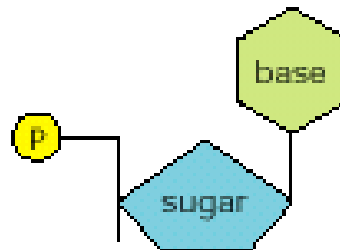


Nucleotides are the subunits of the **nucleic acids**.

# Názvosloví



BASE + SUGAR = NUCLEOSIDE



BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

Nucleotides are abbreviated by three capital letters. Some examples follow:

AMP = adenosine monophosphate

dAMP = deoxyadenosine monophosphate

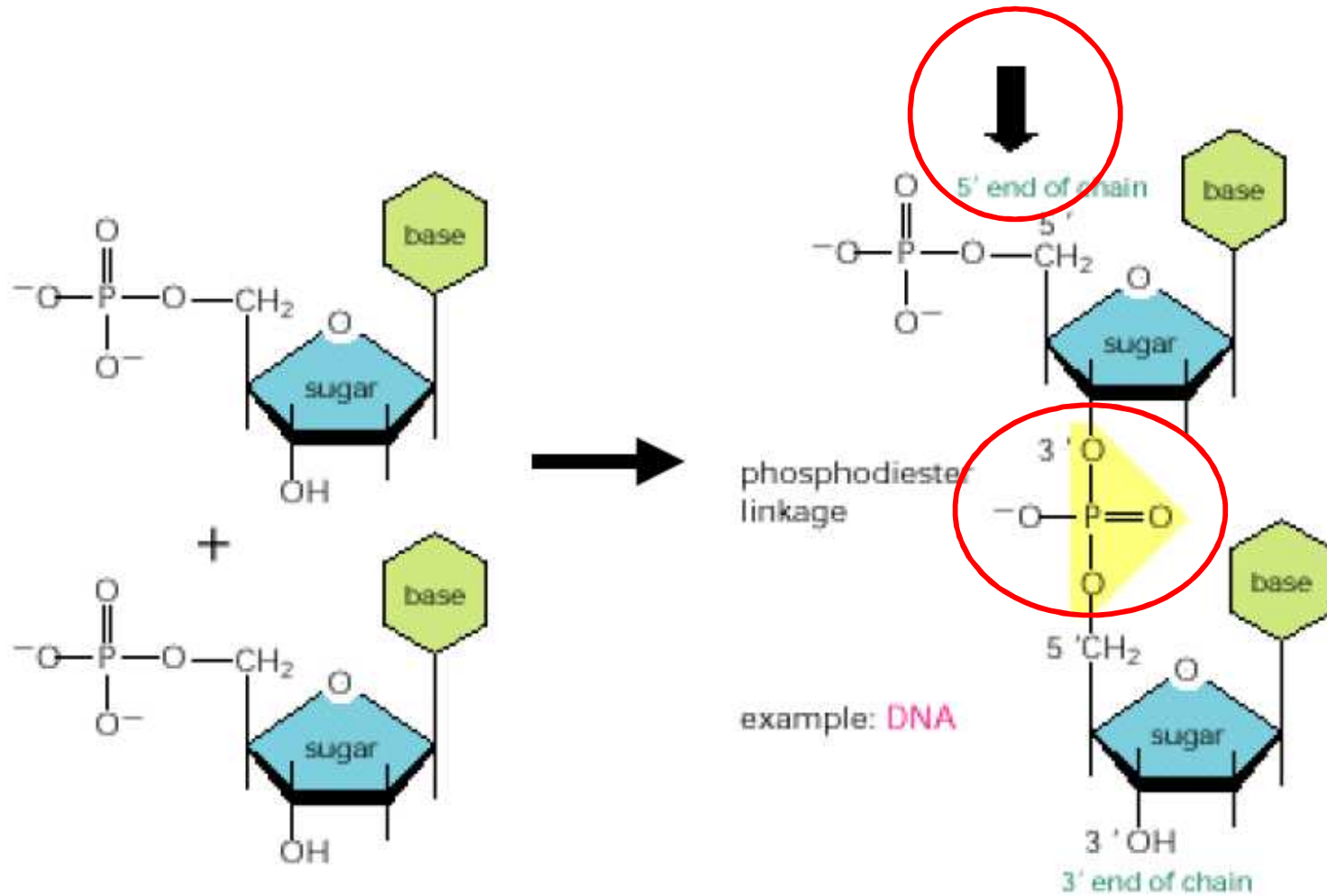
UDP = uridine diphosphate

ATP = adenosine triphosphate

# Funkce nukleotidů

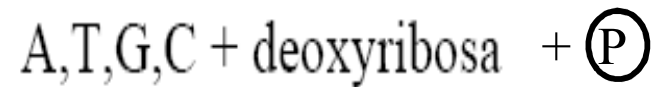
- přenašeče energie (ATP, GTP)
- fosforylační činidla (ATP – kinasy) - kofaktory
- aktivátory meziproduktů biosyntéz – UDP-glukosa, CDP-cholin
- součásti kofaktorů - NAD(P), FAD, PAPS,
- využití v terapii - antivirotika(AIDS, herpes) – AZT
- **stavební složky nukleových kyselin**

# Polynukleotid – nukleová kyselina





## Struktura a funkce DNA



**Primární struktura** – sekvence basí      5' – 3'

**Sekundární struktura** – Watson, Crick (1953) – dvojšroubovice

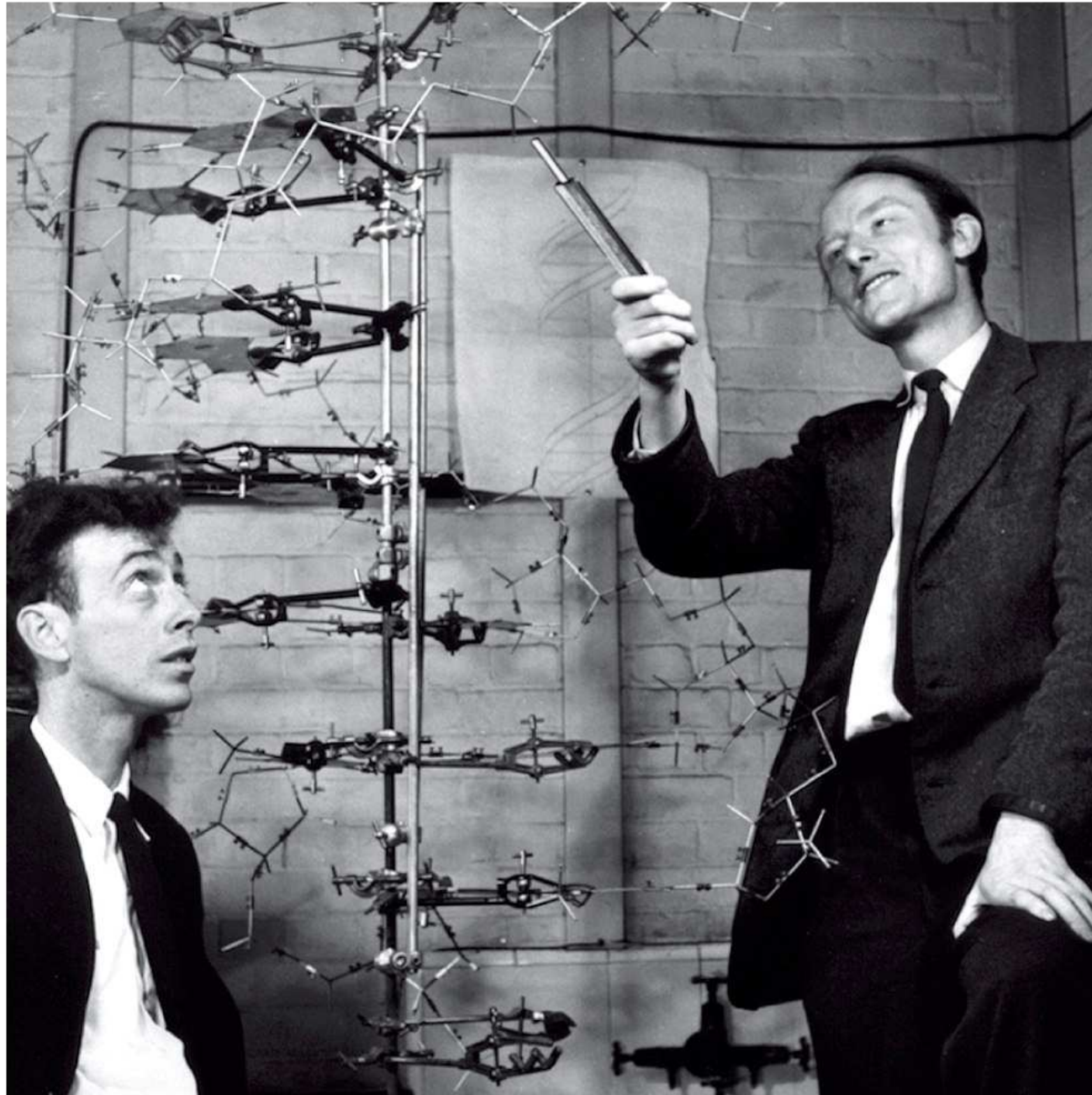
- Chragaffovy pravidla – poměr bazí v DNA



- Donohue – báze v tautomerních ketoformách

- Franklinová – RTG difrakční analýza

Watson

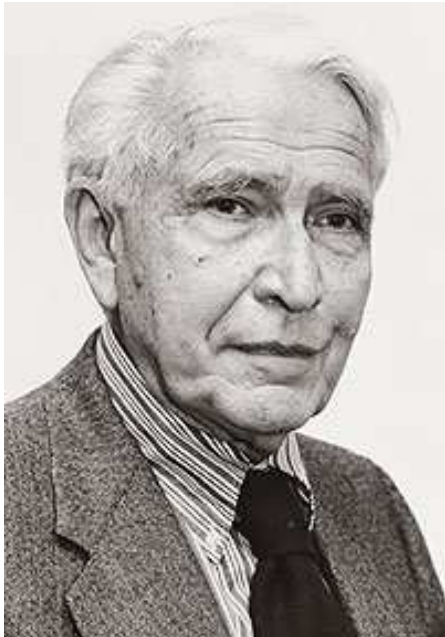


Crick

Unnumbered figure pg 9 Concepts in Biochemistry, 3/e

# Chargaffovy pravidla

Erwin Chargaff



# Chargaffovy pravidla

## Zastoupení basí v DNA (molární %)

<u>Organismus</u>	<u>A</u>	<u>T</u>	<u>G</u>	<u>C</u>
Člověk	30.9	29.4	19.9	19.8
Kuře	28.8	29.2	20.5	21.5
Kobylka luční	29.3	29.3	20.5	20.7
Pšenice	27.3	27.1	22.7	22.8
Kvasinky	31.3	32.9	18.7	17.1
E. coli	24.7	23.6	26.0	25.7

nezávisí na tkáni

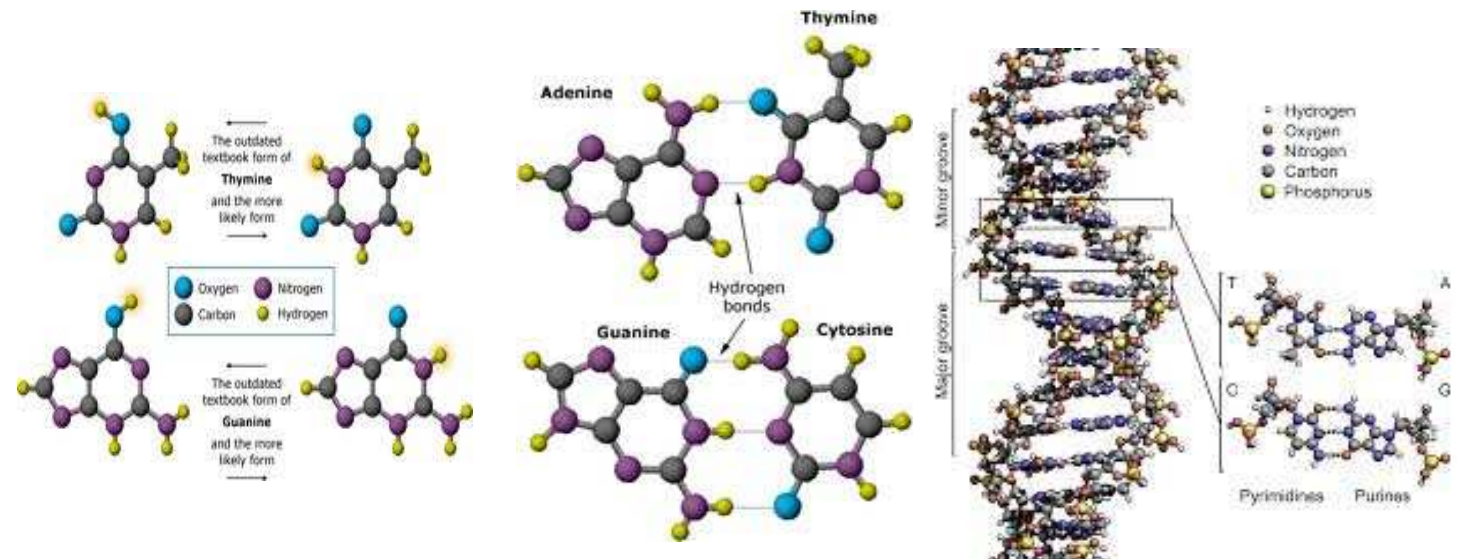
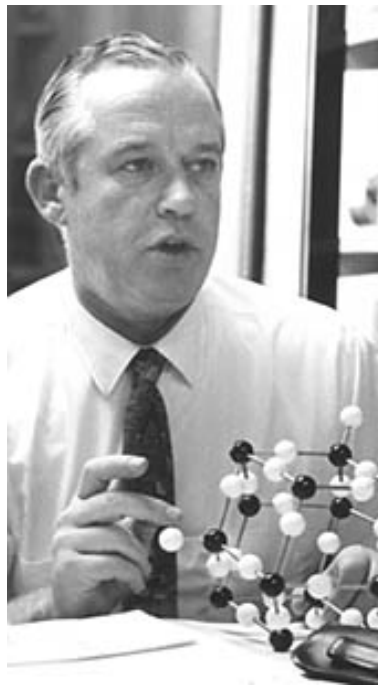
nezávisí na stáří

nezávisí na nutričních faktorech

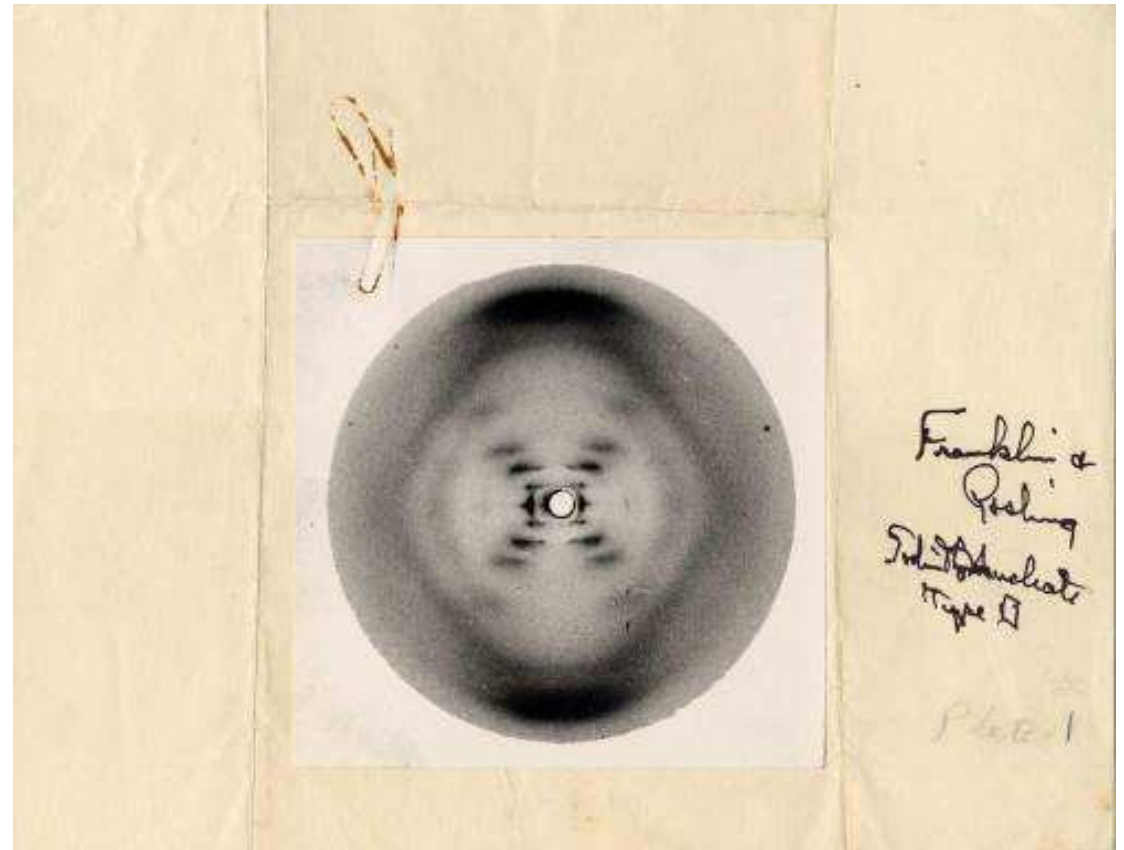
nezávisí na životním prostředí

# Jerry Donohue

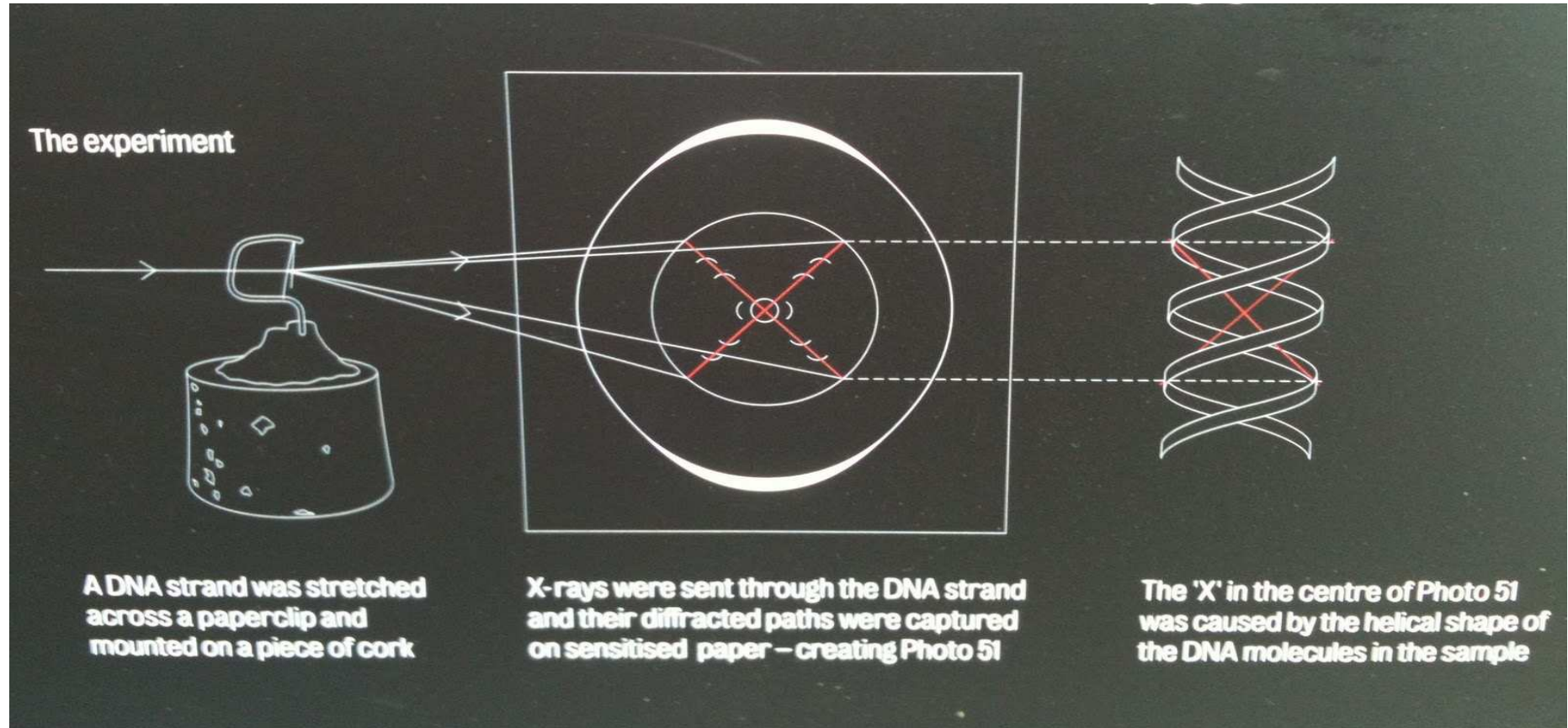
## tautomerní formy bází



# Rosalind Franklin



## Obrázek 51



# Nobelova cena 1962



**Francis Harry  
Compton Crick  
(1916-2004)**

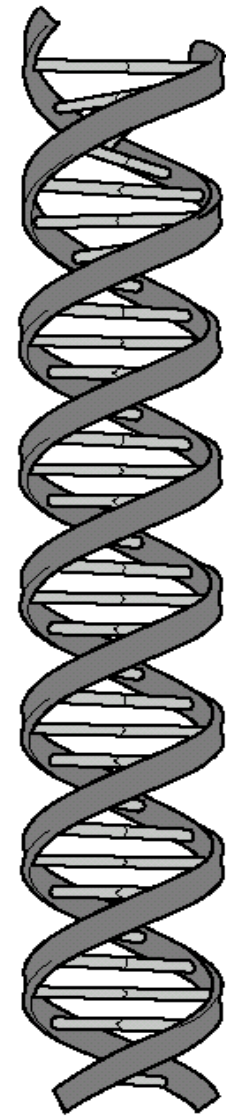
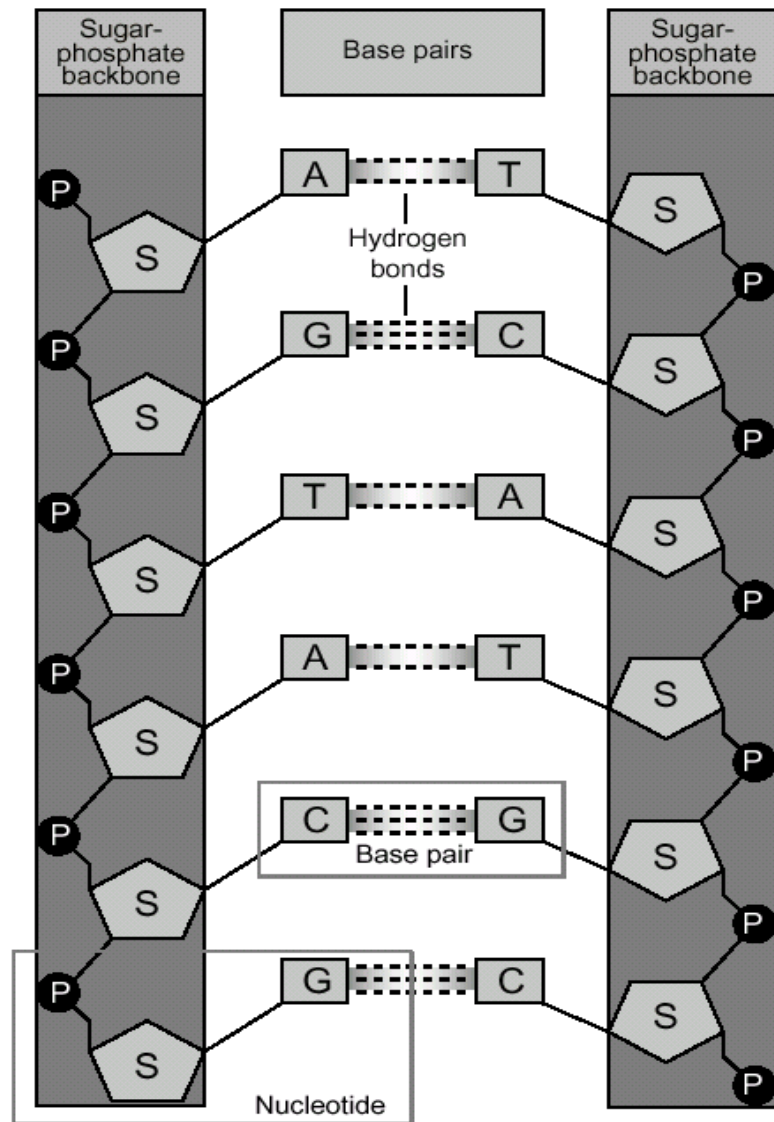


**James Dewey  
Watson  
(1928 - )**

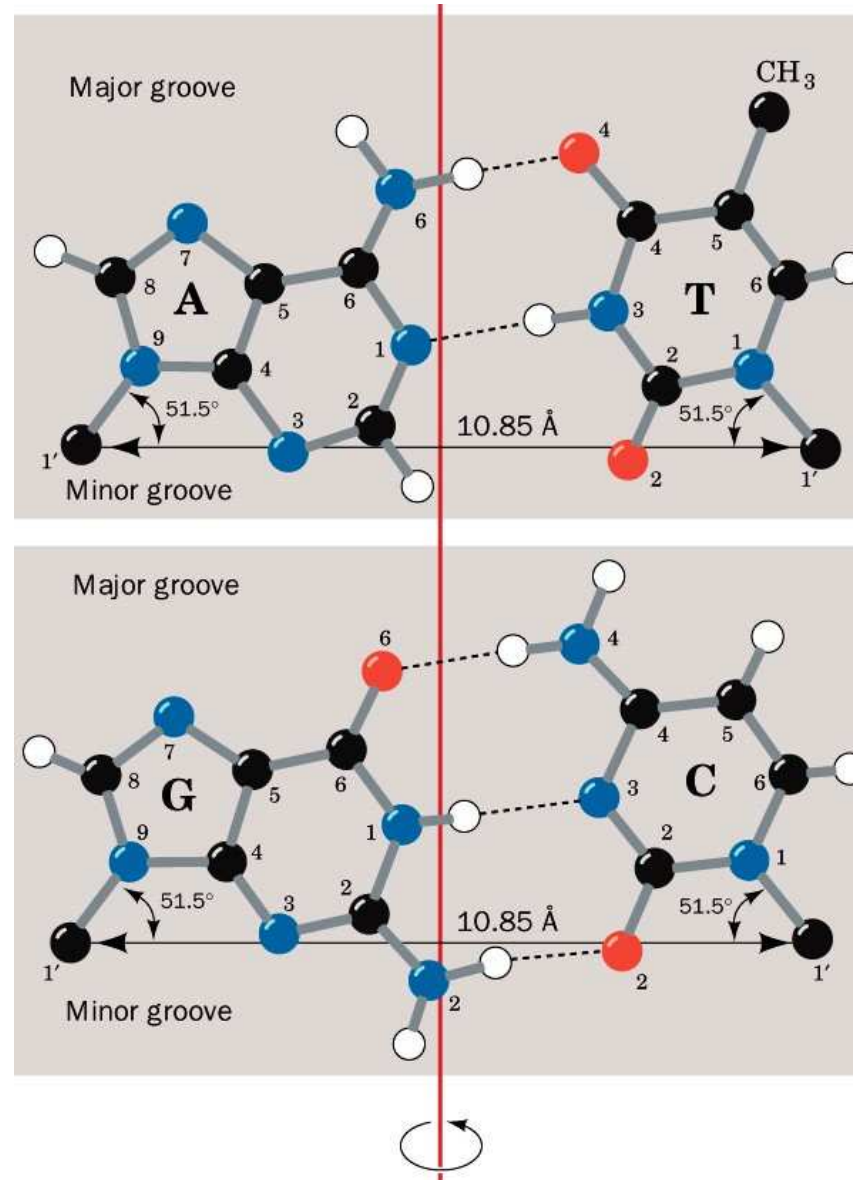


**Maurice Hugh  
Frederick Wilkins  
(1916-2004)**

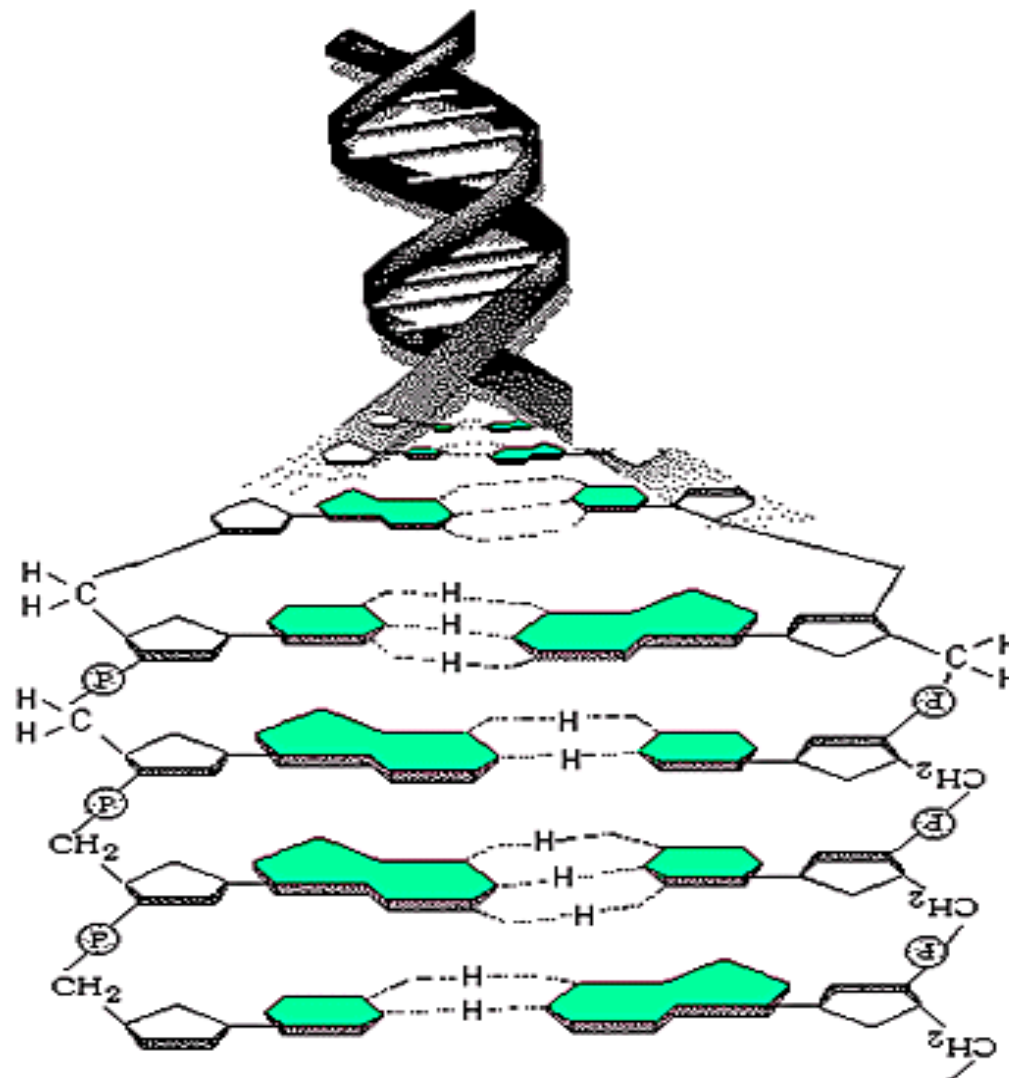




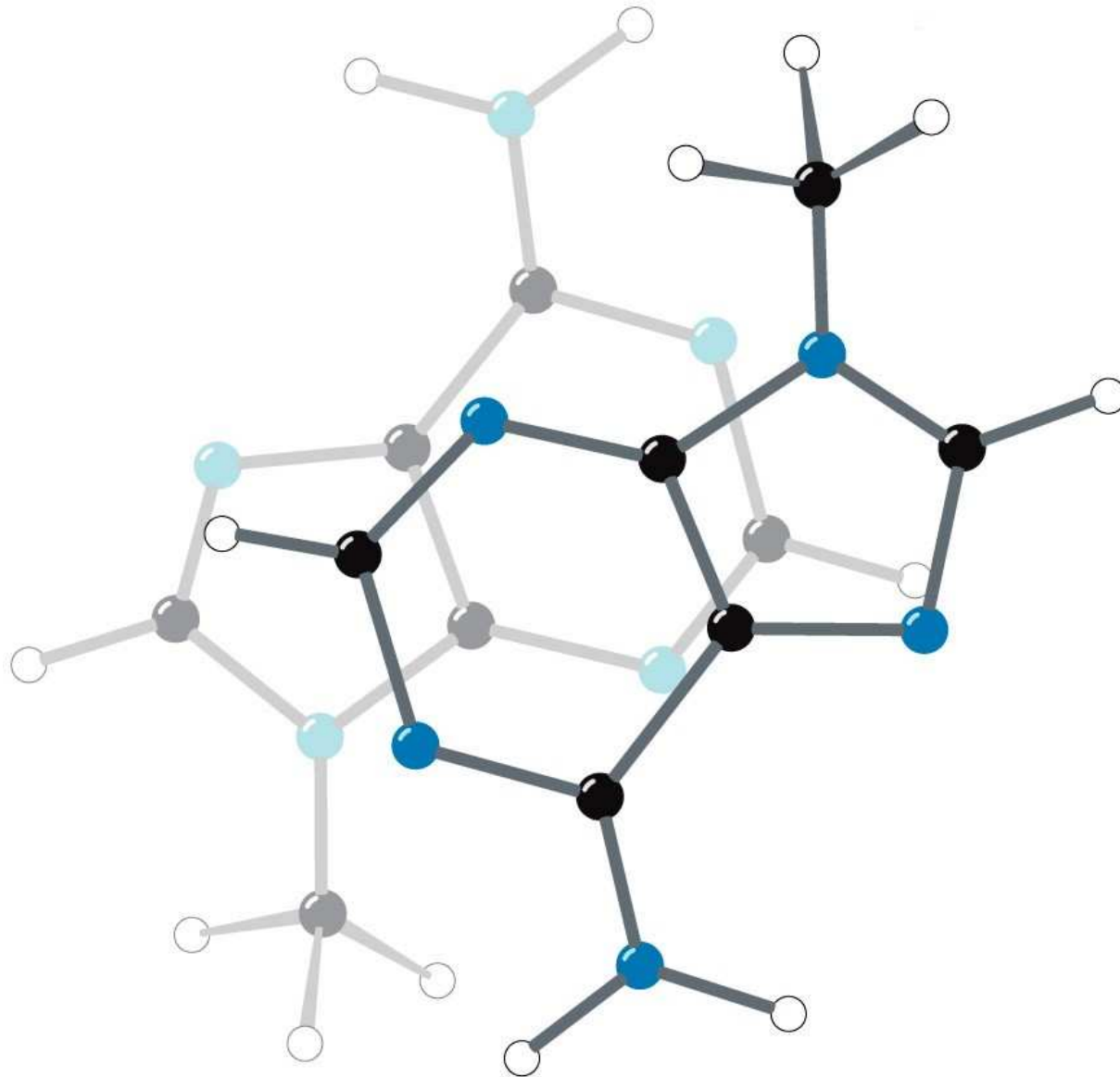
# Párování basí – H můstky



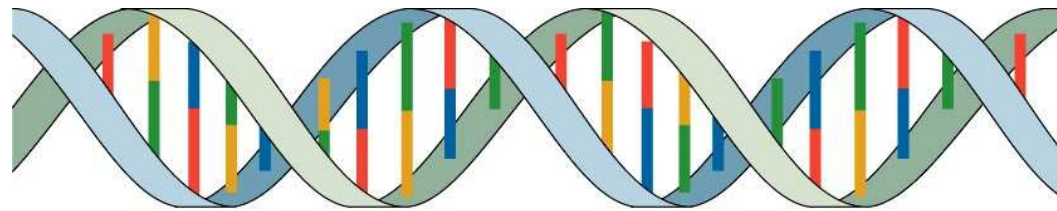
# Stabilizující vazby v DNA



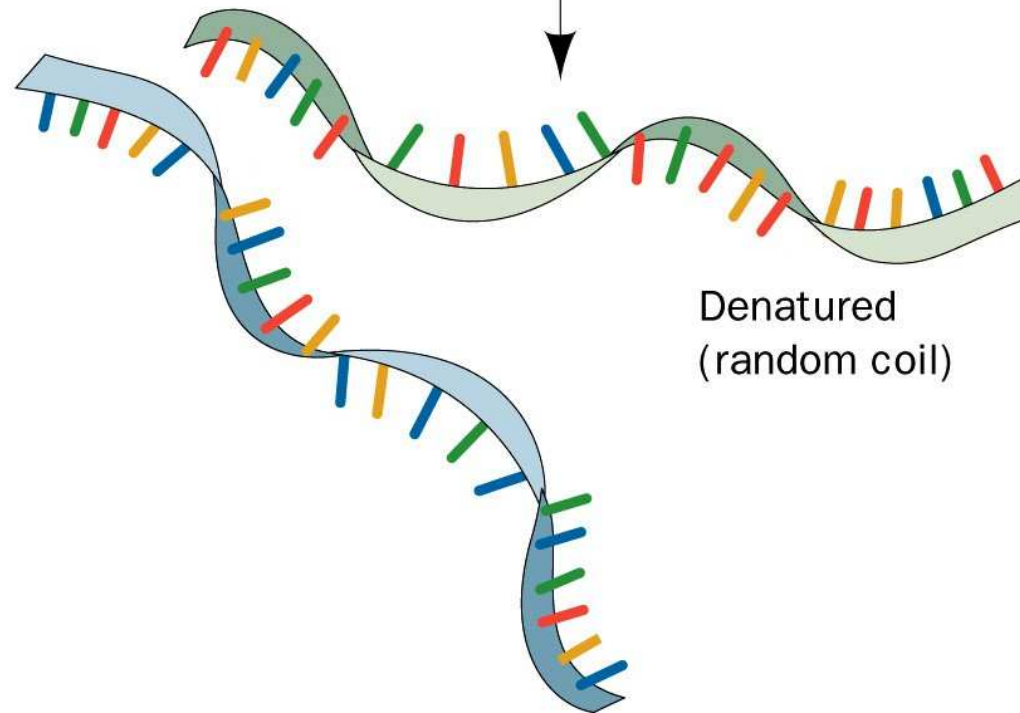
# Stohování bází



# Denaturace - renaturace

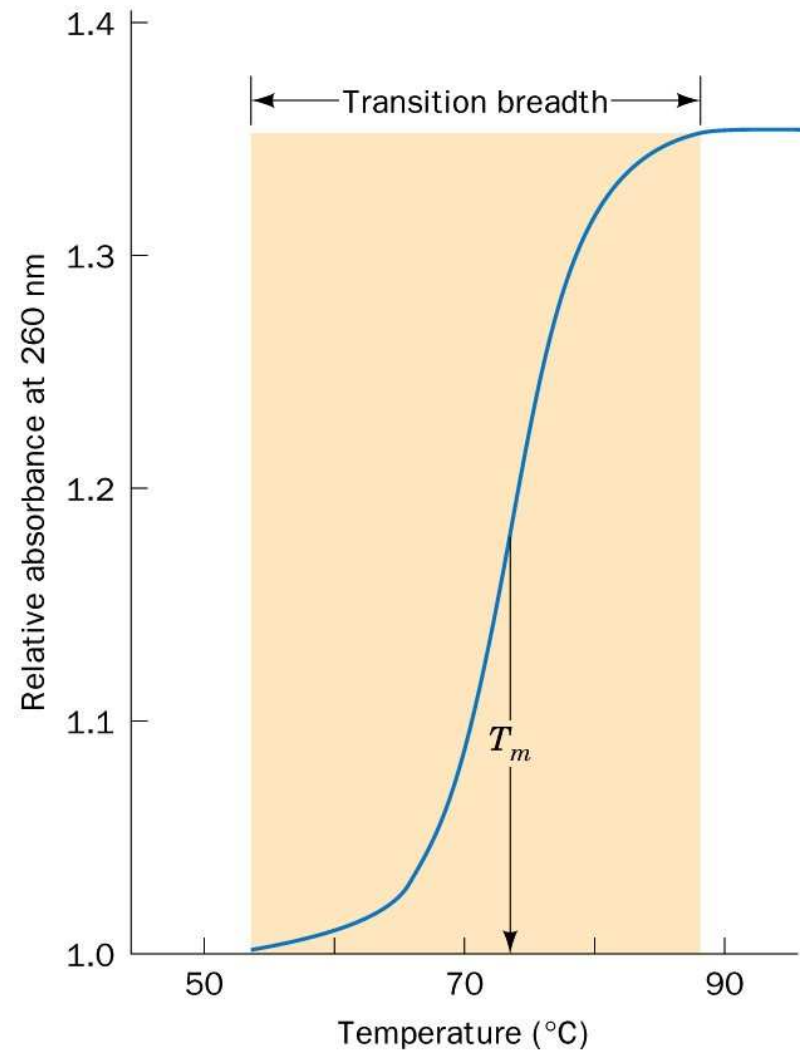
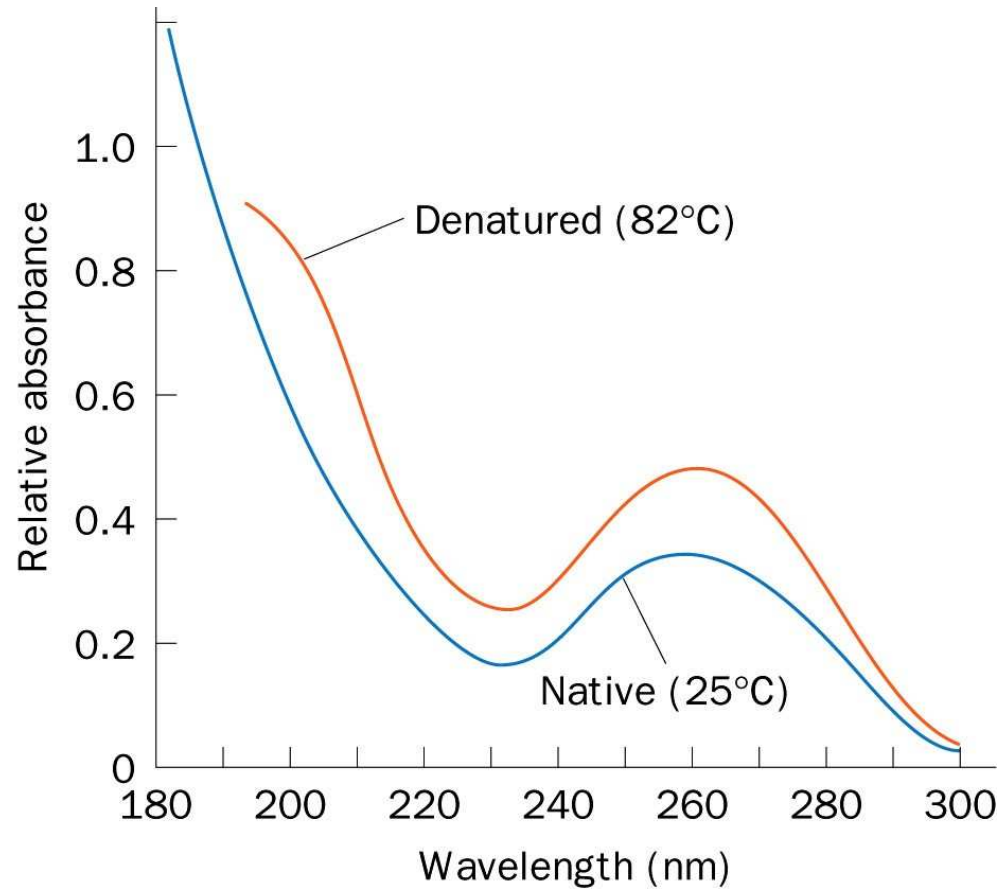


Native (double helix)



Denatured  
(random coil)

# Denaturace - renaturace



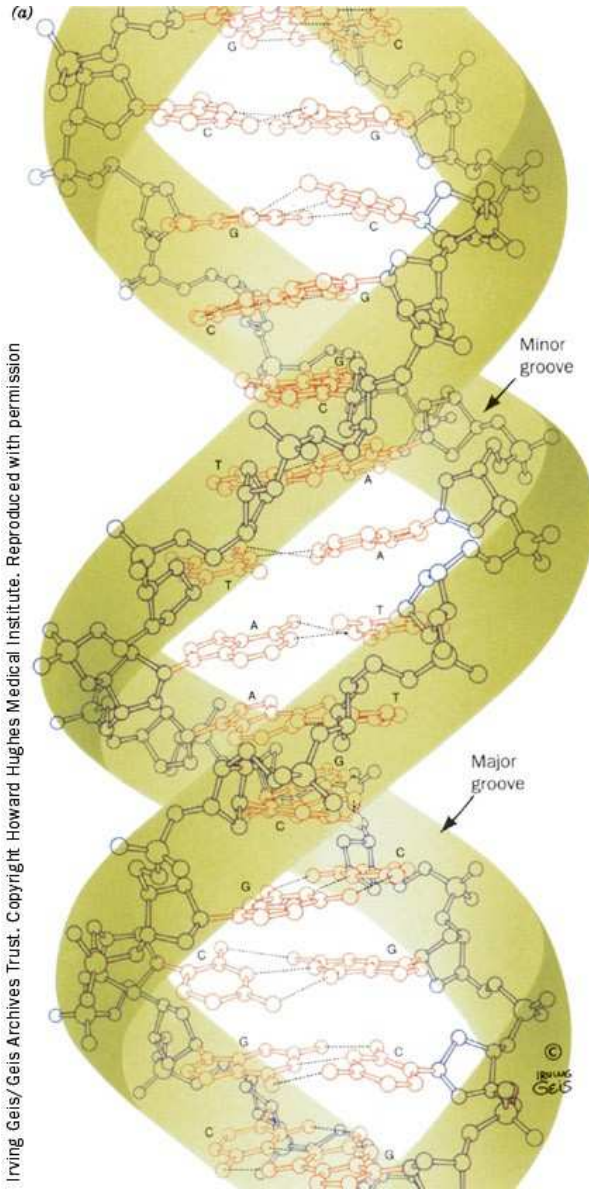
## Formy DNA

• B - DNA - nativní 92 % H<sub>2</sub>O, Na<sup>+</sup>  
pravotočivá - 10 párů bází na závit

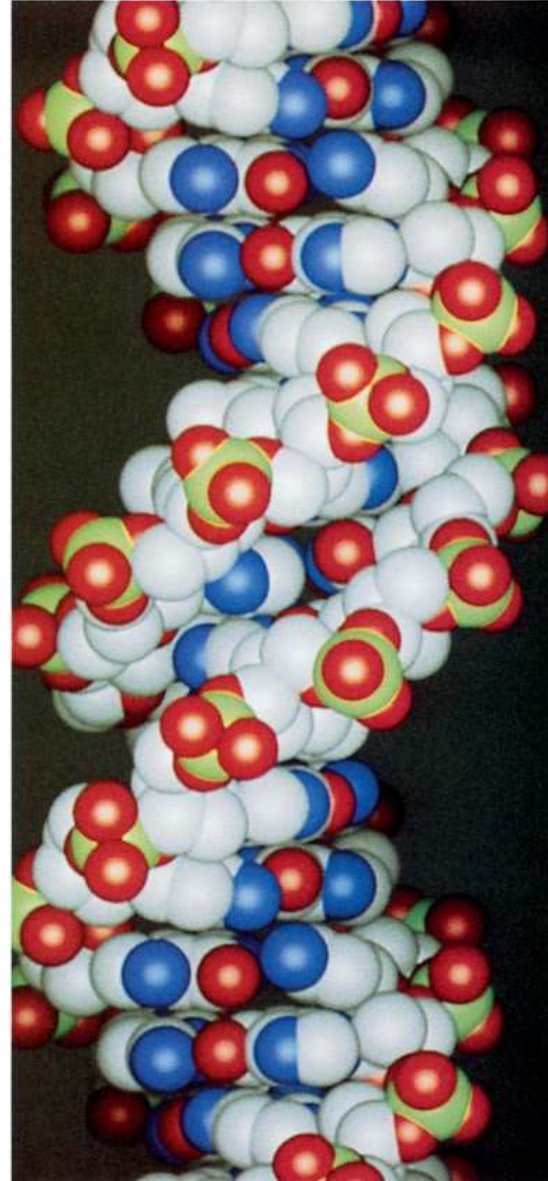
• A - DNA - 75 % H<sub>2</sub>O, rovina bází 20°  
pravotočivá - 11 párů bází na závit

• Z - DNA - d(CGCGCG)  
levotočivá - 12 párů bází na závit

# B DNA

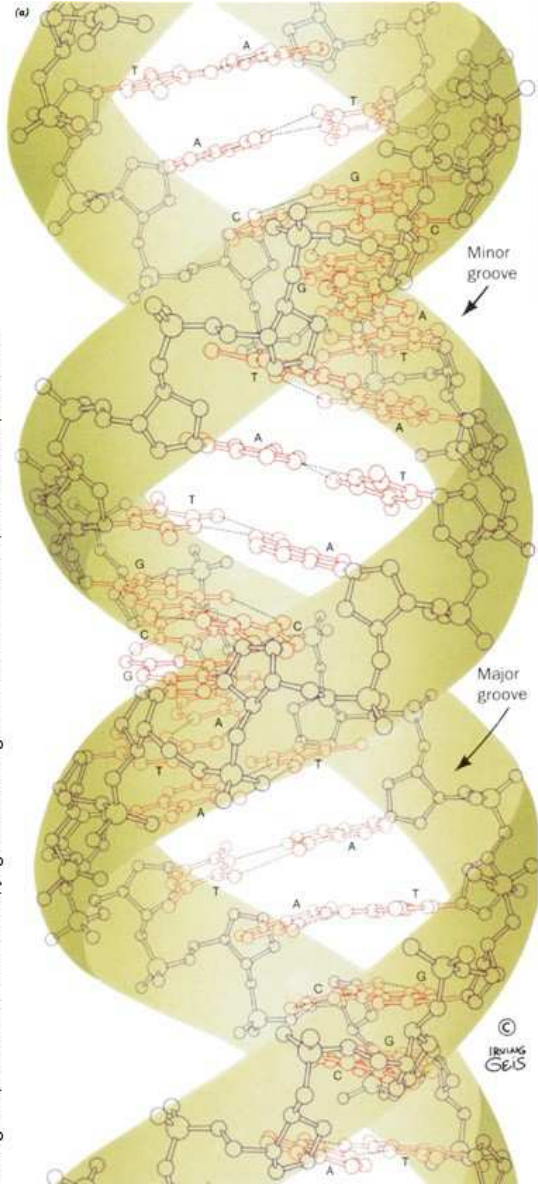


Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with permission

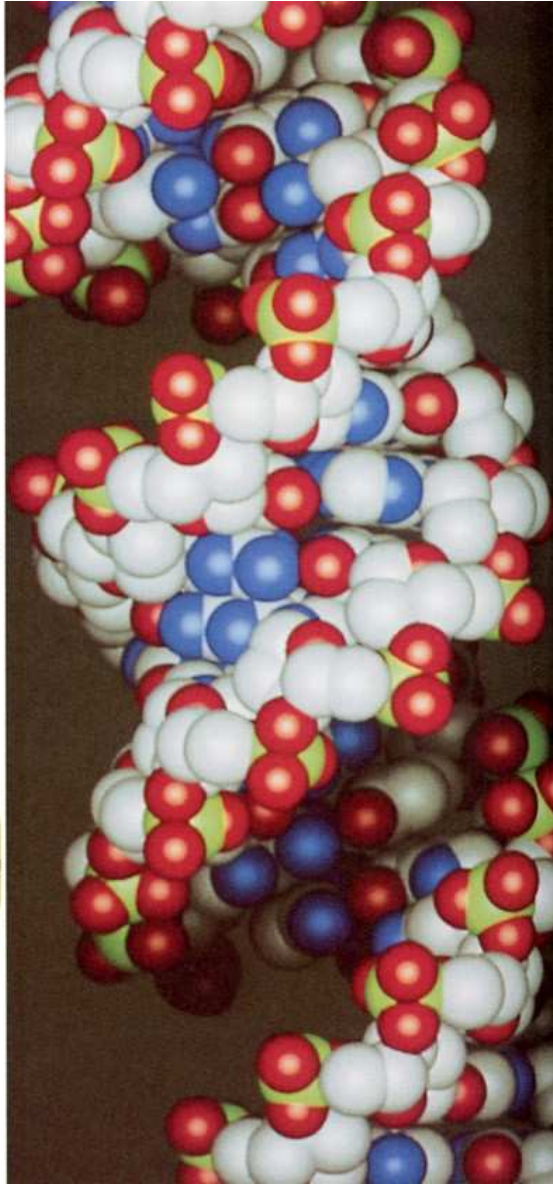




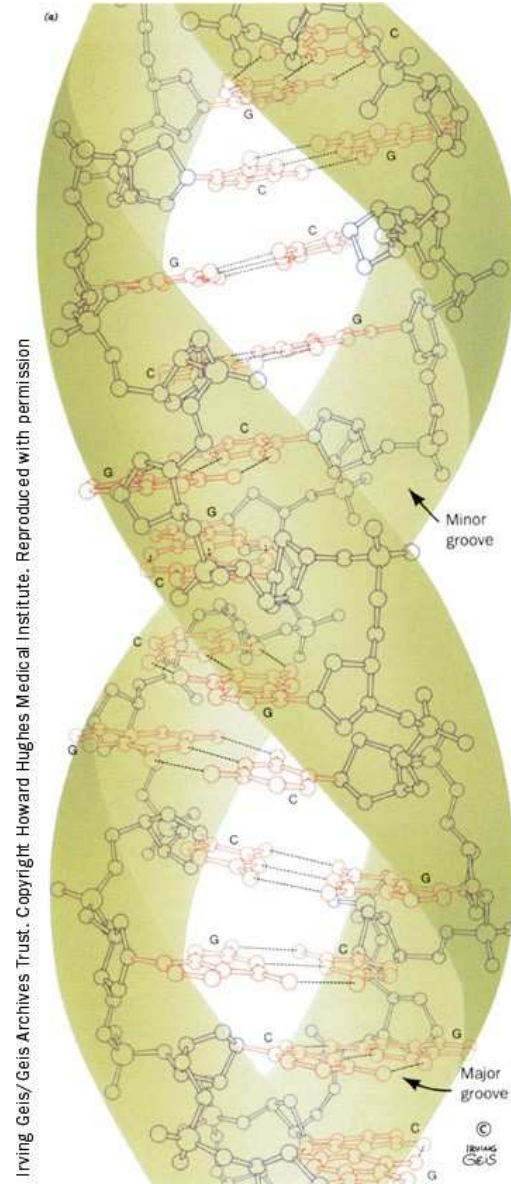
# A DNA



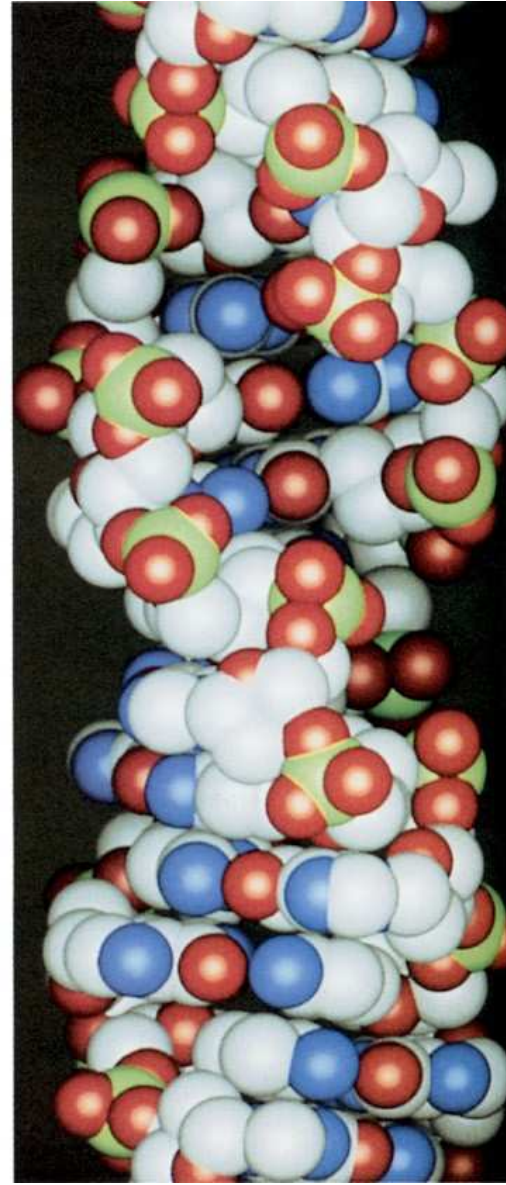
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# Z DNA

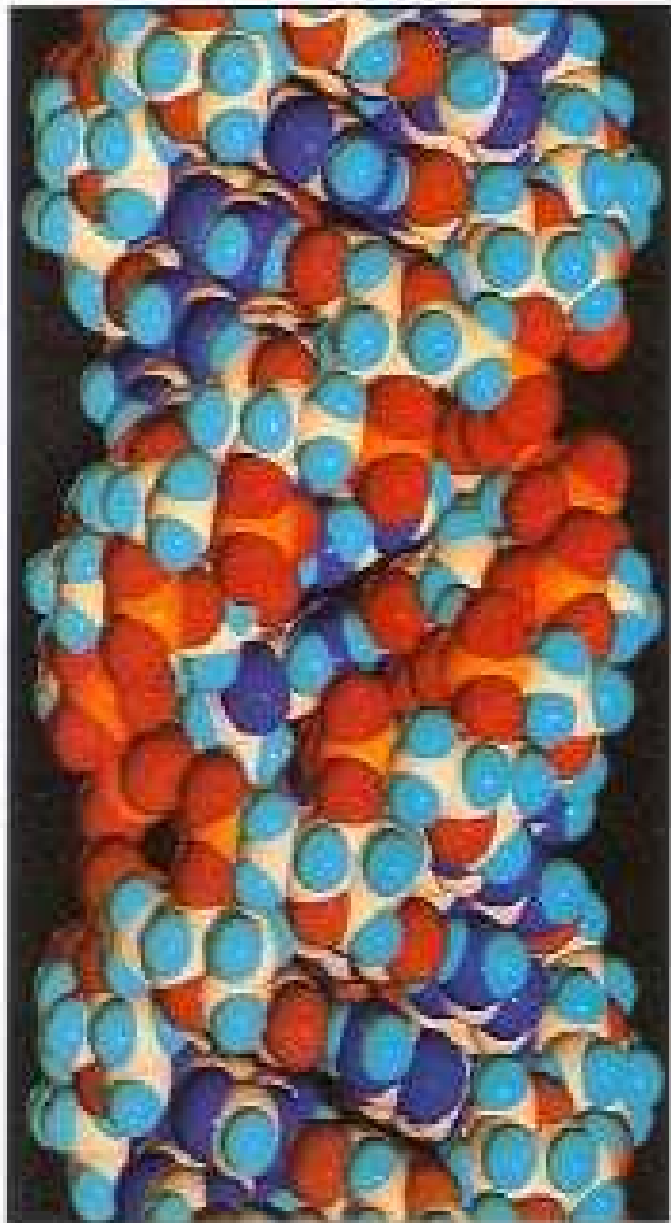


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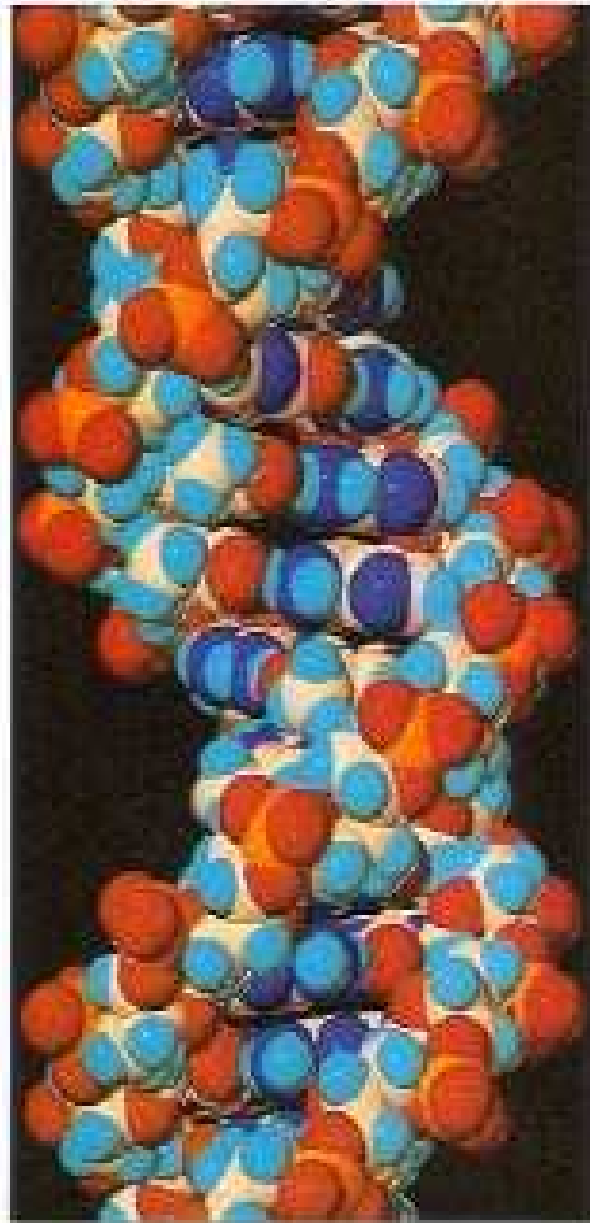


## Základní číselné charakteristiky tří nejznámějších helikálních forem DNA

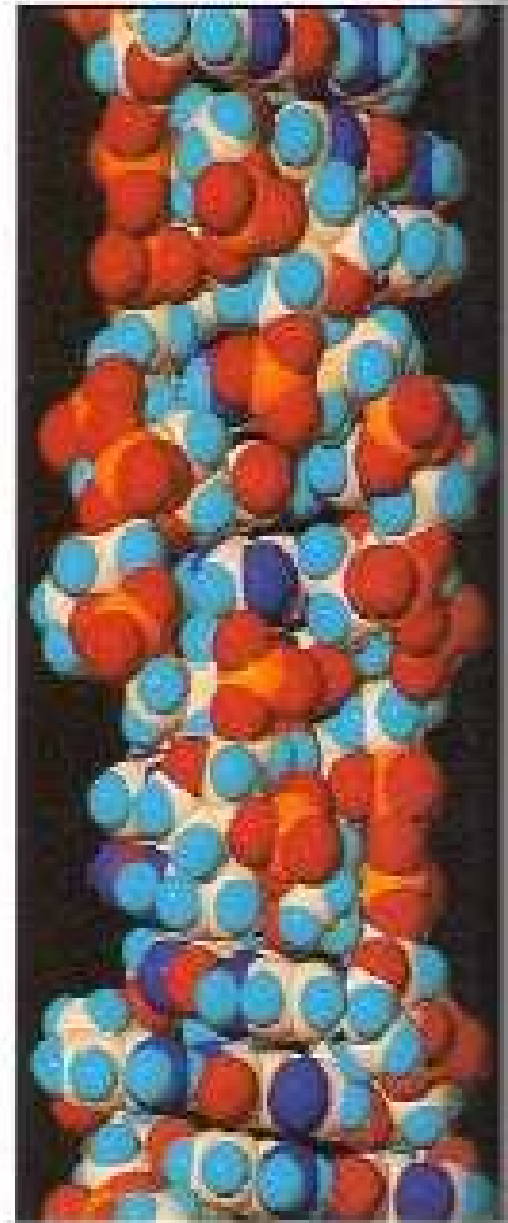
Atribut	A-DNA	B-DNA	Z-DNA
Točivá tendence šroubovice (chiralita) <sup>[1]</sup>	pravotočivá	pravotočivá	levotočivá
Opakování <sup>[2]</sup>	po každém páru	po každém páru	po každých dvou párech
Otočení po každém opakování <sup>[1]</sup>	32,7°	34,3°	
Průměrný počet párů na jedno otočení šroubovice <sup>[1]</sup>	11	10,5	12
Sklon páru k ose <sup>[2]</sup>	20°	6°	7°
Vzestup vůči ose na jeden pár <sup>[1]</sup>	2,55 Å (0,25 nm)	3,4 Å (0,34 nm)	3,7 Å (0,37 nm)
Průměr <sup>[1]</sup>	23 Å (2,3 nm)	20 Å (2,0 nm)	18 Å (1,8 nm)



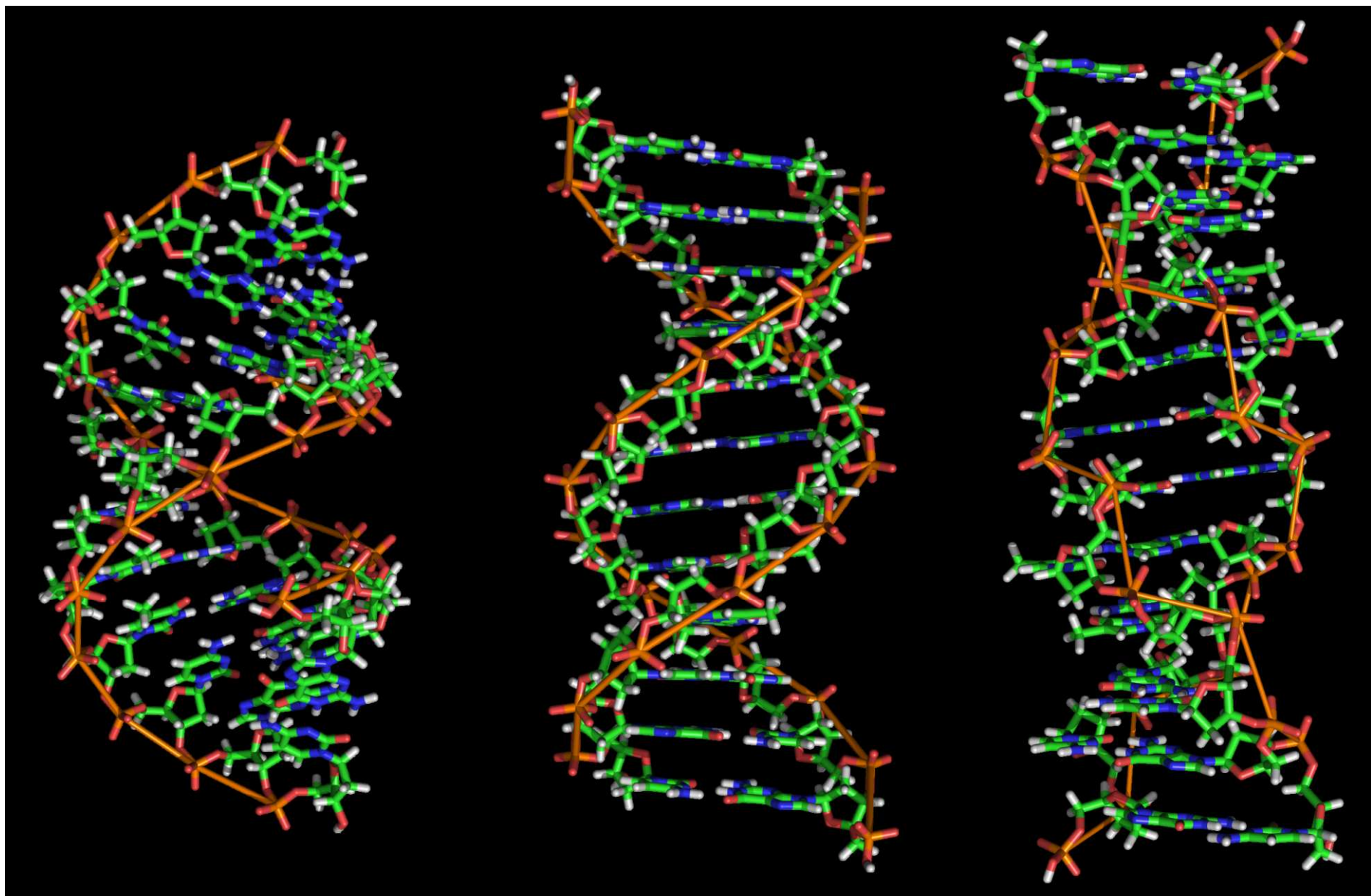
A-DNA



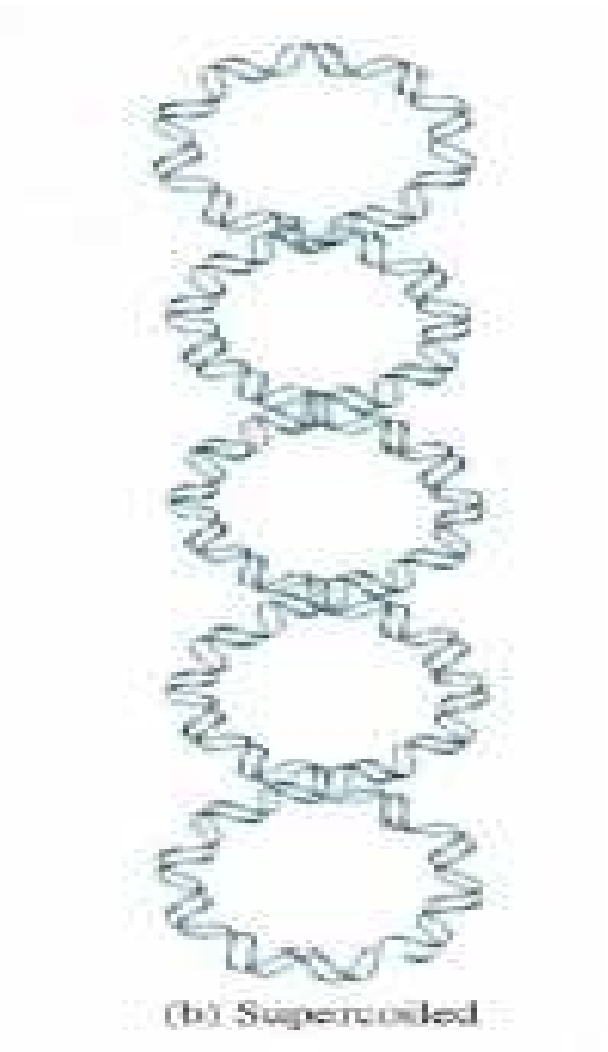
B-DNA



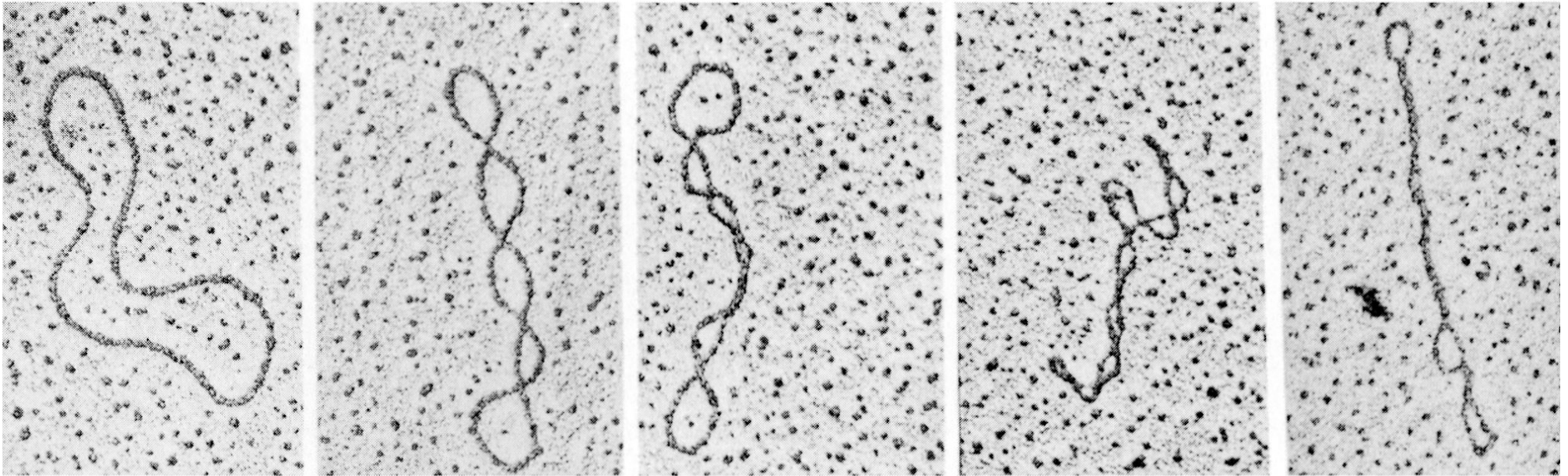
Z-DNA



# Bakteriální DNA

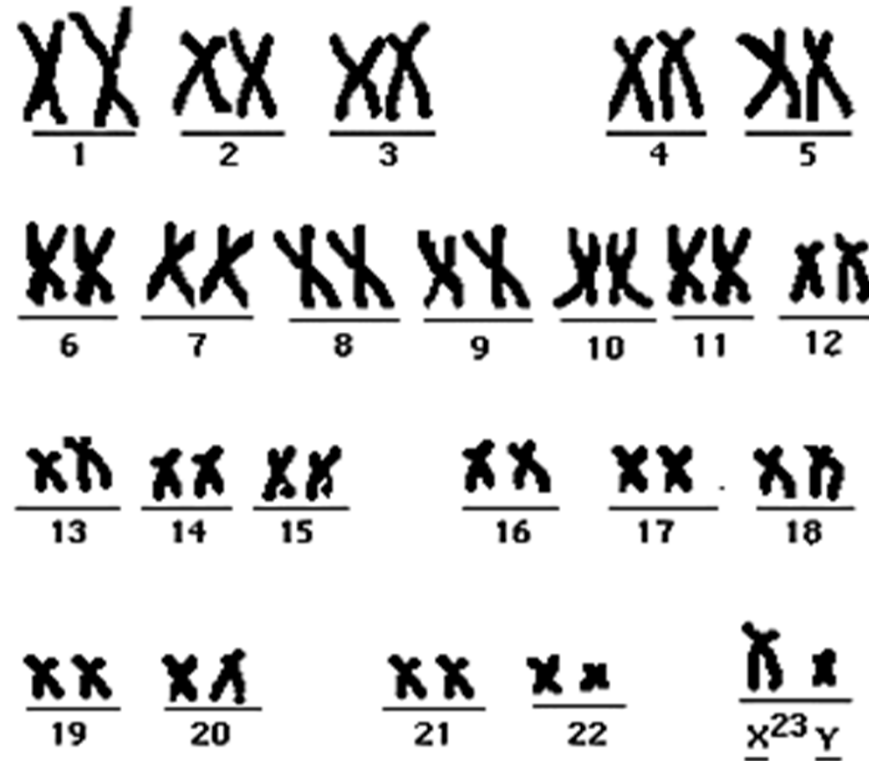


# Bakteriální DNA



Electron micrographs by Laurien Polder. From Kornberg, A. and Baker, T.A., DNA Replication (2nd ed.), p. 36, W.H. Freeman (1992). Used with permission

# Lidské chromozomy



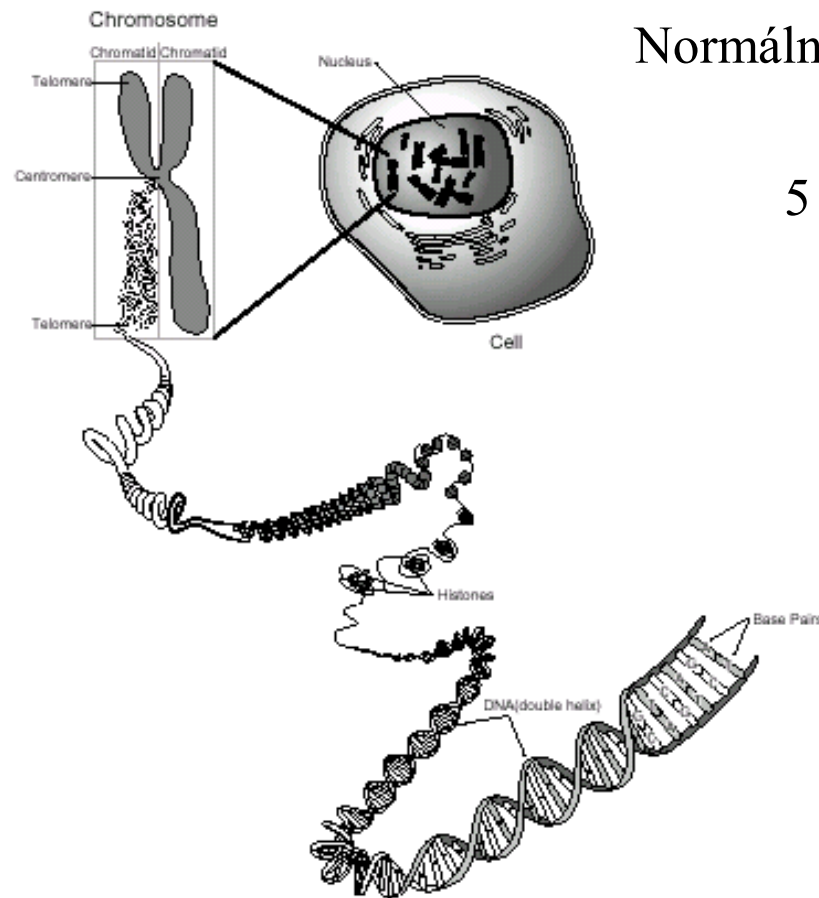


# Jádro – mitotický chromozom

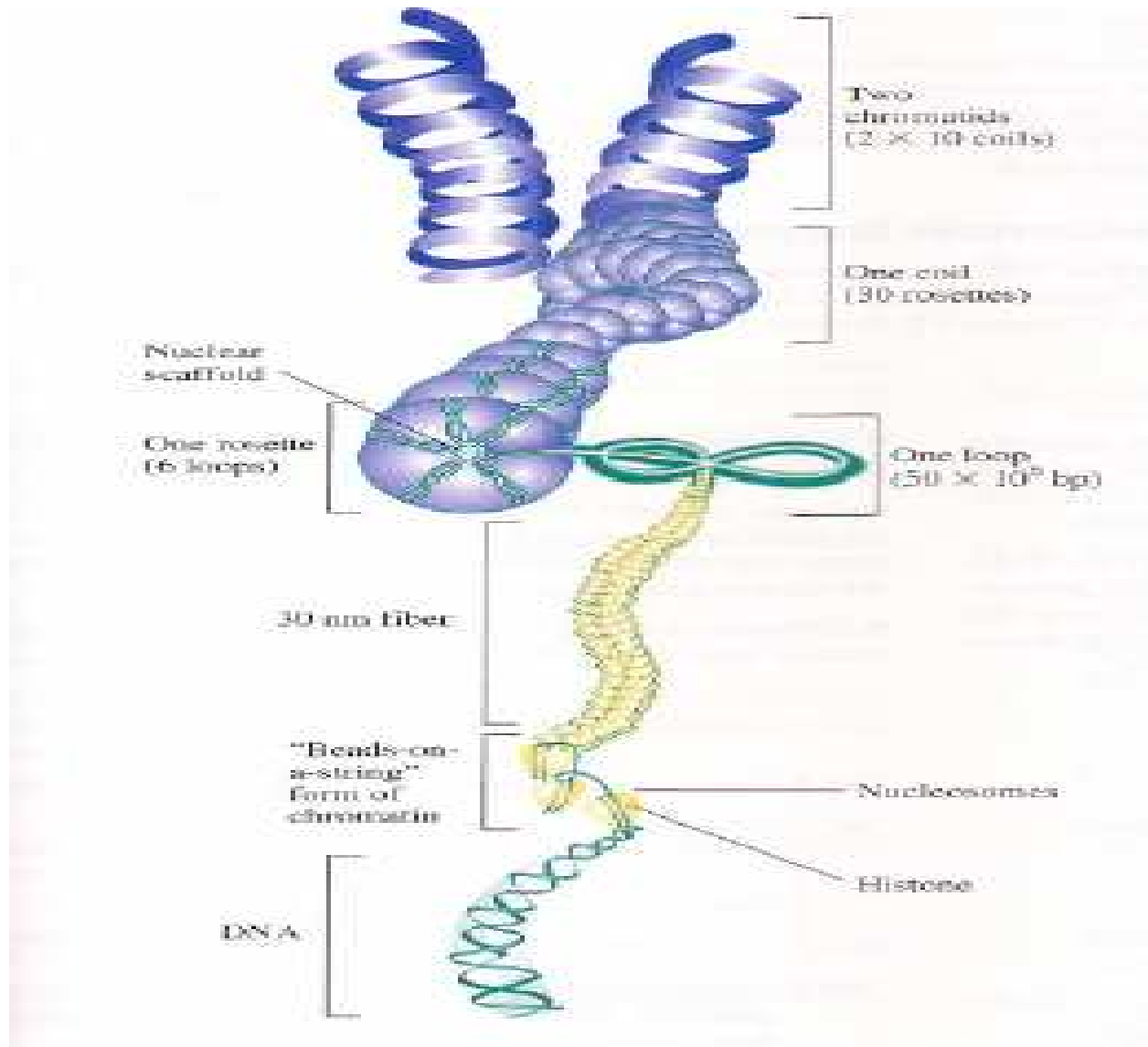
Metafáze - spiralizace

Normálně - despiralizace

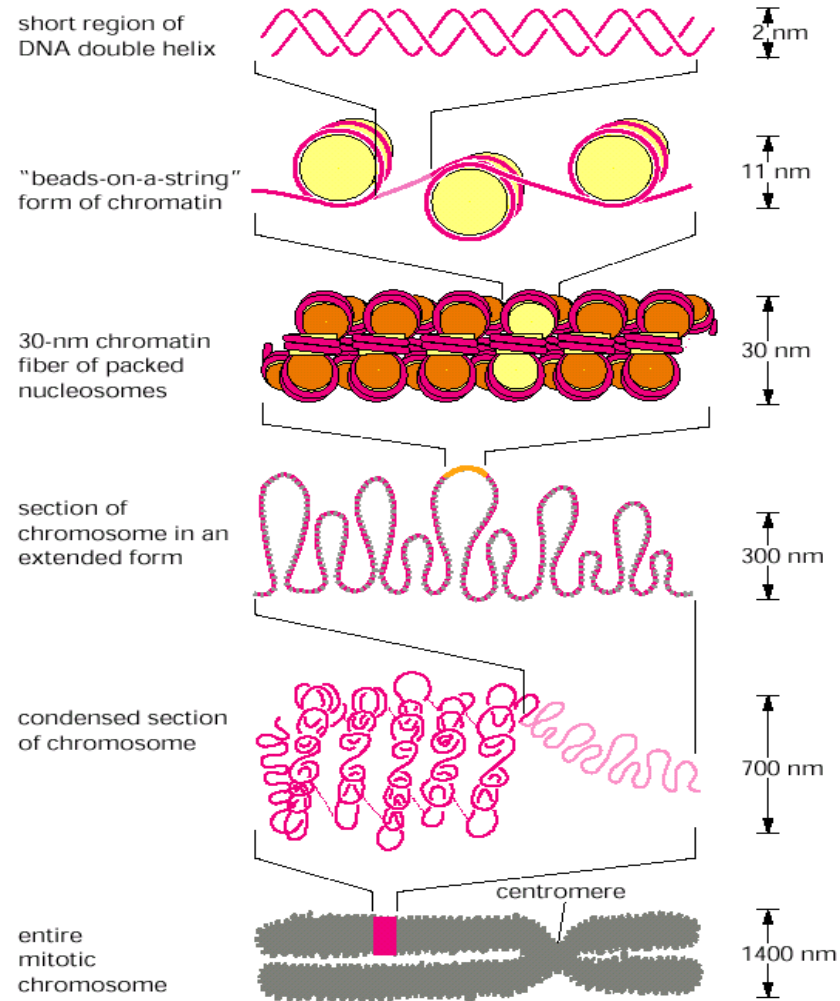
5 – 8  $\mu\text{m}$  – DNA 2 metry



# Chromozom



# Chromatin



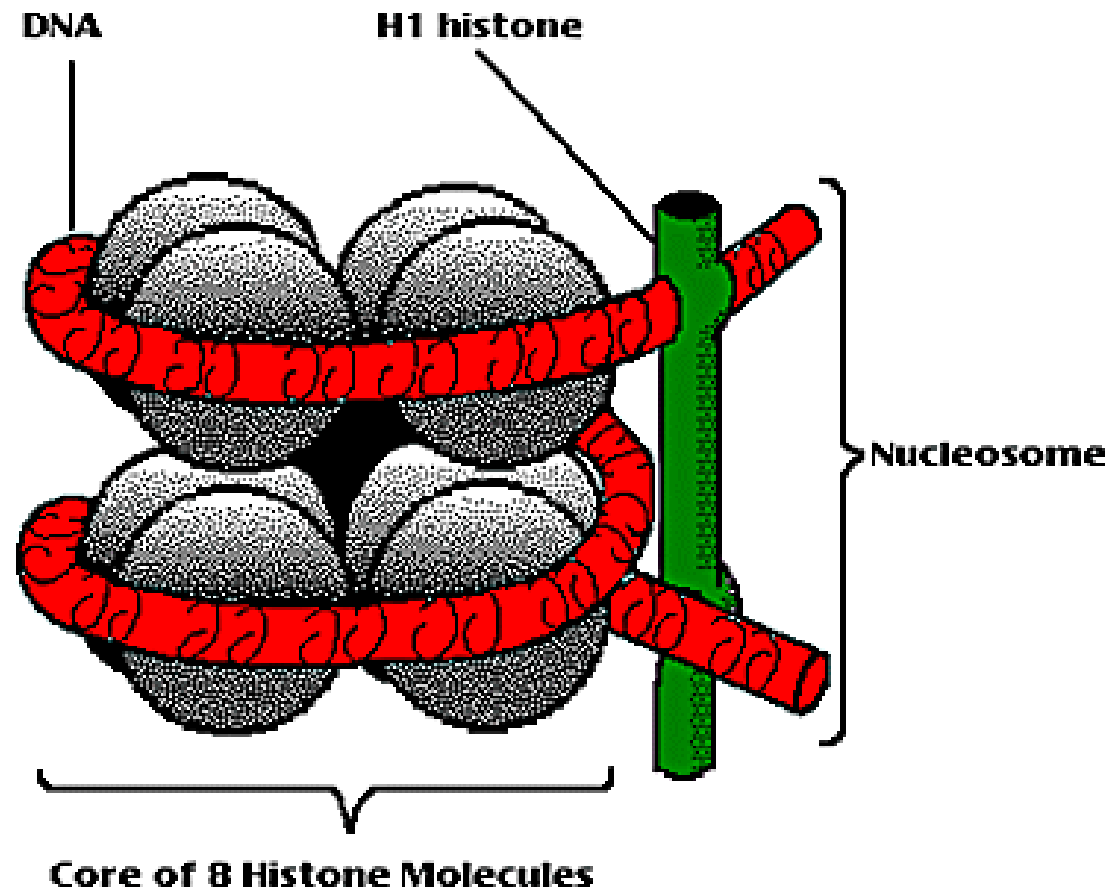
Nukleosom  
(korálky)

(cívka)

(smyčka)

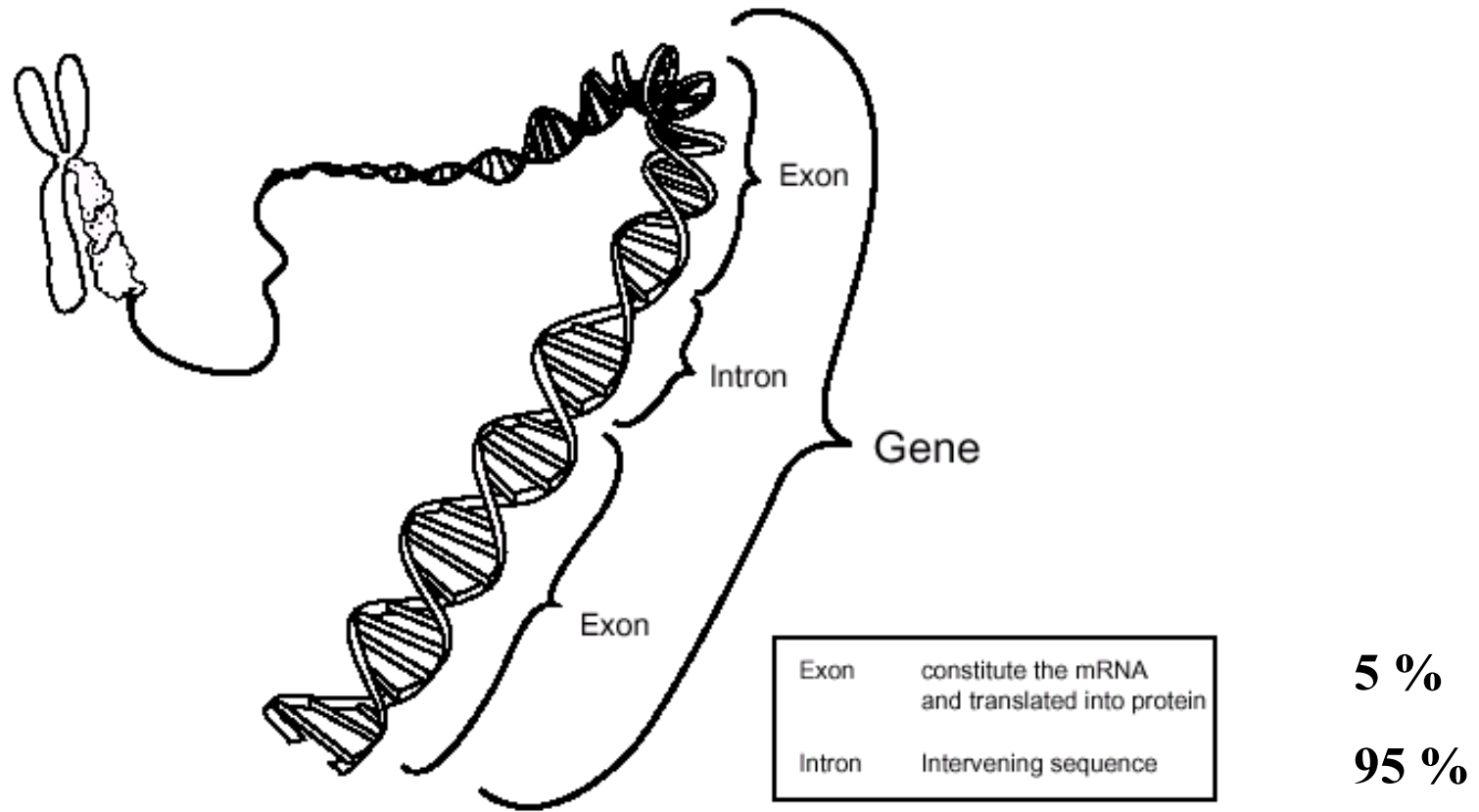
NET RESULT: EACH DNA MOLECULE HAS BEEN  
PACKAGED INTO A MITOTIC CHROMOSOME THAT  
IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

# Nukleosom – DNA + 5 tříd histonů

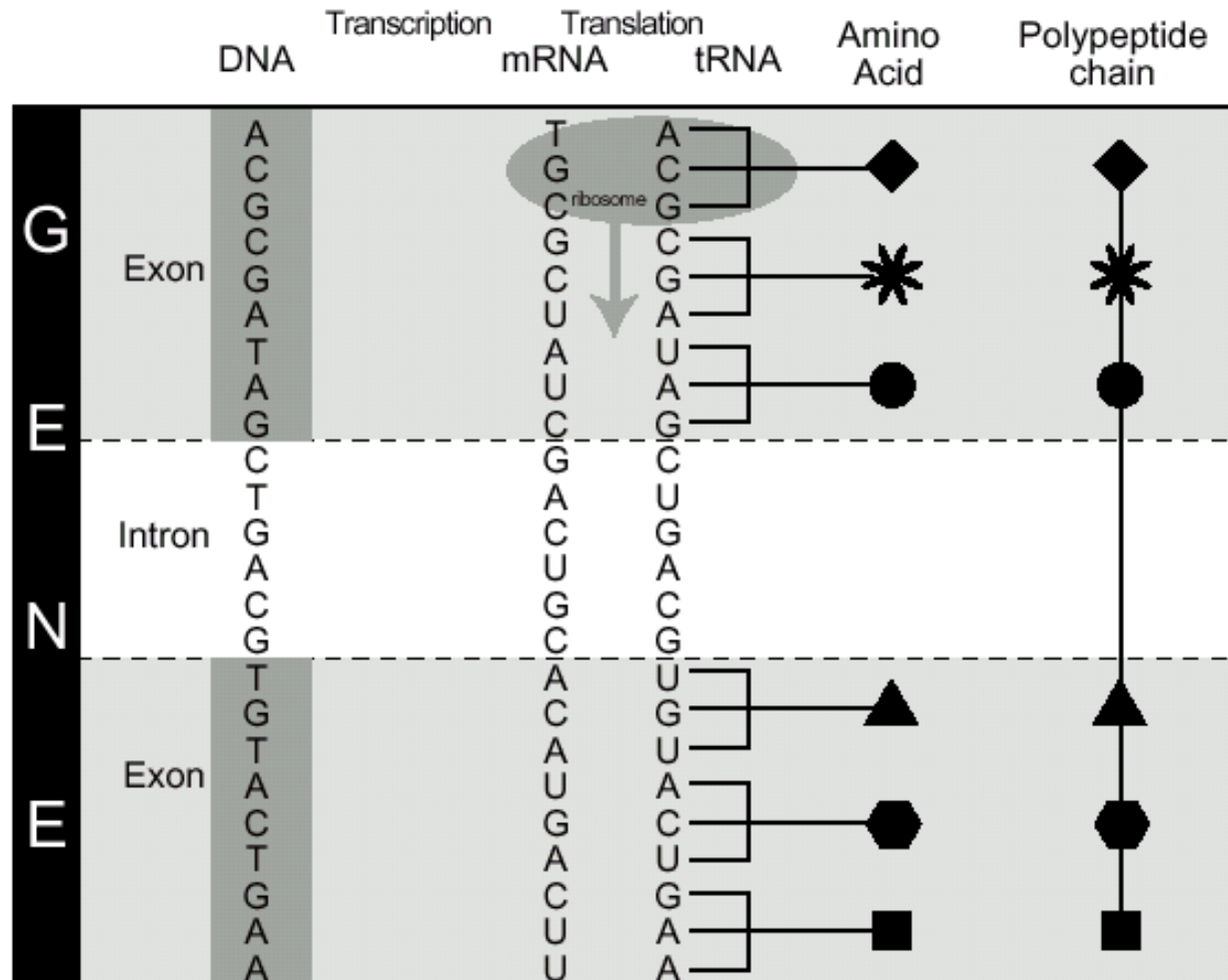


2 x H2A - 2 x H2B - 2 x H3 - 2 x H4

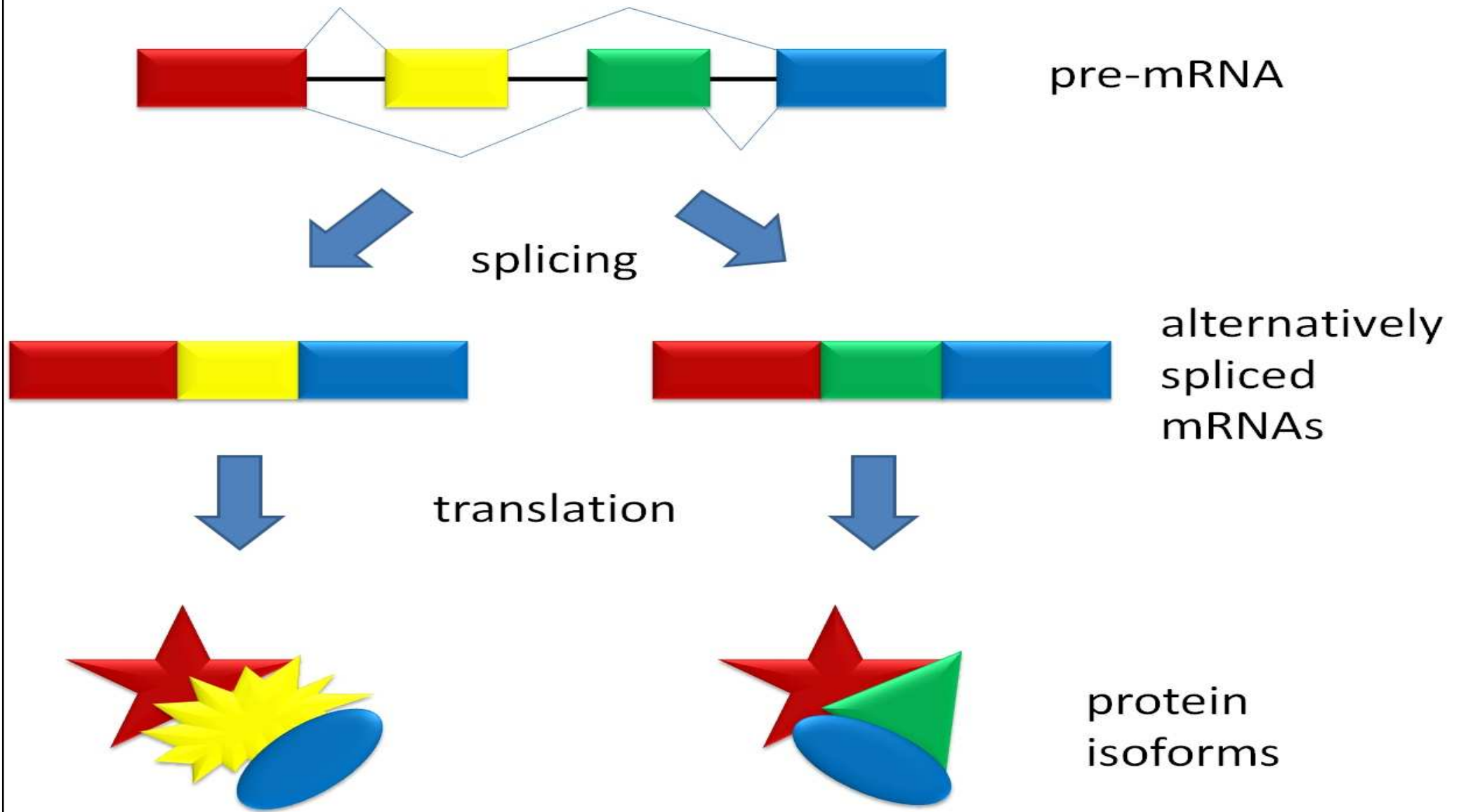
# Exon a intron



# Exon a intron



# Význam intronů



# Stanovení sekvence DNA

- Restrikční enzymy
- Chemické štěpení – Maxam Gilbertovo metoda
- Enzymová metoda
- Pyrosekvenování (enzymová metoda)

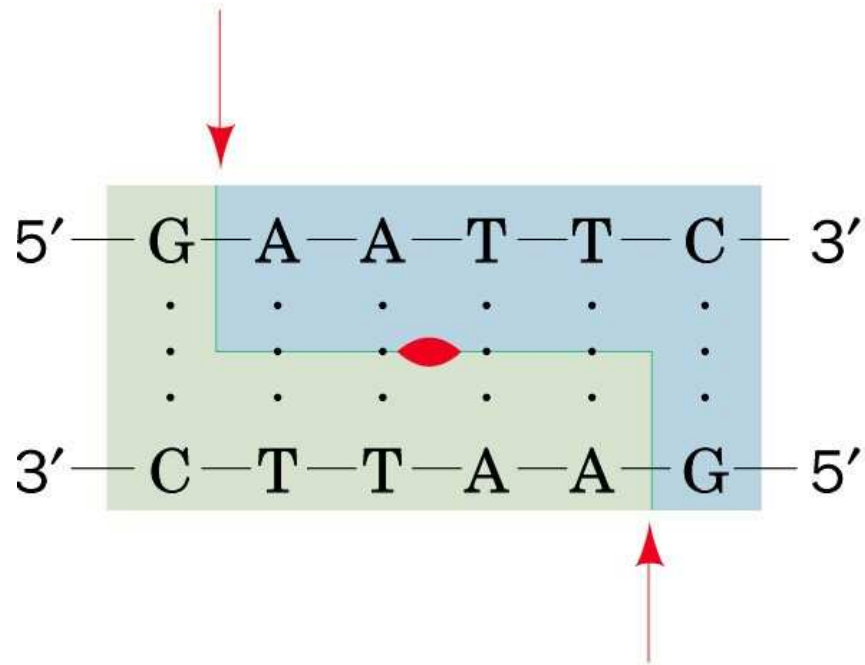


# Restrikční enzymy

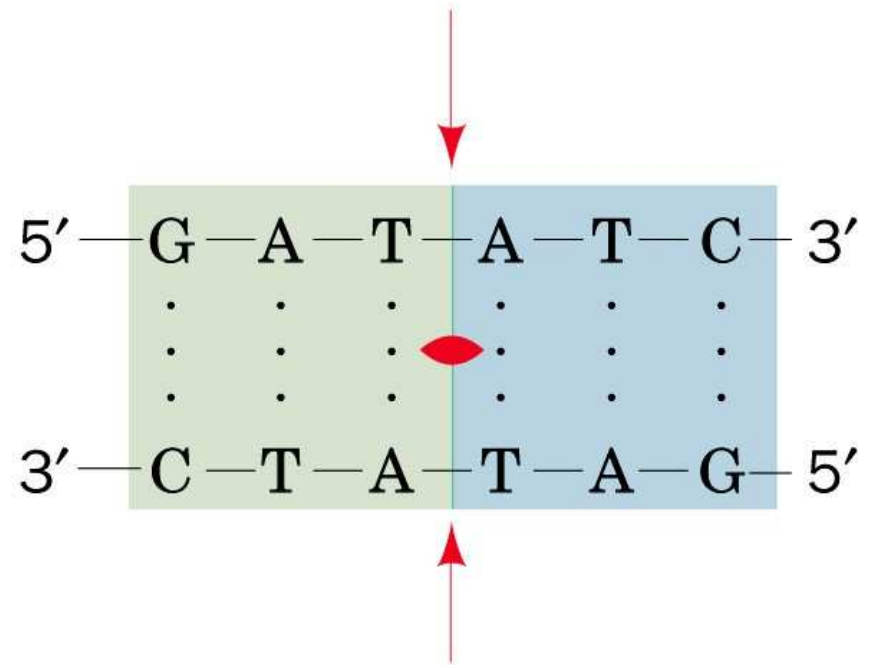
Enzyme	Recognition Sequence*	Microorganism
<i>AclI</i>	AGIC* <sup>T</sup>	<i>Arthrobacter luteus</i>
<i>BamHI</i>	G↓GATC* <sup>C</sup>	<i>Bacillus amyloliquefaciens</i> H
<i>BglI</i>	GCCNNNN↓NGCC	<i>Bacillus globigii</i>
<i>BglII</i>	A↓GATCT	<i>Bacillus globigii</i>
<i>EcoRI</i>	G↓AA* <sup>TT</sup> C	<i>Escherichia coli</i> RY13
<i>EcoRII</i>	↓CC*( <sup>?</sup> )GG	<i>Escherichia coli</i> R245
<i>EcoRV</i>	GA* <sup>T</sup> ↓ATC	<i>Escherichia coli</i> J62 pLG74
<i>HaeII</i>	RGCGC↓Y	<i>Haemophilus argyritus</i>
<i>HaeIII</i>	GG↓C* <sup>C</sup>	<i>Haemophilus argyritus</i>
<i>HindIII</i>	A* <sup>↓</sup> AGCTT	<i>Haemophilus influenzae</i> R <sub>13</sub>
<i>HpaII</i>	C↓A* <sup>CG</sup>	<i>Haemophilus parainfluenzae</i>
<i>MspI</i>	C* <sup>↓</sup> CGG	<i>Moraxella</i> species
<i>PvuI</i>	CTGCA* <sup>↓</sup> AG	<i>Providencia stuartii</i> 164
<i>PvuII</i>	CAG↓C* <sup>GT</sup>	<i>Providencia vulgaris</i>
<i>SalI</i>	G↓TCGAC	<i>Streptomyces albus</i> G
<i>TaqI</i>	T↓CGA* <sup>C</sup>	<i>Thermus aquaticus</i>
<i>XbaI</i>	C↓TCCAG	<i>Xanthomonas badrii</i> 101

# Restrikční enzymy

(a) *EcoRI*



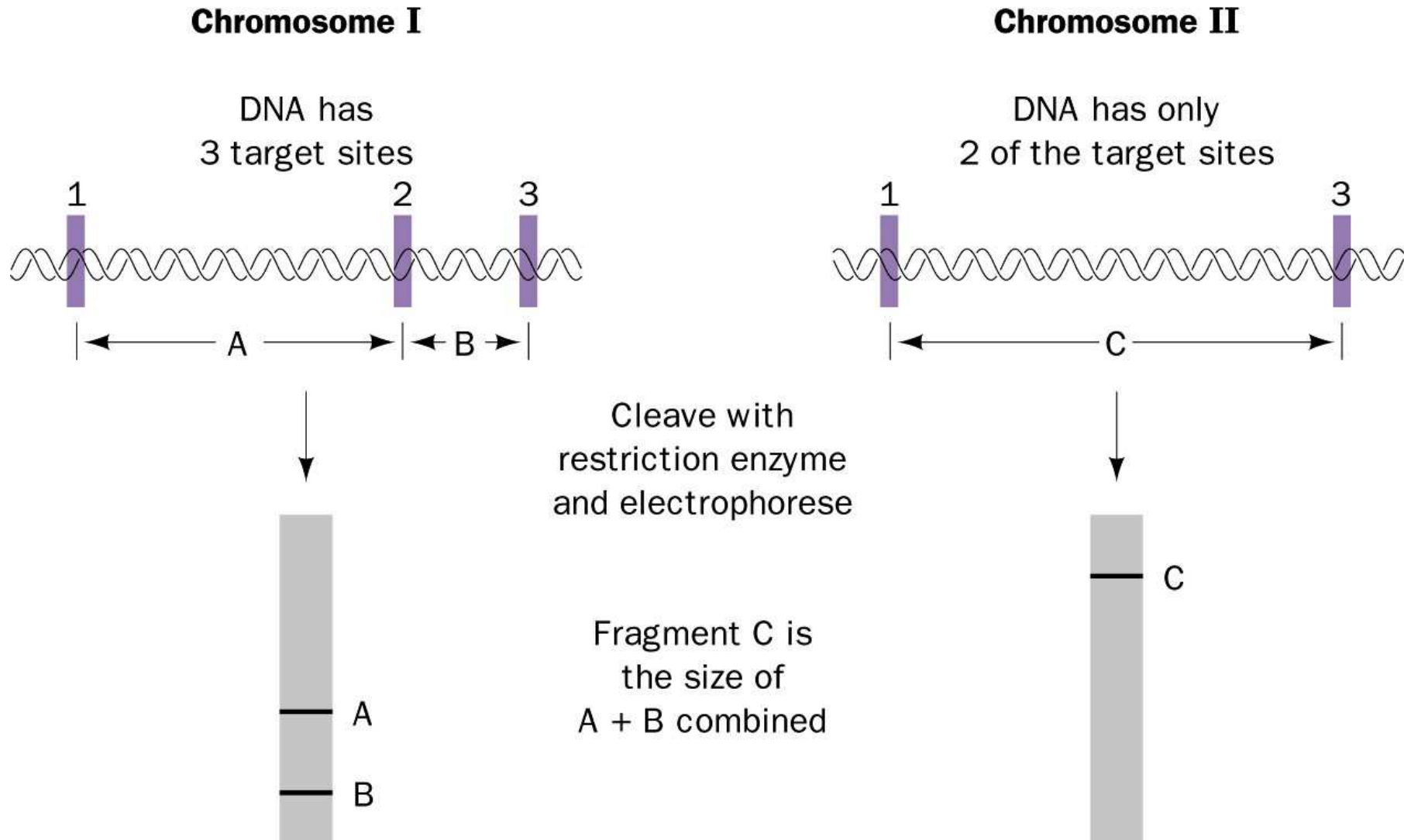
(b) *EcoRV*



↓ Cleavage site

● Twofold symmetry axis

# Restrikční enzymy



# Restrikční enzymy

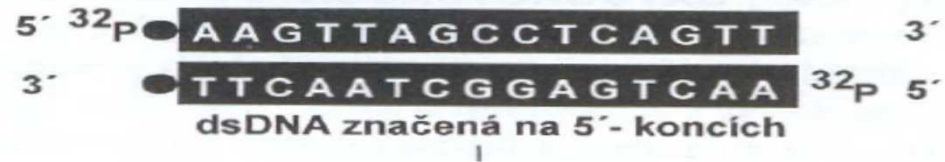


# Maxam Gilbertova metoda

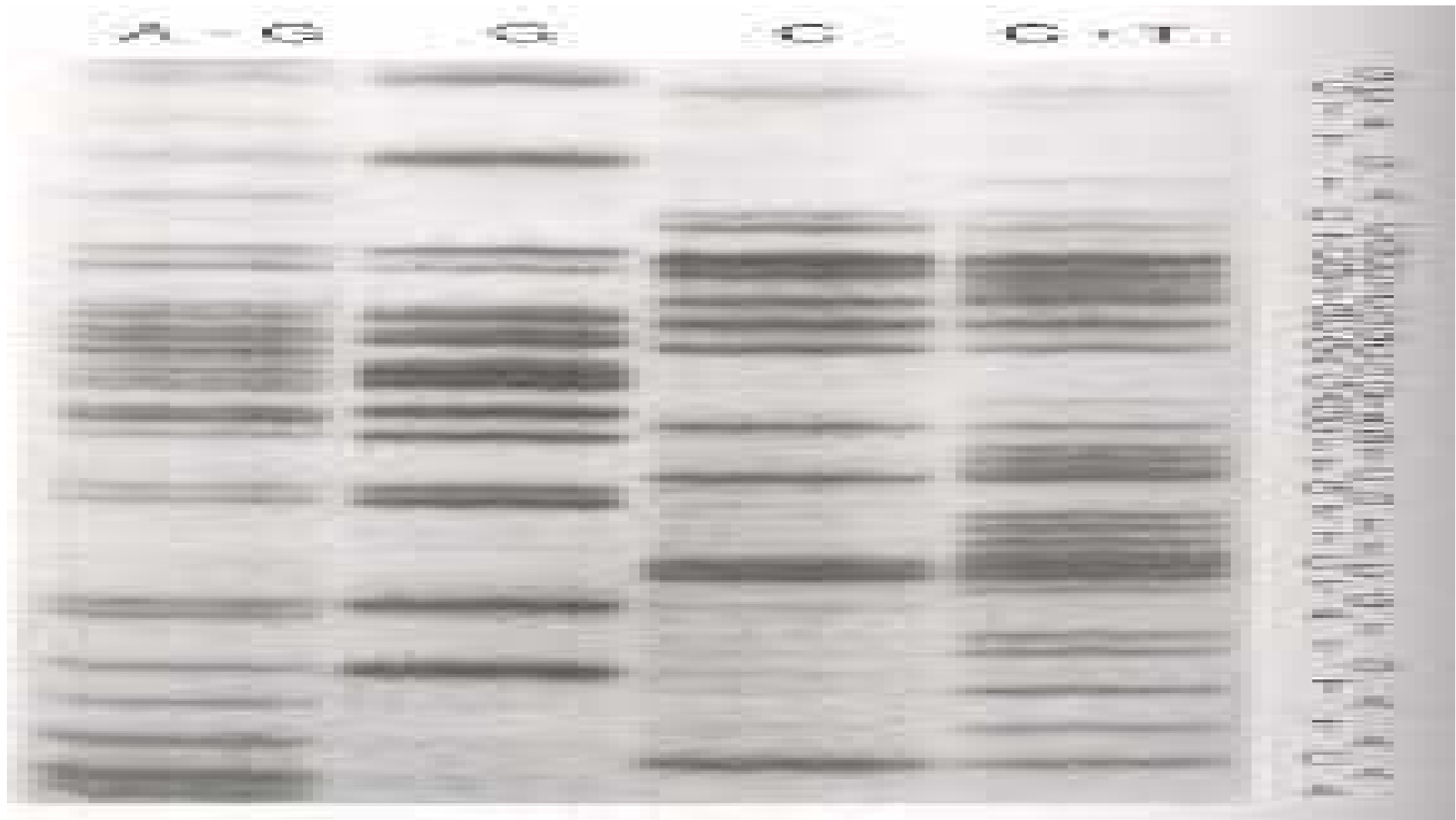
## Maxam-Gilbertova metoda

- značení 5' konce  $^{32}\text{P}$
- specifické chemické štěpení
- elektroforéza

# Maxam Gilbertova metoda



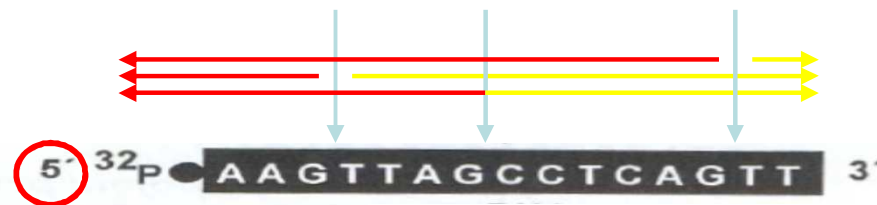
# Maxam Gilbertova metoda



**kyselina a piperidin   dimetylsulfát a piperidin   kyselina, NaCl a piperidin   hydrazin a piperidin**

# Maxam Gilbertova metoda

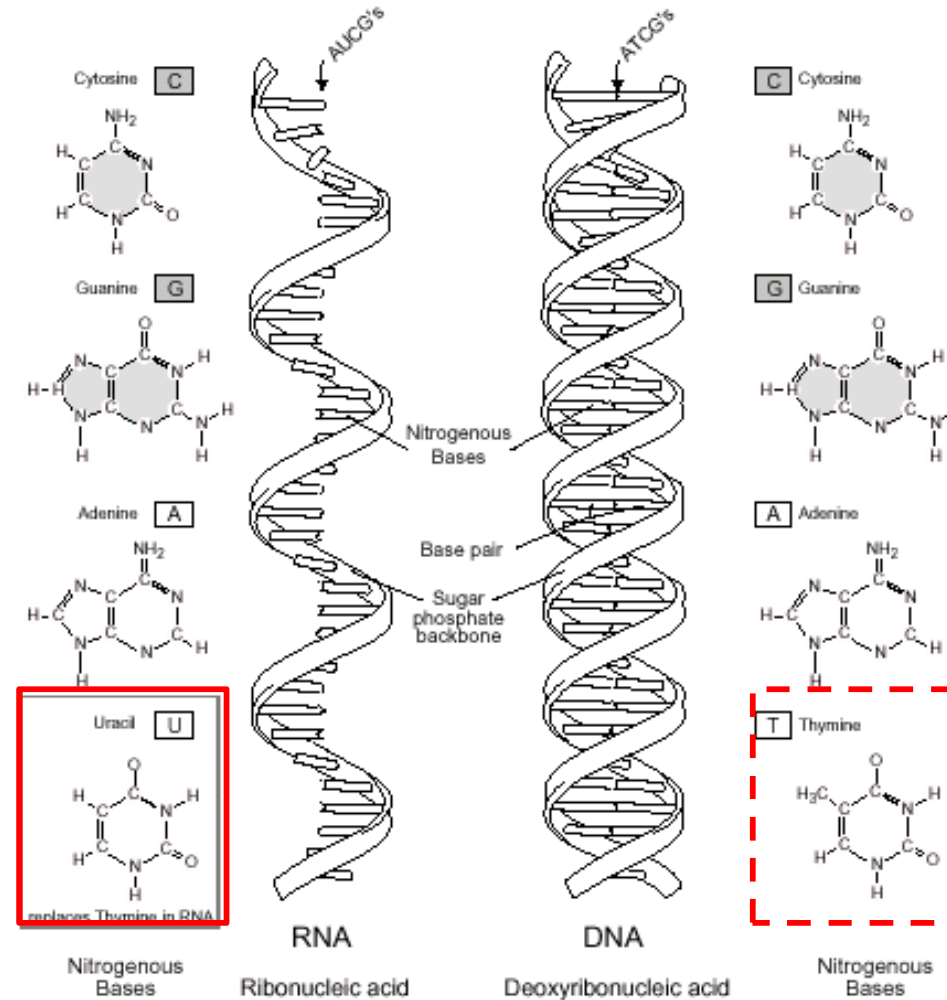
**dimetylsulfát a piperidin**





# FUNKCE RNA versus DNA

## Ribosa - Deoxyribosa



**Jednovláknová - Dvouvláknová**

## Formy RNA

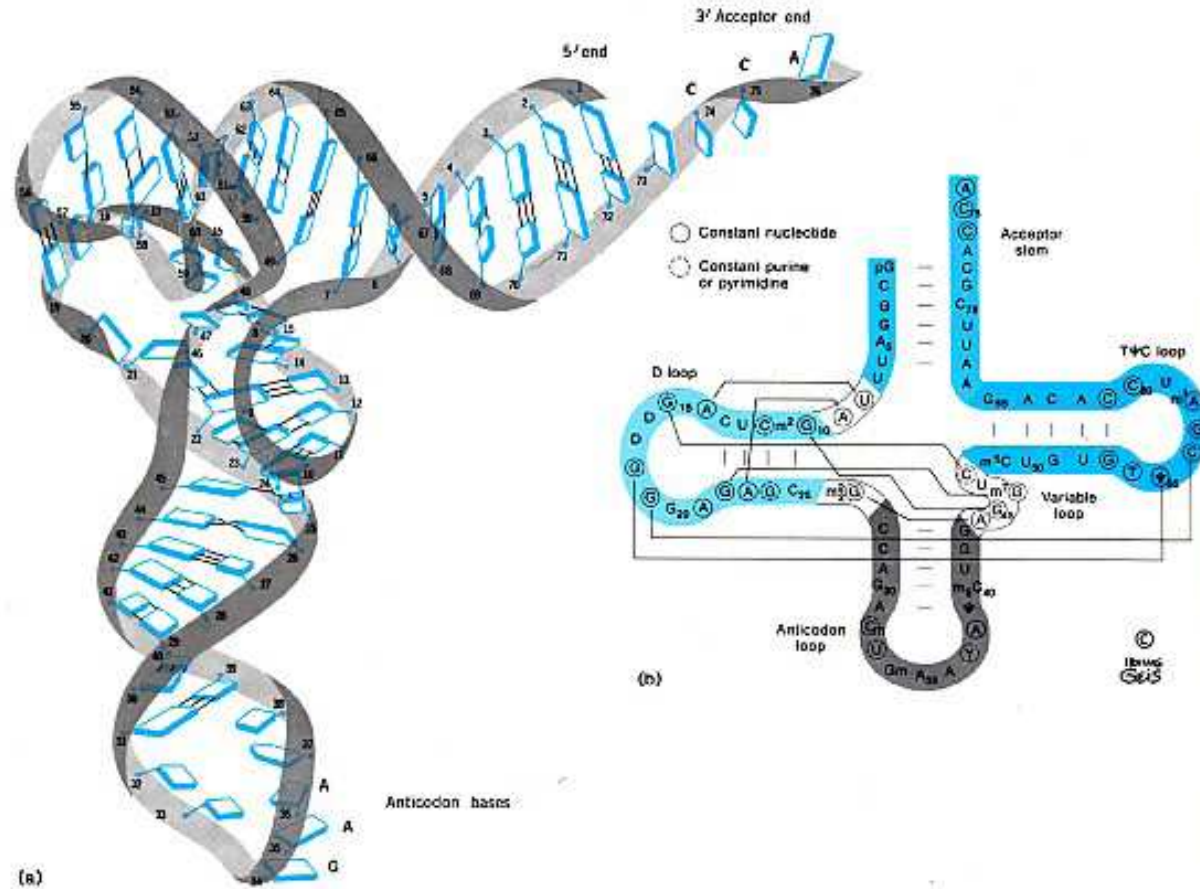
- mRNA – mediátorová, messenger,  
informační – 5-10 %r

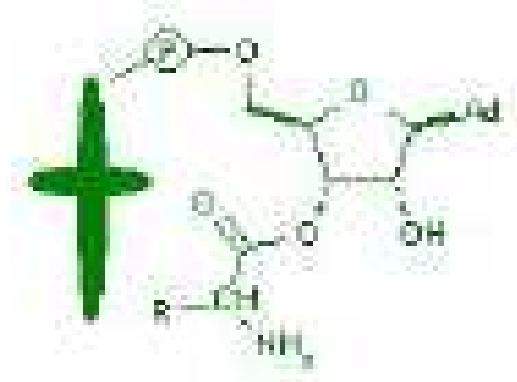
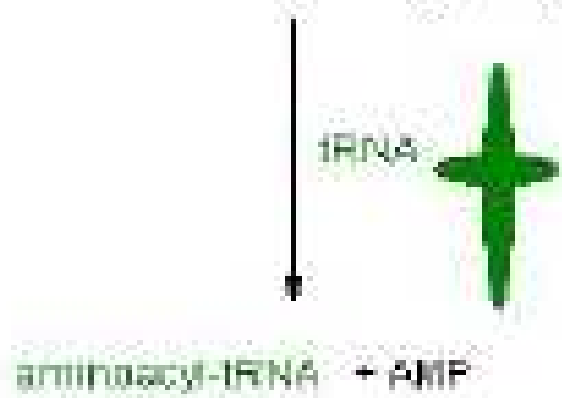
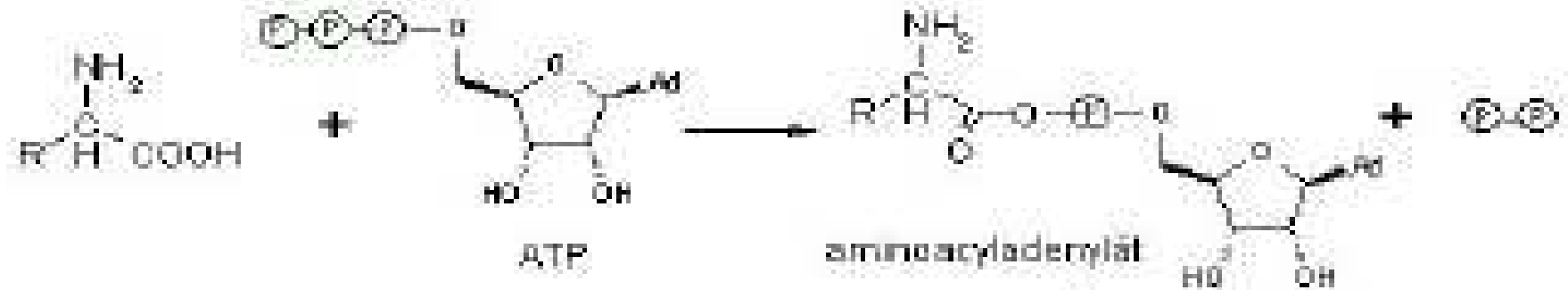
- rRNA – ribosomální – 80 %

- tRNA – transferová, přenosová – 10-15 %

60 tRNA

# tRNA





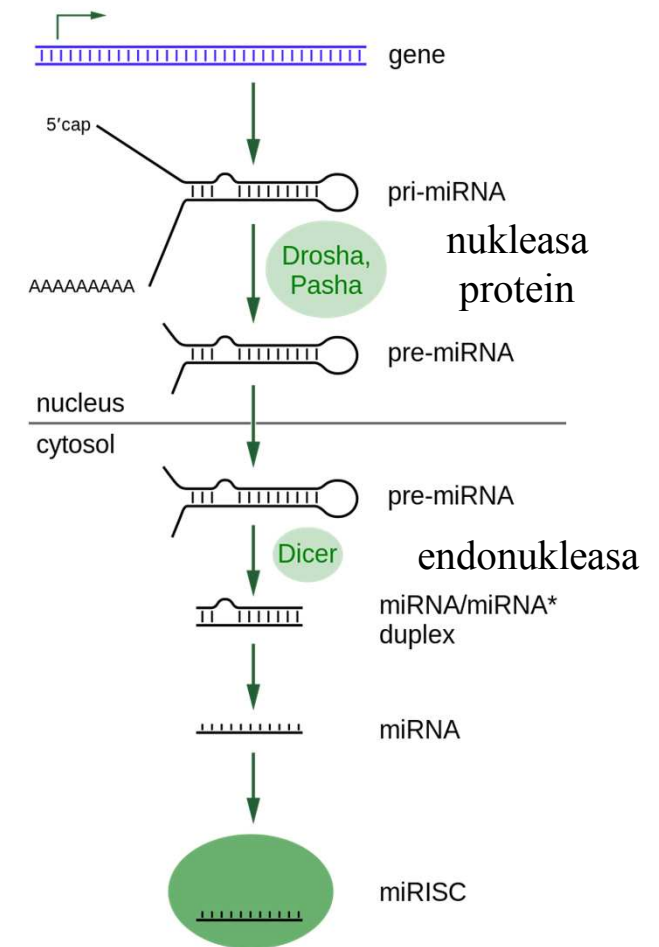
## Formy RNA

- mRNA – mediátorová, messenger,  
informační – 5-10 %of
- RNA – ribosomální – 80 %
- tRNA – transferová, přenosová – 10-15 %

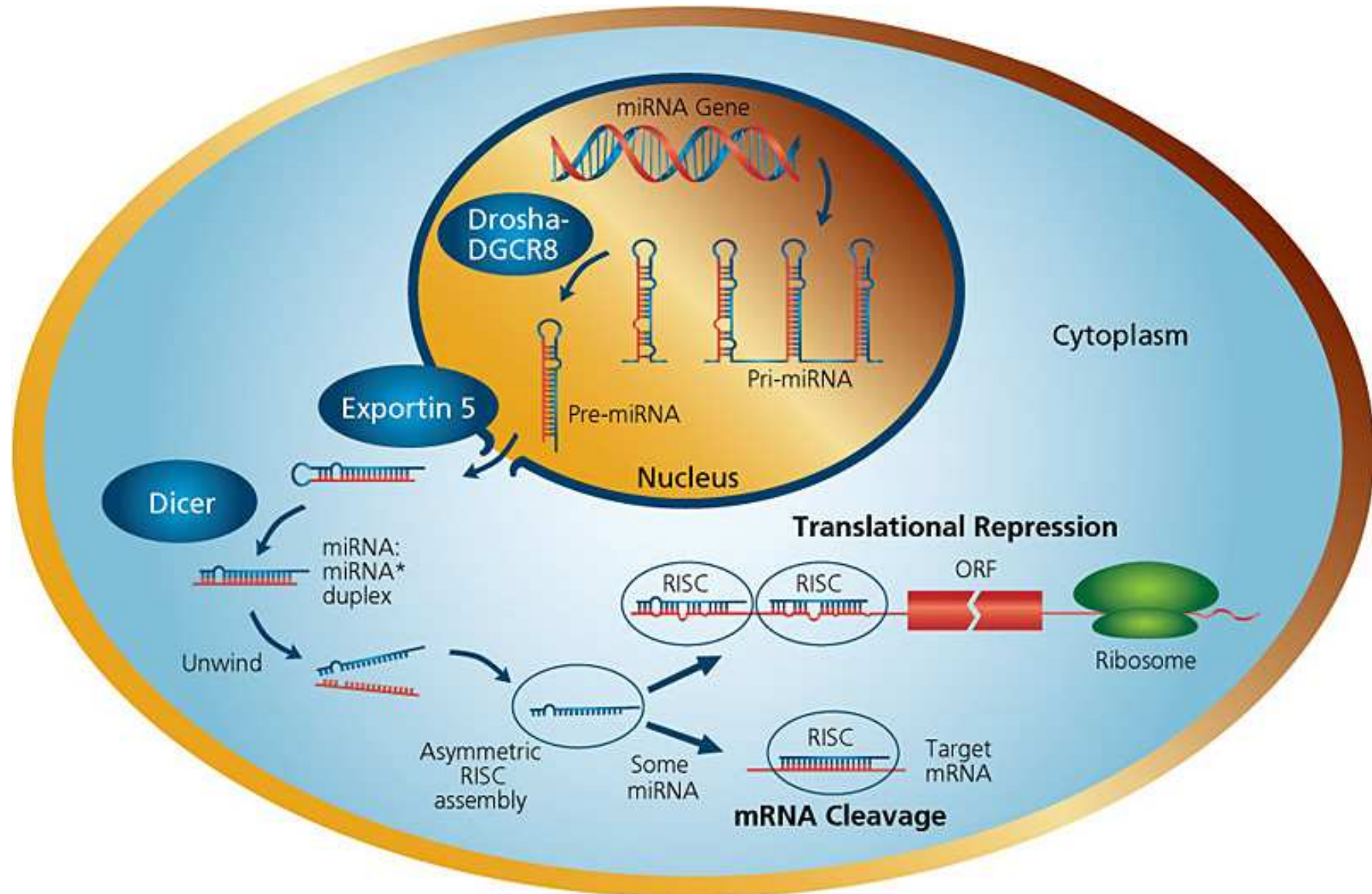
60 tRNA

1993 Victor Ambrose

miRNA - microRNA



# Micro RNA



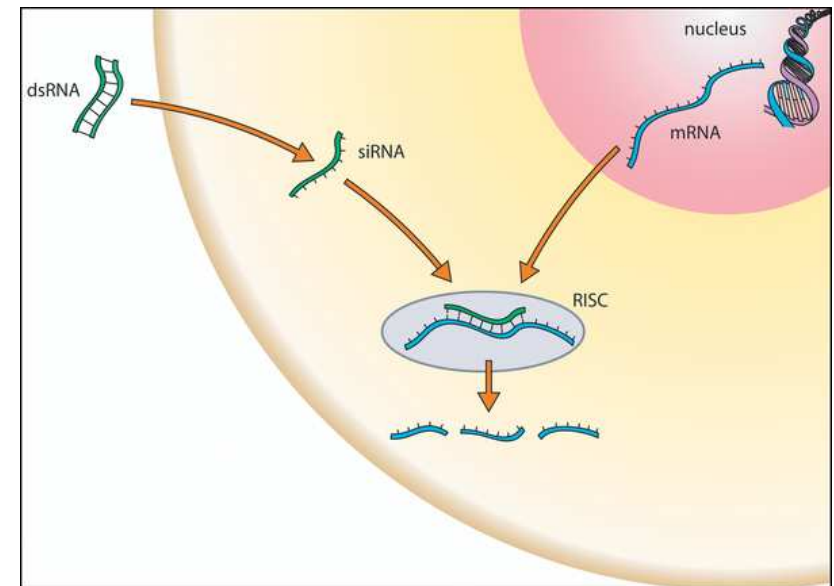
## Formy RNA

- mRNA – mediátorová, messenger,  
informační – 5-10 %r
- r RNA – ribosomální – 80 %
- tRNA – transferová, přenosová – 10-15 %

60 tRNA

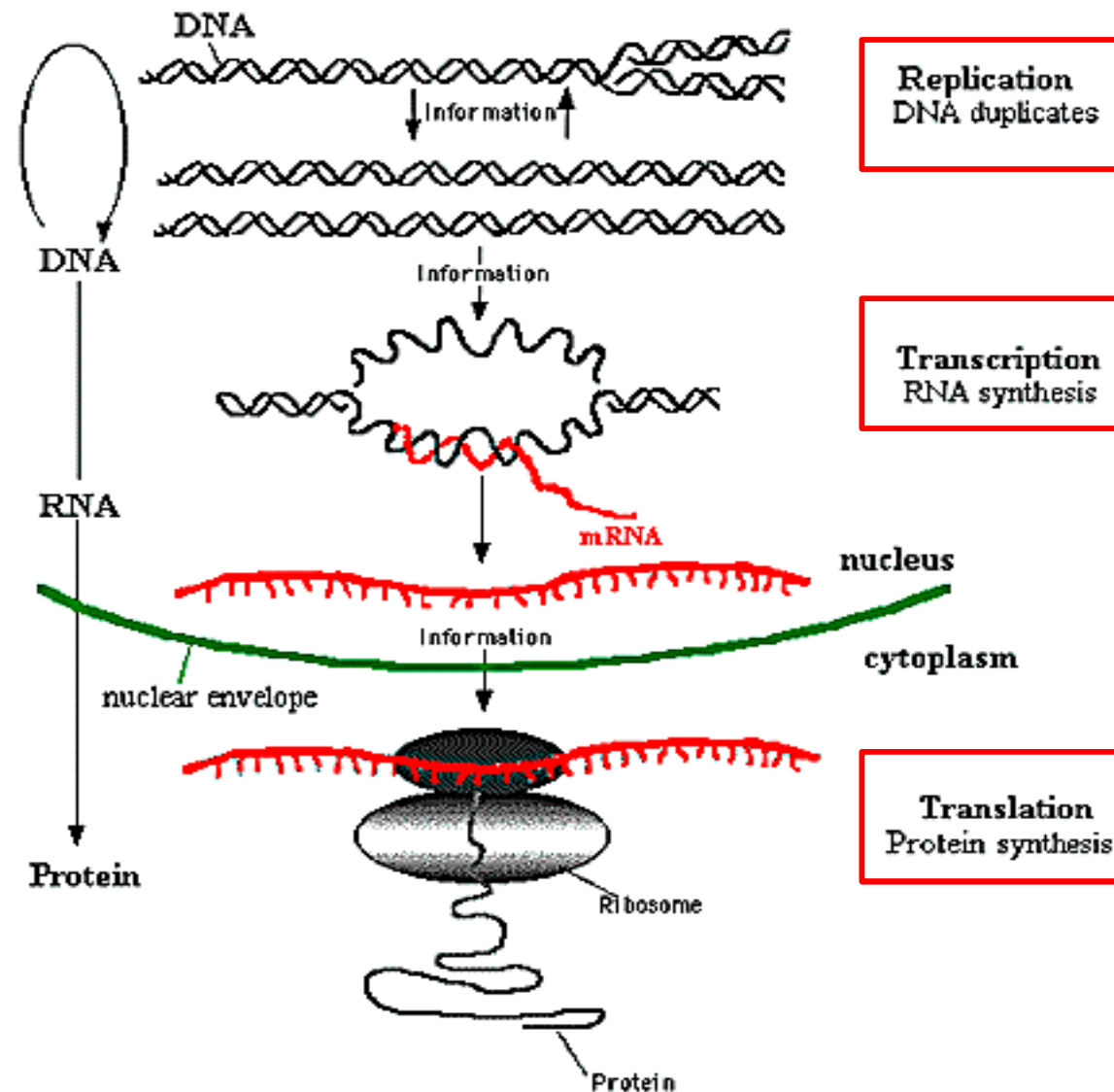
miRNA - microRNA

siRNA – small interfering RNA



# Centrální dogma mol.biologie

**Crick 1958 - cesta od NK k proteinu**





# Centrální dogma mol.biologie

DNA 5' — A-G-A-G-G-T-G-C-T — 3'  
3' — T-C-T-C-C-A-C-G-A — 5'

↓  
Přepis

mRNA 5' — A-G-A-G-G-U-G-C-U — 3'

tRNAs

U-C-U C-C-A C-G-A

Arginine Glycine Alanine

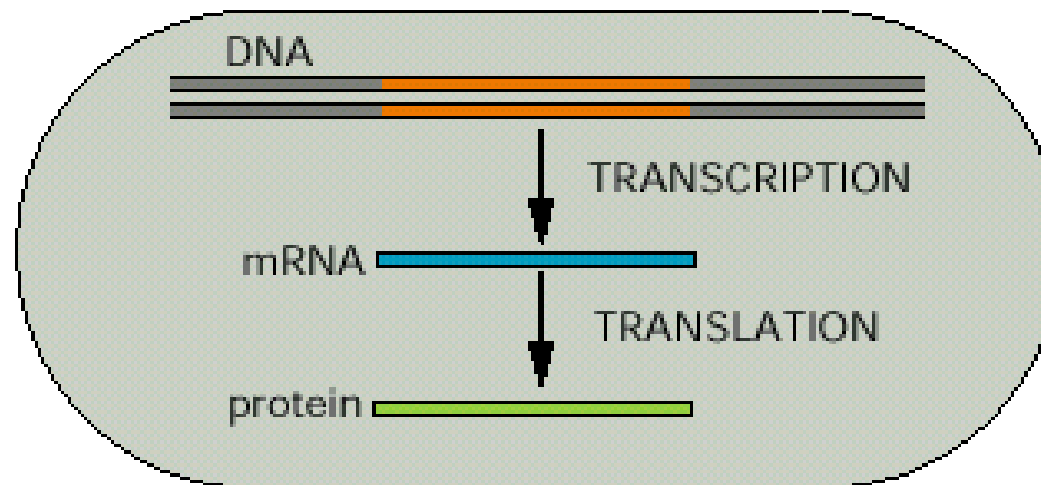
↓  
Překlad

Polypeptide

—Arg—Gly—Ala—

# Prokaryota

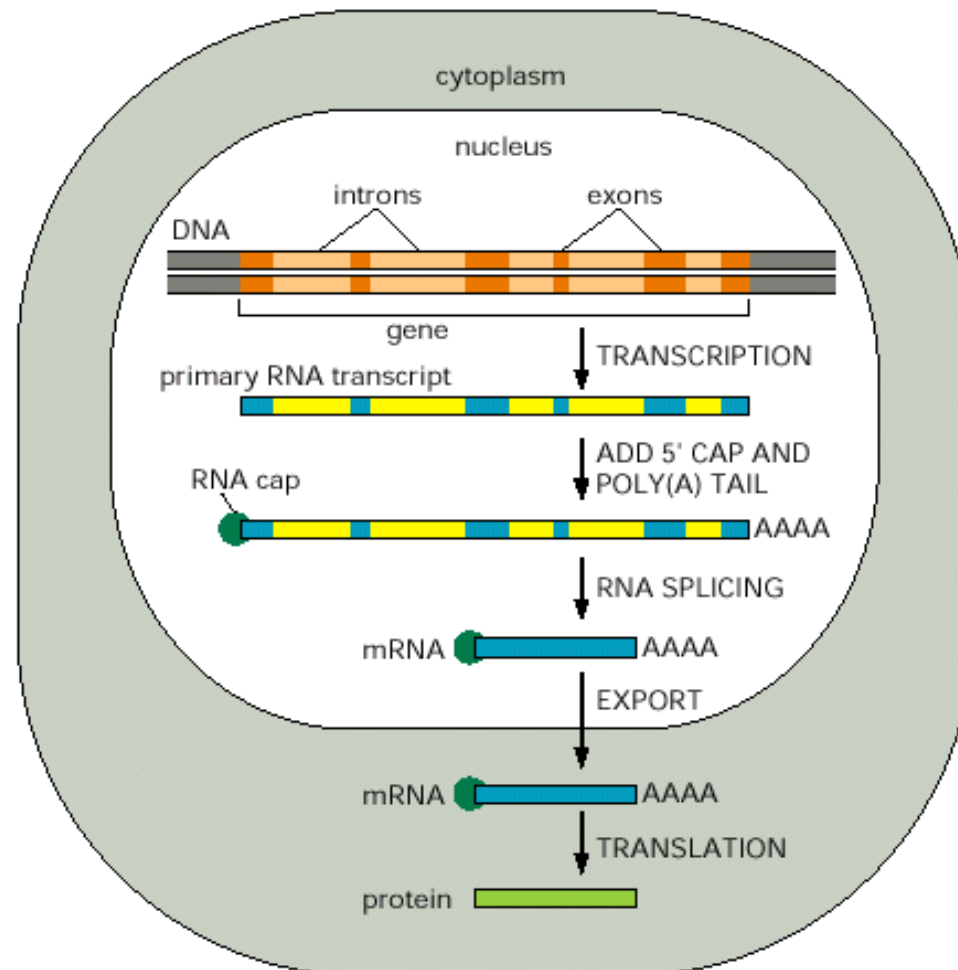
(B) PROCARYOTES



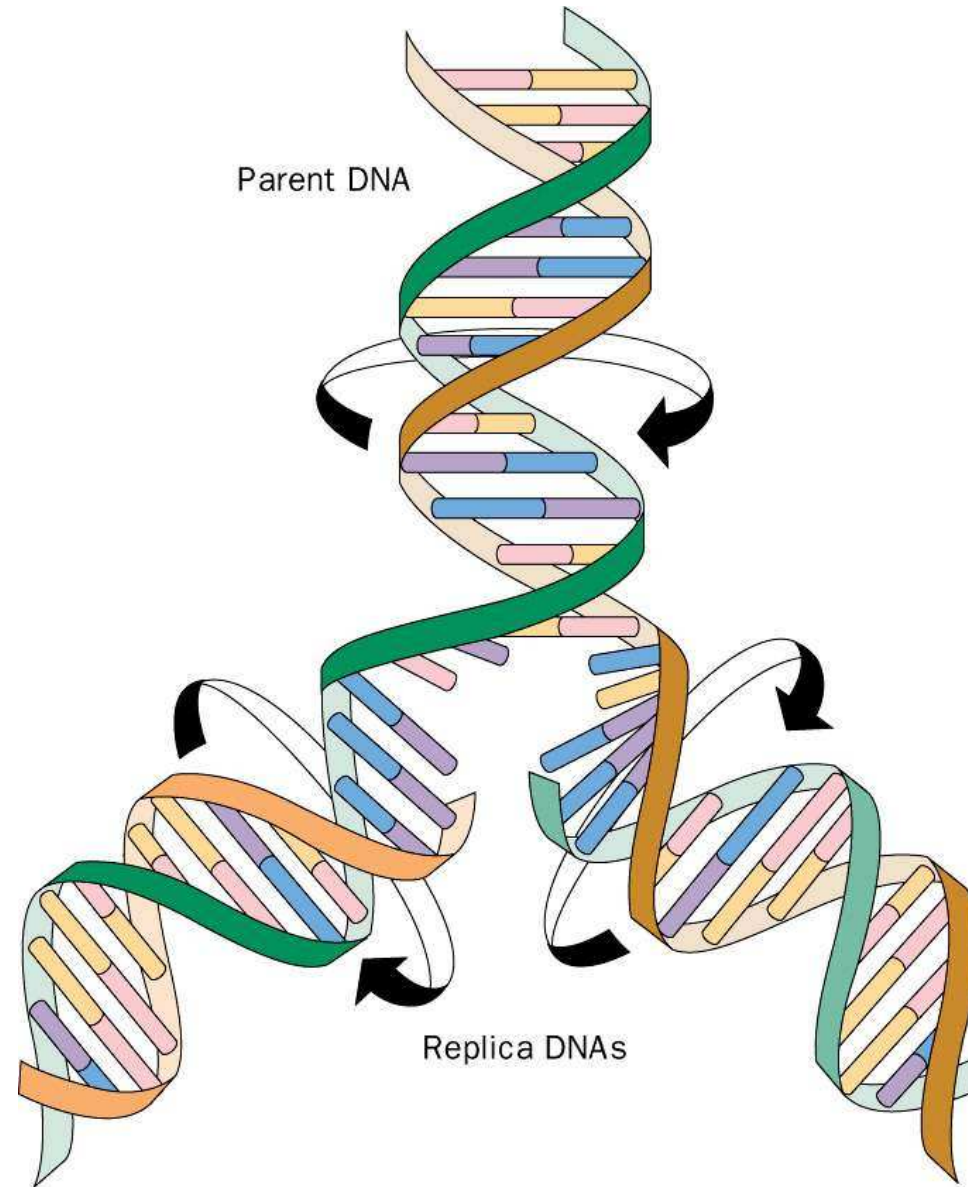
# Eukaryota

(A) EUCARYOTES

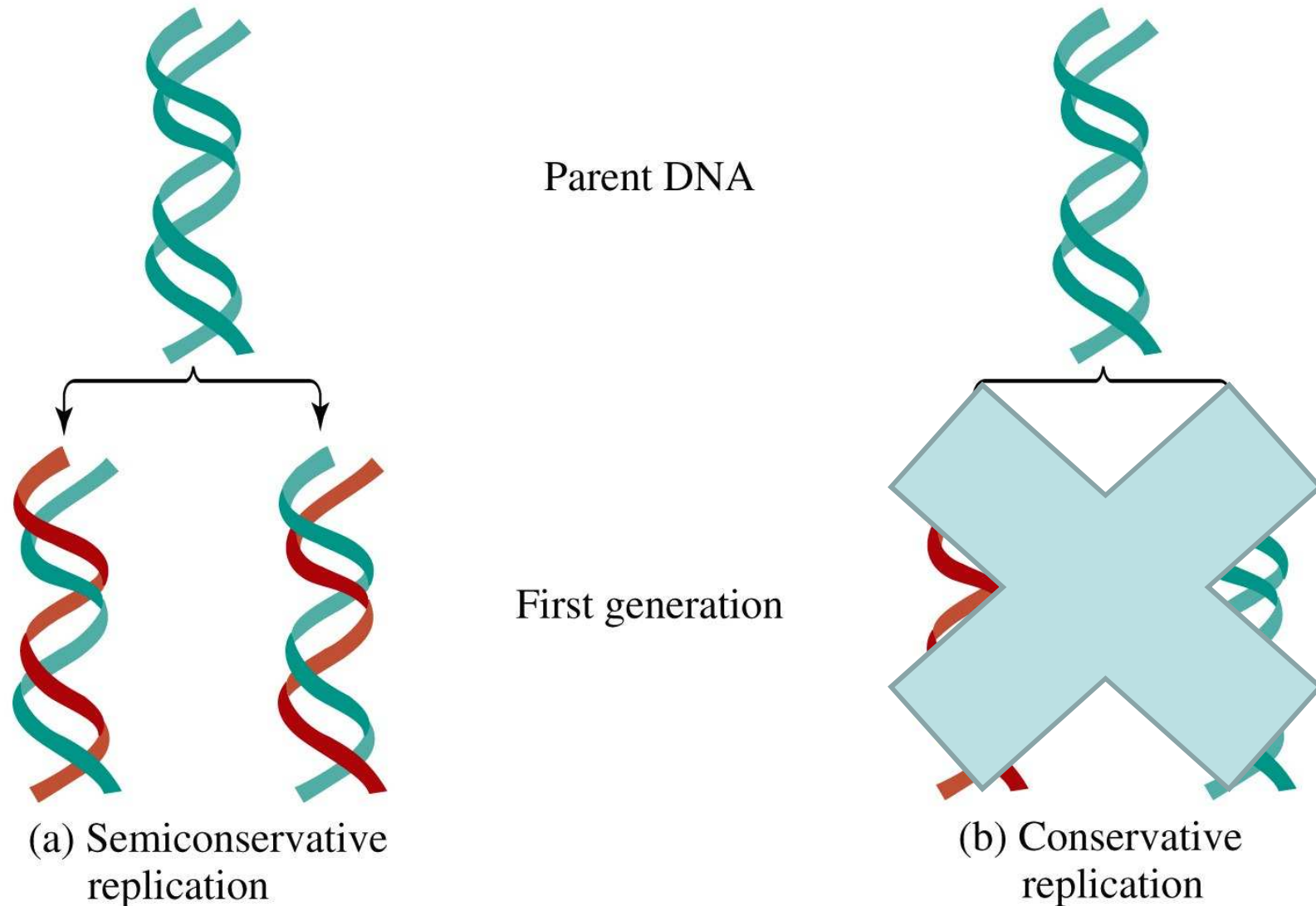
(B)



# Replikace – zdvojení - DNA → DNA tvorby identických kopií DNA



# Replikace DNA



# Meselson a Stahlův experiment

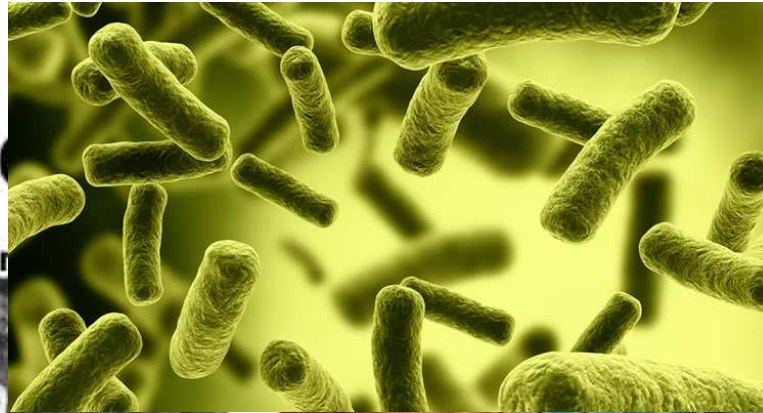
Mes

Matthew Meselson

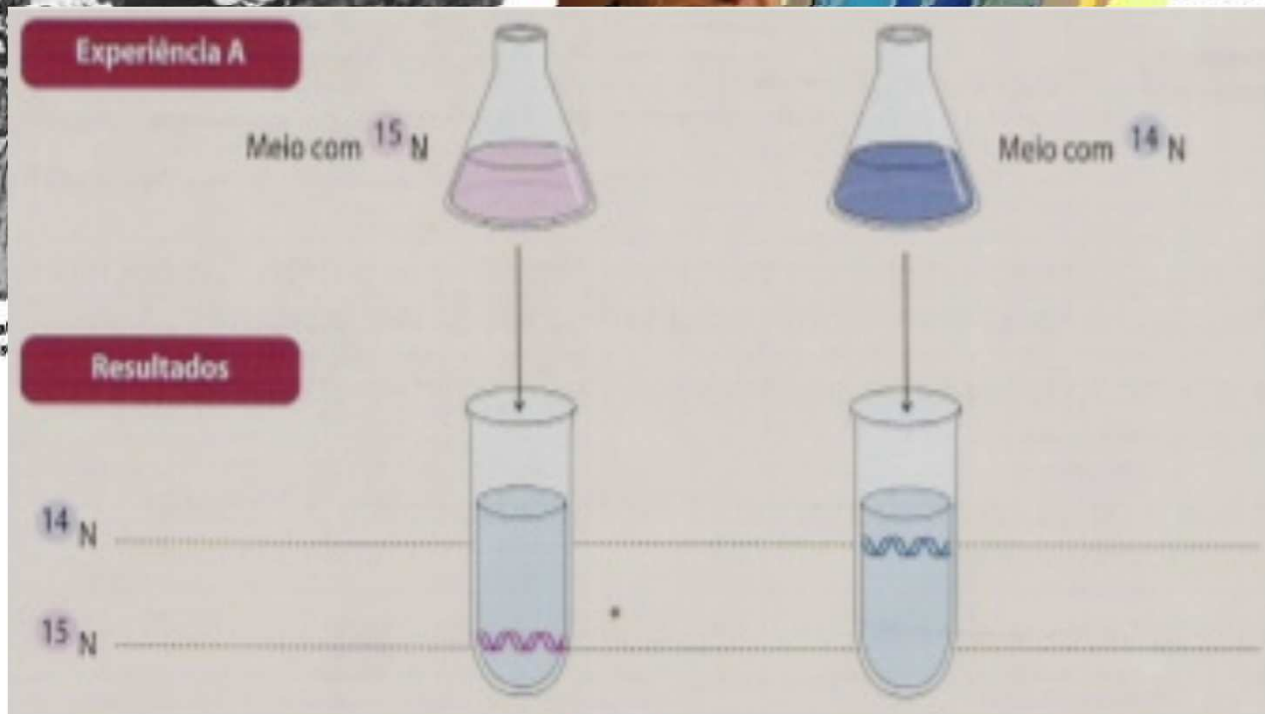
Fr



FIGURE 9-3. (Left) Ma  
[Courtesy of M. Mesel



Franklin Stahl



Courtesy of Dr. M. Meselson, Harvard University.

# Centrifugace

20. léta 20. století - **Svedberg** - počátky laboratorních centrifug a analytické centrifugace; teoretické základy metody

50. léta - Brakke - centrifugace v gradientu hustoty

# Theodor Svedberg (1884-1971)



**Nobelova cena za chemii 1926**

pojmenována po něm  
Svedbergova jednotka pro vyjádření  
sedimentačního koeficientu



# Centrifugace

- Odstranění hrubých částic z roztoku  
Sediment (pelet) – supernatant
- Izolace organel nebo biomakromolekul
- Stanovení základních parametrů – MW, hustota, sedimentační koeficient

# Použití

Centrifugace

Preparativní

Analytická

# Ultracentrifugace

**150 000 ot/min**

**1 000 000 g**

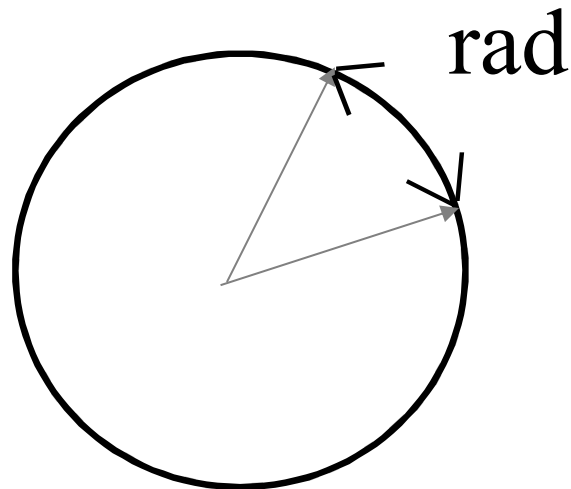
Otáčky  $\rightarrow$  g

$$g = \omega^2 \cdot r$$

$\omega$ - uhlová rychlost  
(rad/s)

$$\omega = 2\pi \cdot f$$

f – otáčky/min



# Preparativní ultracentrifuga

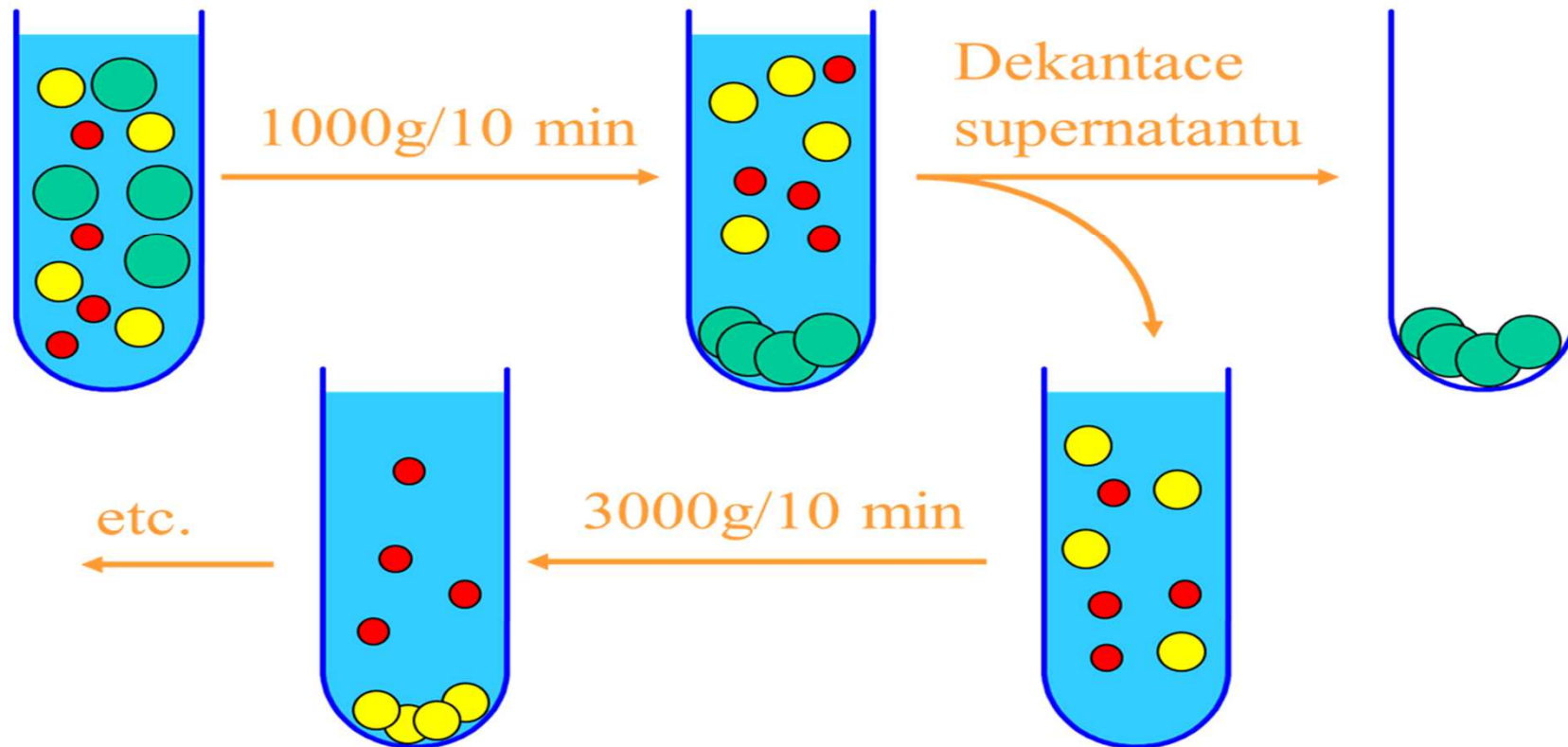


# Rotory

- Úhlový – diferenciální centrifugace
- Výkyvné – zonální centrifugace

# Diferenciální centrifugace

- opakovaná centrifugace se zvyšující se rychlostí otáček = gravitací



# Úhlový rotor

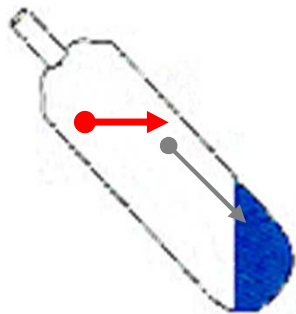
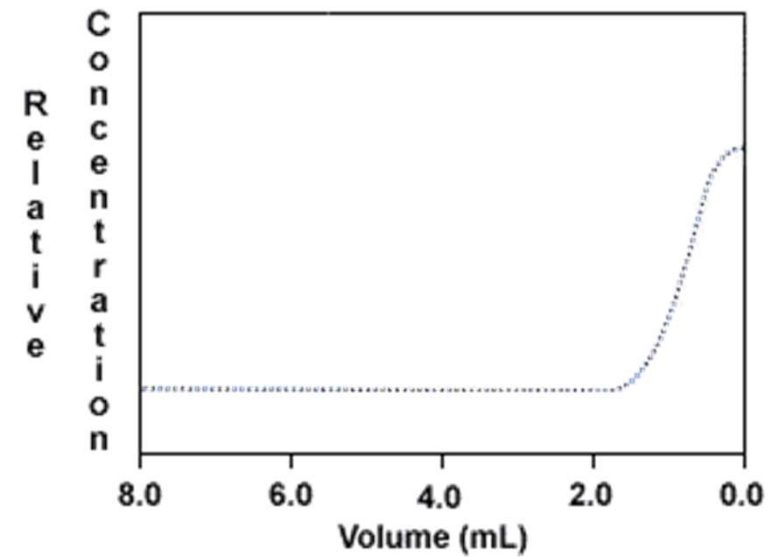


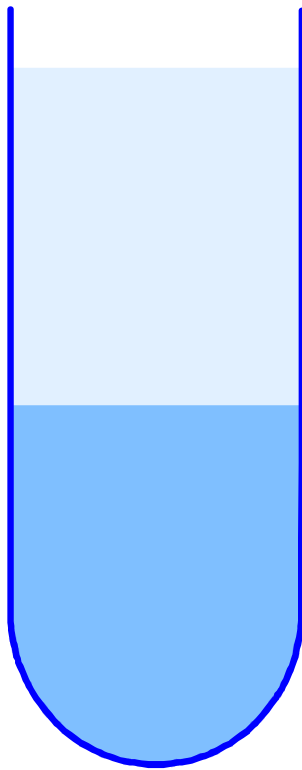
Figure 4



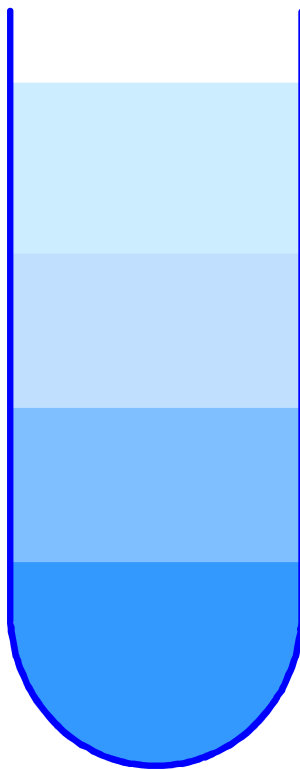


# Gradientová centrifugace

**Hustotní bariera**



**Diskontinuální**

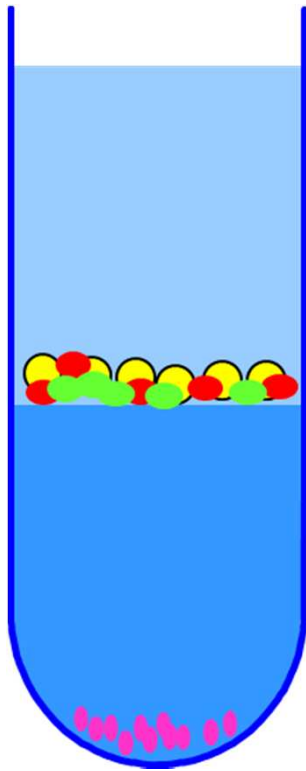


**Kontinuální**

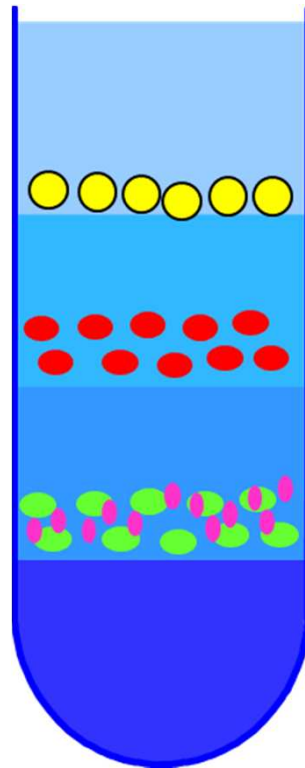


# Gradientová centrifugace

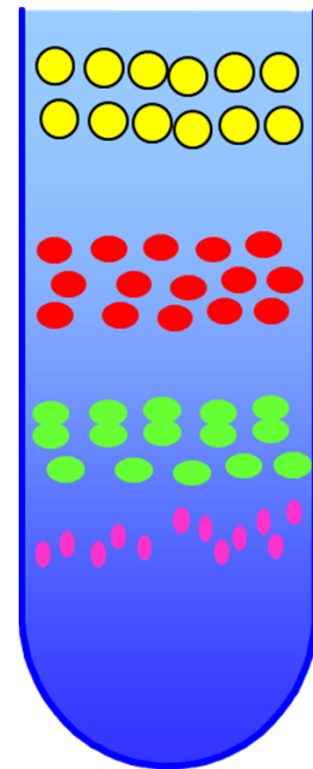
**Hustotní bariera**



**Diskontinuální**



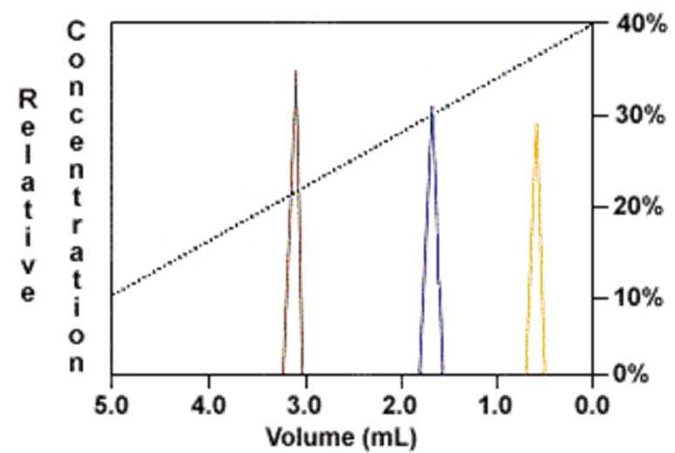
**Kontinuální**



# Výkyvný rotor



**Figure 2**



# Gradientová centrifugace média

- Sacharosa

- Glycerol

- Ficoll - dextran

- Percoll – SiO<sub>2</sub>

Hypertonické prostředí

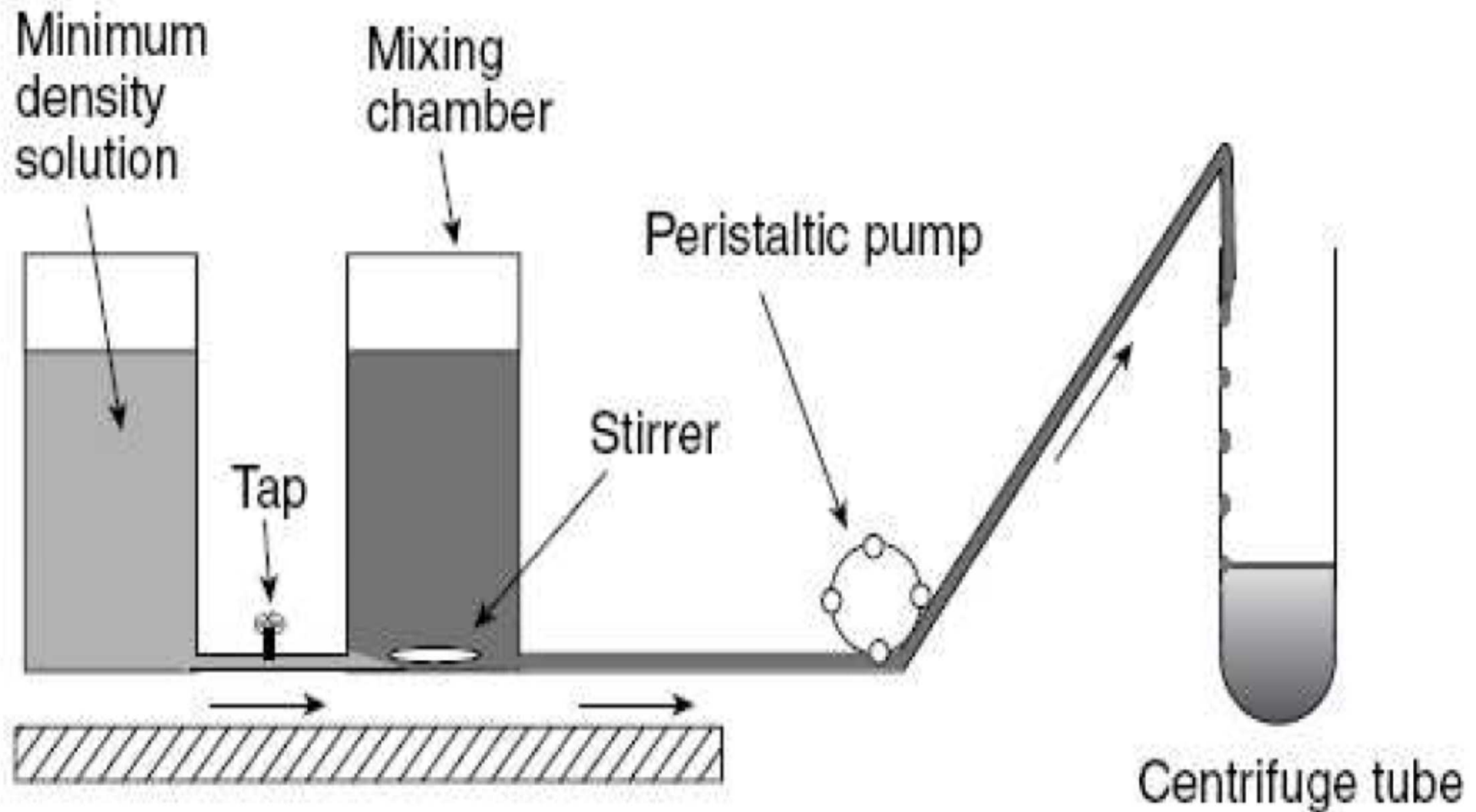
Nutno připravit gradient

- CsCl

- Cs<sub>2</sub>SO<sub>4</sub>

Gradient vzniká během centrifugace

# Gradientová centrifugace



# Gradientová centrifugace

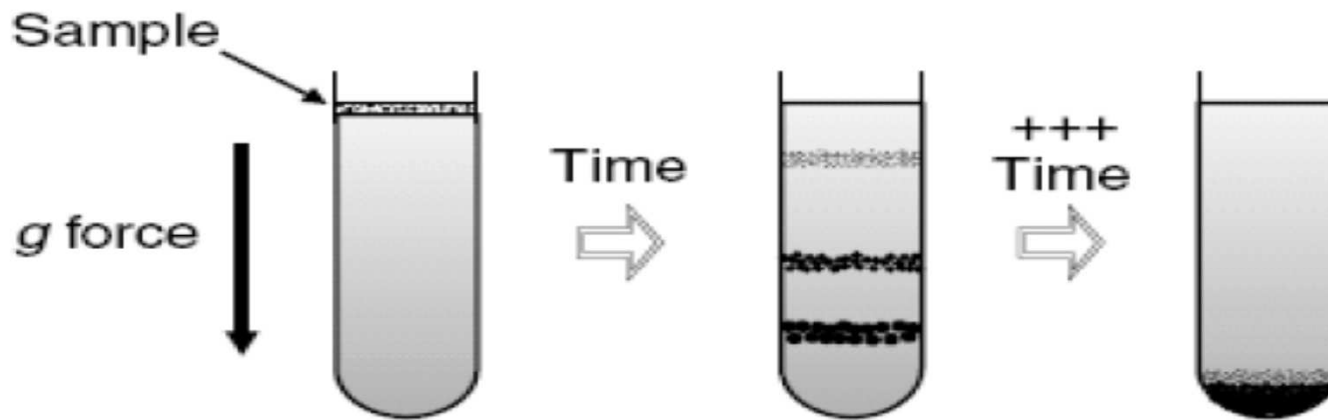
Metoda

Izopyknická

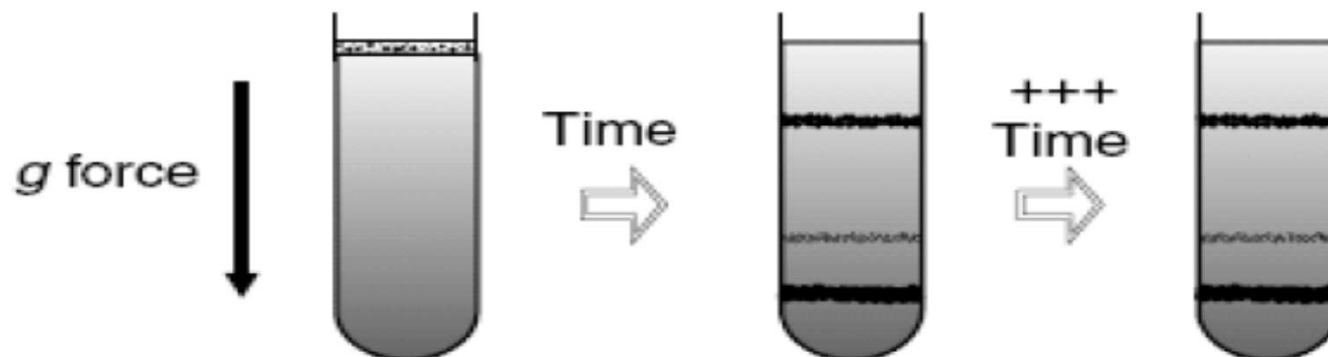
Nerovnovážná

# Gradientová centrifugace

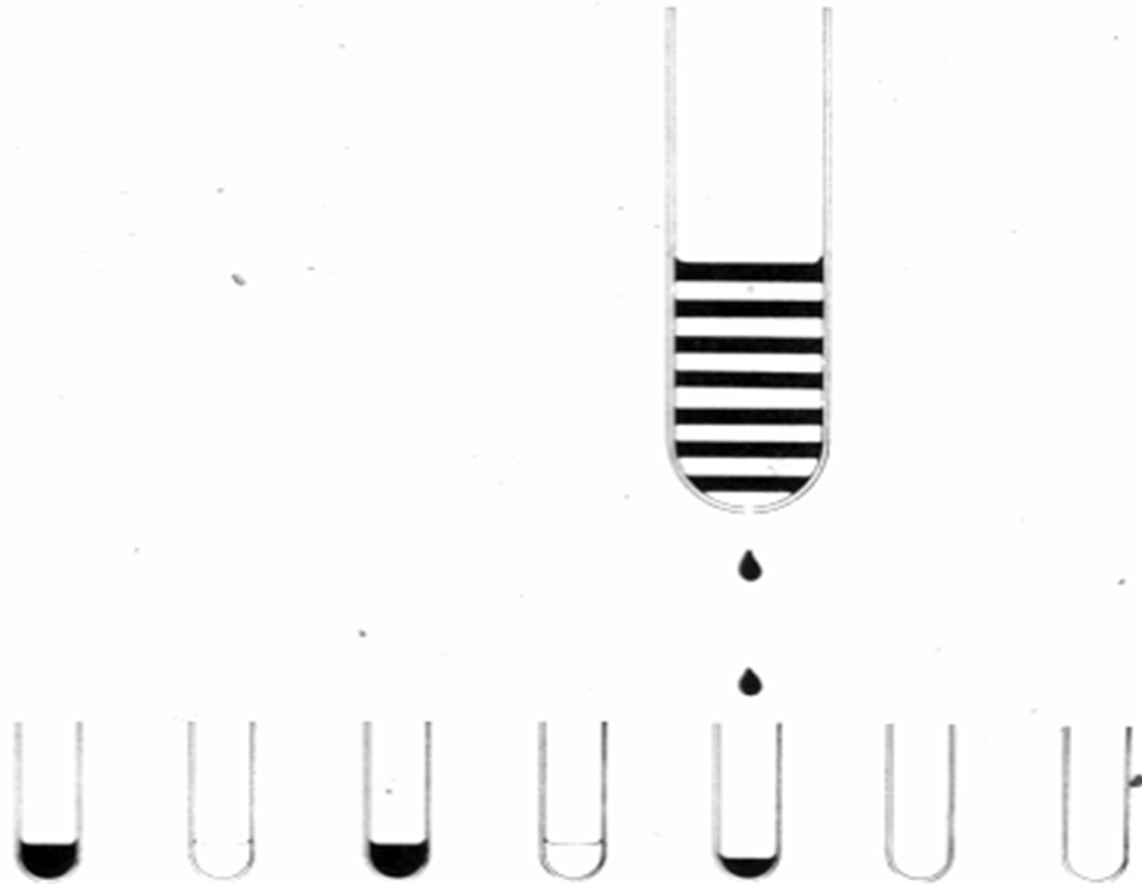
## Rate-zonal centrifugation



## Isopycnic centrifugation

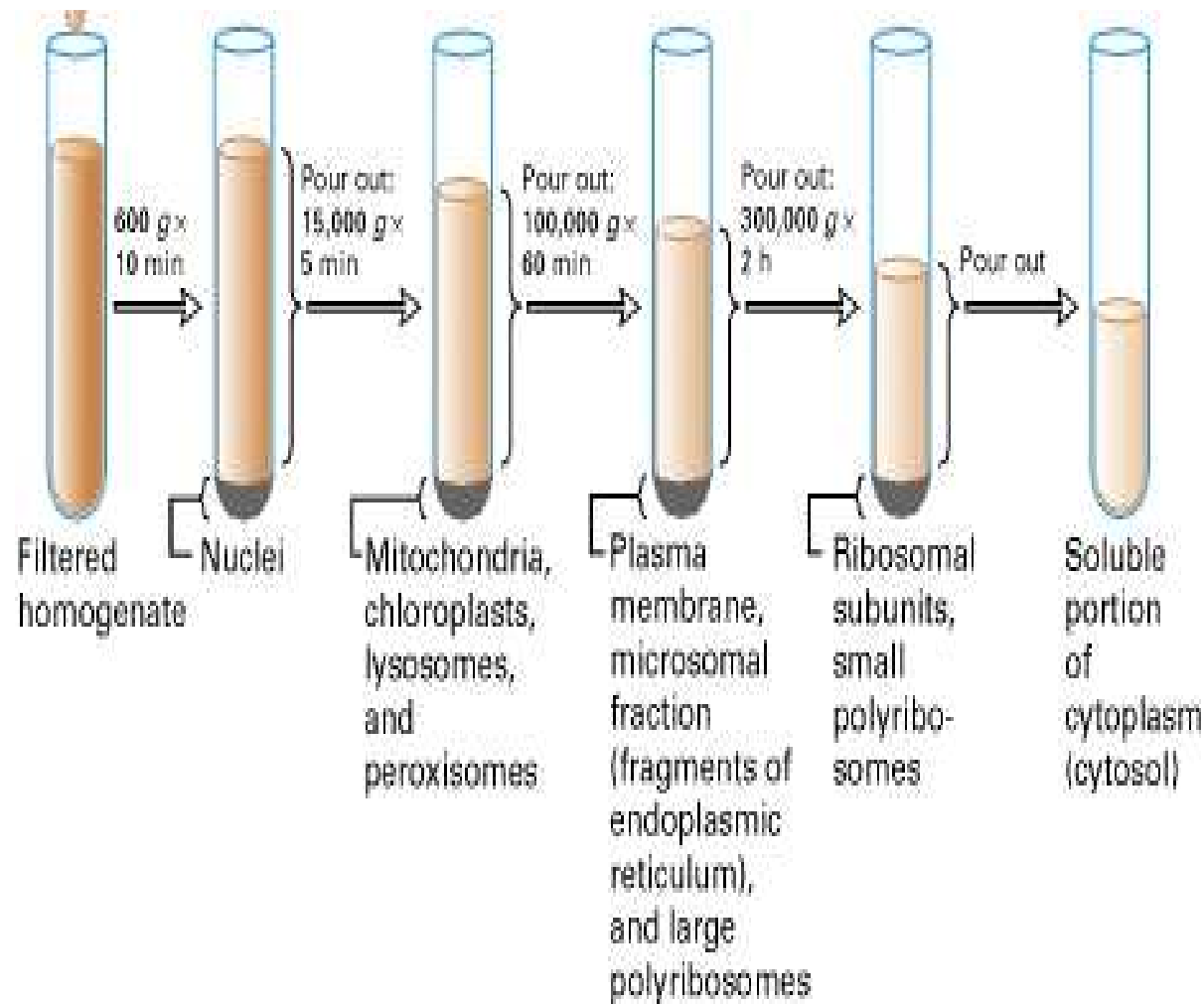


# Gradientová centrifugace

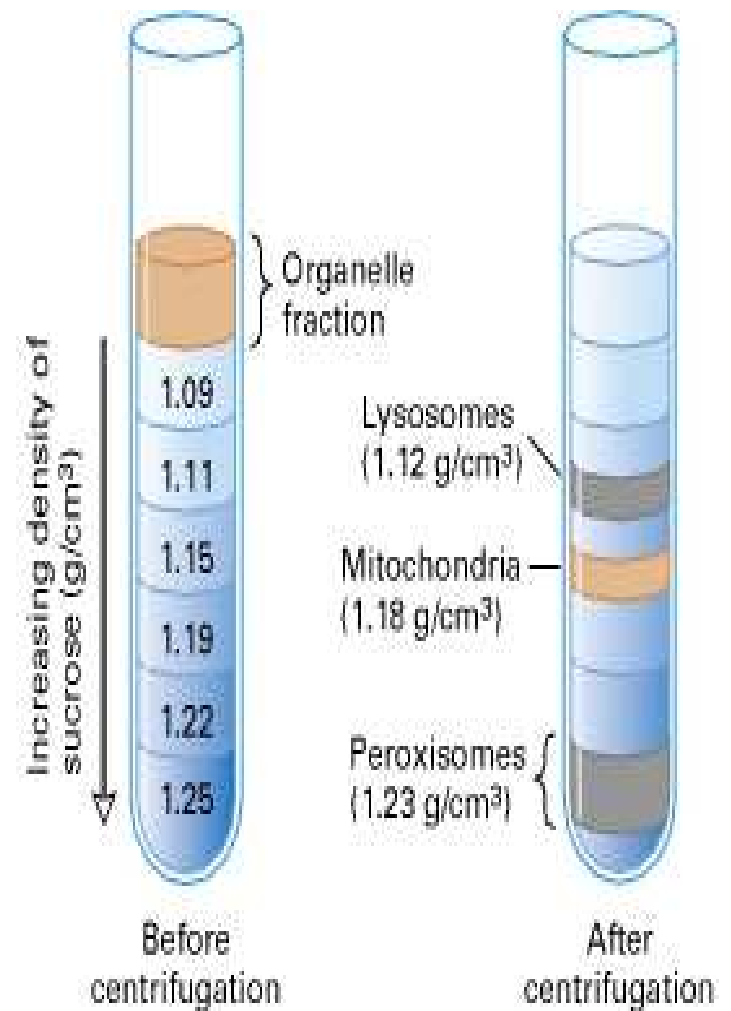




# Diferenciální versus gradientová centrifugace



4 x



1 x

# Meselsonův a Stahlův experiment

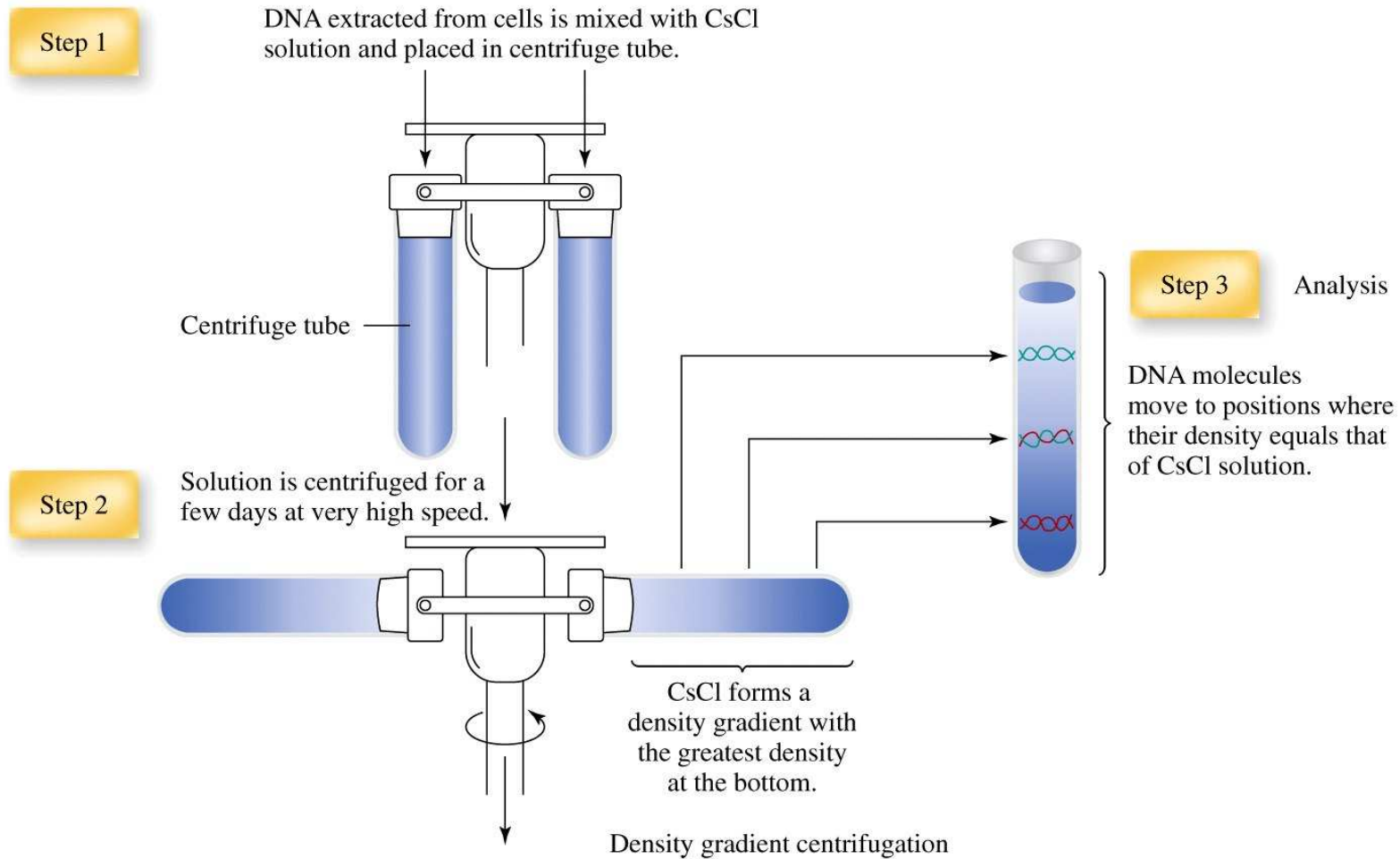


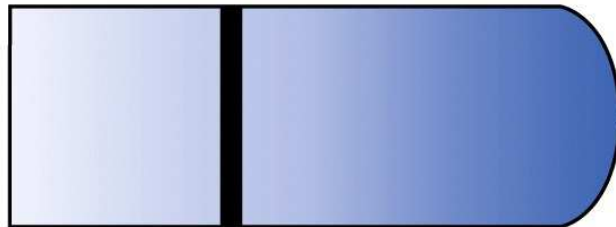
Figure 11-2a Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

# Meselsonův a Stahlův experiment

Direction of sedimentation



Heavy DNA



Light DNA



Parent  $^{15}\text{N}$ -DNA  
(both strands  
heavy)

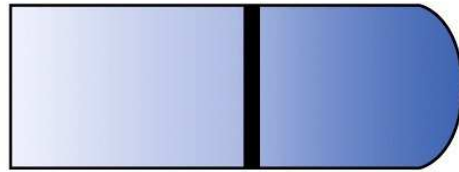


Normal  $^{14}\text{N}$ -DNA  
(with two light  
strands)

Preliminary experiment

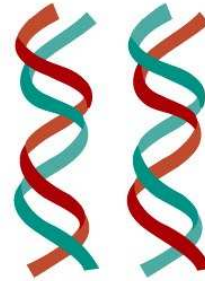
# Meselson and Stahl experiment

## Experimental results



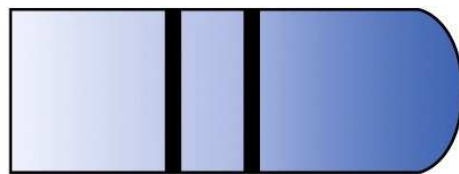
Hybrid DNA

After one generation on  $^{14}\text{N-NH}_4\text{Cl}$



## Conclusions

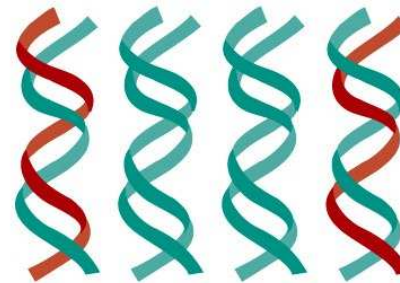
First generation:  
Both DNAs contain one light and one heavy strand.



Light DNA

Hybrid DNA

After two generations on  $^{14}\text{N-NH}_4\text{Cl}$

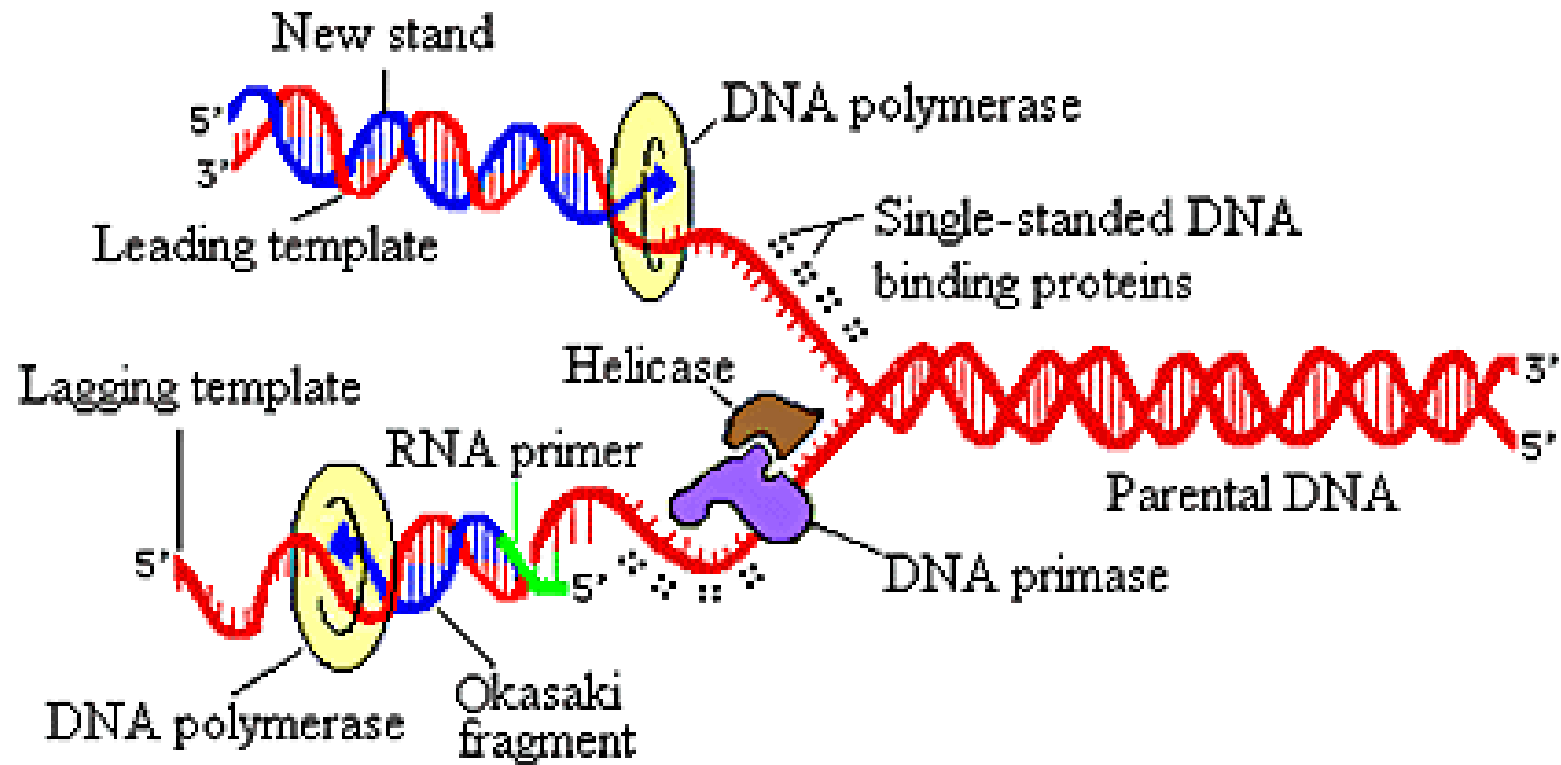


Second generation:  
Two hybrid DNAs and two light DNAs are formed.

Actual experiment

# Replikace

**DNA + NTP + Mg<sup>2+</sup> + 7 enzymů**



# Účast enzymů na replikaci

**Table 11.2**

**Proteins necessary for DNA replication in *E. coli***

Protein	Function
Helicase	Begins unwinding of DNA double helix
DNA gyrase	Assists unwinding
SSB proteins	Stabilize single strands of DNA
Primase	Synthesis of RNA primer
DNA polymerase III	Elongation of chain by DNA synthesis
DNA polymerase I	Removal of RNA primer and filling in gap with DNA
DNA ligase	Closes last phosphoester gap to form phosphodiester bond

Table 11-2 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

# Prokaryontní replikace

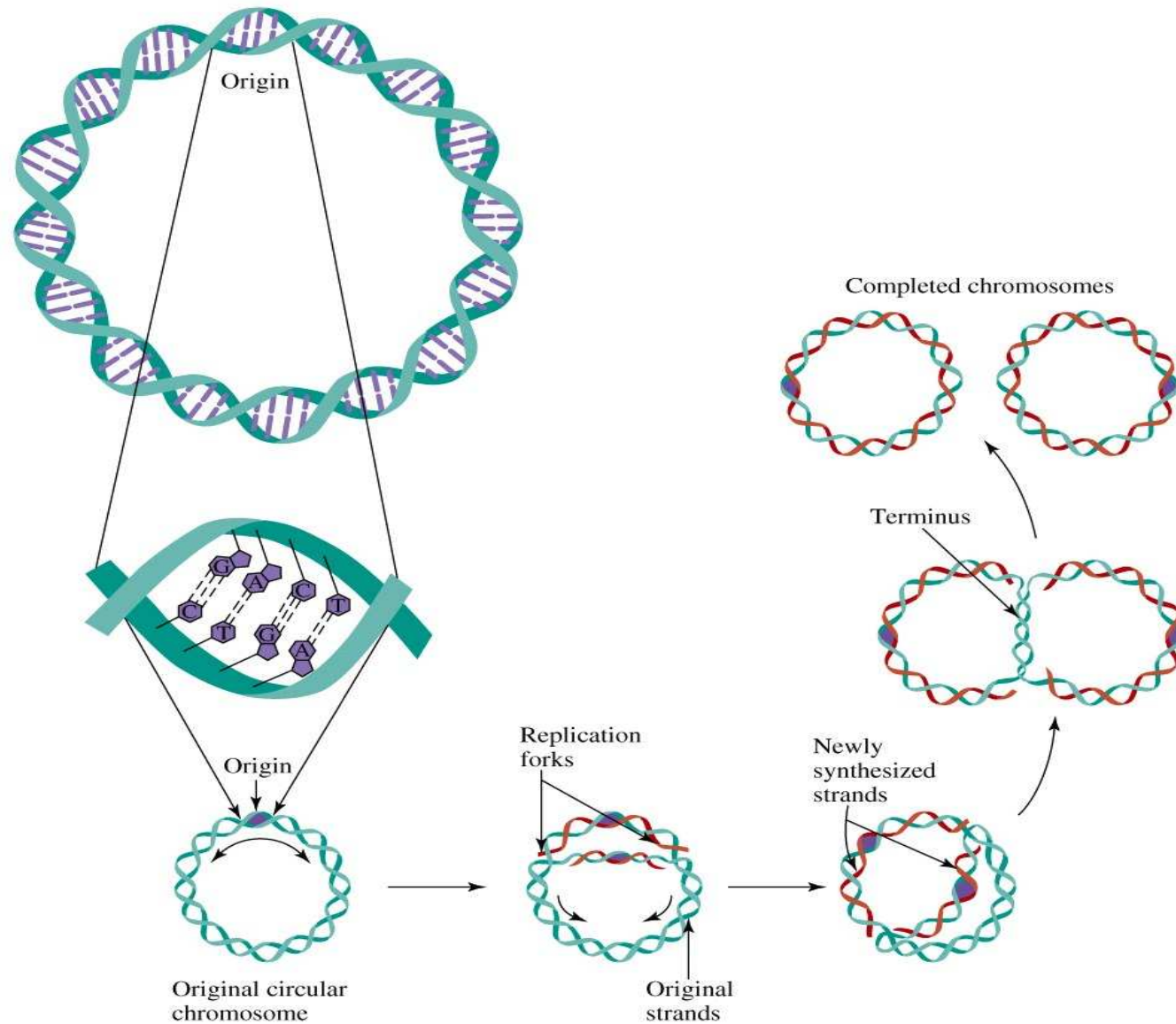
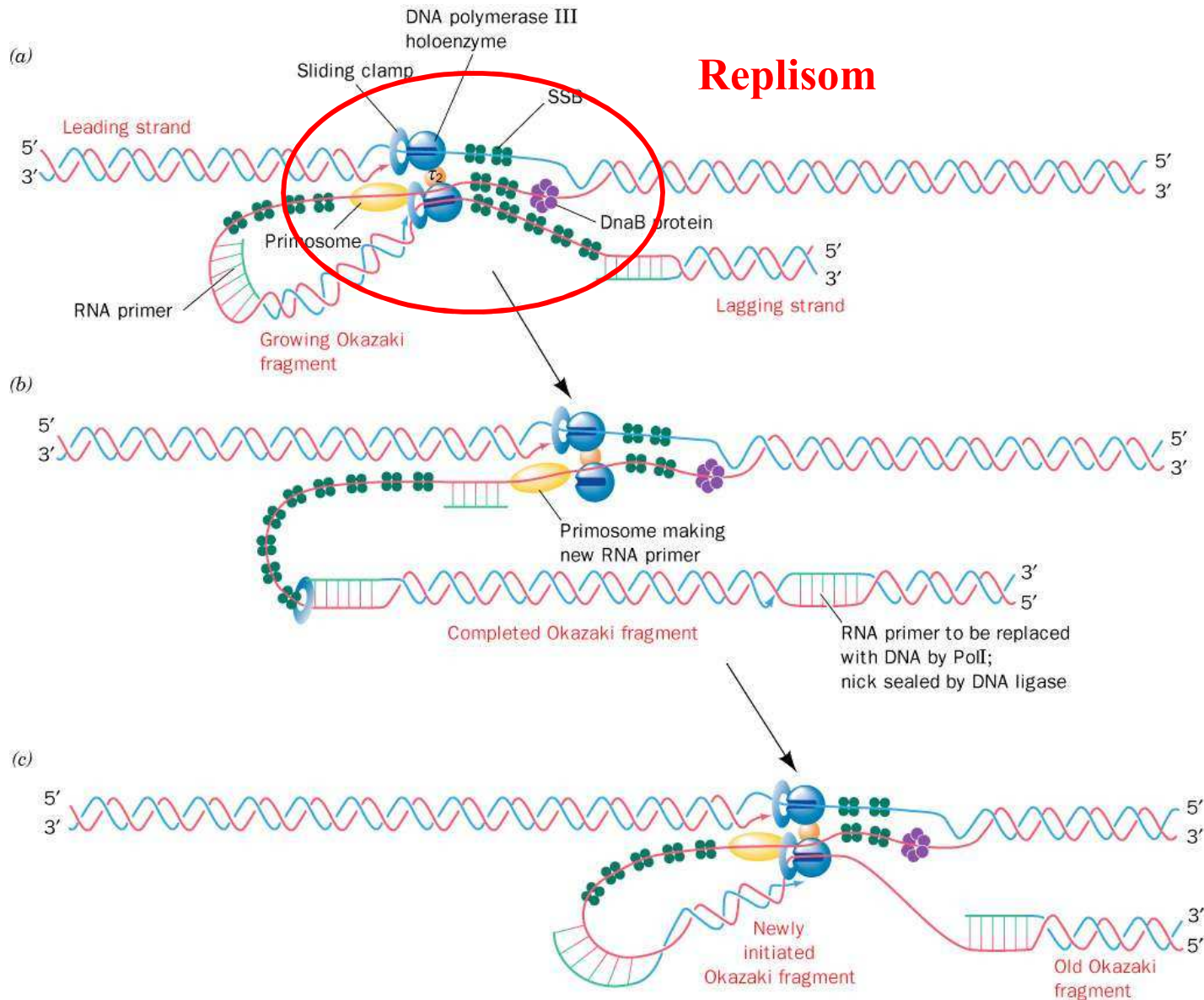


Figure 11-4 Concepts in Biochemistry, 3/e  
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# Prokaryontní replikace





# Replikace u E.coli

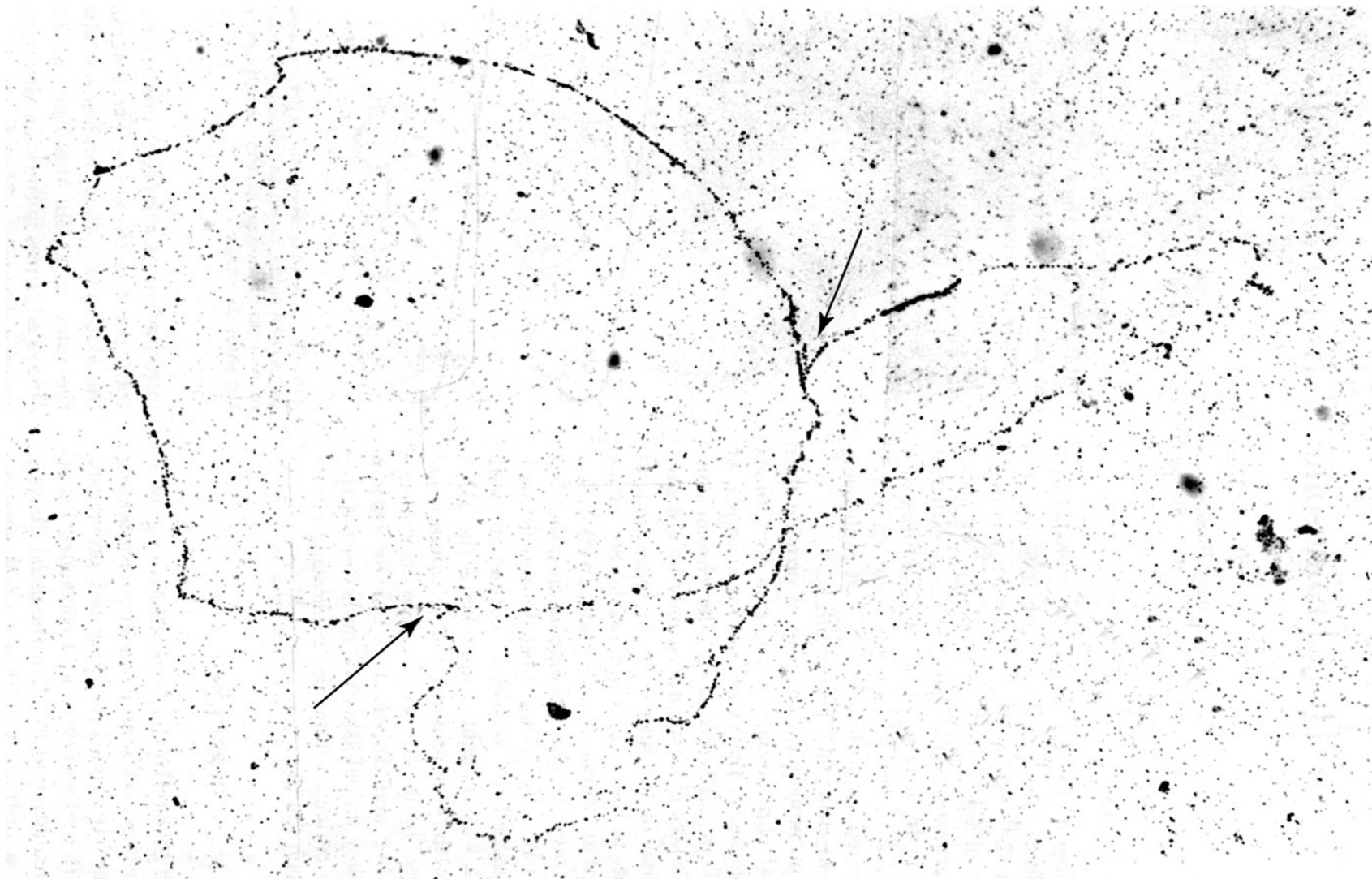


Figure 11-3 Concepts in Biochemistry, 3/e

# Eukaryontní replikaci

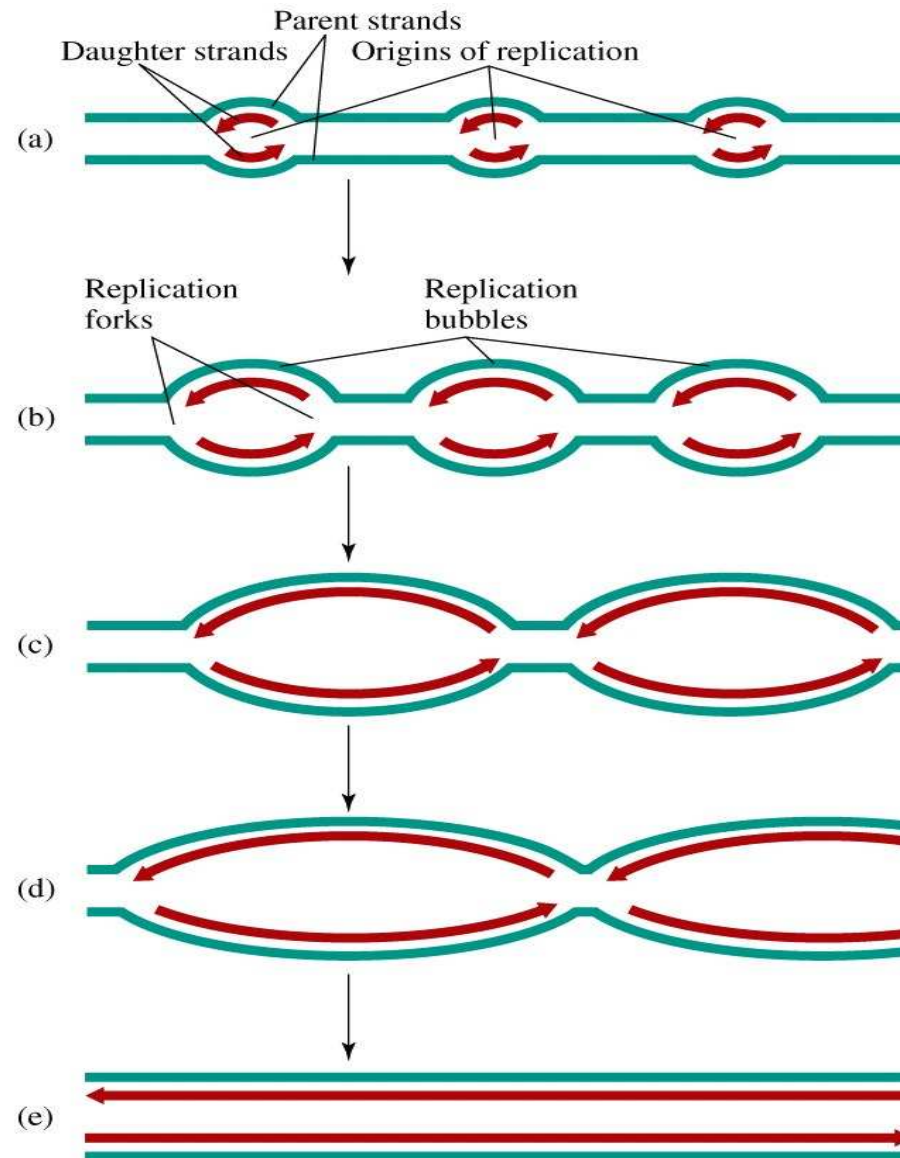
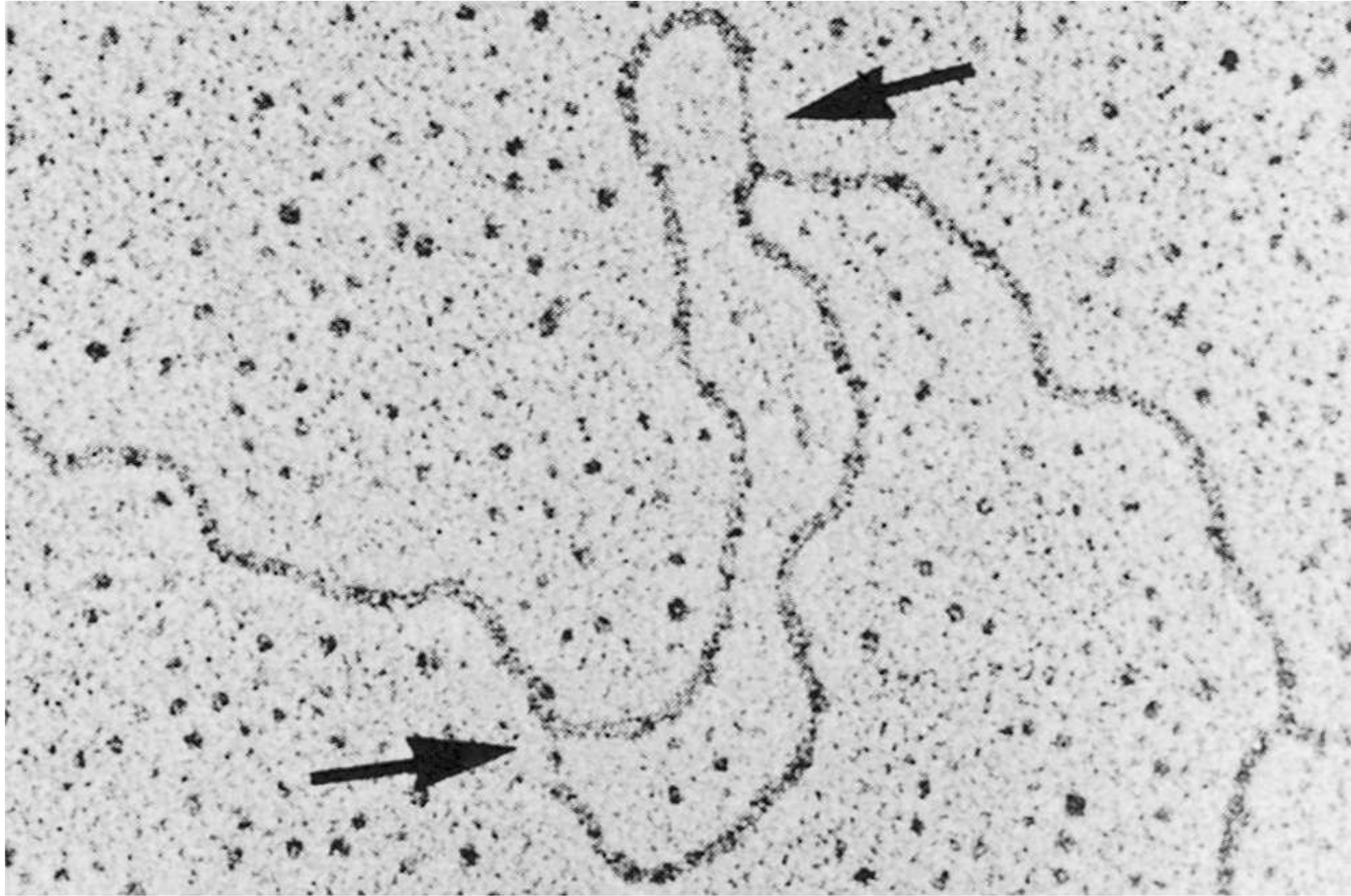


Figure 11-5 Concepts in Biochemistry, 3/e  
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# Replikace u drosofilý



From Kreigstein, H.J. and Hogness, D.S., *Proc. Natl. Acad. Sci.* **71**, 173 (1974)

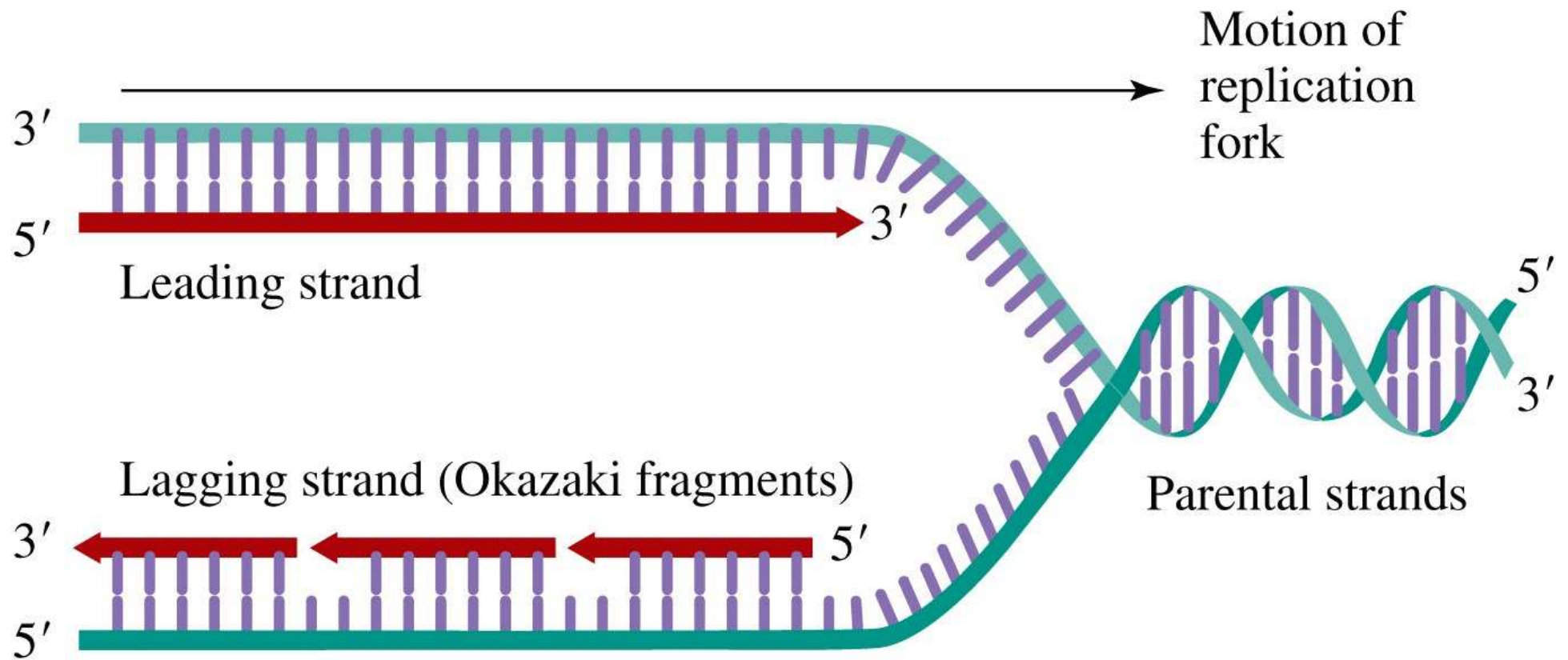
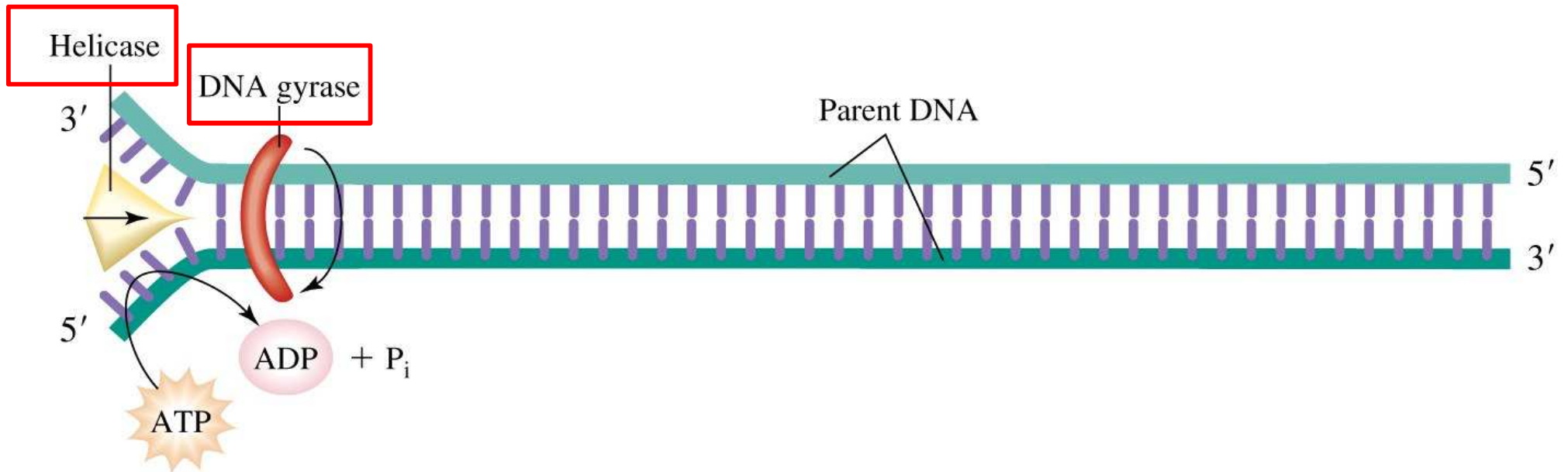
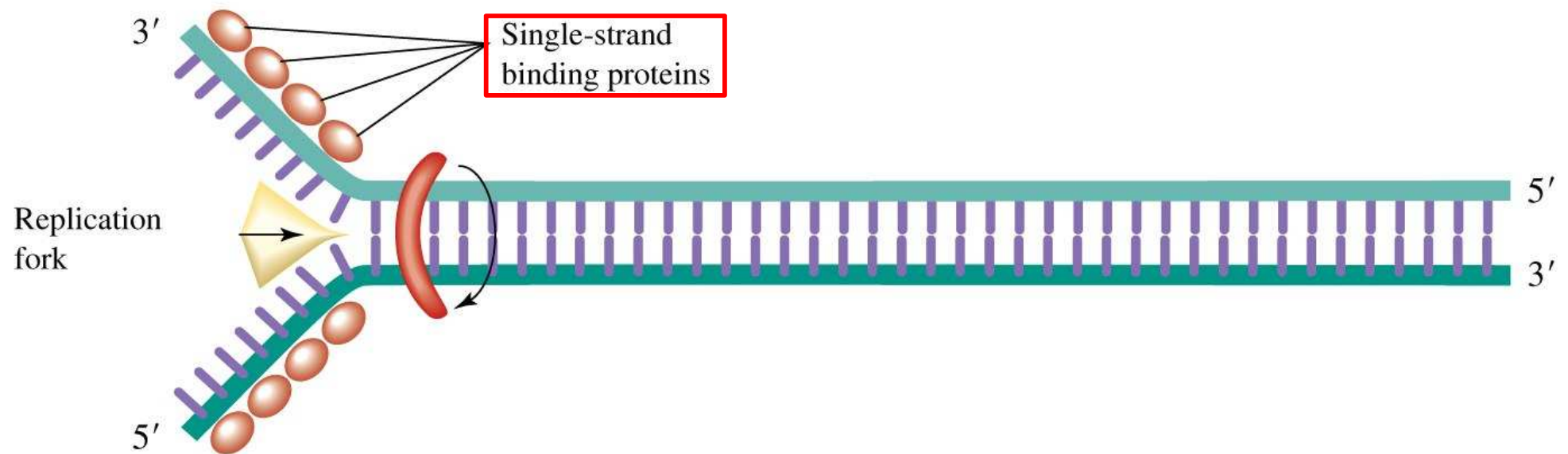


Figure 11-9 Concepts in Biochemistry, 3/e  
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**Figure 11-10a** Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons



**Figure 11-10b** Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

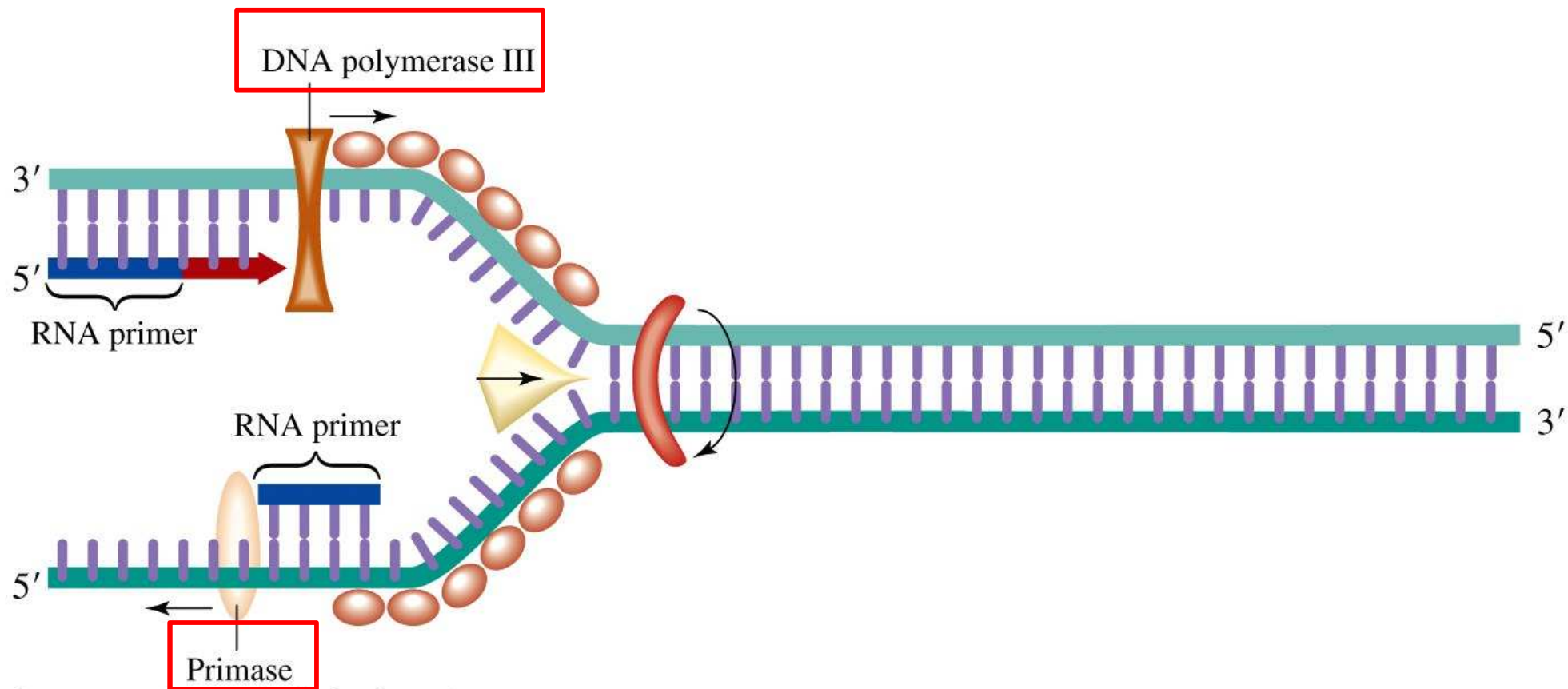
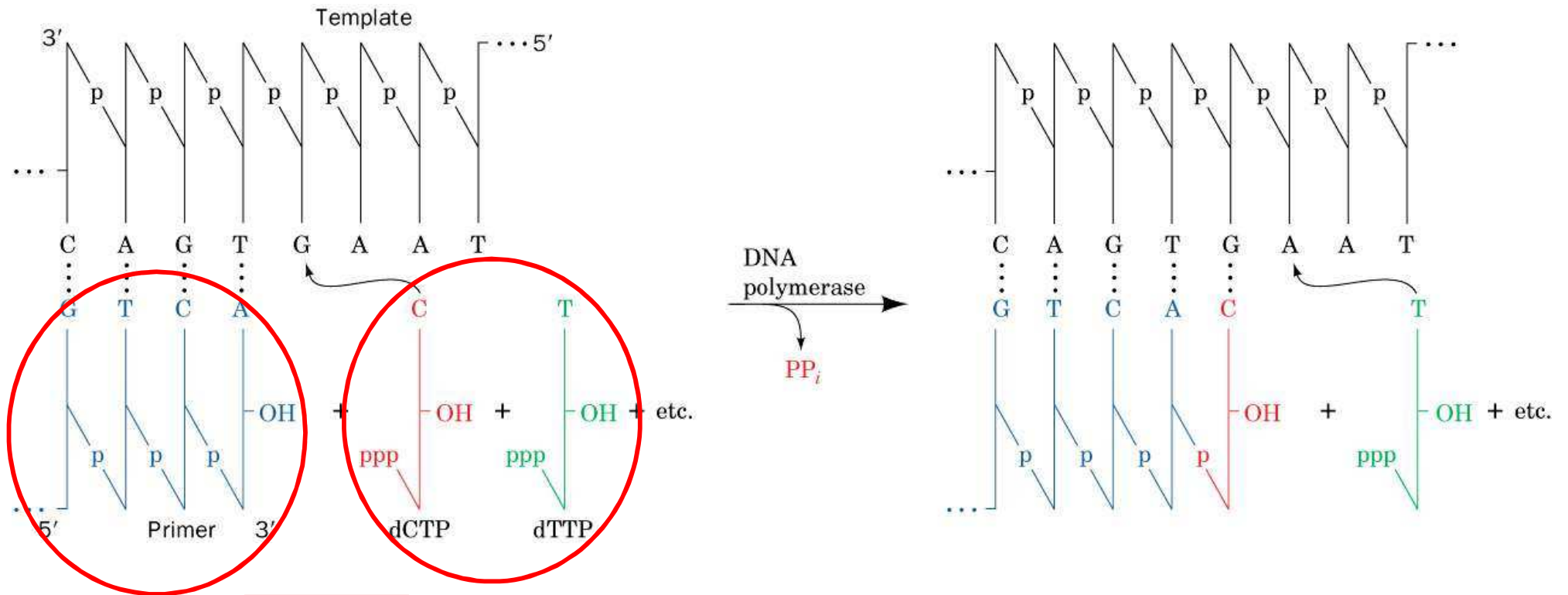


Figure 11-10c Concepts in Biochemistry, 3/e  
 © 2006 John Wiley & Sons

# DNA polymeráza III



5' → 3'

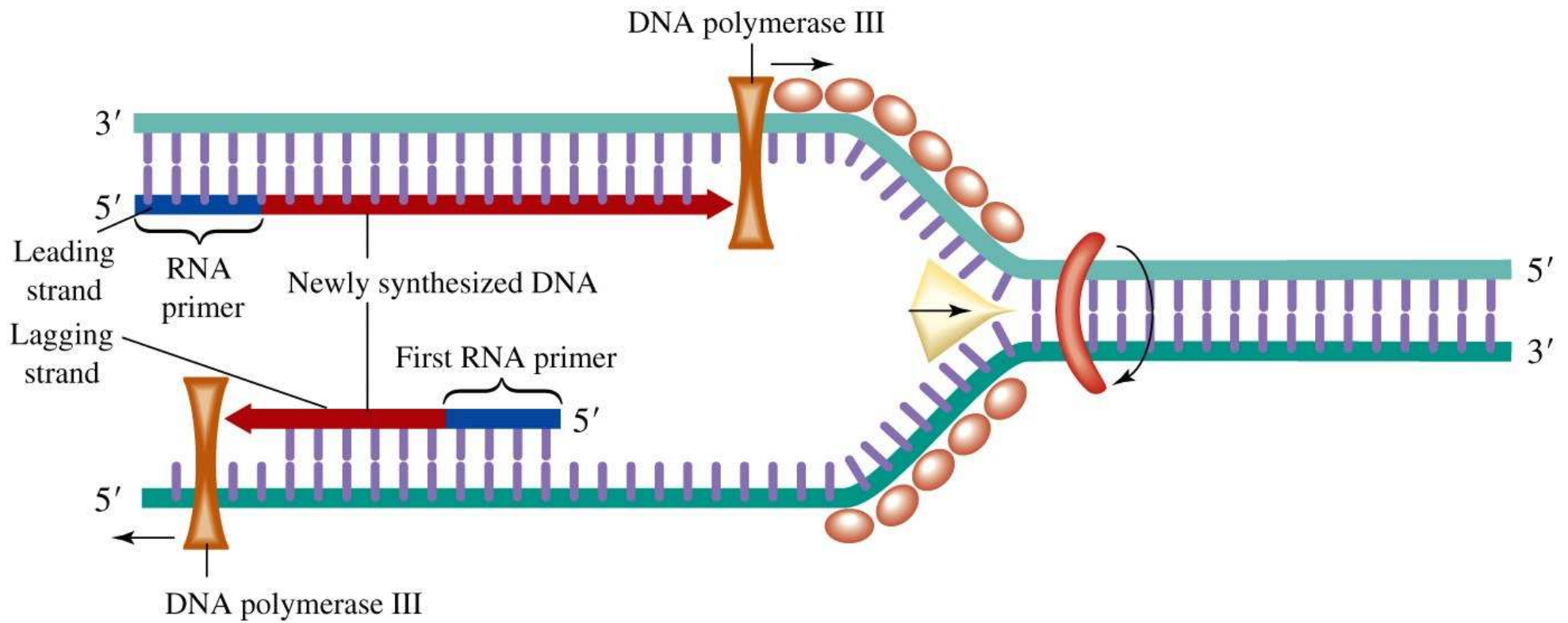


Figure 11-10d Concepts in Biochemistry, 3/e  
 © 2006 John Wiley & Sons



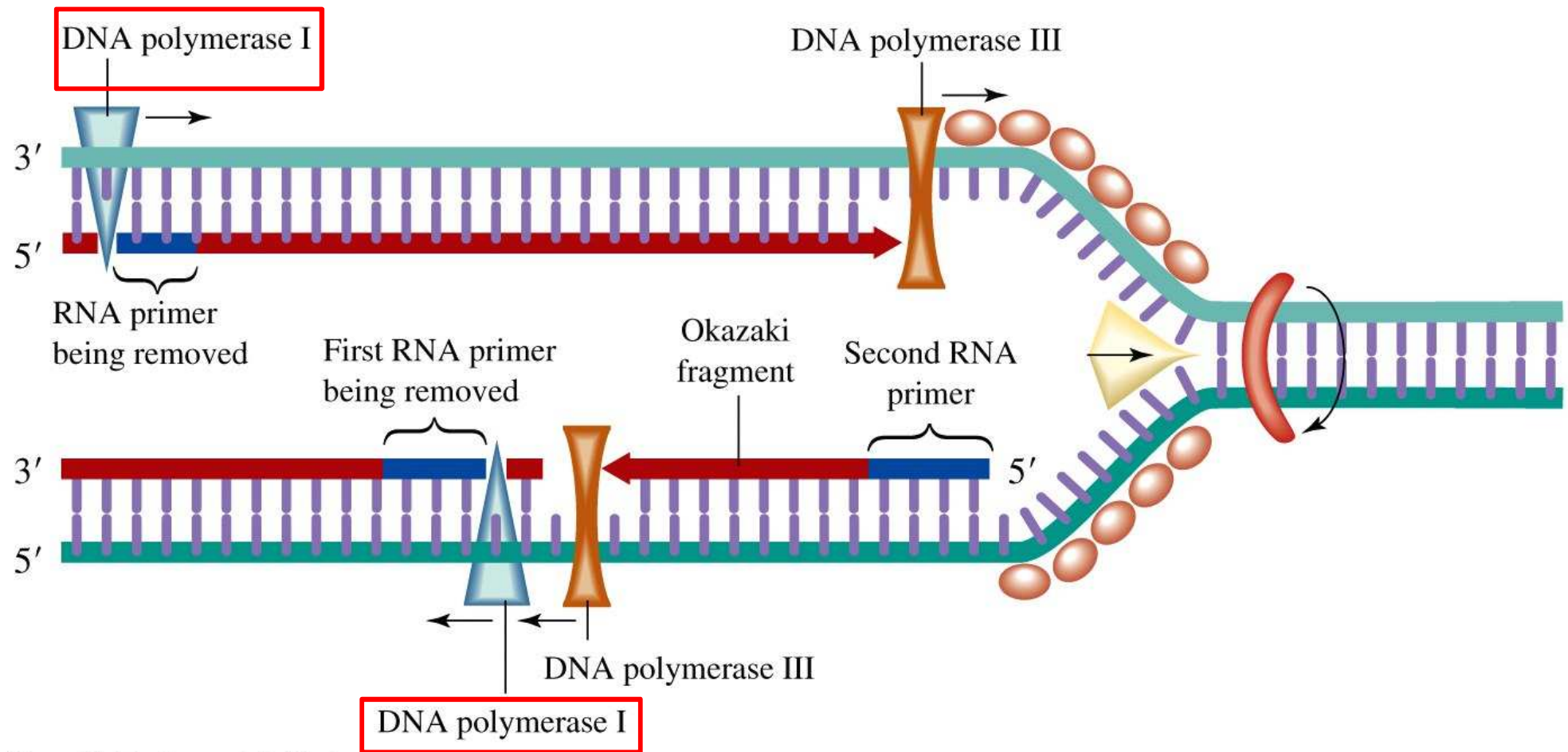


Figure 11-10e Concepts in Biochemistry, 3/e  
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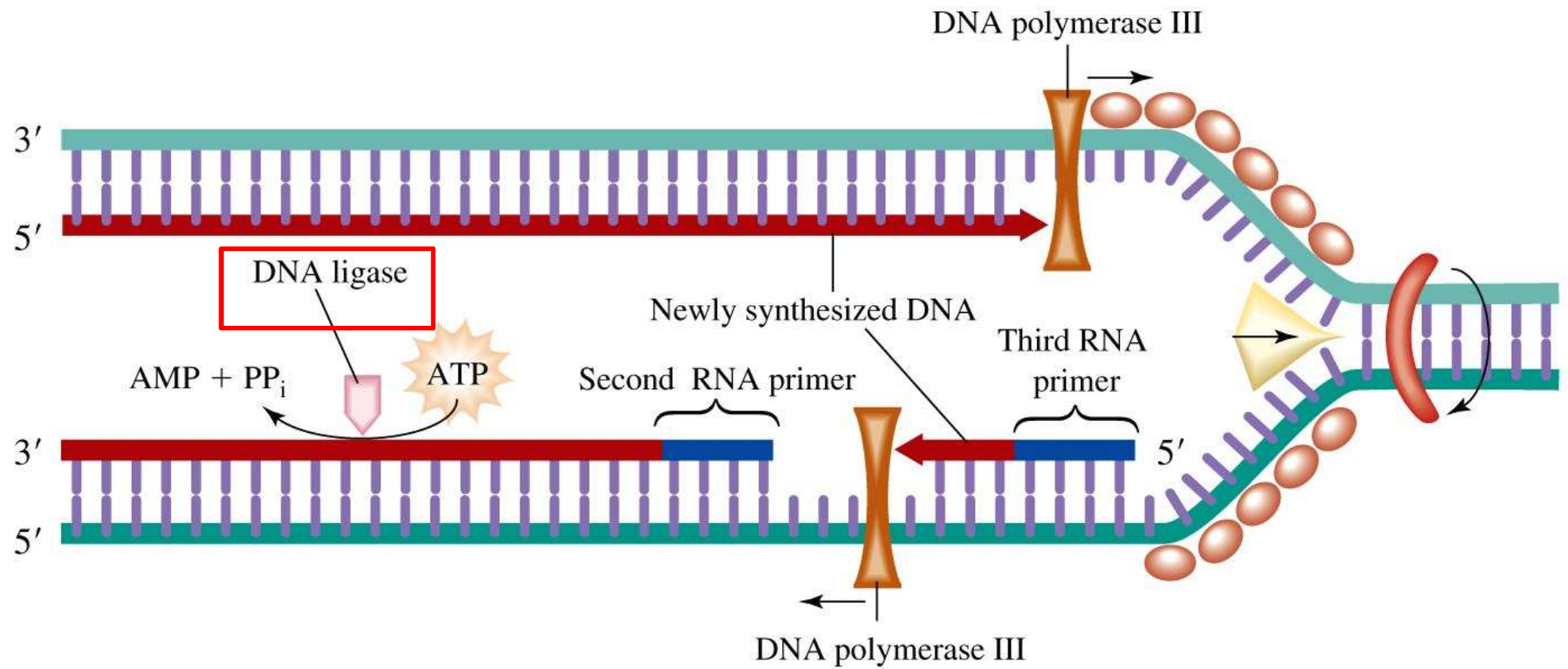
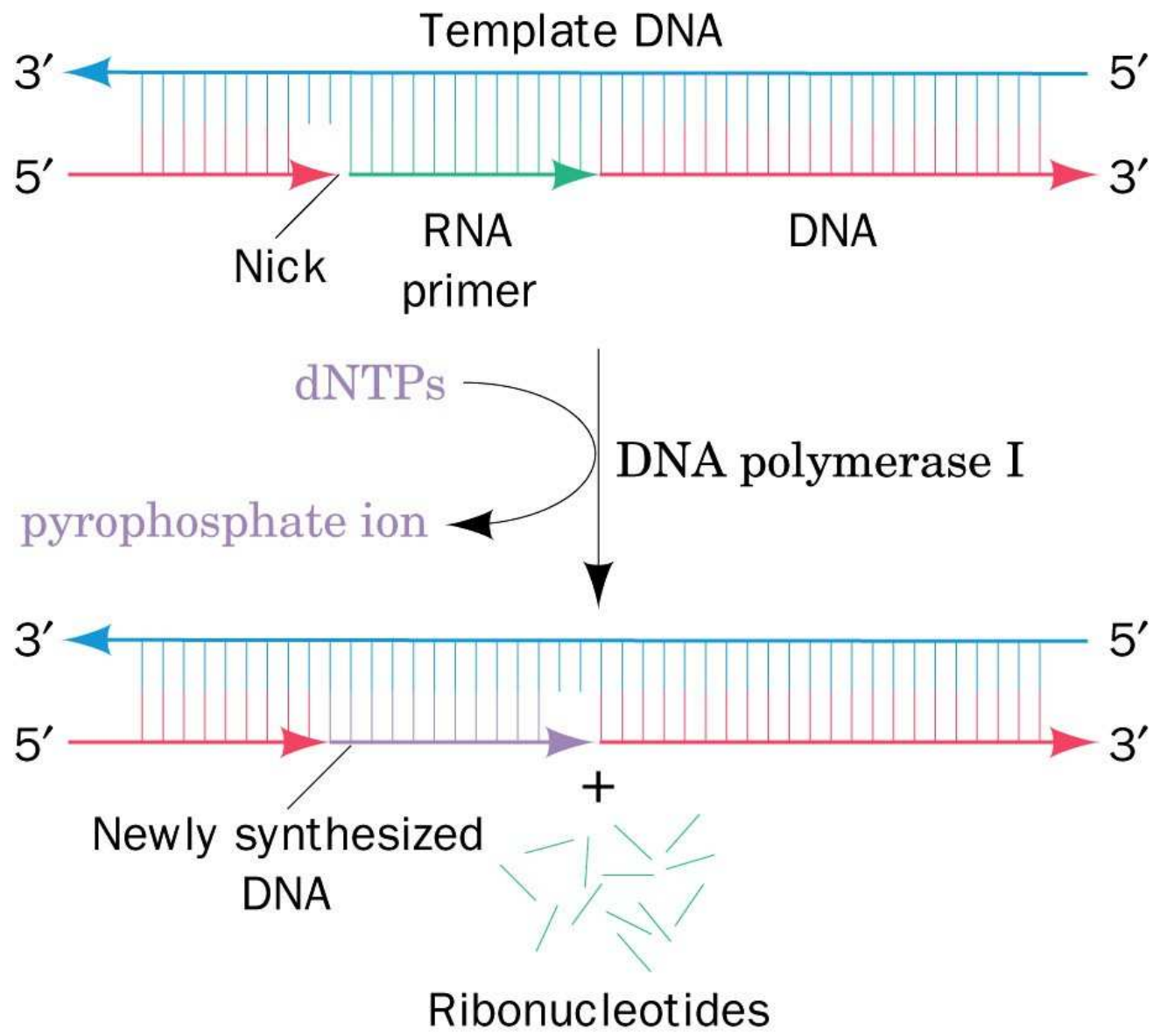
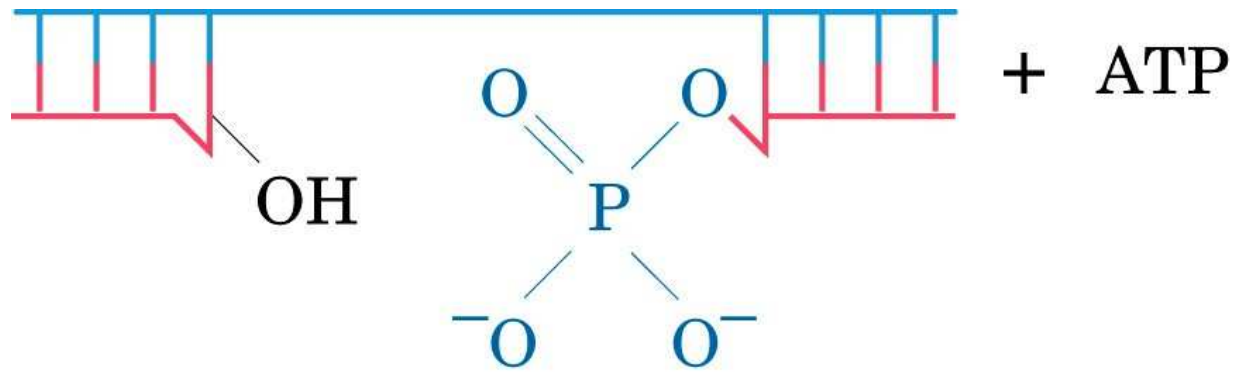
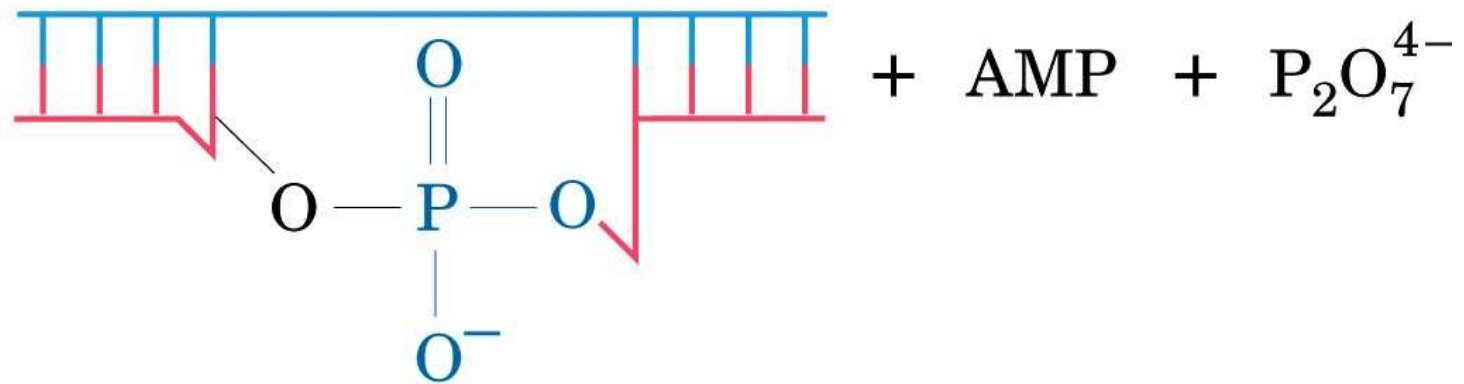


Figure 11-10f Concepts in Biochemistry, 3/e  
 © 2006 John Wiley & Sons





DNA ligase



# Enzymová metoda sekvenace DNA

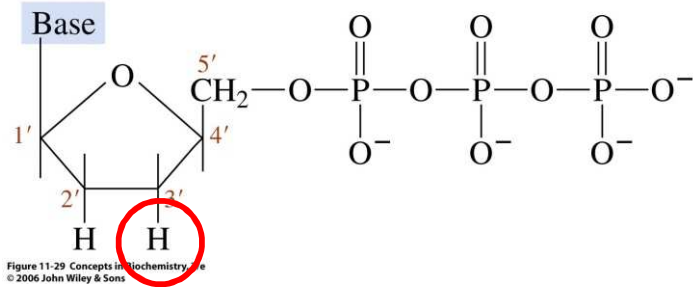
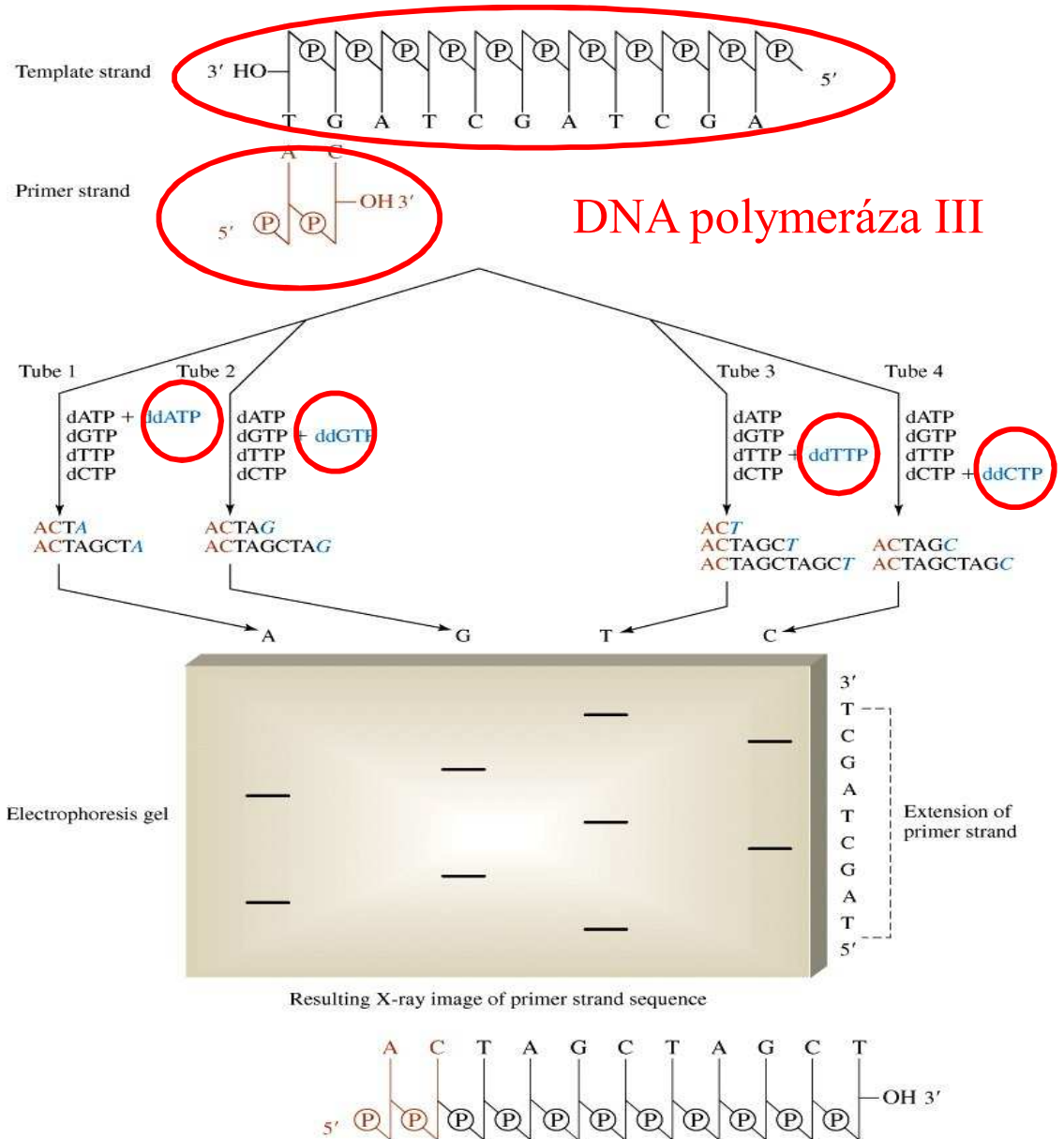


Figure 11-29 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons

Figure 11-30 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons

A G C T

A G C T

140

AGGTTCTTGG  
CTTTATGTAT  
GTAACAGAG  
GCATTCGCG  
90 ATCTGACTATA  
TAATAATATG  
TCCAAGTTTA



80

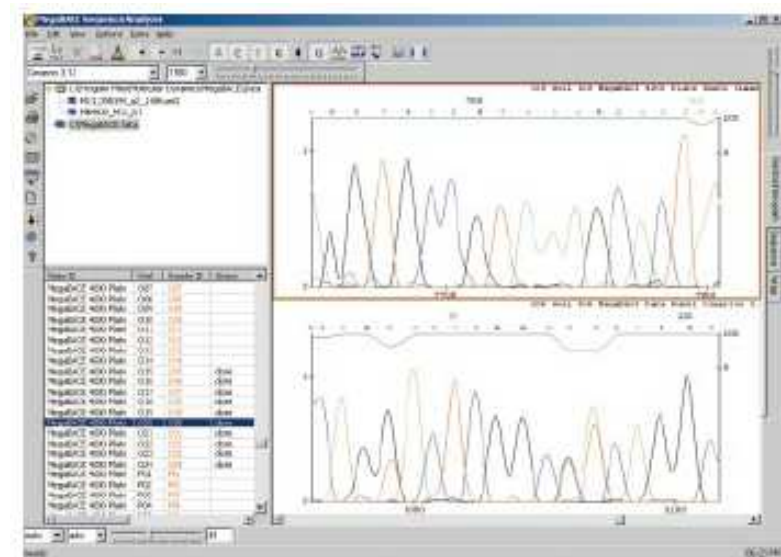
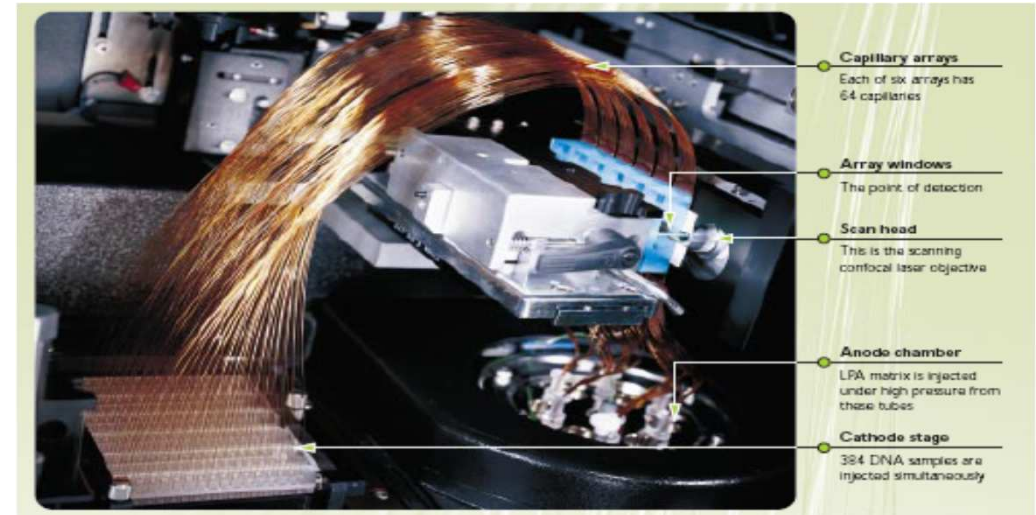
ATTGAACT  
CAGATAGTAA  
TTCCCTGAGTT  
CCTGGGAGAA  
AACATCCGAA  
TGAACCTTTG  
TACCCATCAT  
TCGTACTTGT

# 1989- 2003 Projekt lidského genomu

## Paralelní sekvencování



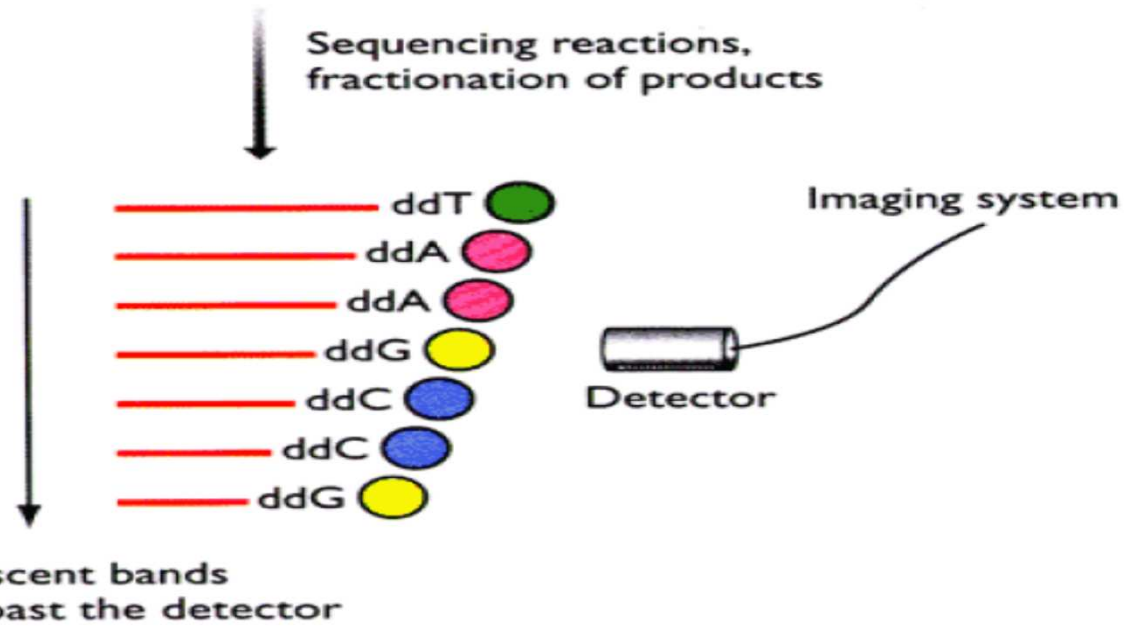
## Automatizace



# 1 DNA – 1 vzorek

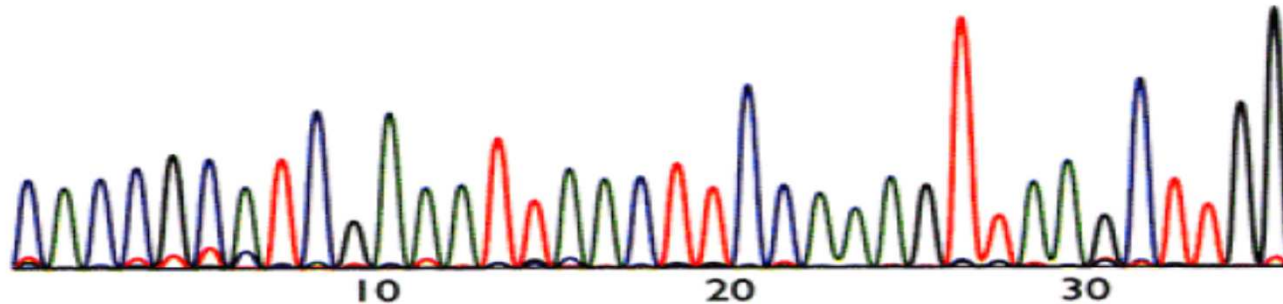
(A)

ddA  ddC  ddNTPs – each with a  
different fluorescent label  
ddT  ddG 



(B)

CACCGCATCGAAATTAAC TTCCAAAGTTAAGCTTGG





## 300 sekvenátorů

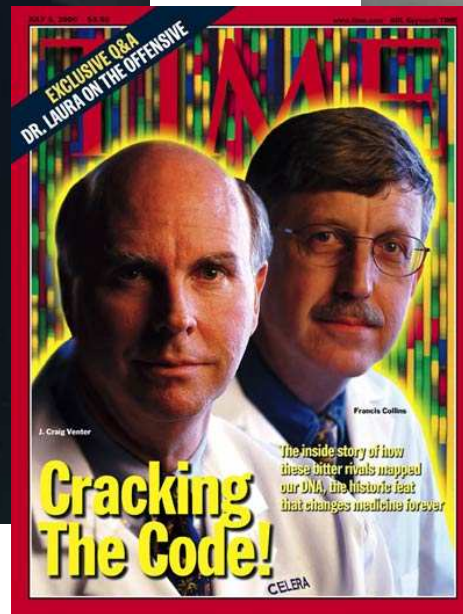
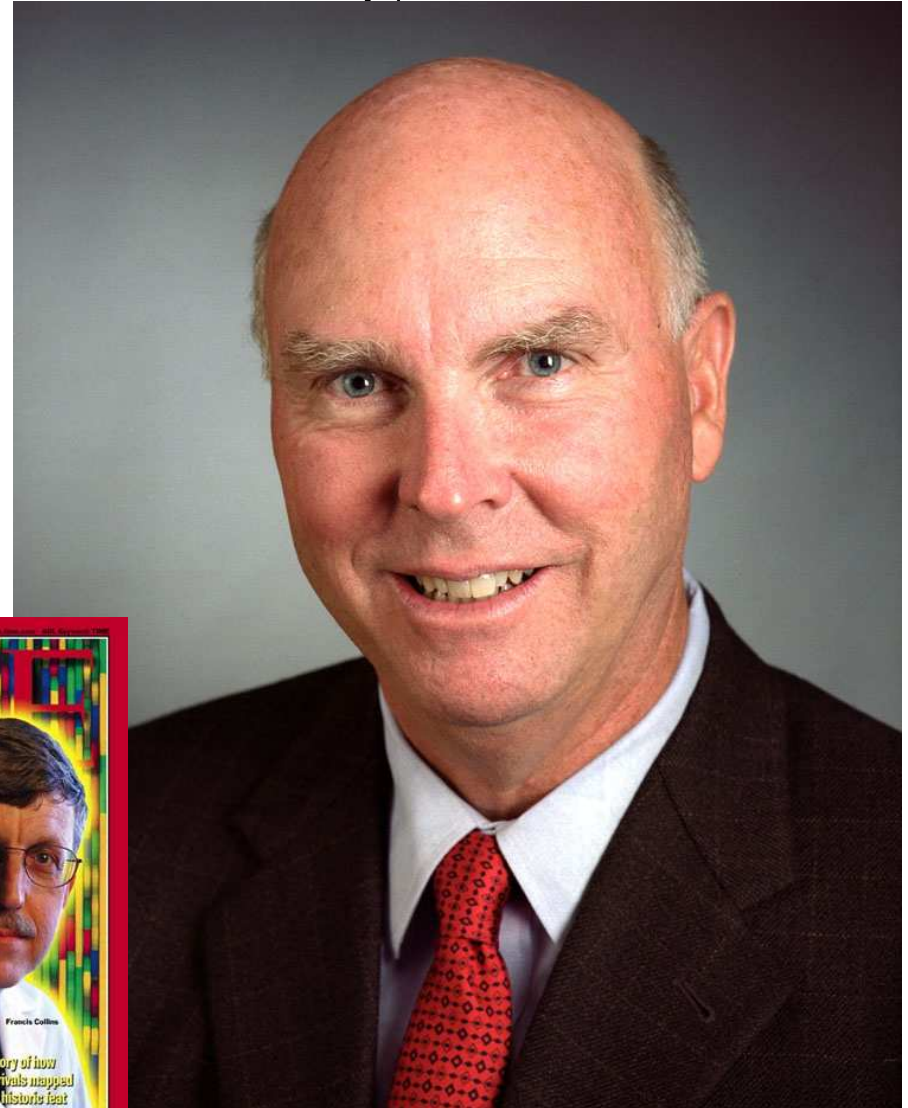


# Veřejné consortium x Celera Genomics

Francis Collins



Craig Venter

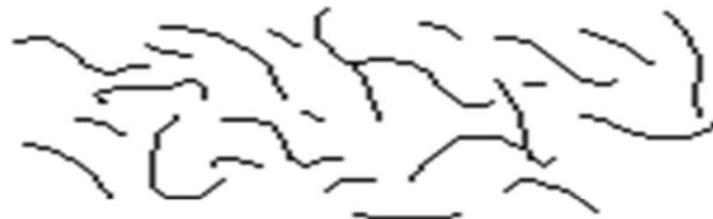


# *Clone-by-clone*

## Hierarchical Shotgun Sequencing Method

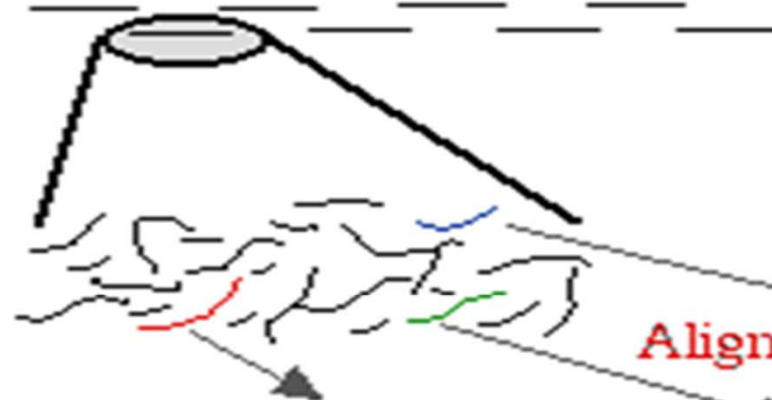


Genomic DNA



BAC Library

Create Contig Map



Sequence Each Contig  
with Shotgun Approach

Align Contiguous Sequences

GCATTTTCGAGTTACCTGGACAACCAAGTG

CCAGTGGTACTGAGGACGCAGAGGGCTTGA

GCTTGATTGGCCAATAATAGTATAT

GCATTTTCGAGTTACCTGGACAACCAAGTGGTACTGAGGACGCAGAGGGCTTGATTGGCCAATAATAGTATAT

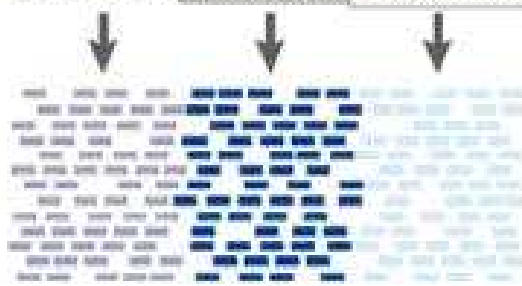
Generate Finished Sequence



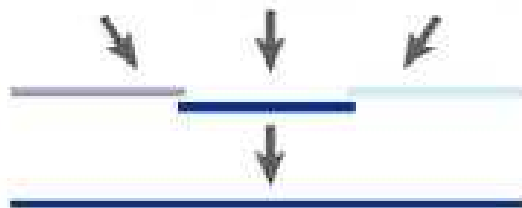
Construct clone map and select mapped clones

AGTTGGTACCTA	TGGCAATGTAGA	CGATCGATGACTA
ATTTGACTTGGAA	TAACTTGCATGCT	CACTAGCCTGAT
CGATCGATGACTG	TGATCGATGTACT	ATCTGACTGTAG
CTTGAATCGATGA	GGATCTTSCAGGT	ATRACCTGCTTTG
ACTGGGATCCTAC	GGATTAAAACCA	CGAGCCTTGGCG
TGGCCTATAGCCC	AACTTAAATCGA	ATCGATGTACTGG
AACTCGATGTGAT	TAGCACATGGGCT	ATTTTACRAGTAA
ATACAGCTTCTAT	ATAGCCCTAGAT	CGTTAGATCGATA
TGATCGATGAAT	CTGTATCGATAT	GCACATGGGCTAT

Generate several thousand sequence reads per clone



Assemble



# ***Whole-genome shotgun***

## Whole Genome Shotgun Sequencing Method



Genomic DNA



Sequence Each Fragment  
with Shotgun Approach

GCATTTTCGAGTTACCTGGACACCAGTG

CCAGTGGTACTGAGGACGCARAGAGGCTTGA

GCTTGATTGGCCAAATATAGTATAT

**Align Contiguous Sequences**

GCATTTTCGAGTTACCTGGACACCAGTGGTACTGAGGACGCARAGAGGCTTGAATTGGCCAAATATAGTATAT

**Generate Finished Sequence**



↓ Generate tens of millions  
of sequence reads



↓ Assemble



## **únor 2001:**

- **International Human Genome Sequencing Consortium publikuje draft lidského genomu v časopisu Nature 15.2.2001.**
- **Celera Genomics, Inc. publikuje svou sekvenci lidského genomu v časopisu Science 16.2.2001.**

# Lidský genom - 2003

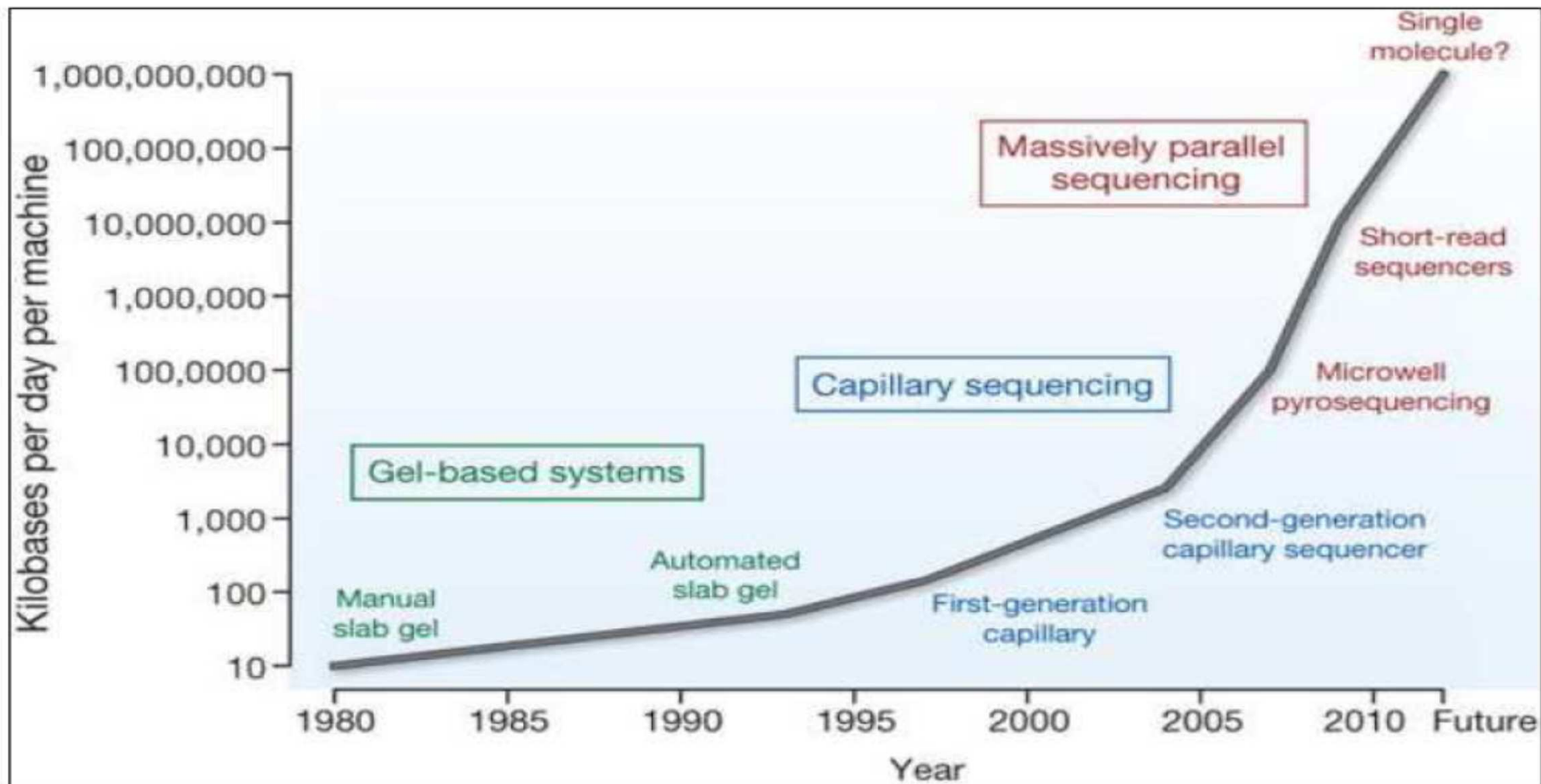
- Geny tvoří cca čtvrtinu genomu, jen asi 1,5% ale kóduje proteiny, rRNA a tRNA, zbytek jsou introny
- u bakterií 90 % genomu kóduje proteiny, 10 % jsou regulační oblasti
- Drosophila 13 %



**Table 13.3**  
**Some Sequenced Genomes**

Organism	Genome size (kb)	Number of Chromosomes
<i>Mycoplasma genitalium</i> (human parasite)	580	1
<i>Borrelia burgdorferi</i> (agent of Lyme disease)	1444	1
<i>Haemophilus influenzae</i> (human pathogenic bacterium)	1830	1
<i>Mycobacterium tuberculosis</i> (cause of tuberculosis)	4412	1
<i>Escherichia coli</i> (bacterium)	4639	1
<i>Saccharomyces cerevisiae</i> (yeast)	11,700	16
<i>Drosophila melanogaster</i> (fruit fly)	137,000	4
<i>Oryza sativa</i> (rice)	430,000	12
<i>Homo sapiens</i> (human)	3,200,000	23

# Sekvenování



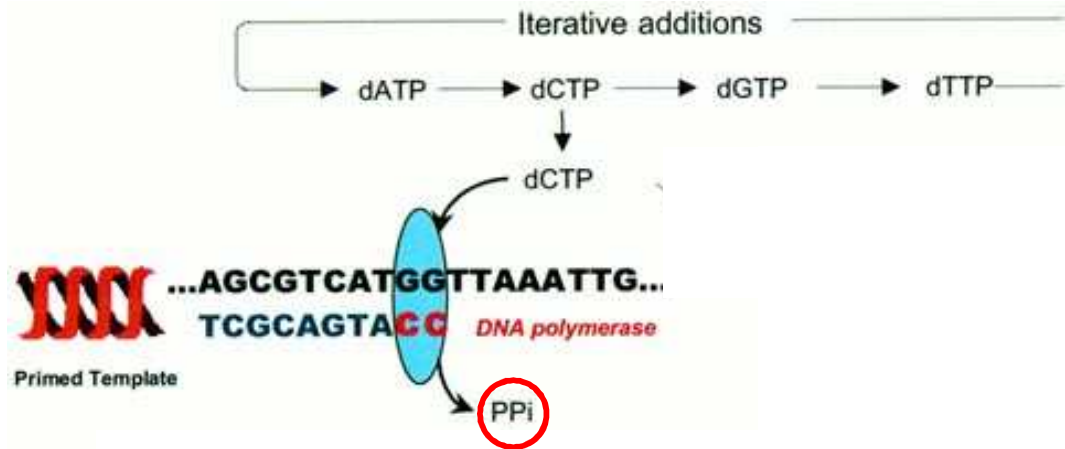
# Pyrosekvenování

## sekvenování druhé generace

- První reakcí je DNA polymerace pomocí DNA polymerasa, kdy dochází k zařazení příslušného deoxynukleotid trifosfátu (dNTPs) za uvolnění pyrofosfátu.



# Pyrosekvenování



# Pyrosekvenování

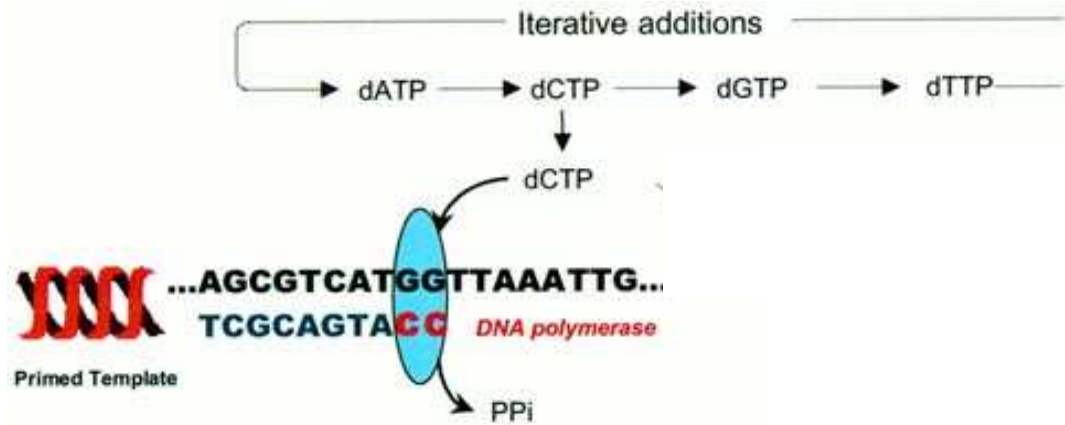
- První reakcí je DNA polymerace pomocí DNA polymerasy, kdy dochází k zařazení příslušného deoxynukleotid trifosfátu (dNTPs) za uvolnění pyrofosfátu.



- Vzniklý pyrofosfát je uvolněn z polymerasy a může sloužit jako substrát pro ATP sulfurylasu. Při této reakci dojde ke kvantitativnímu převedení pyrofosfátu na ATP.



# Pyrosekvenování



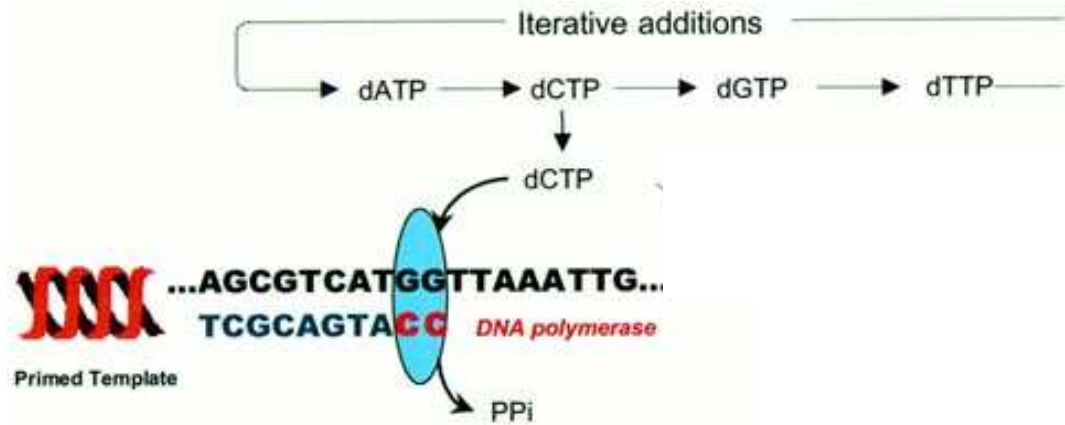
# Pyrosekvenování

- Během třetí a čtvrté reakce je ATP převedeno na světelný signál pomocí enzymu luciferasy a následně je světelný signál detekován a vyhodnocen programem.

Luciferáza + D-luciferin + ATP → Luciferasa-luciferin-AMP + PPi

Luciferáza-luciferin-AMP + PPi + O<sub>2</sub> → Luciferasa + Oxyluceferin + AMP + CO<sub>2</sub> + světlo

# Pyrosekvenování



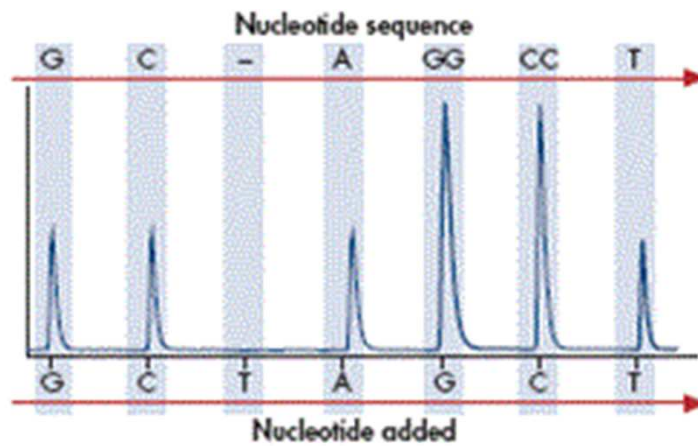
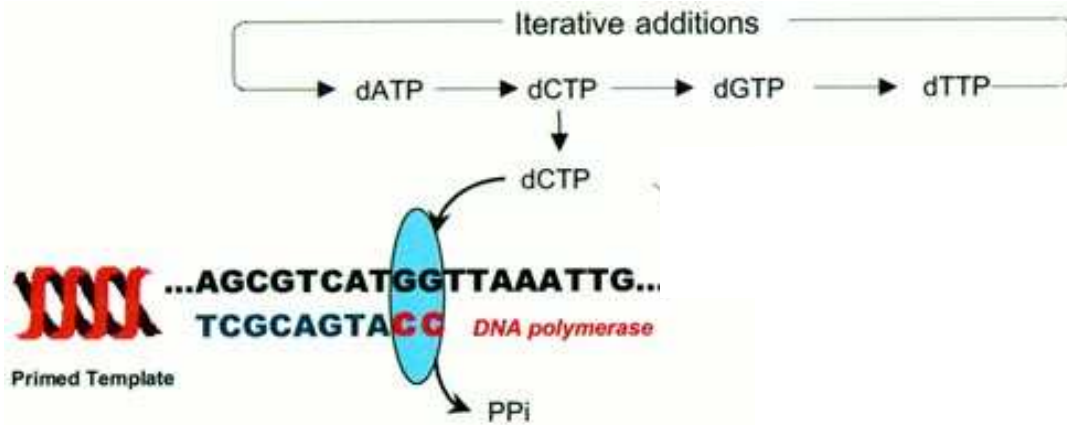


# Pyrosekvenování

- Poslední enzymatickou reakcí je reakce apyrasy, která odstraní nezainkorporované nukleotidy a ATP, aby následně mohlo dojít k zopakování celého výše popsaného procesu a mohlo být analyzováno zařazení dalšího nukleotidu. Tato degradace je nezbytná, aby bylo zajištěna synchronizace mezi syntézou a detekcí světelného signálu.



# Pyrosekvenování



# 454 pyrosequencing

Technologie využívá paralelní sekvenace: více než

-2 milion sekvencí zároveň.

-Lze získat až 1GB (gigabázi) informace během jedné analýzy (cca 4.5 h).

Využití:

-sekvenace genomů (náhodně naštěpená genomová DNA je sekvenována a sestavena)

- studium metagenomů (tj. souhrn všech genů, přítomných v daném prostředí, používá se DNA extrahovaná ze vzorku půdy, vody, sedimentu, mikroflóry střeva ad.)

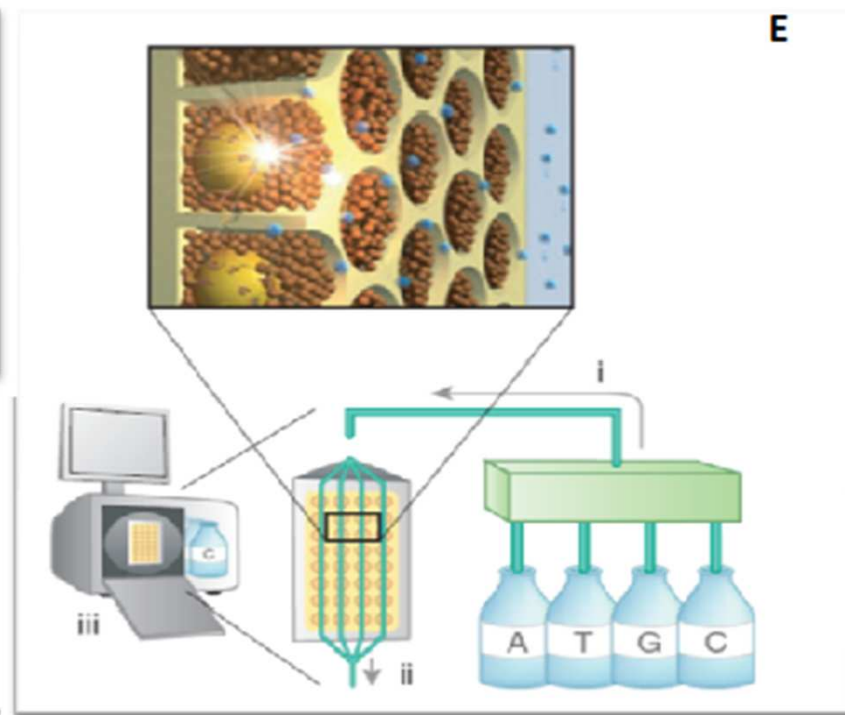
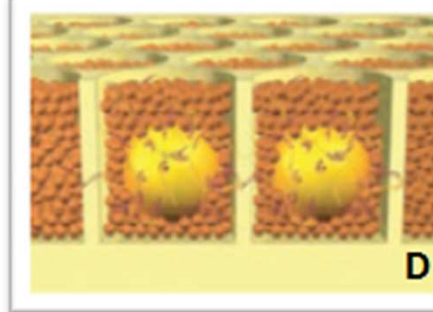
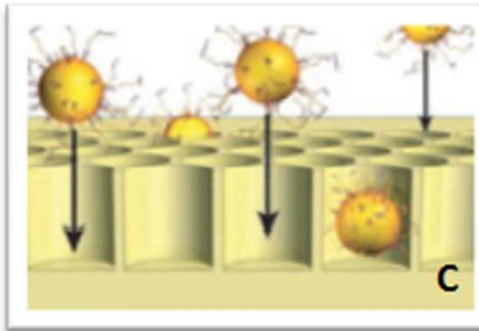
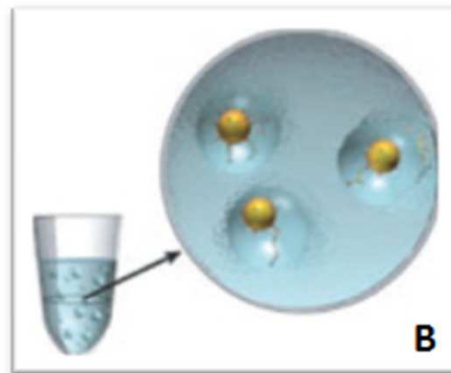
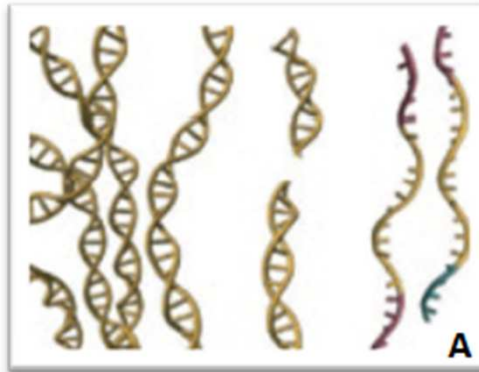
- tzv. ampikonové sekvenování. Vlastní sekvenaci předchází PCR zacílená na 16S nebo 18S geny prokaryot a eukaryot

- analýza typu „shotgun“ – veškerá DNA / RNA, získaná ze vzorku

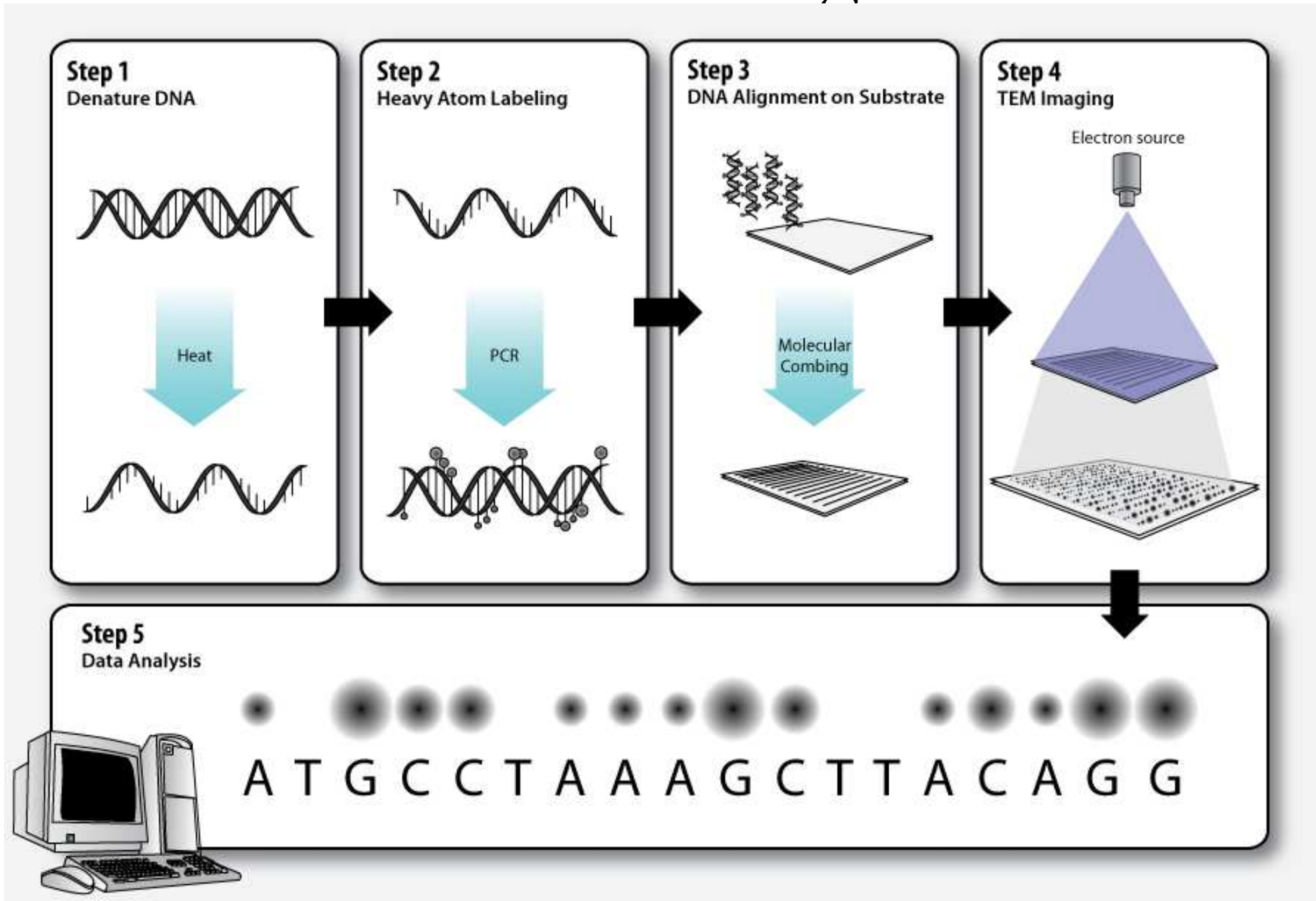


Zařízení je velmi nákladné (cca 17 mil. Kč), analýzy jsou ale dostupné komerčně, takže většina laboratoří v současnosti využívá služeb externích sekvenačních středisek.

# 454 Pyrosekvenování

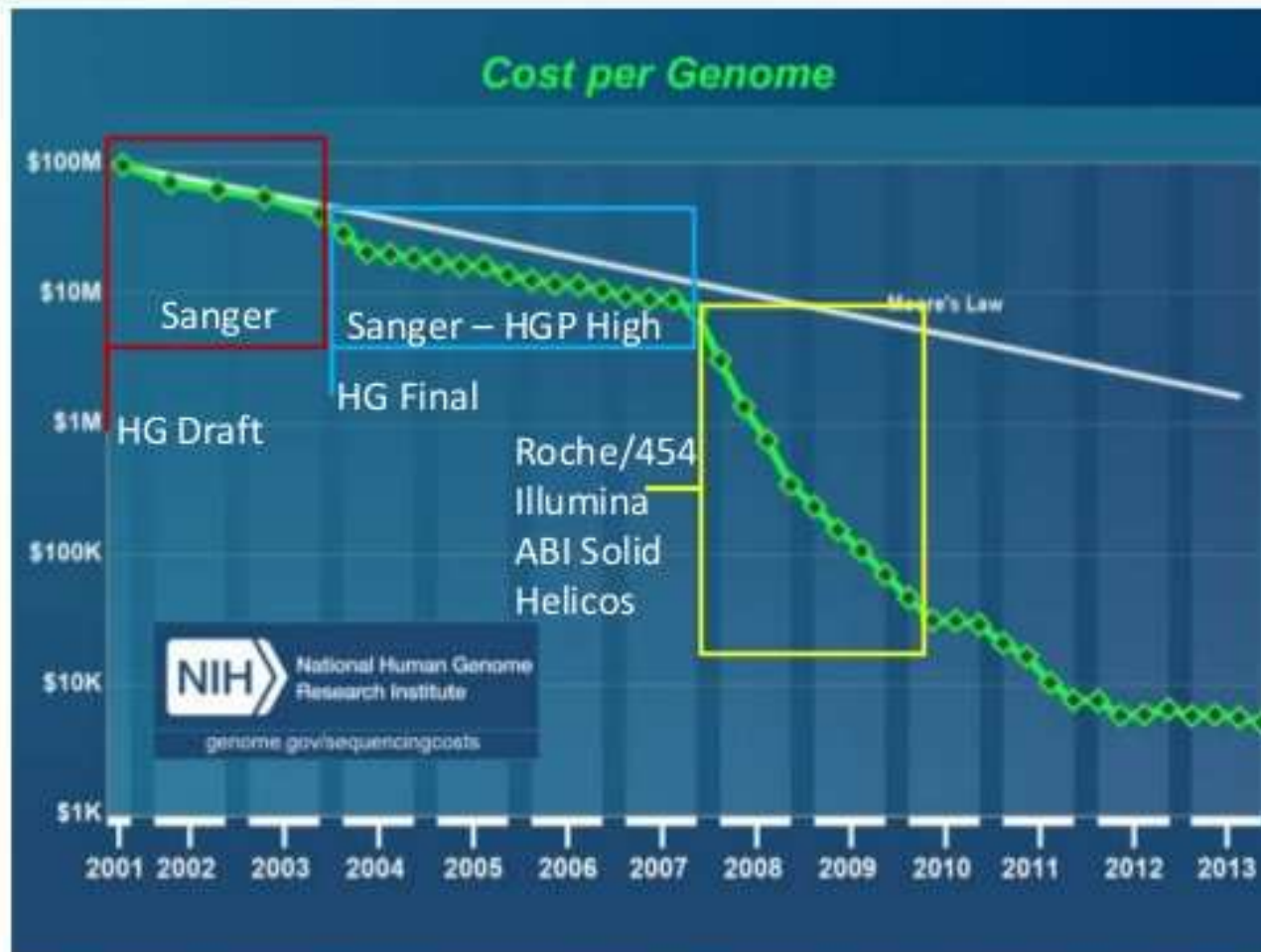


# Sekvenování třetí generace



# Sekvenování

## Sequencing Costs in the Genome Era



# PCR Mullis NC1993

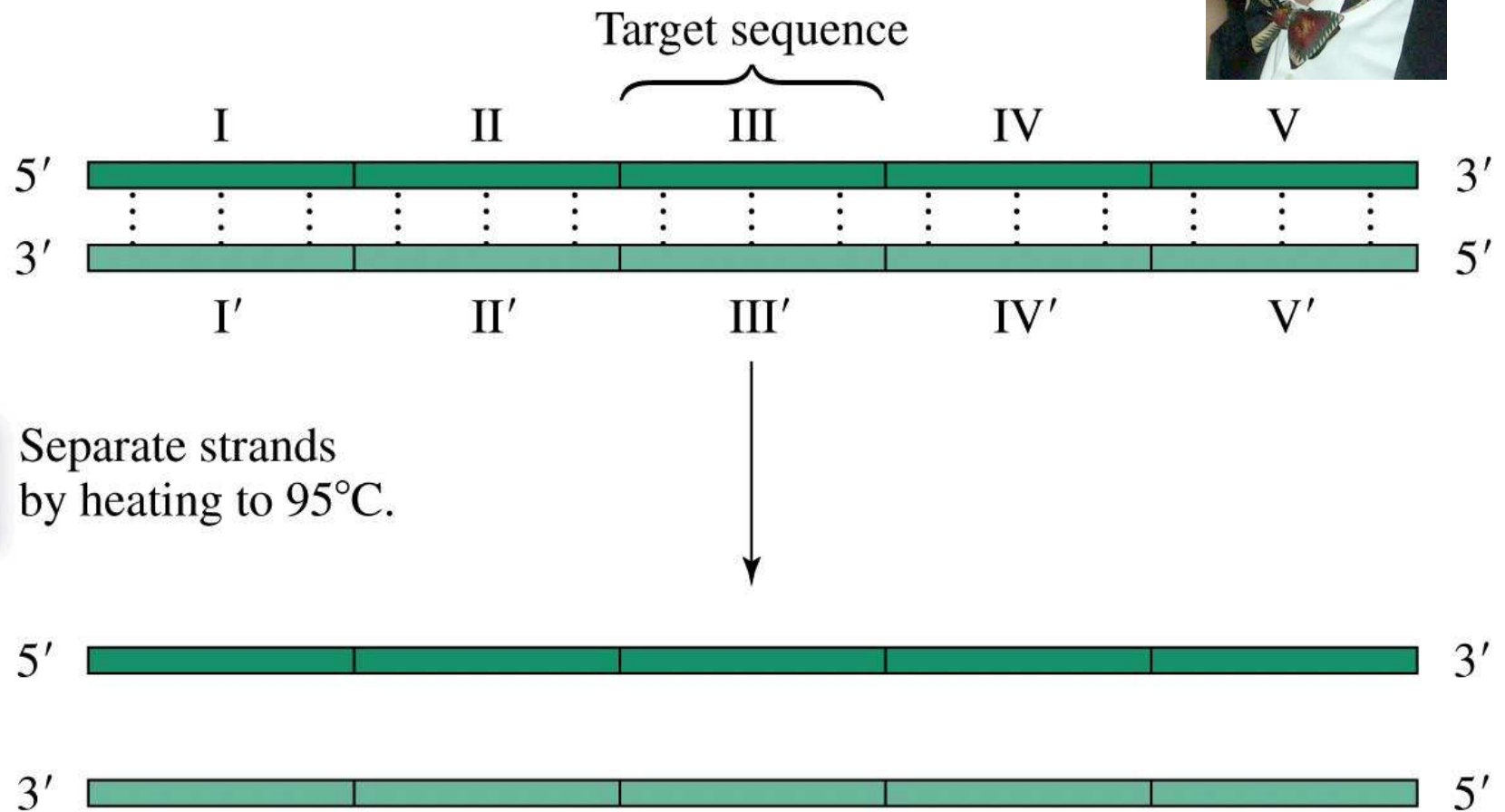
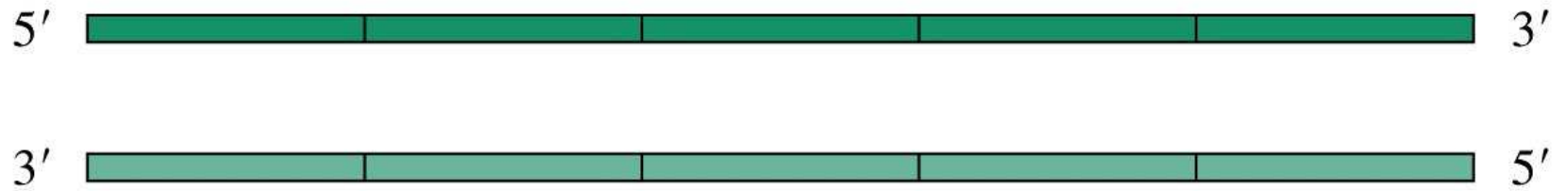


Figure 13-11 part 1 Concepts in Biochemistry, 3/e  
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Step 2

Hybridize primers by cooling to 50°C.

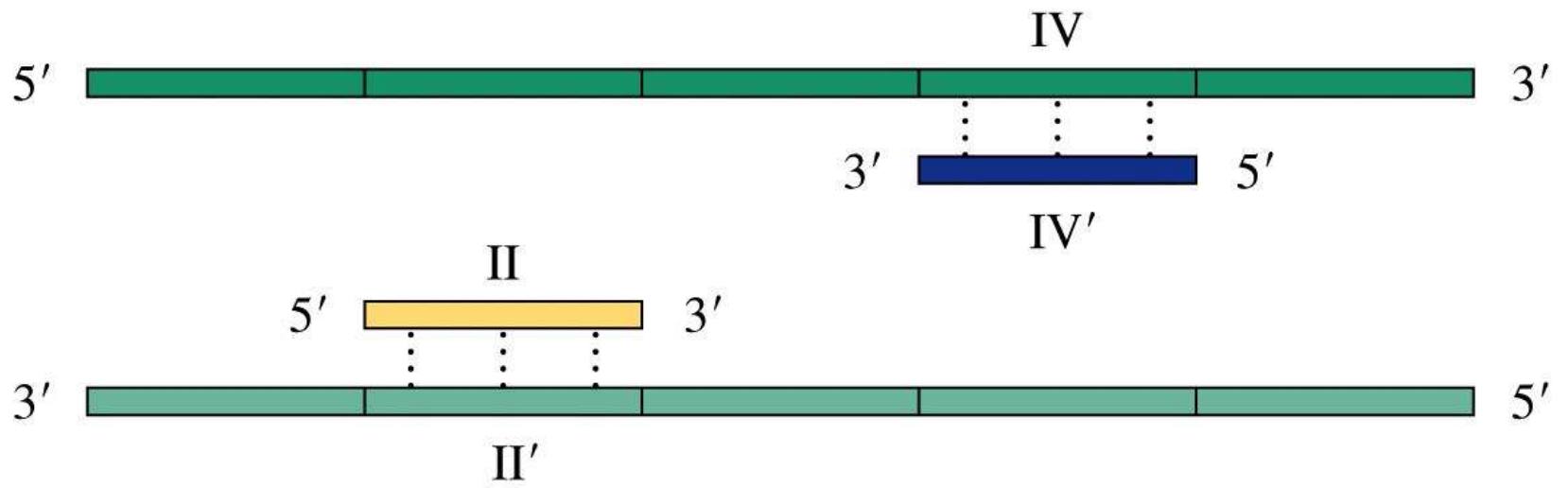
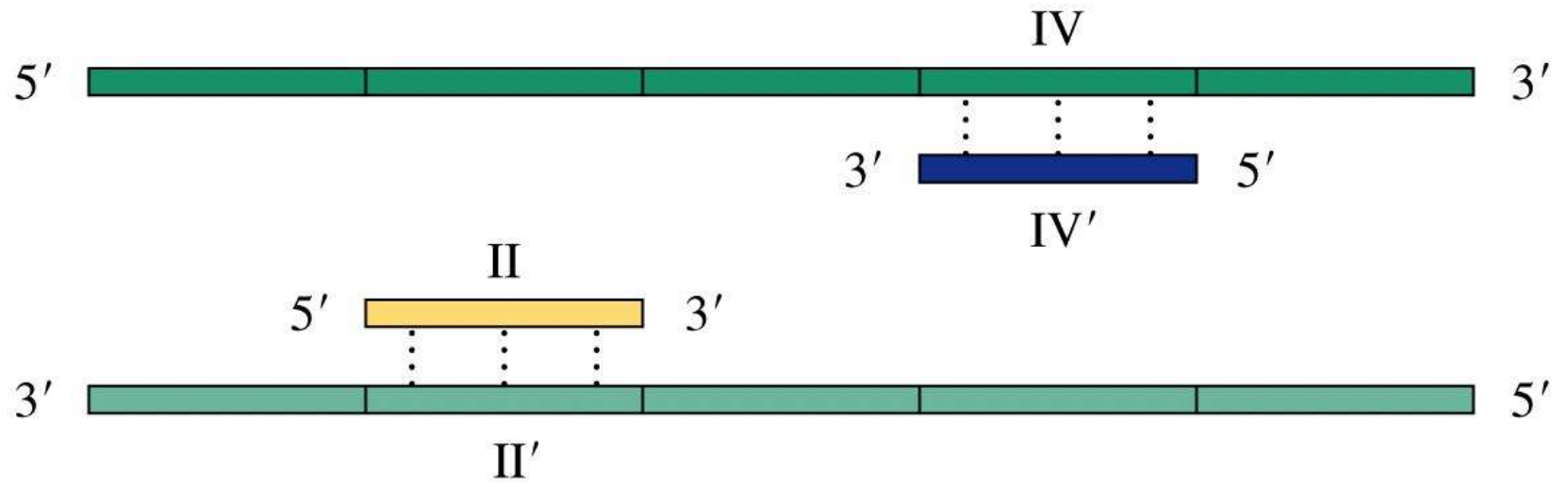


Figure 13-11 part 2 Concepts in Biochemistry, 3/e  
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Step 3

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

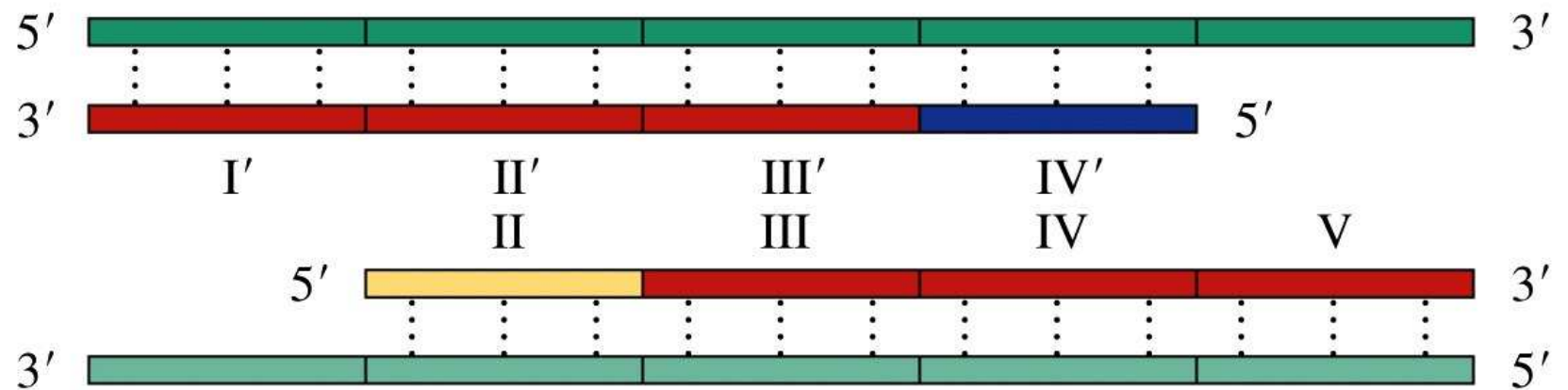
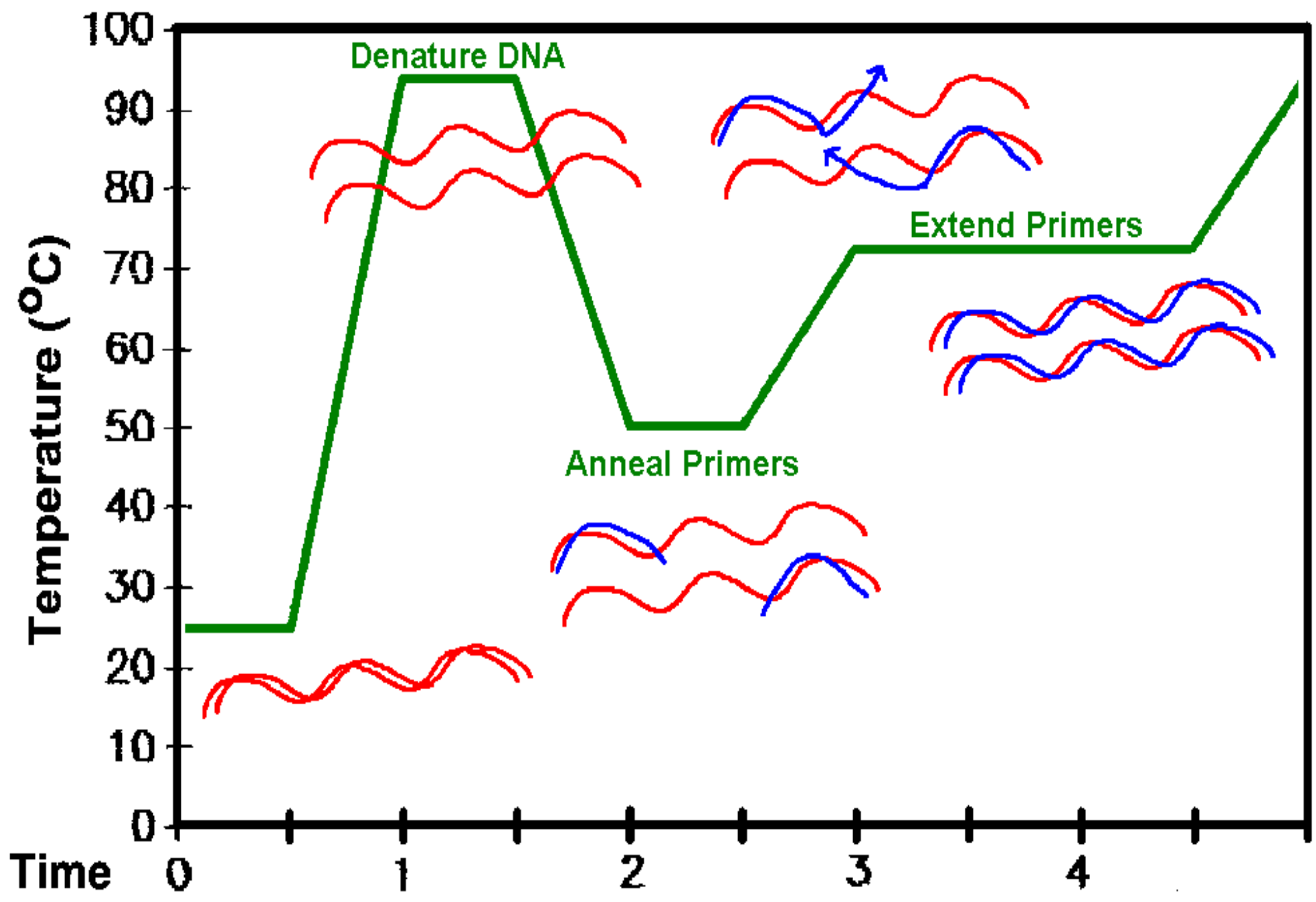
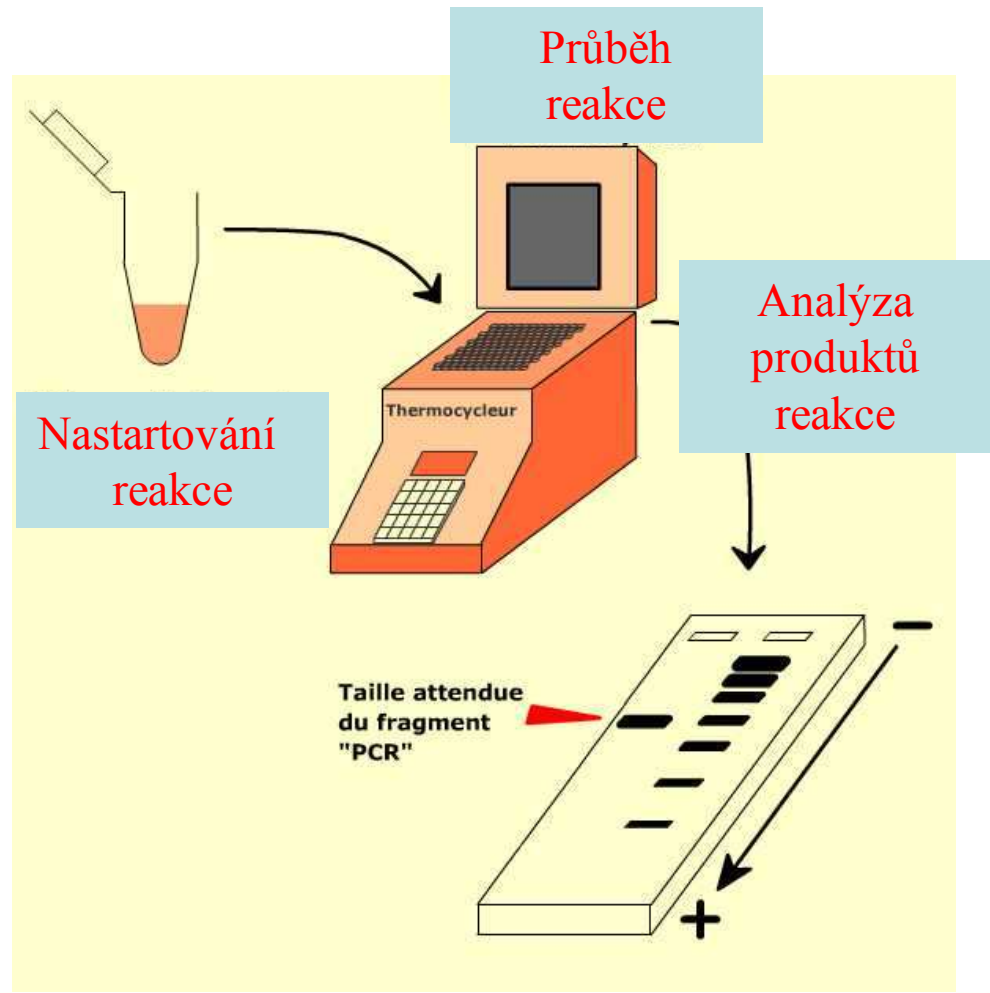


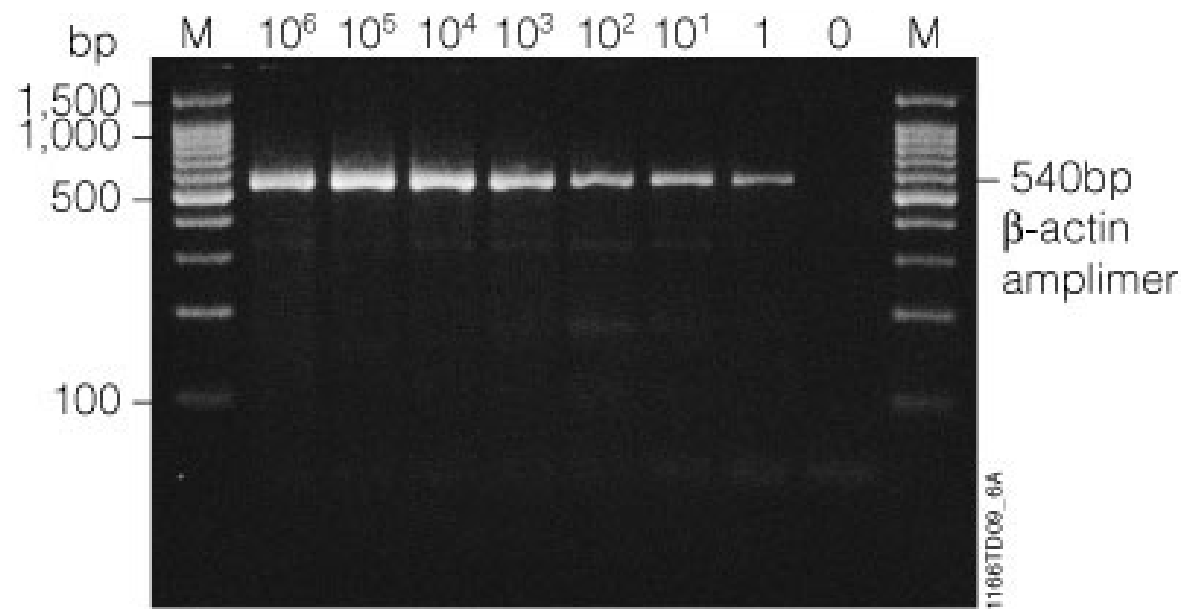
Figure 13-11 part 3 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons





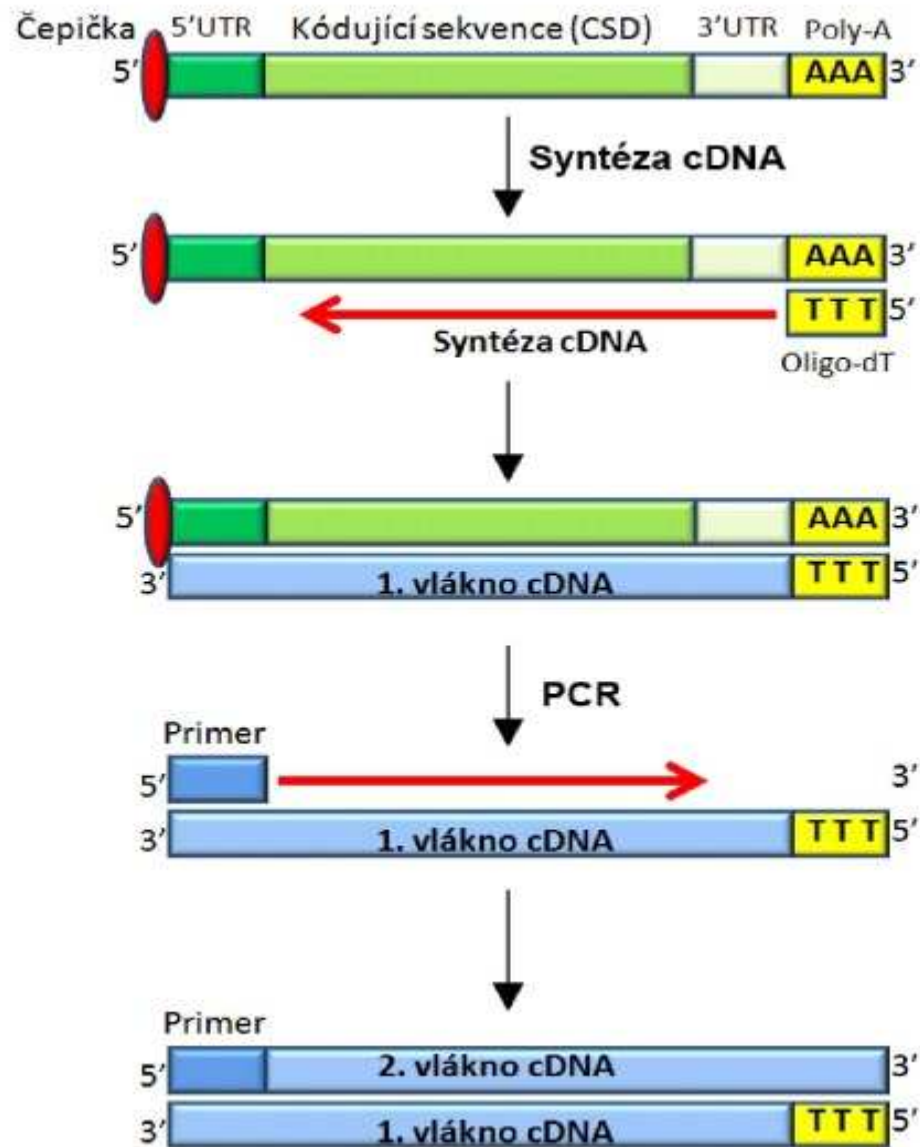
# Thermocycler





<http://www.dnalc.org/resources/animations/pcr.html>

# PCR mRNA – reverzní transkripce

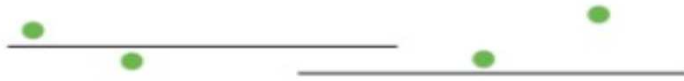


# Real time PCR

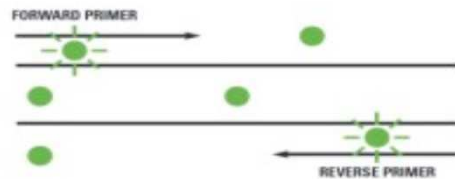
1. **Navázání:** SYBR® Green I se váže během každého cyklu na dvouvláknovou DNA.



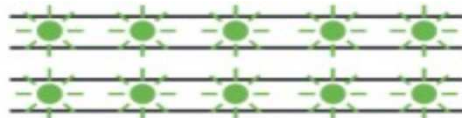
2. **Denaturace:** Ve fázi denaturace DNA je SYBR® Green I uvolněn z vazby na DNA a celková fluorescence dramaticky klesá.



3. **Polymerizace:** Během annealingu primerů a elongace řetězce se Sybr Green opět začíná navazovat na vznikající dvouvláknovou DNA - fluorescence stoupá.

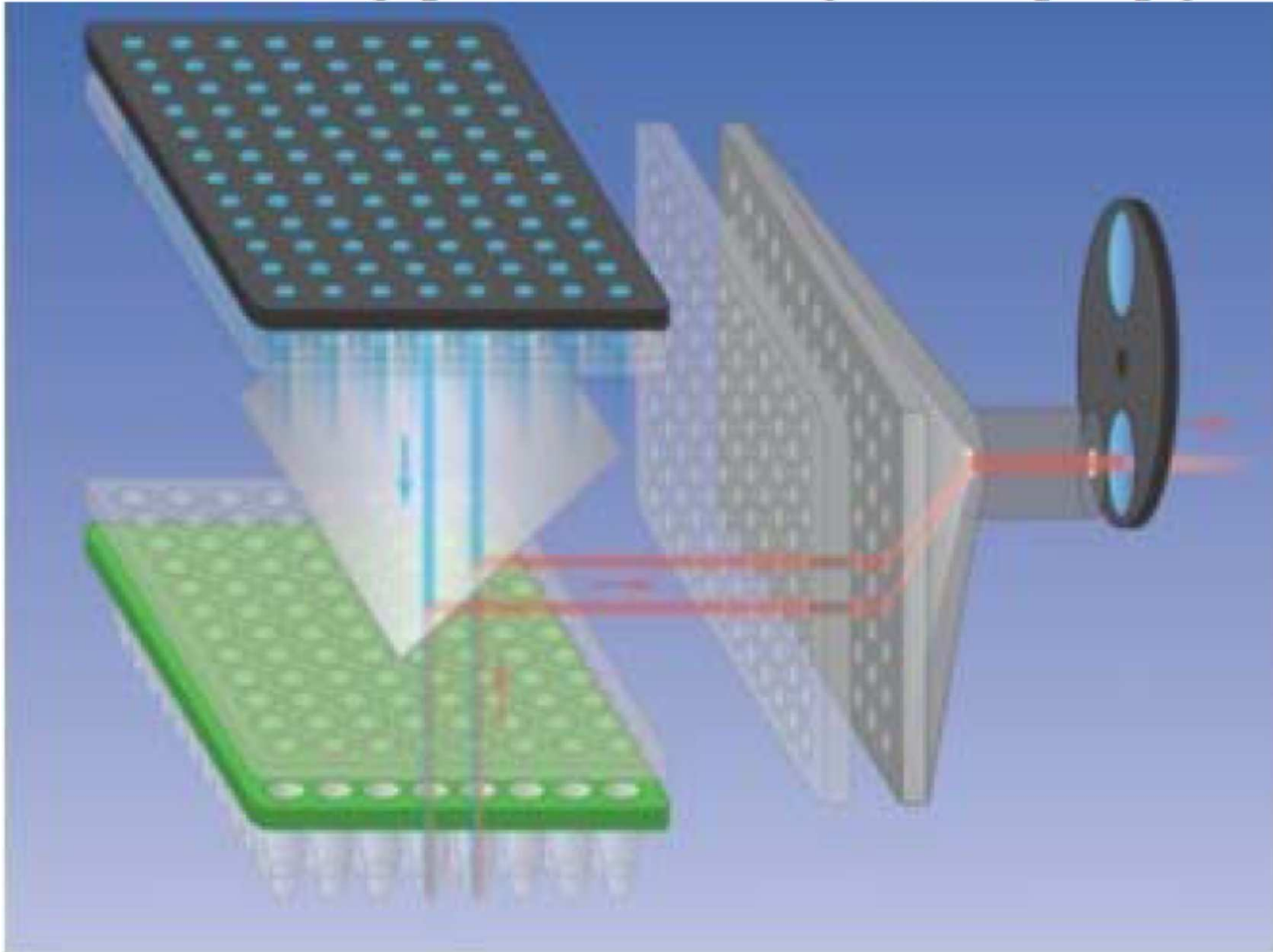


4. **Ukončení polymerizace:** Emitovaná fluorescence dosahuje maxima.





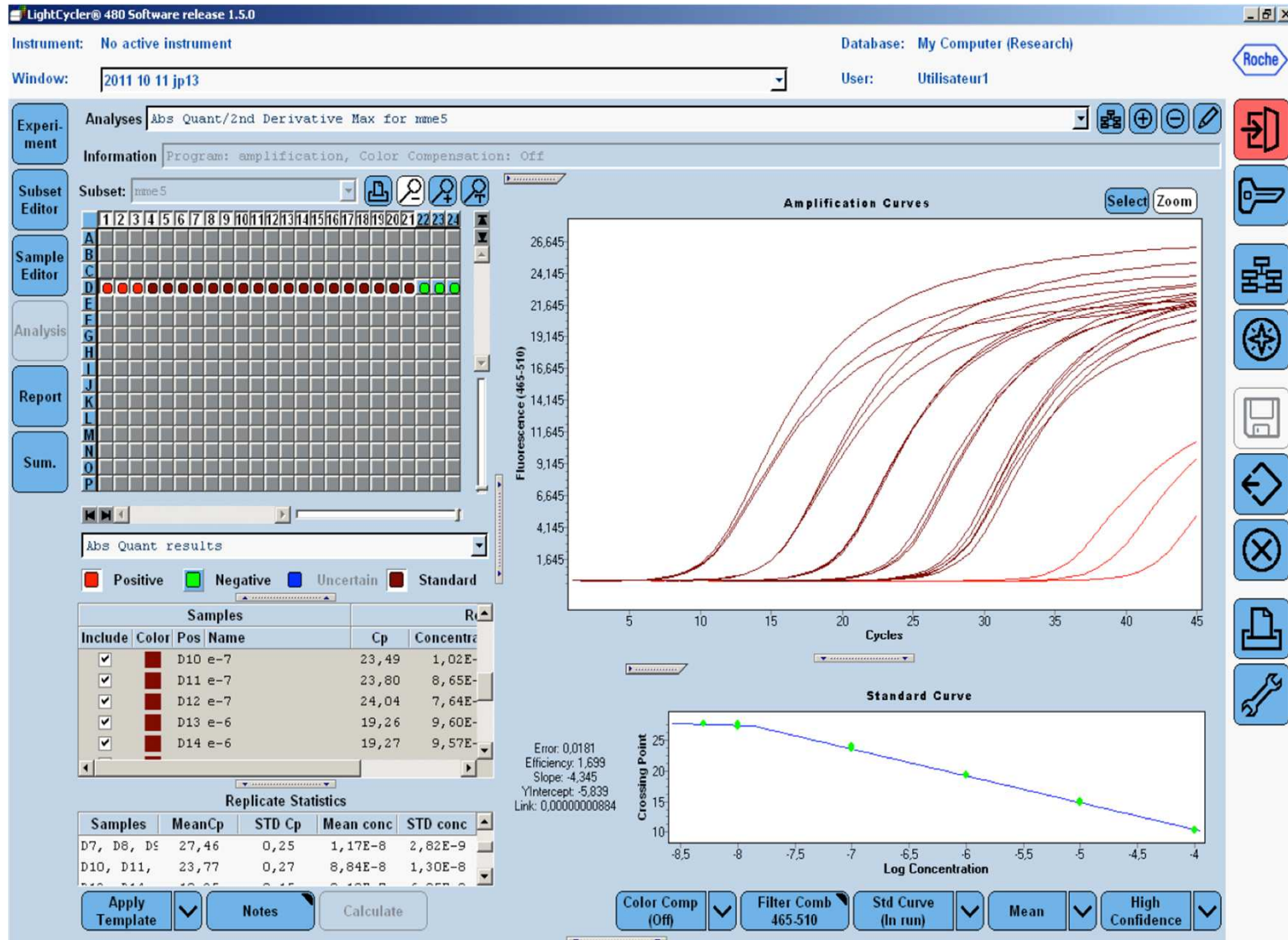
# Real time PCR

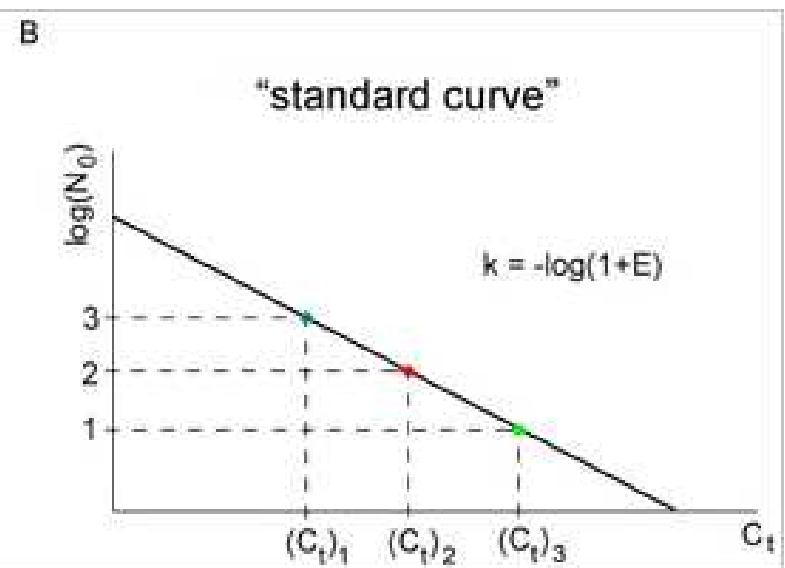
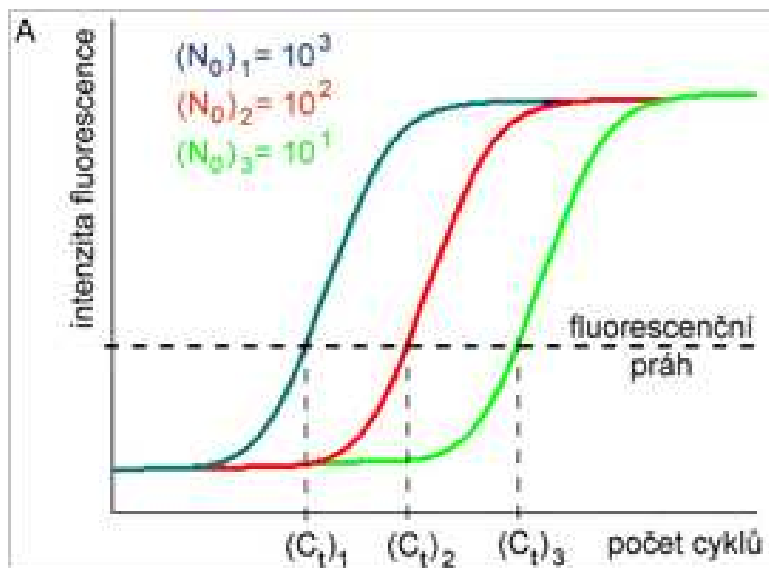


# Real time PCR



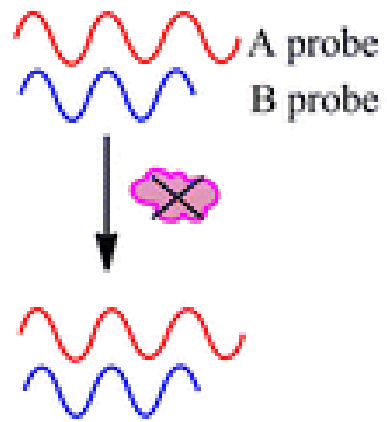
# Real time PCR



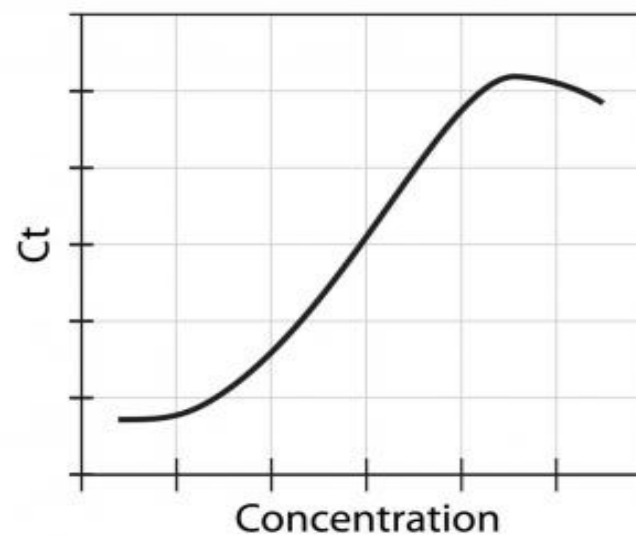
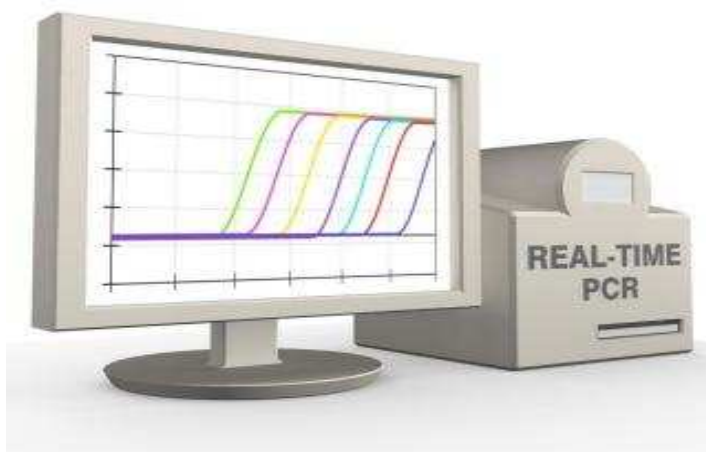
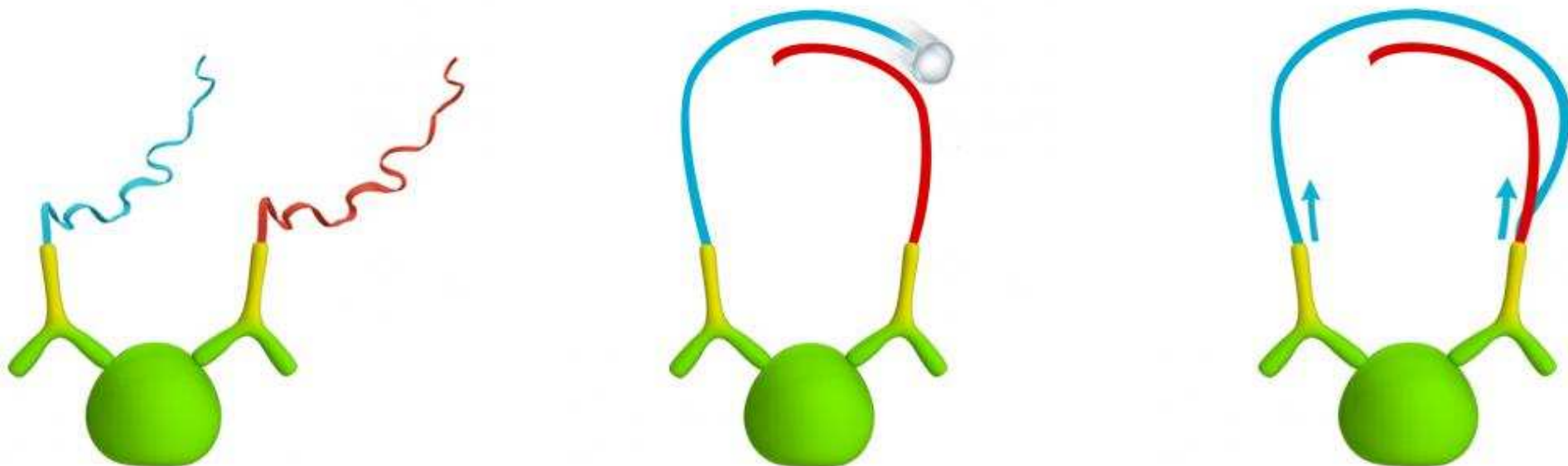


# Real time PCR proteinů

Protein → DNA

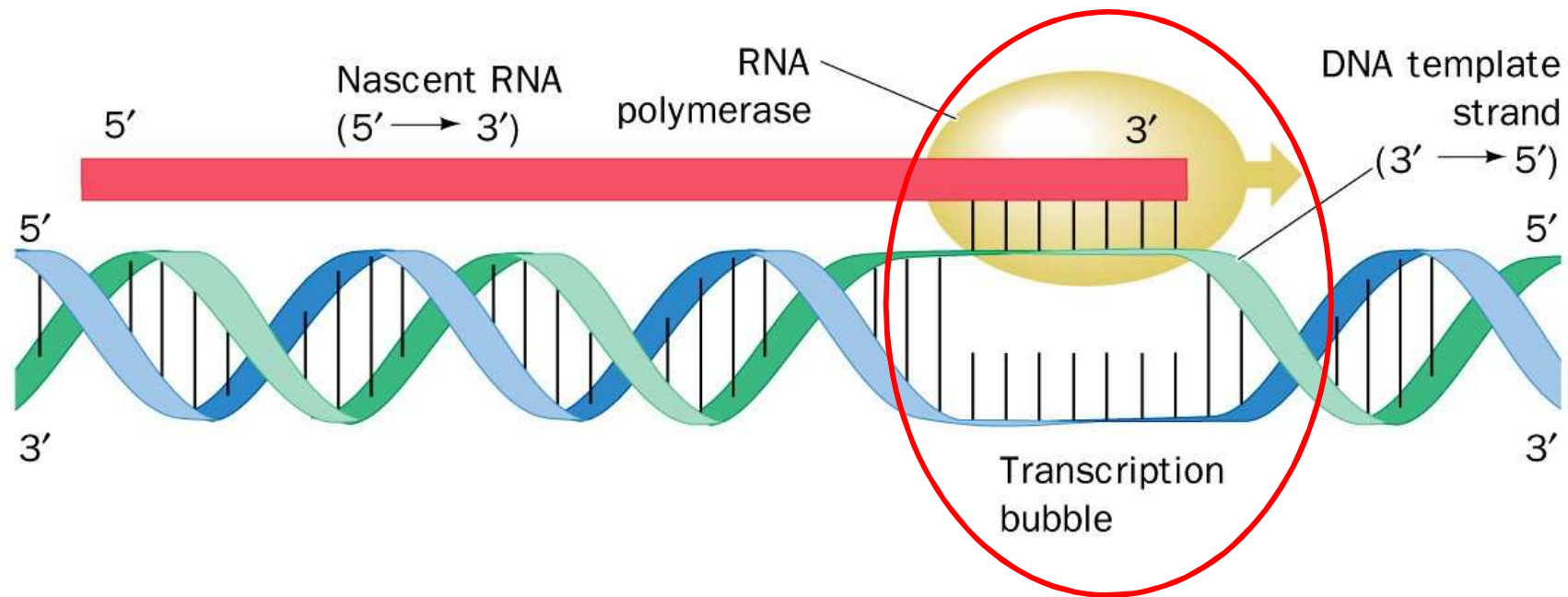


# Real time PCR proteinu



# Transkripce – přepis - DNA → RNA

**DNA + NTP + 1 enzym**







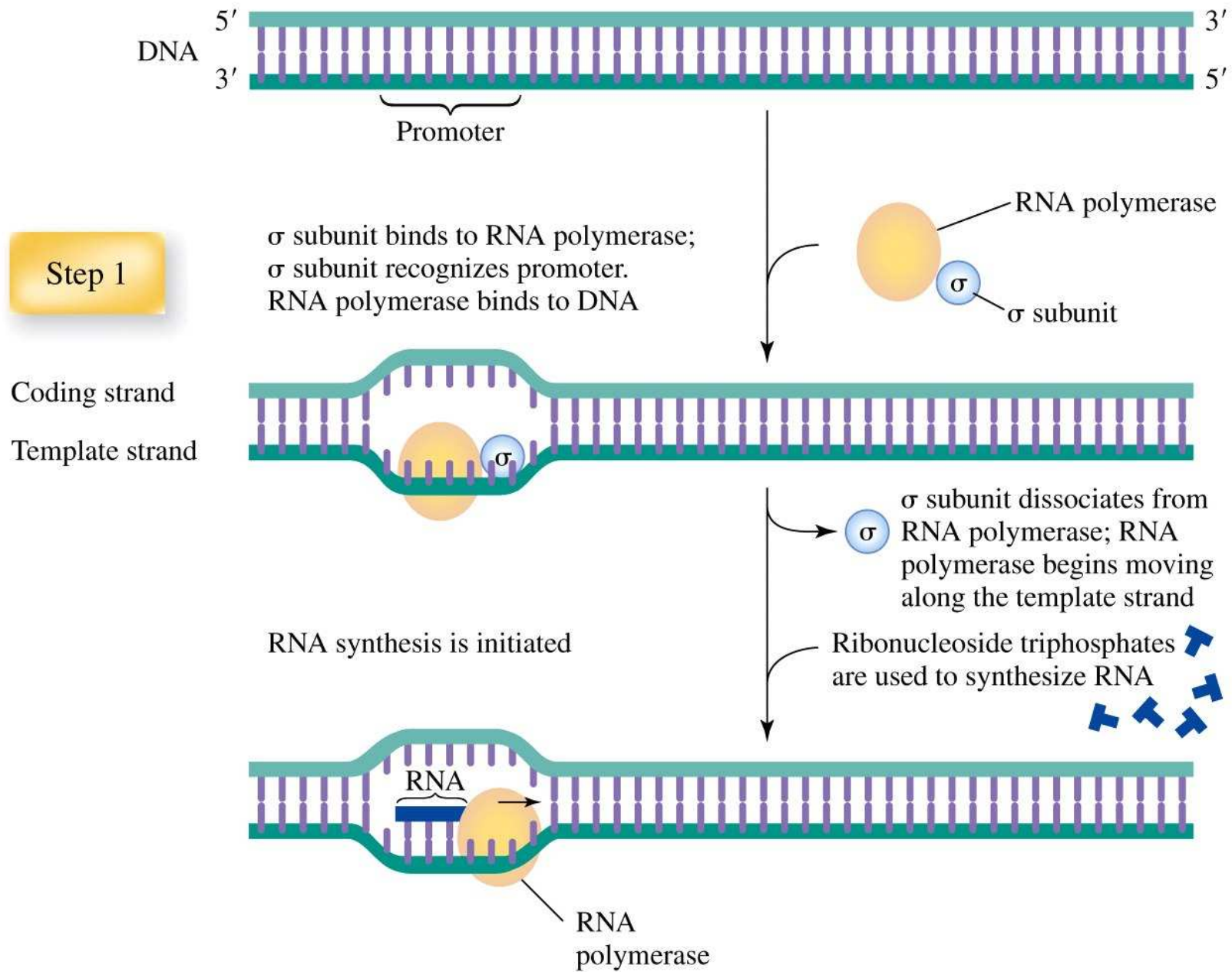


Figure 11-22 part 1 Concepts in Biochemistry, 3/e  
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Step 2

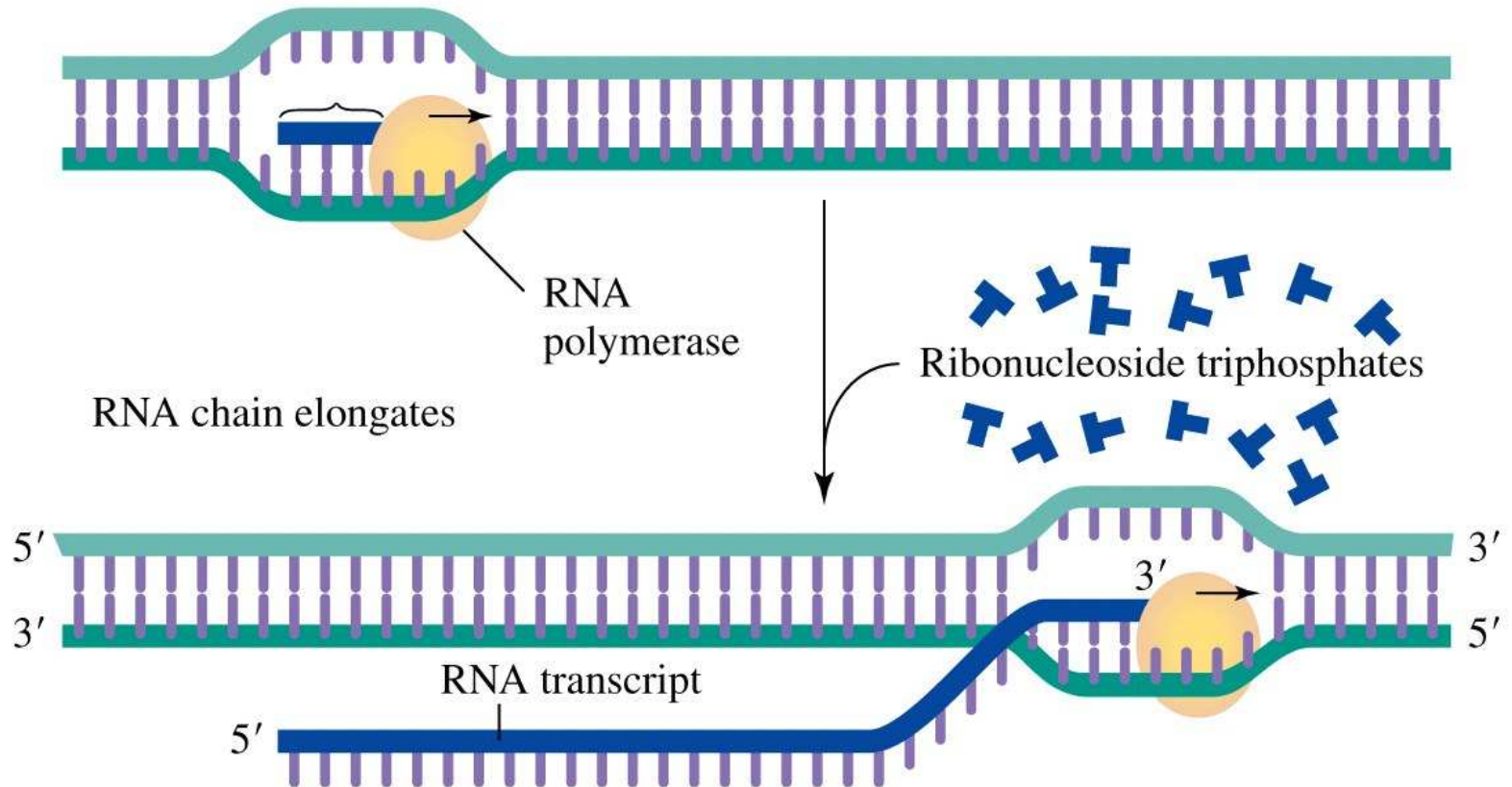


Figure 11-22 part 2 Concepts in Biochemistry, 3/e  
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Step 3

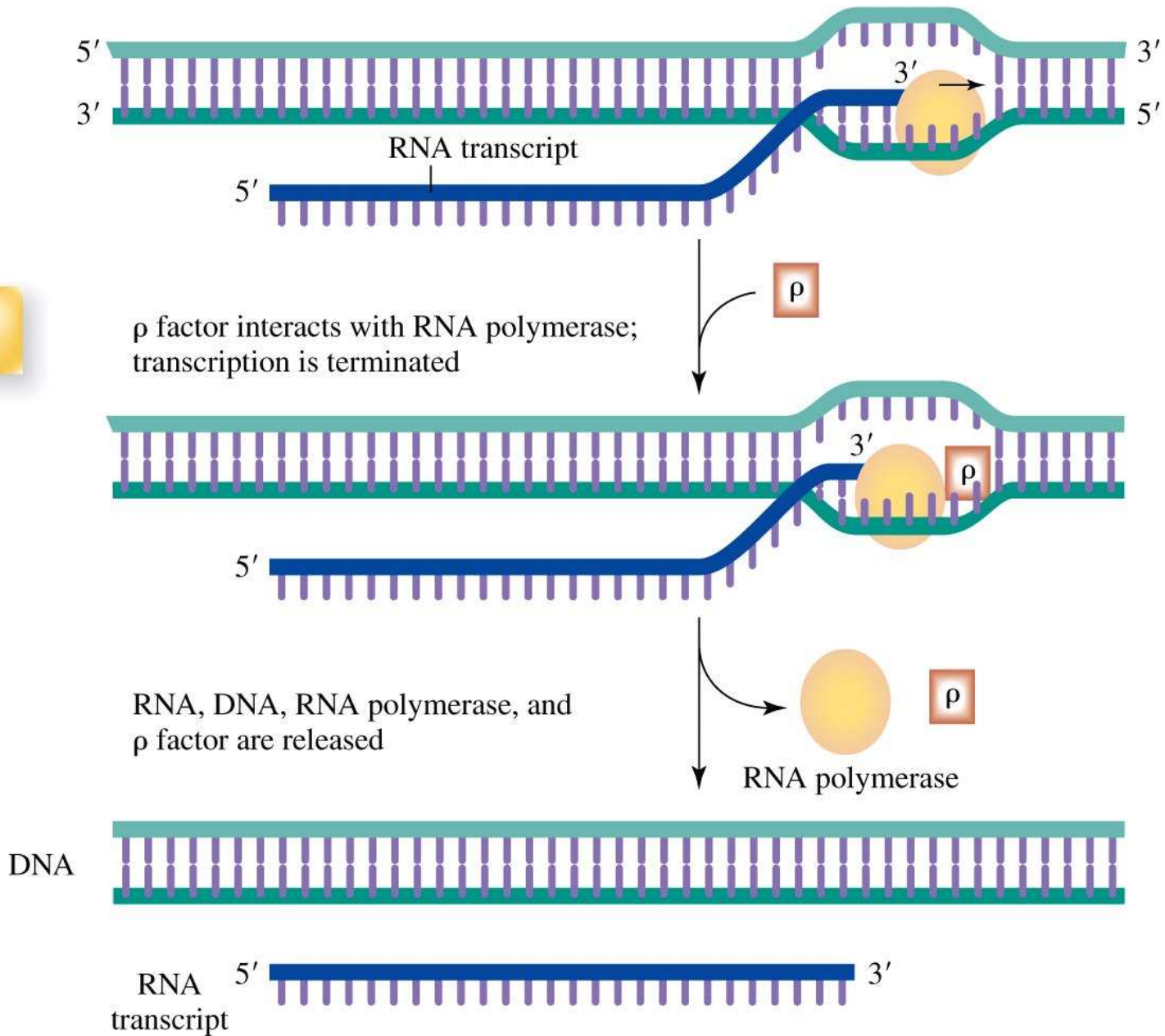
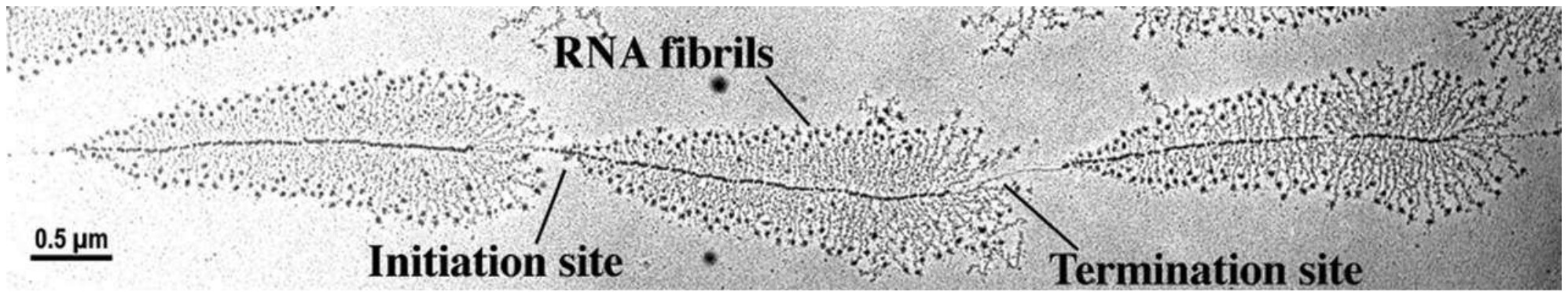
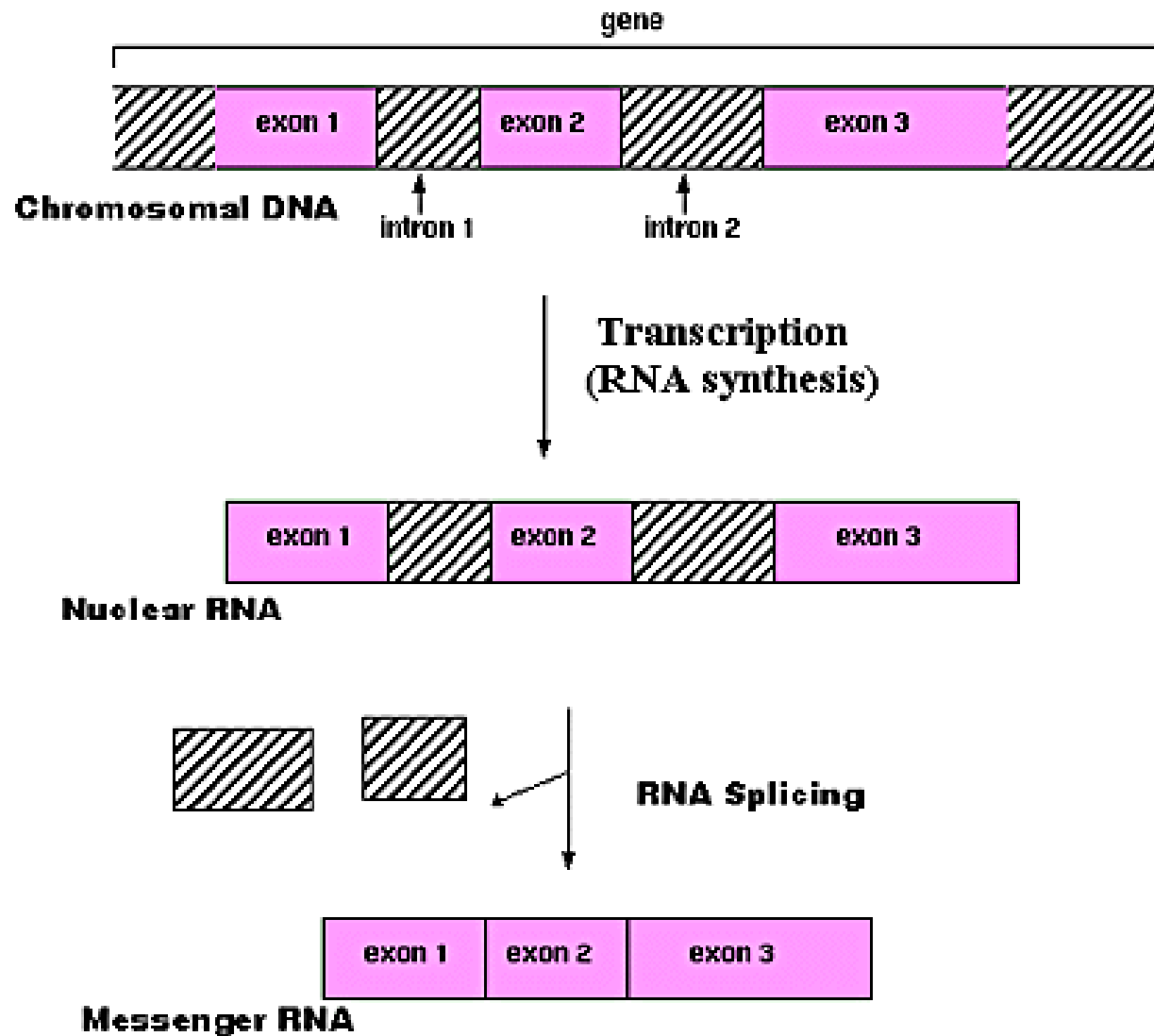


Figure 11-22 part 3 Concepts in Biochemistry, 3/e  
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Courtesy of Ulrich Scheer, University of Würzburg, Germany

# Syntéza eukaryontní RNA



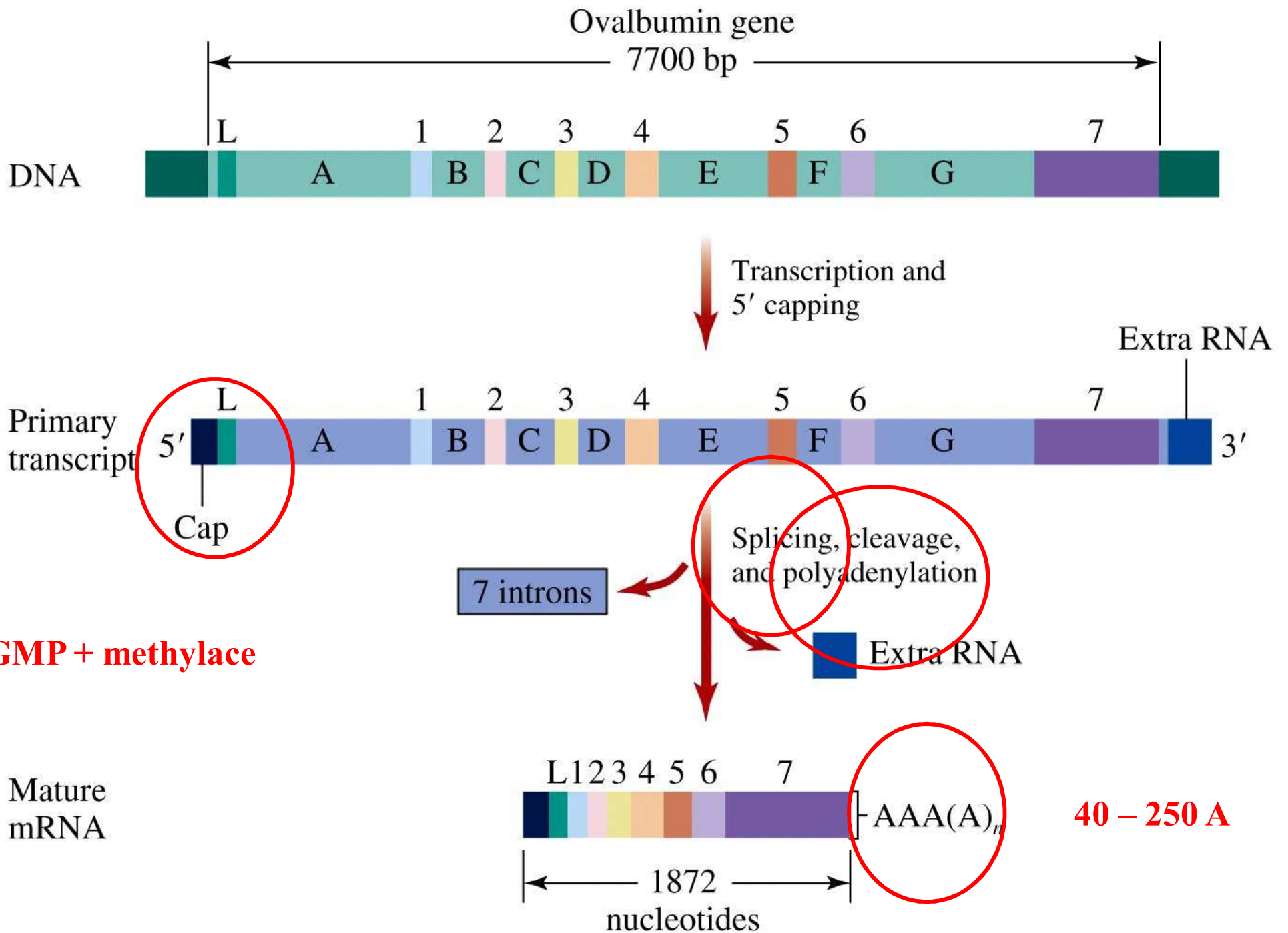
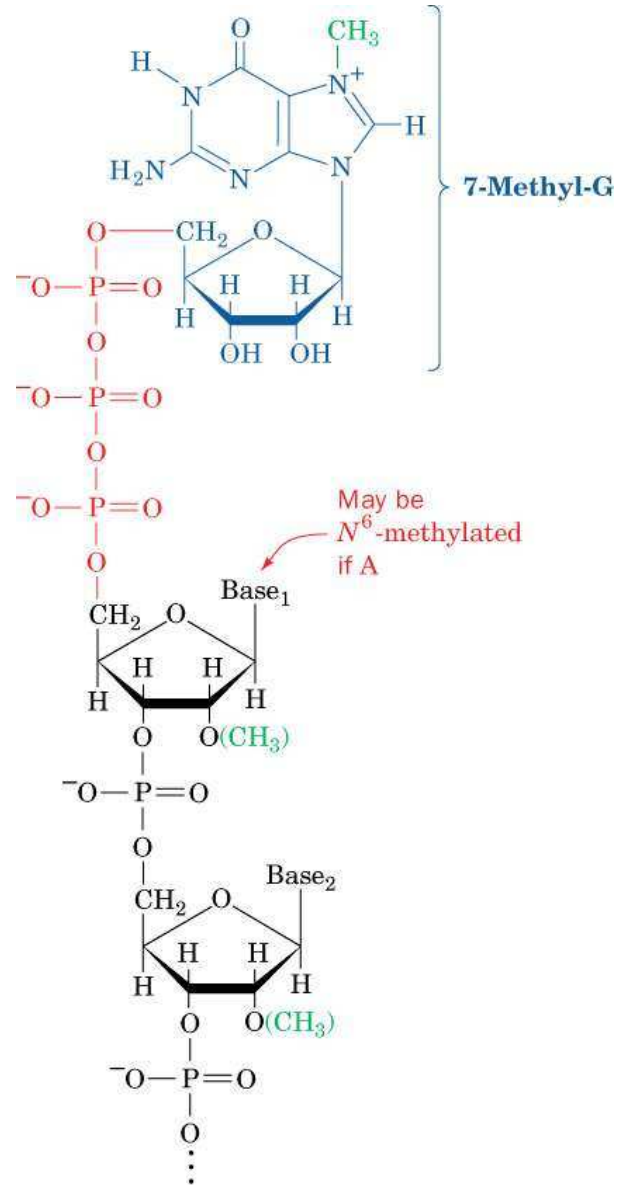


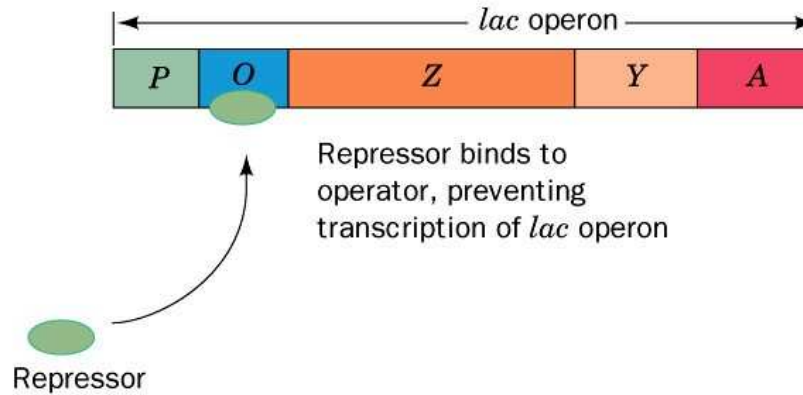
Figure 11-28 Concepts in Biochemistry, 3/e  
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# Cap

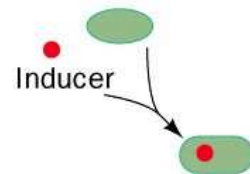
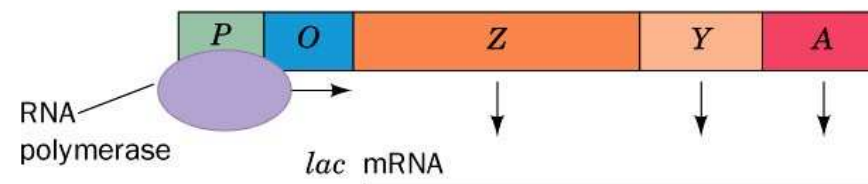


# Regulace translace

(a) Absence of inducer



(b) Presence of inducer

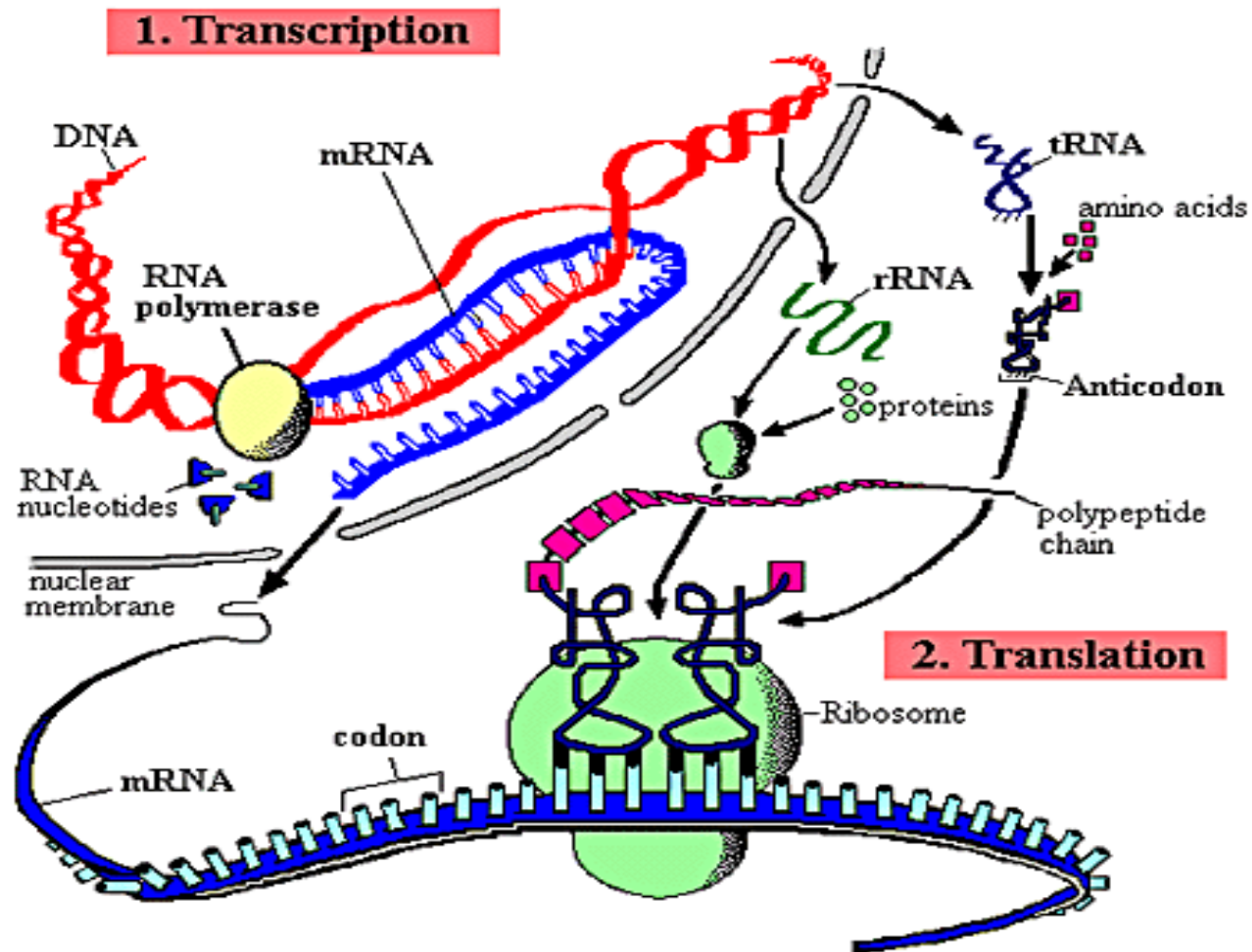


Transcription of *lac* structural genes

Inducer-repressor complex does not bind to operator

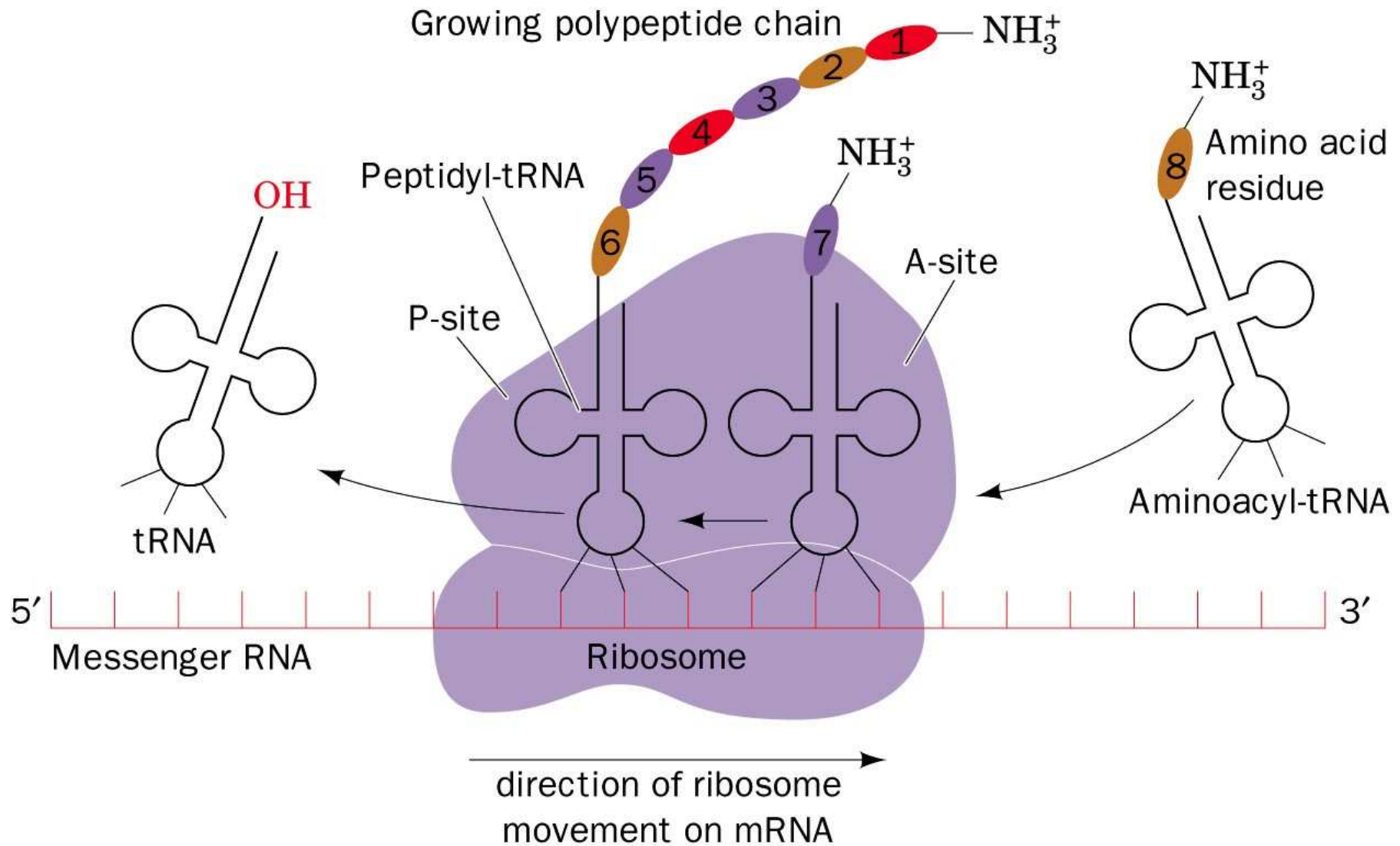


# Syntéza bílkovin



# Translaci – překladi - RNA → protein

**mRNA + ribozomy + (tRNA-AMK)<sub>n</sub> + IF<sub>n</sub> + EF + RF<sub>n</sub>**



# Ribozomy (rRNA + proteiny)

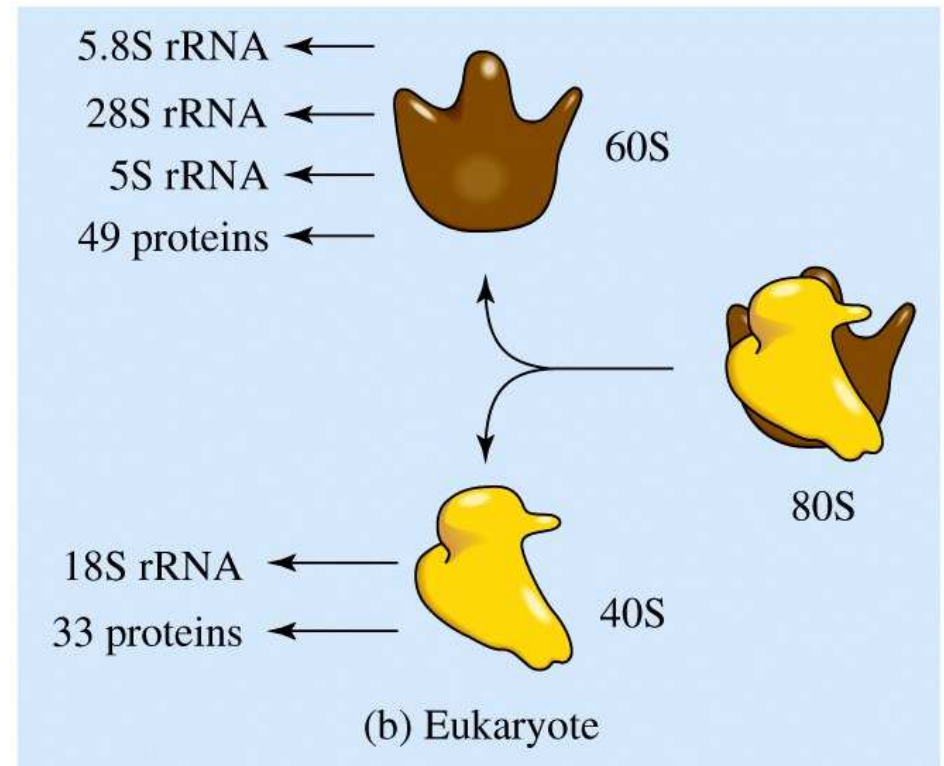
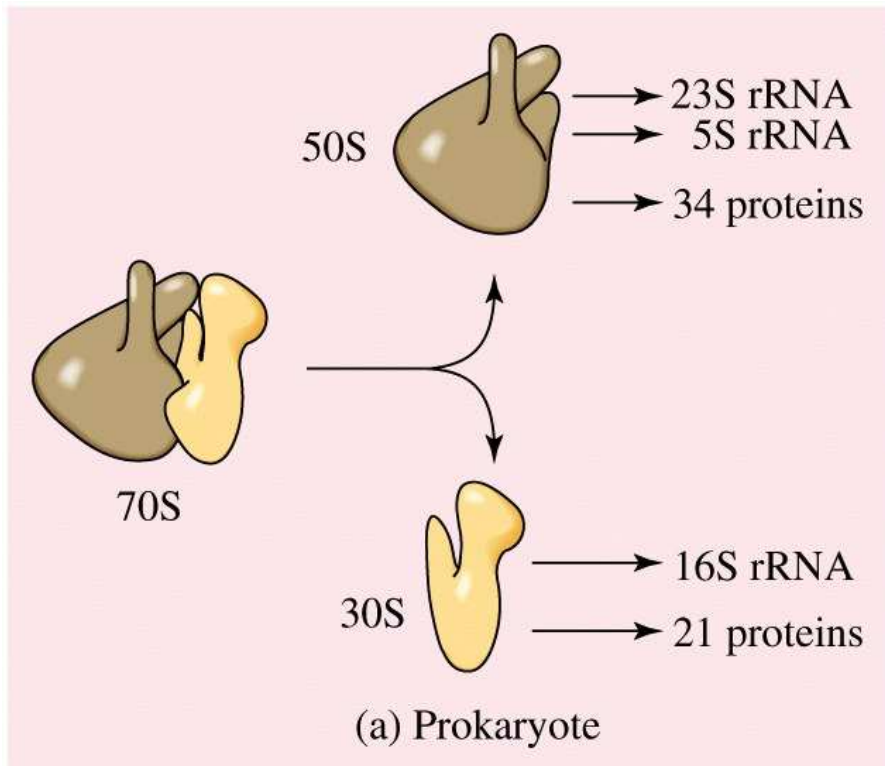
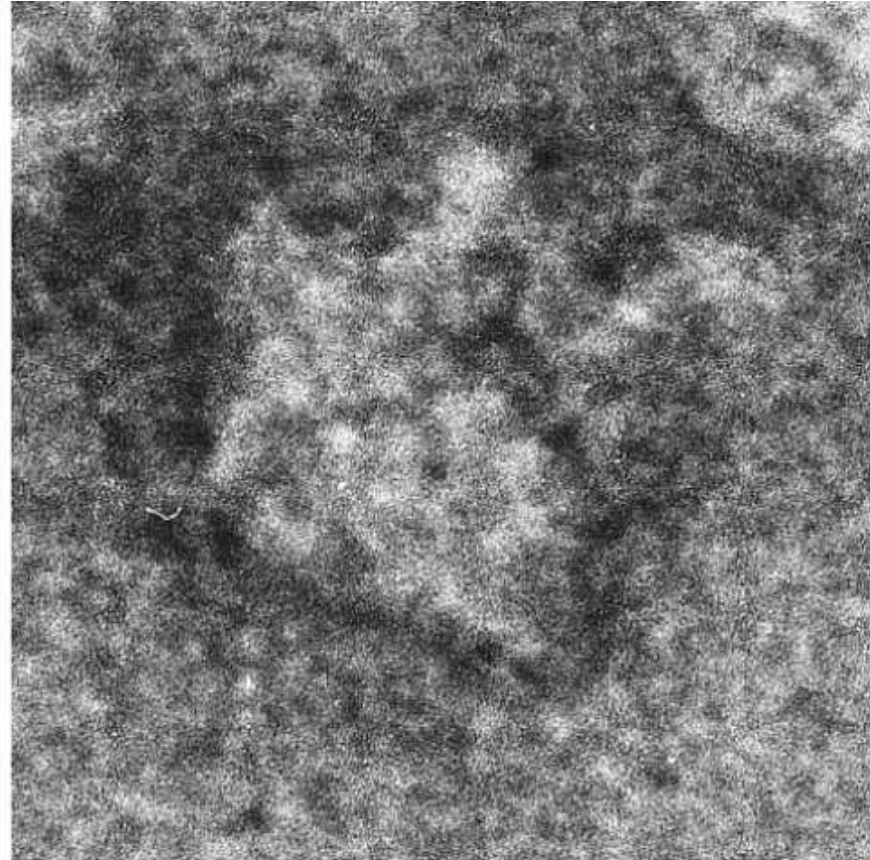
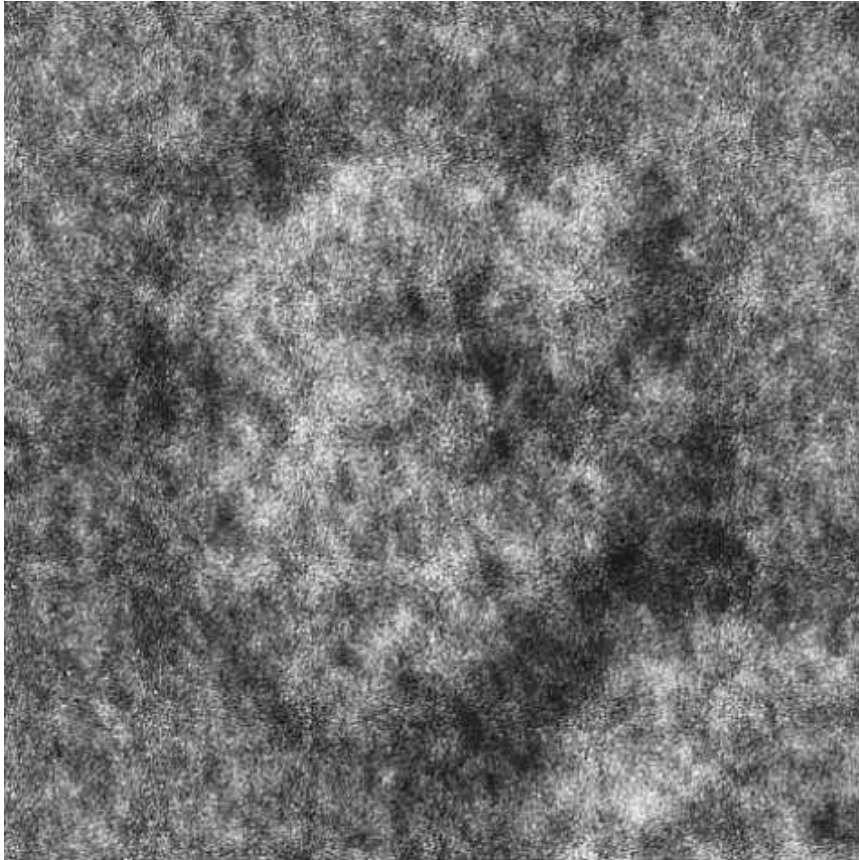


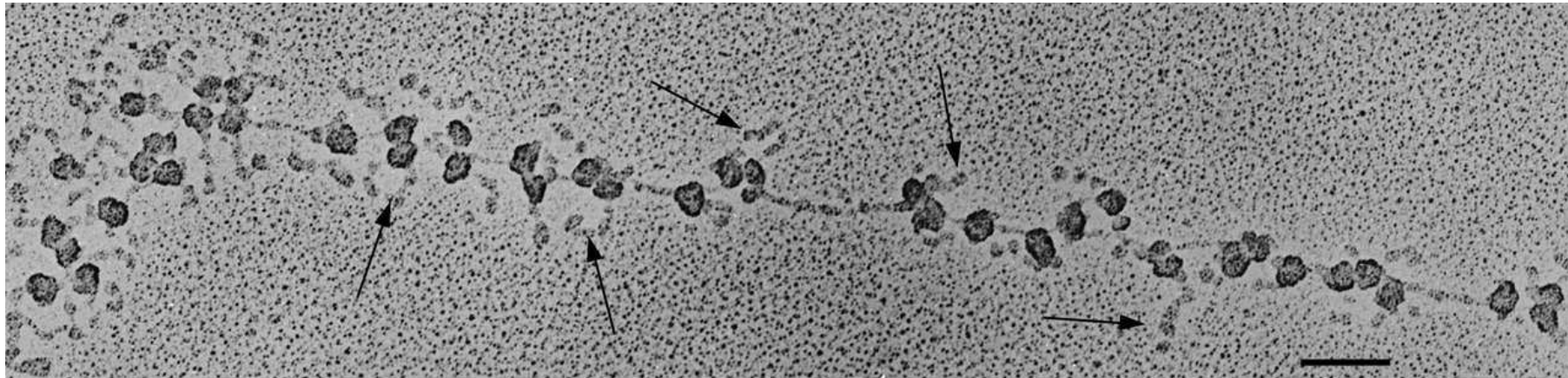
Figure 12-1 Concepts in Biochemistry, 3/e  
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# Ribozomy E.coli



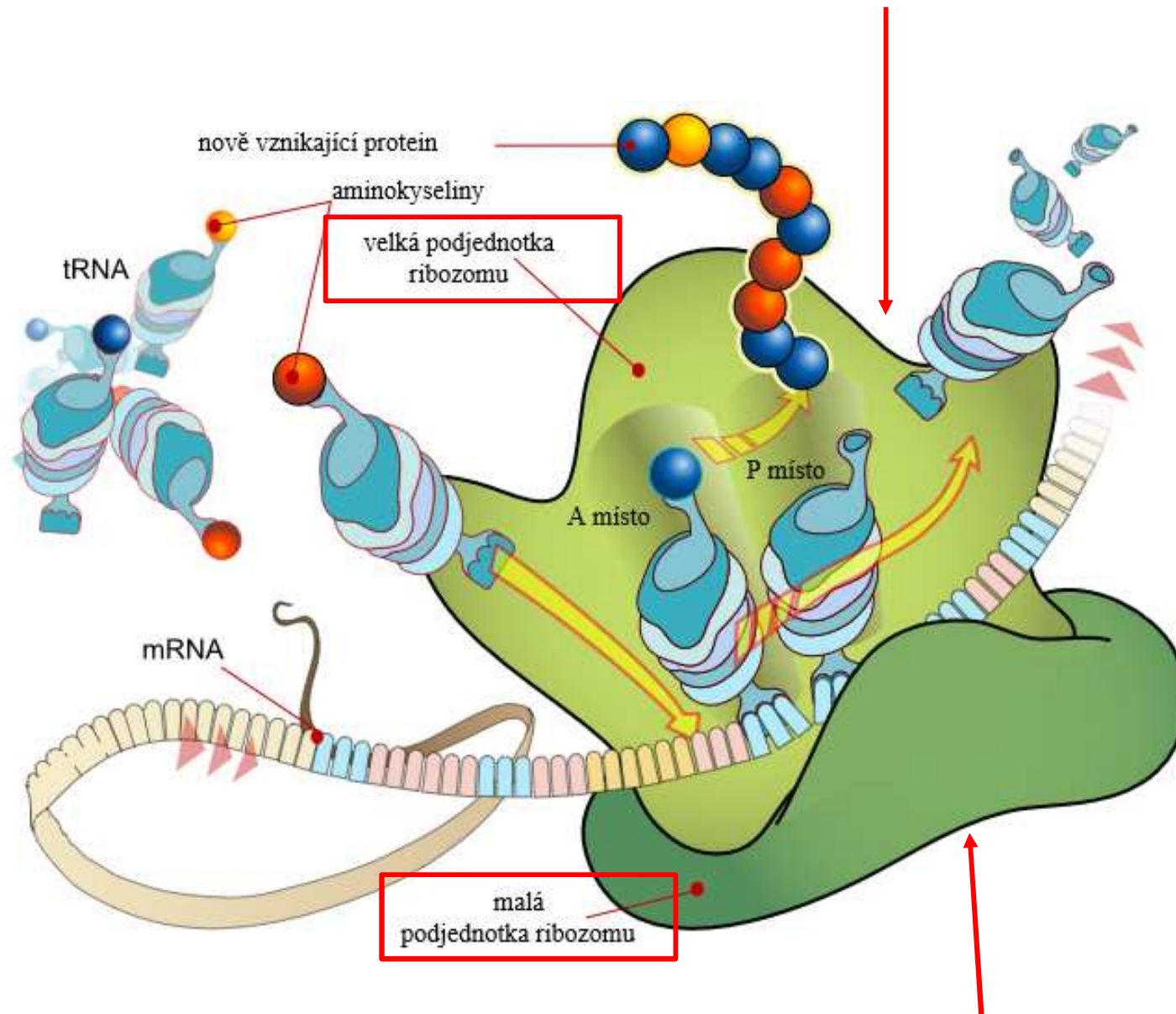
Courtesy of James Lake, UCLA

# Polyribozomy *Bombyx mori*



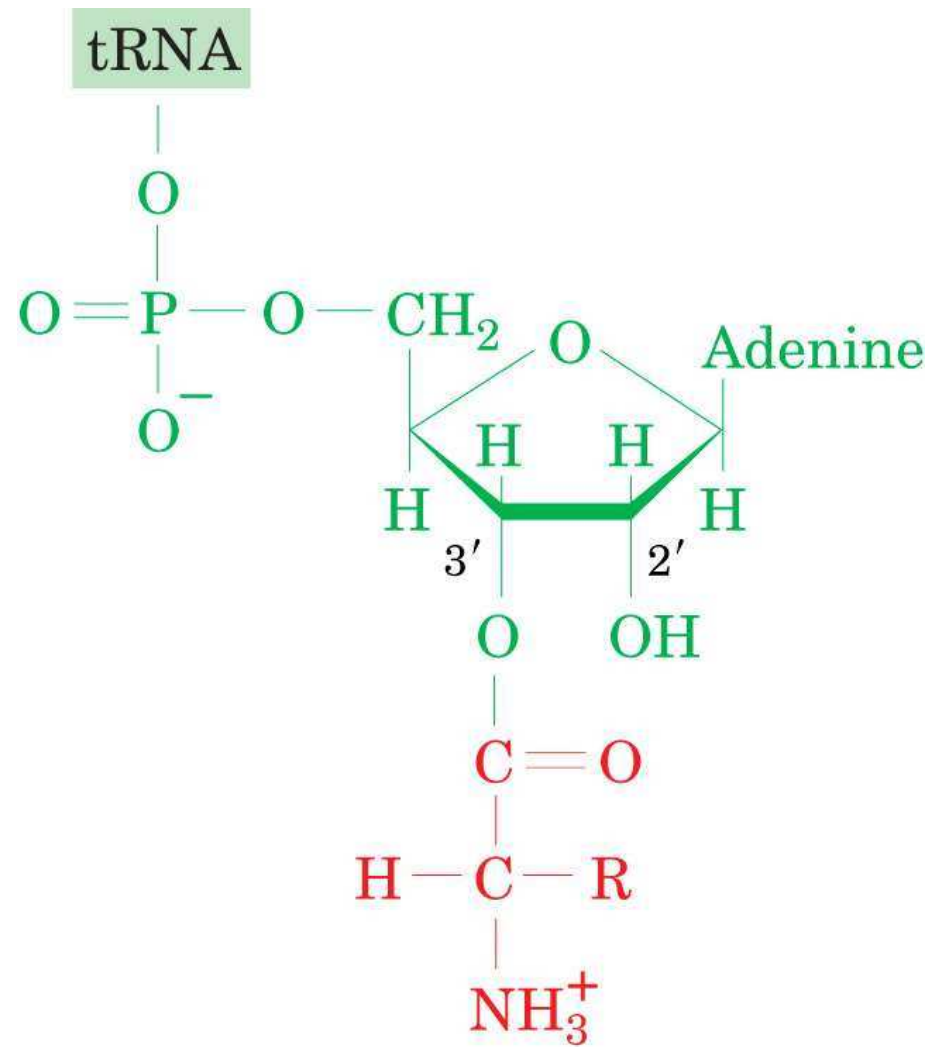
Courtesy of Oscar L. Miller, Jr. and Steven L. McKnight, University of Virginia

## Katalytická funkce – peptidyltransferáza



Rozpoznávací funkce

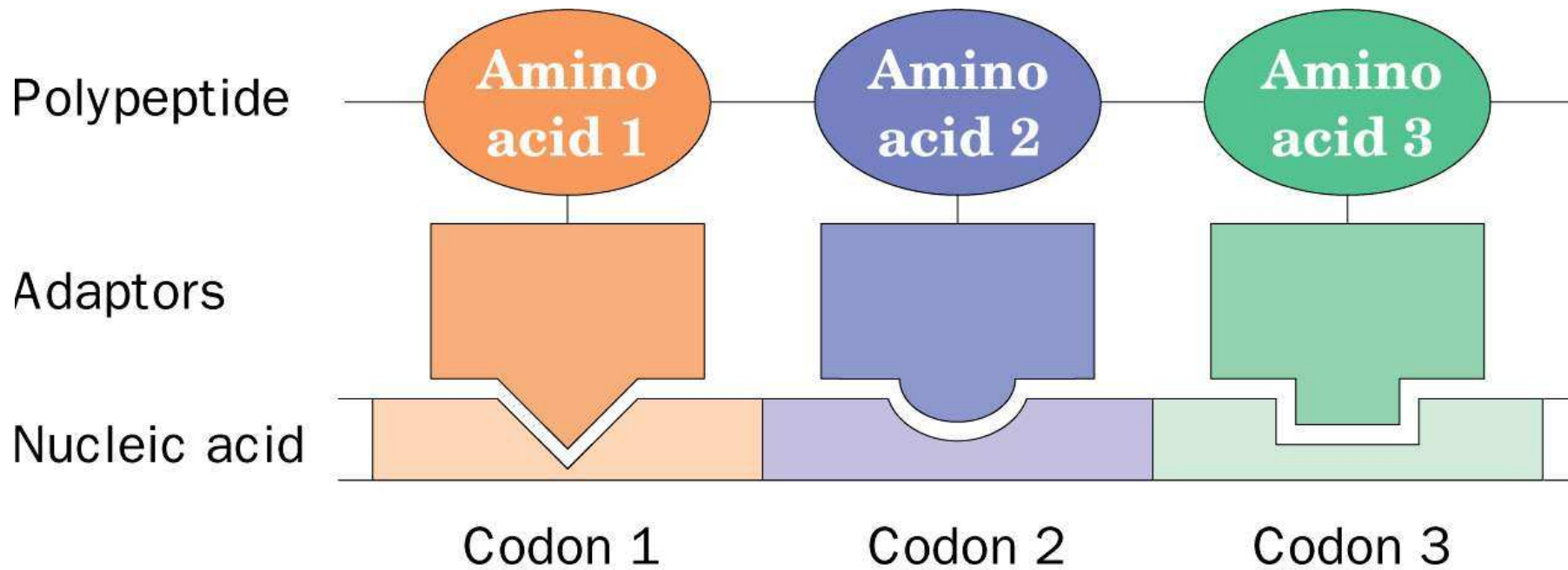




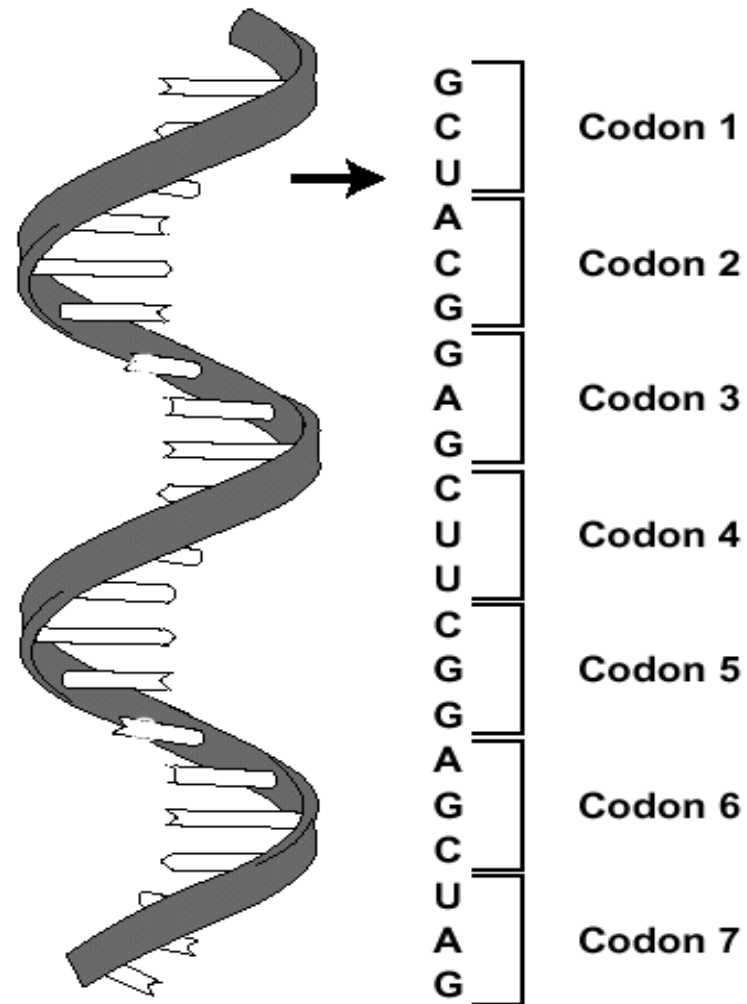
**Aminoacyl-tRNA**





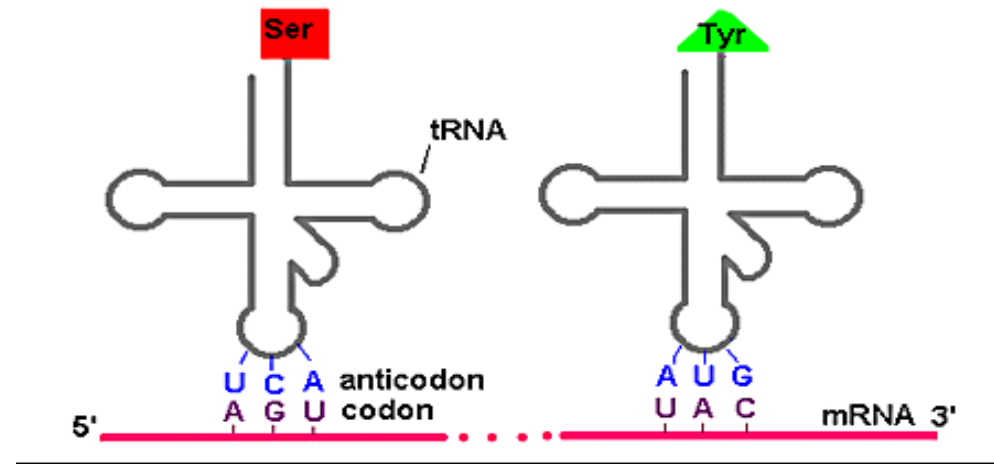


# Kodón



# Genetický kod

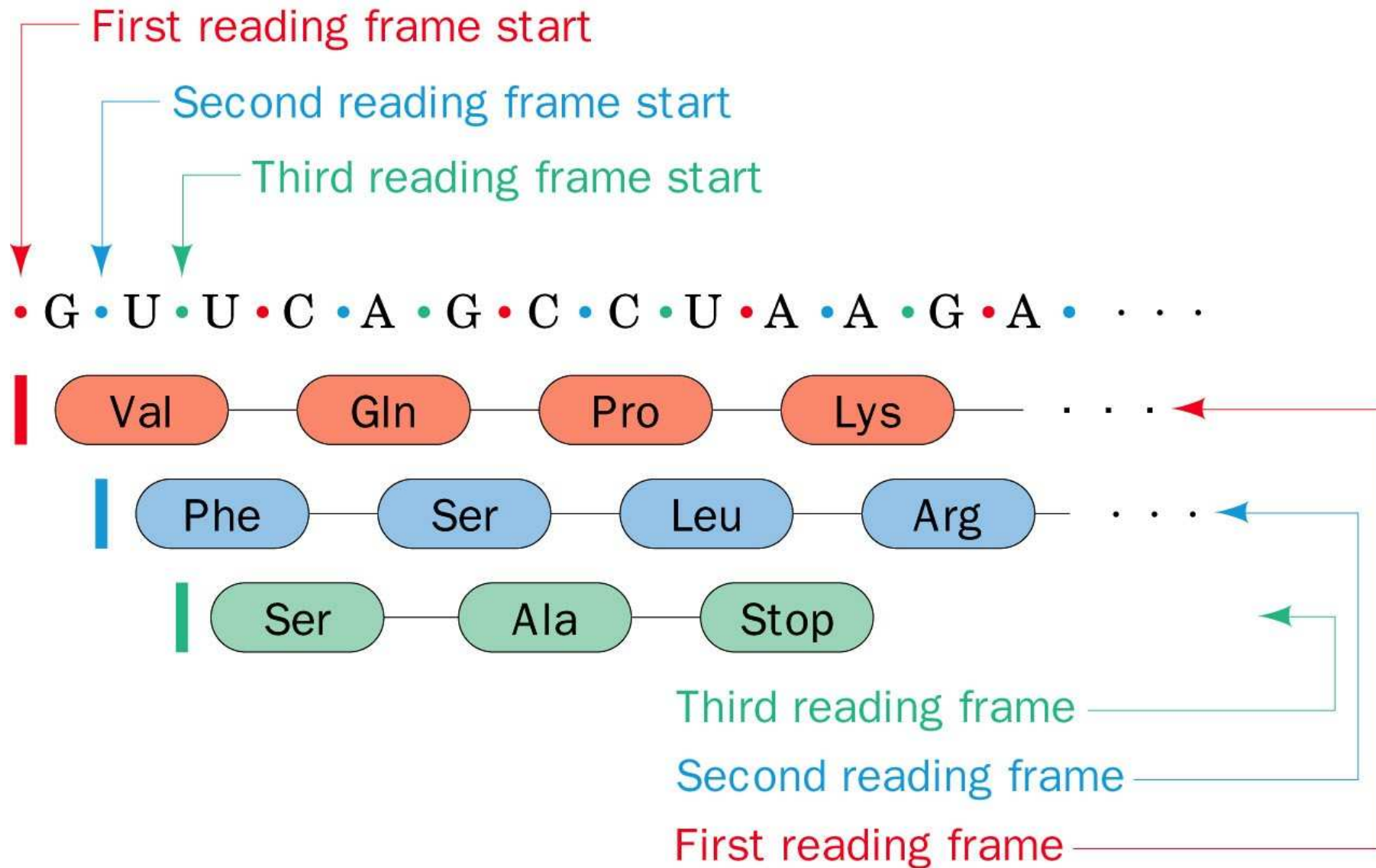
- Tripletový  $4^3 = 64$
- Degenerovaný
- Universální
- Nepřekrývající se



2nd base in codon

		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

# Reading Frame



First reading frame start

Second reading frame start

Third reading frame start

• G • U • U • C • A • G • C • C • U • A • A • G • A • . . . .

Val — Gln — Pro — Lys — . . . .

Phe — Ser — Leu — Arg — . . . .

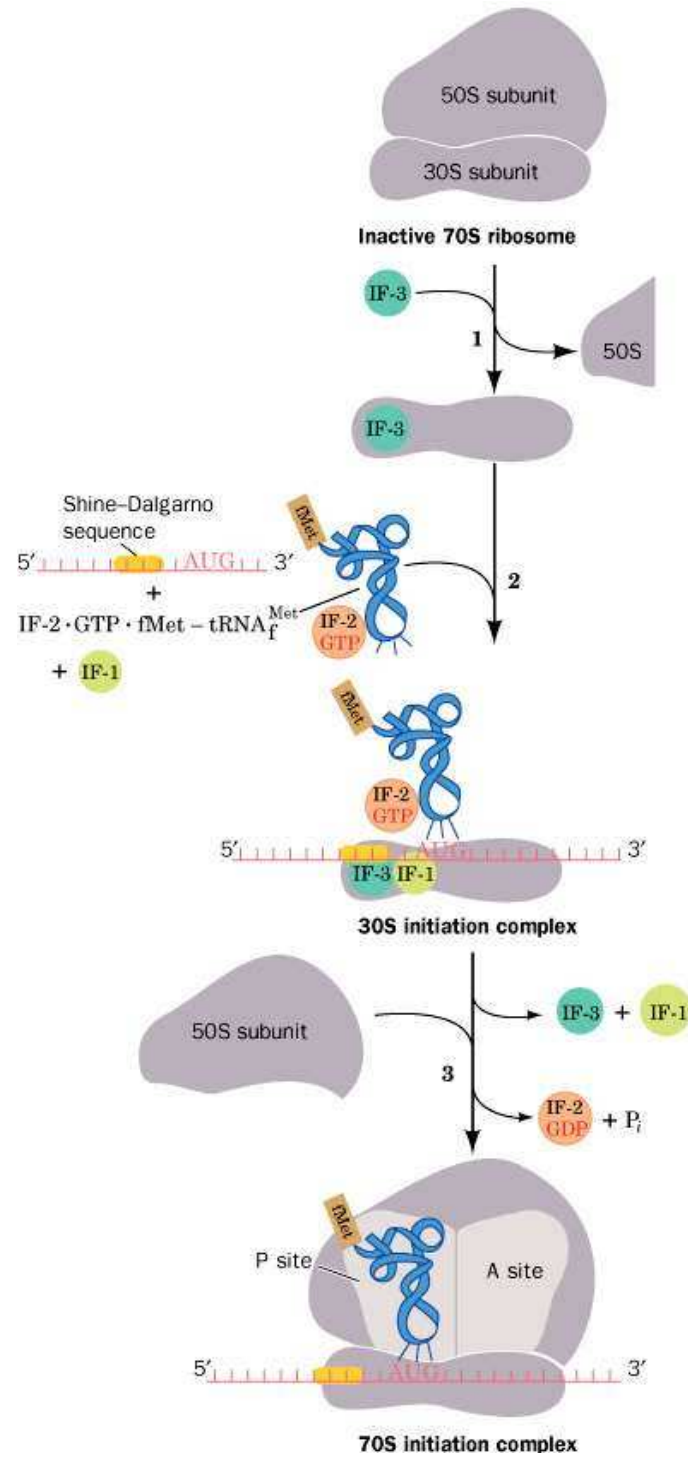
Ser — Ala — Stop

Third reading frame

Second reading frame

First reading frame

# Inicializace



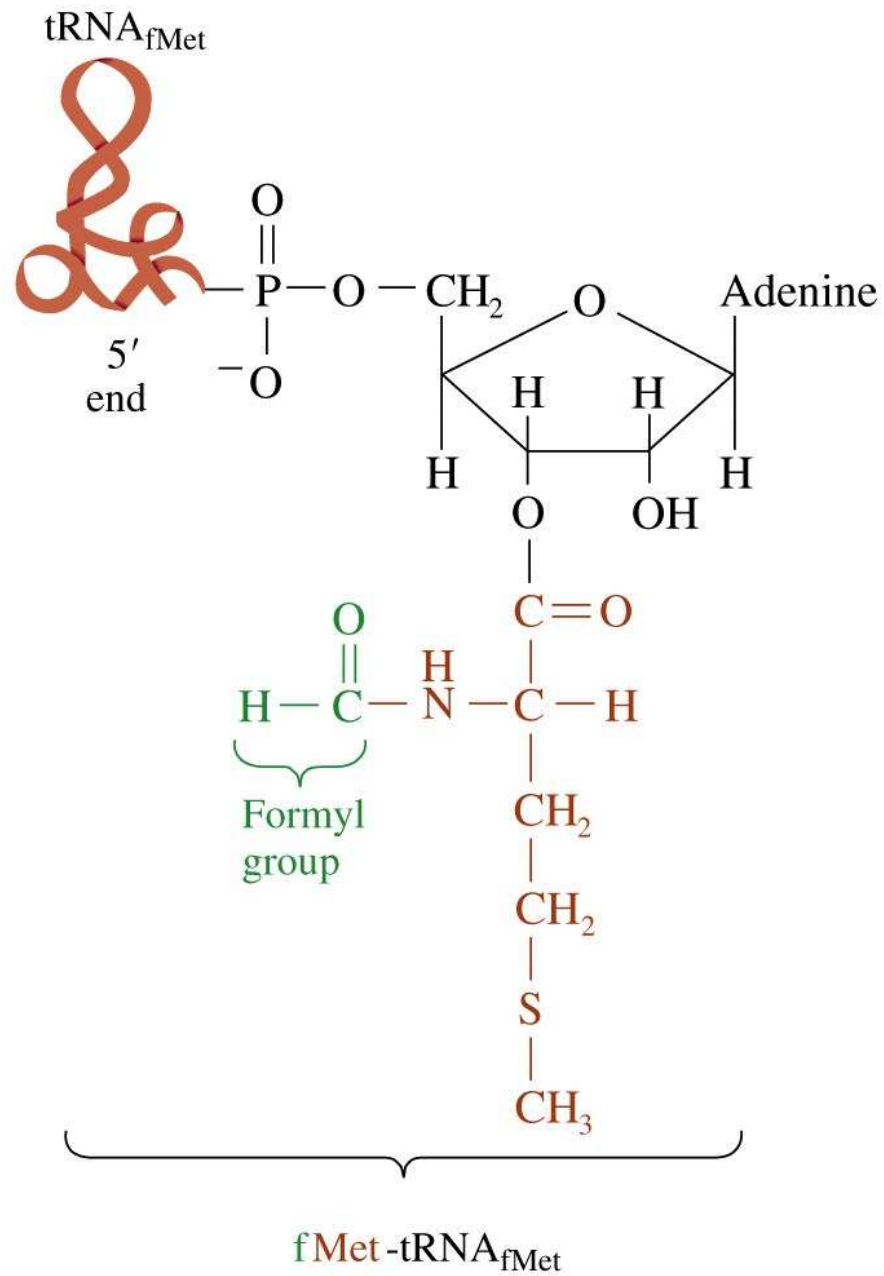
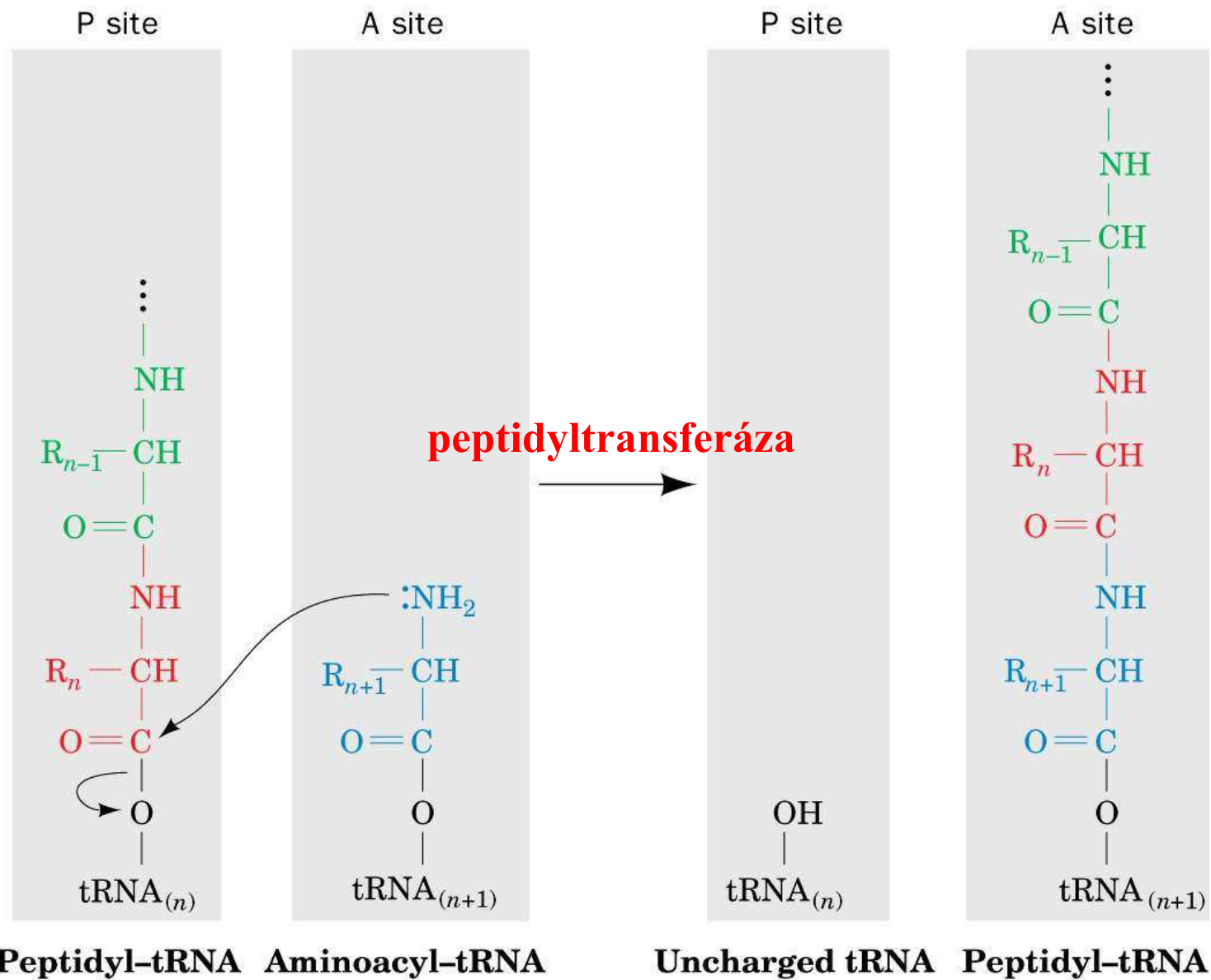


Figure 12-6 Concepts in Biochemistry, 3/e  
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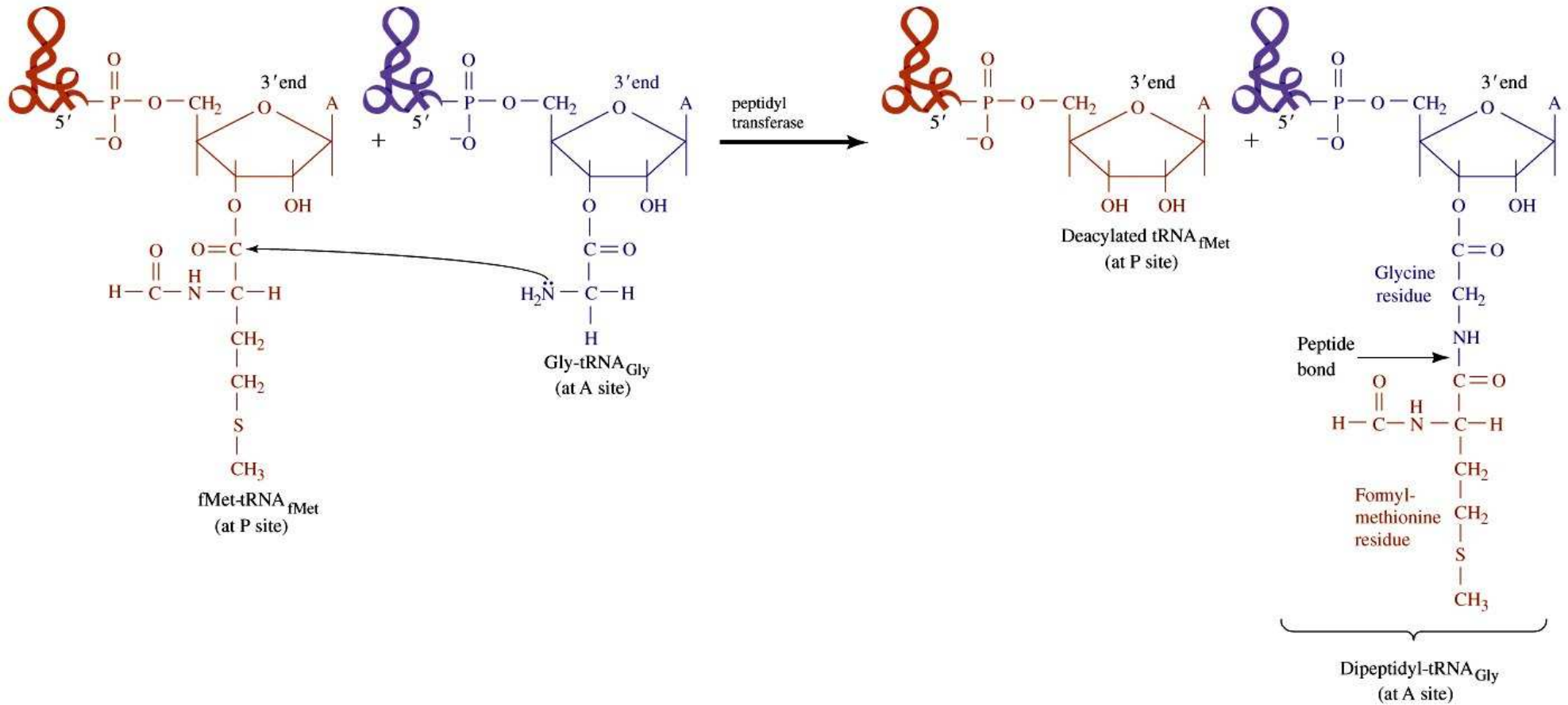
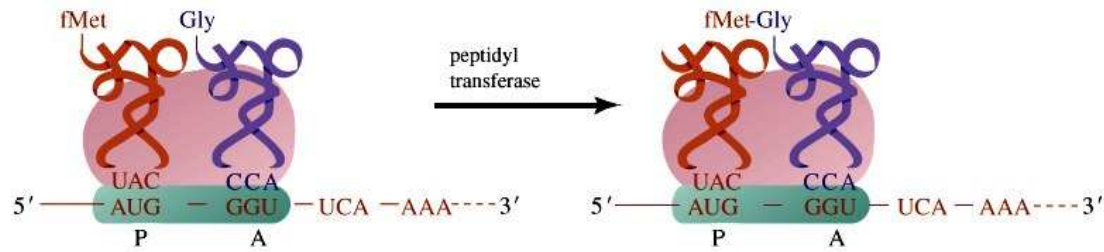
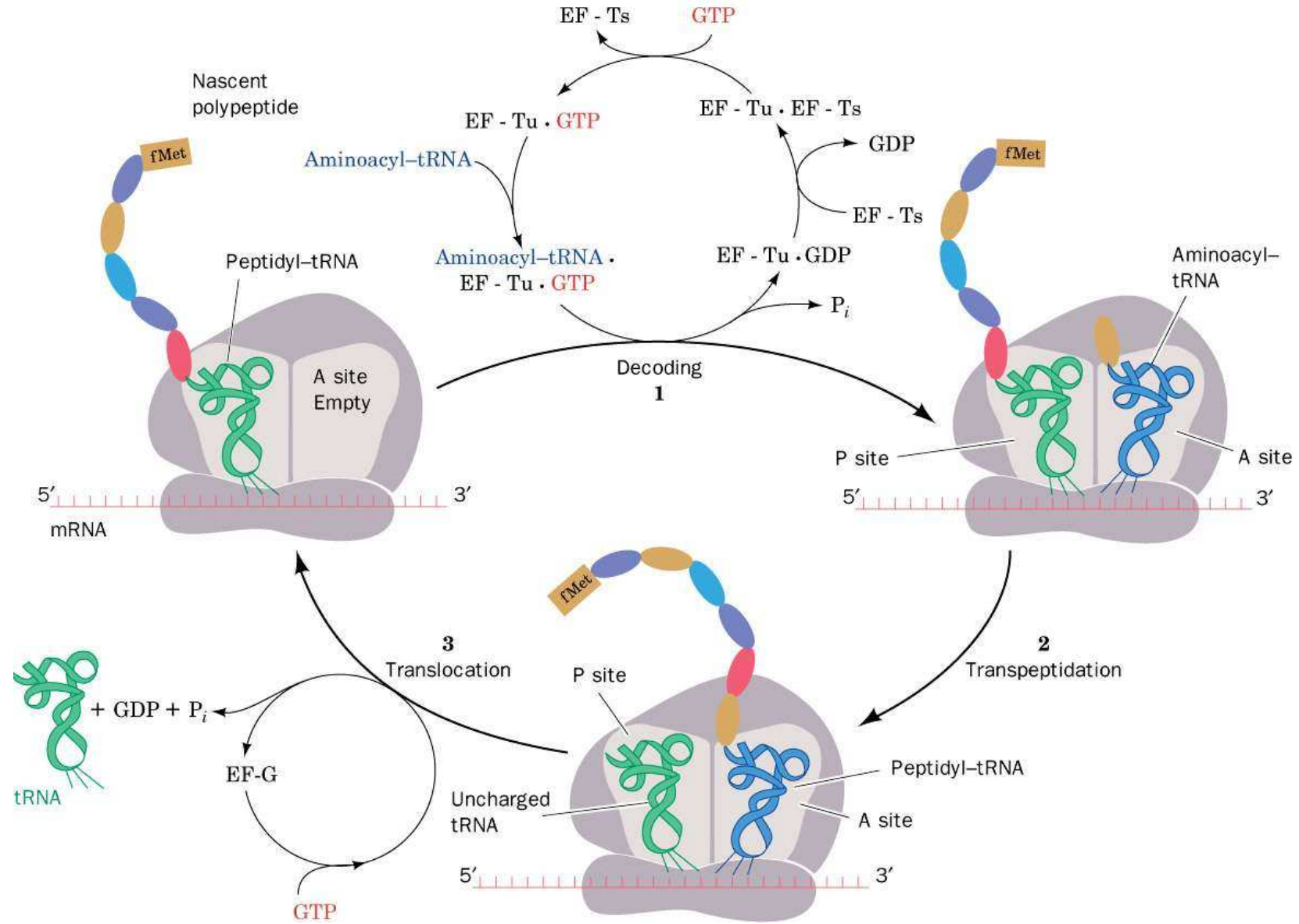
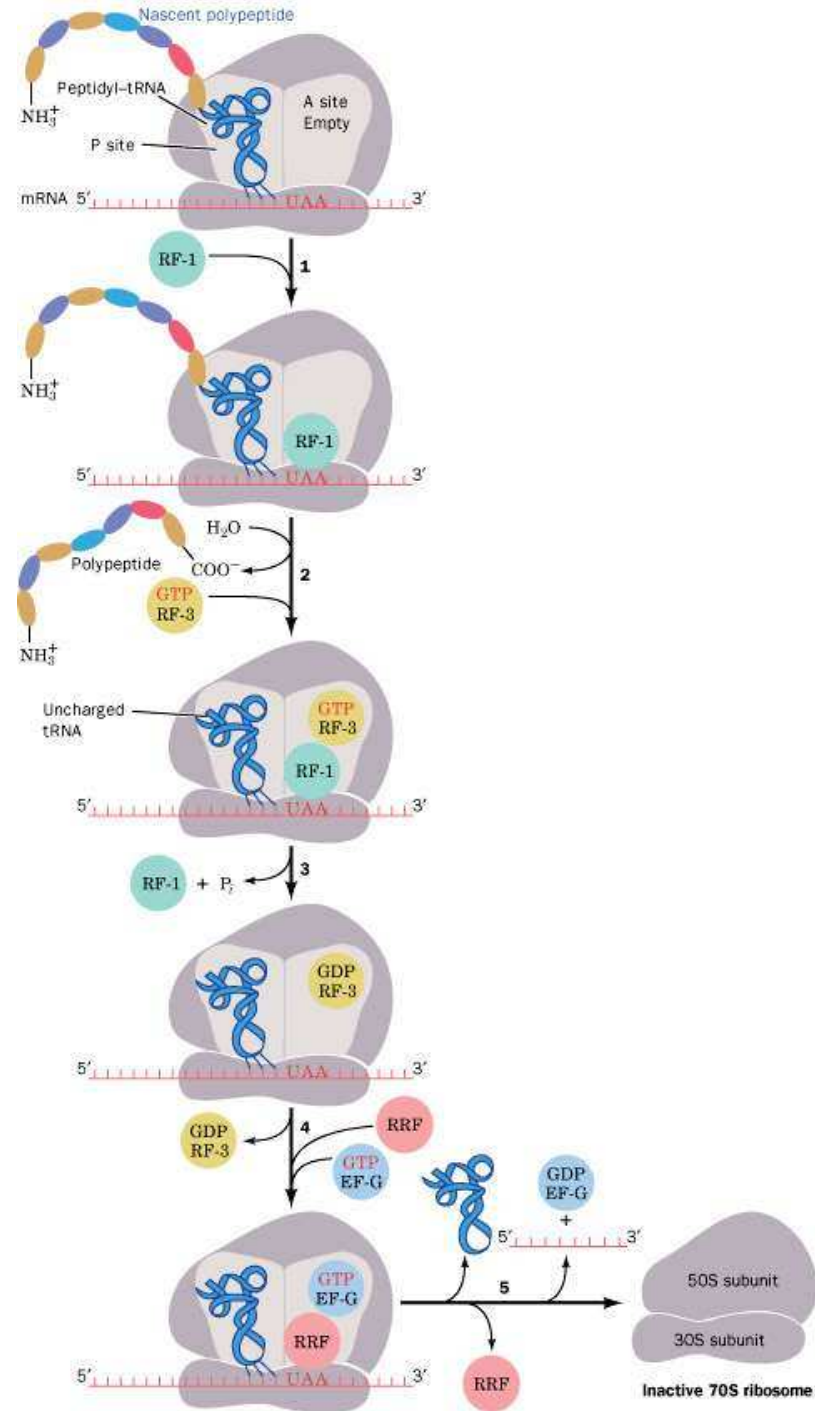


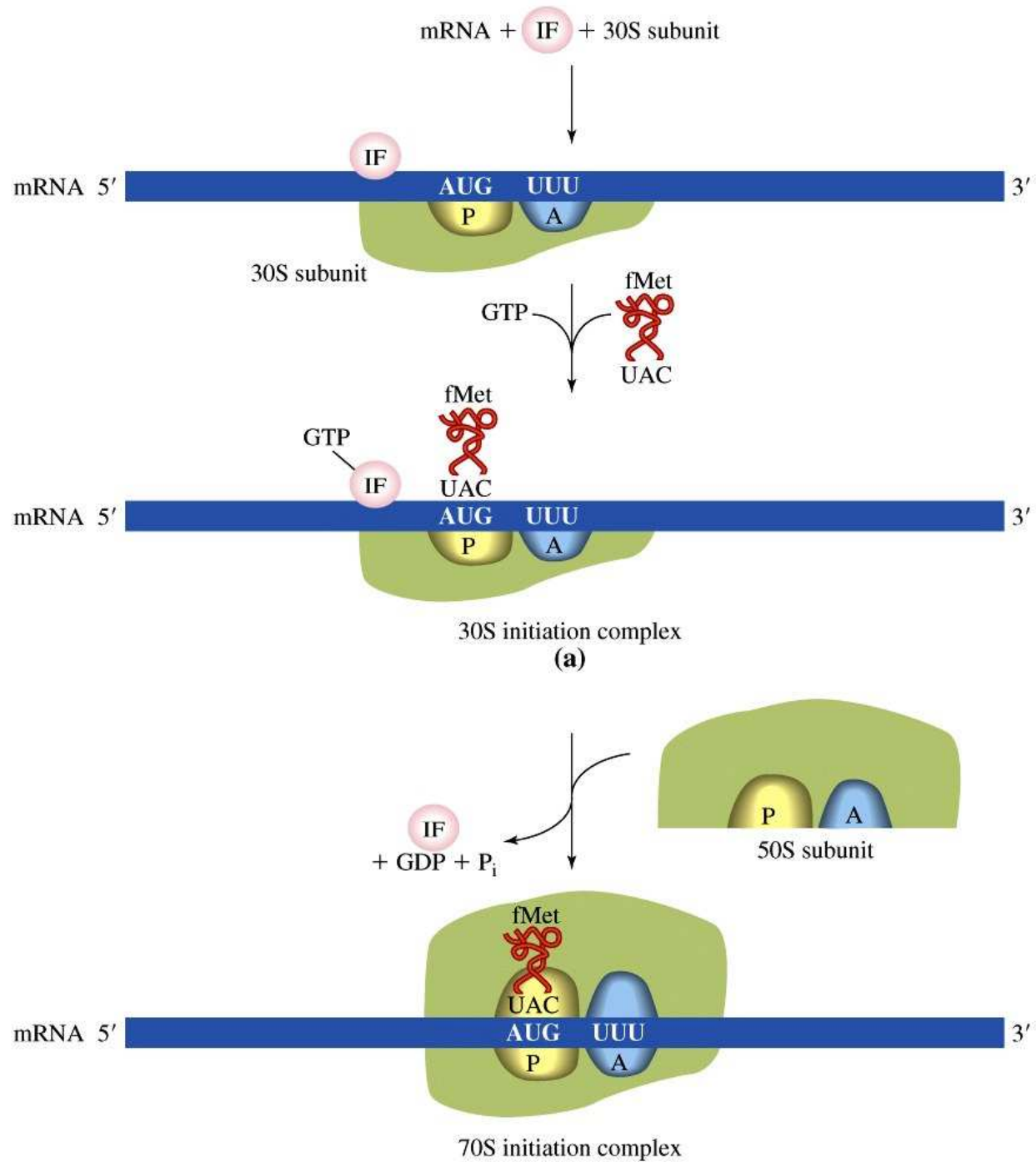
Figure 12-7 Concepts in Biochemistry, 3/e  
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# Elongate

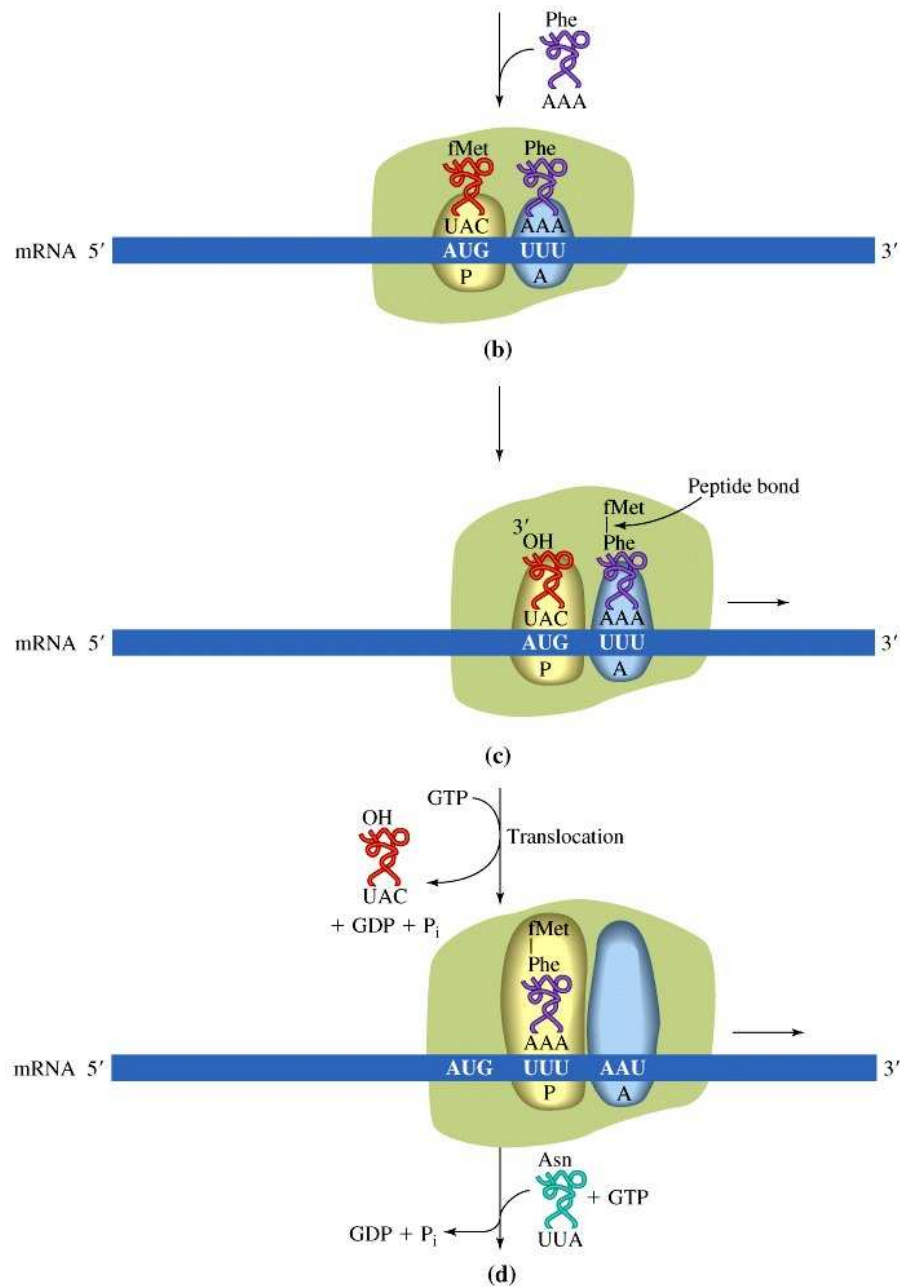


# Terminace

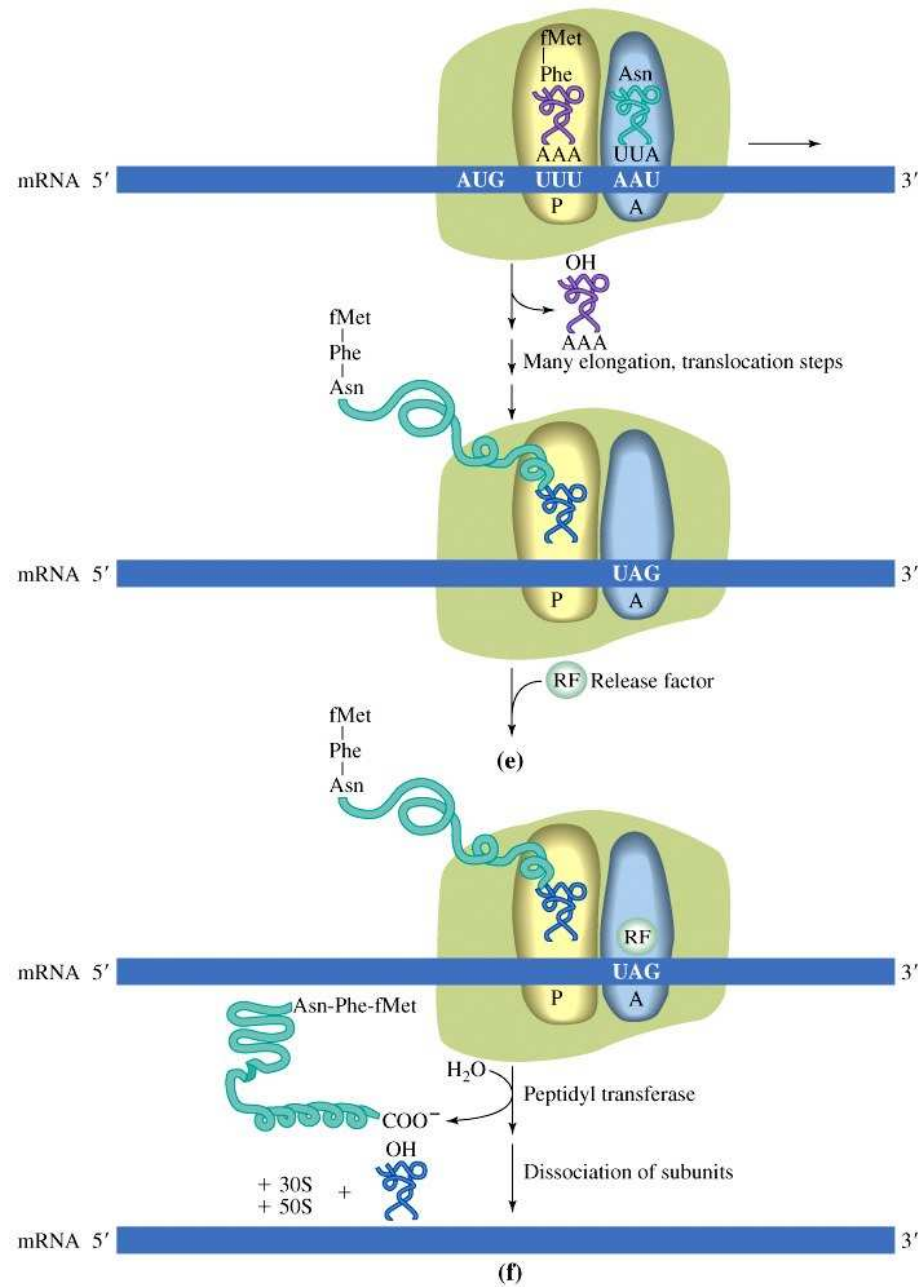




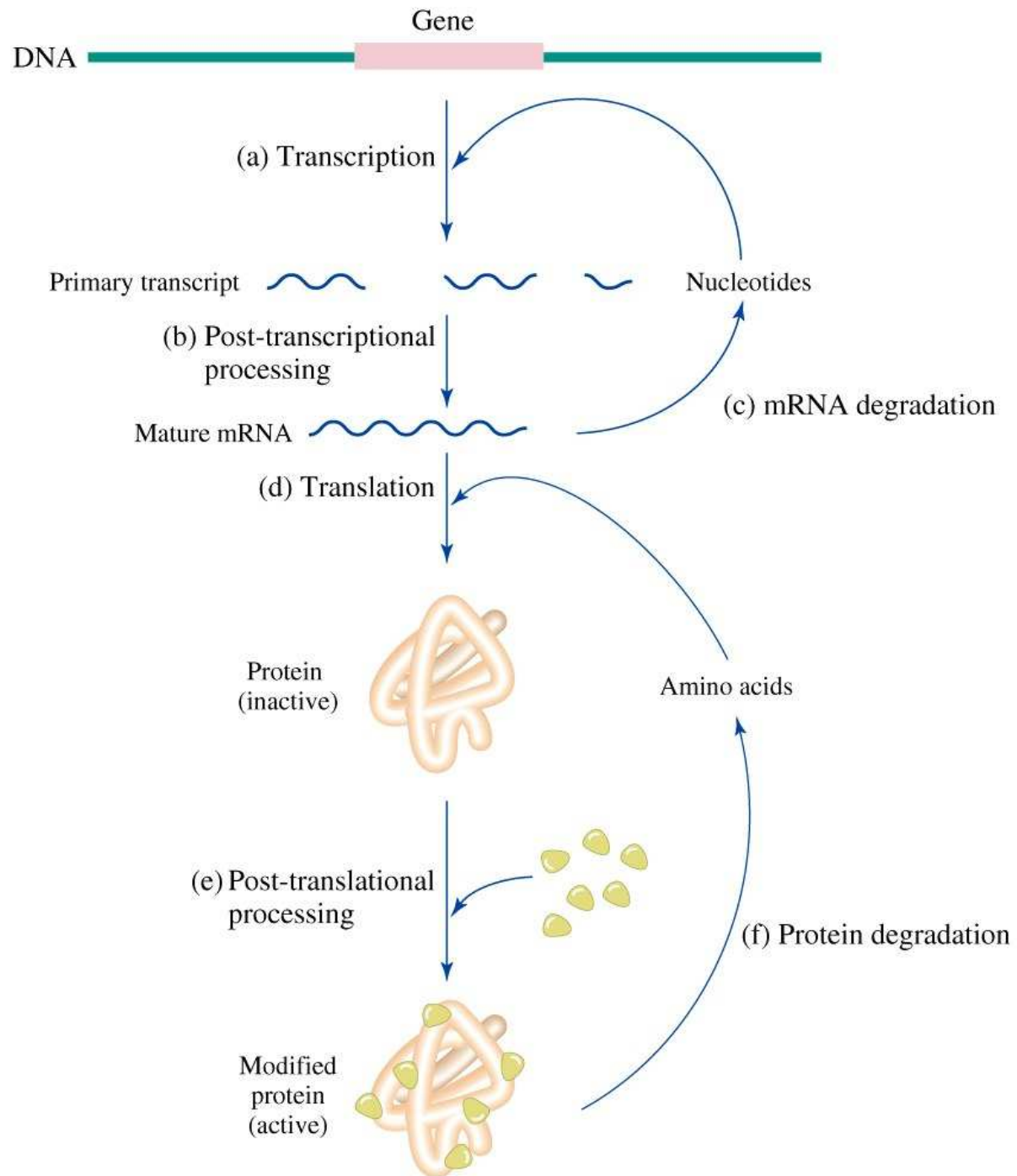
**Figure 12-5a Concepts in Biochemistry, 3/e**  
 © 2006 John Wiley & Sons



**Figure 12-5b Concepts in Biochemistry, 3/e**  
 © 2006 John Wiley & Sons



**Figure 12-5c Concepts in Biochemistry, 3/e**  
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**Figure 12-16 Concepts in Biochemistry, 3/e**  
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# Posttranslační modifikace

- fosforylace/defosforylace – enzymy kinázy a fosfatázy připojují či odpojují fosfátovou ( $\text{PO}_4^{3-}$ ) skupinu k proteinu na jeho serinové / threoninové zbytky nebo tyrosinové zbytky. Fosforylace/defosforylace často působí jako přepínač mezi aktivní a neaktivní formou proteinu.
- glykosylace – napojování sacharidů na protein. Sacharidové zbytky jsou nejčastěji připojovány na serin/ threonin – v případě tzv. O-glykoproteinů, nebo asparagin v případě N-glykoproteinů. Navázání sacharidů může stabilizovat konformaci proteinů; sacharidové složky mnoha proteinů se účastní rozpoznávacích interakcí (protein-sacharidové a nově objevené sacharid-sacharidové interakce)



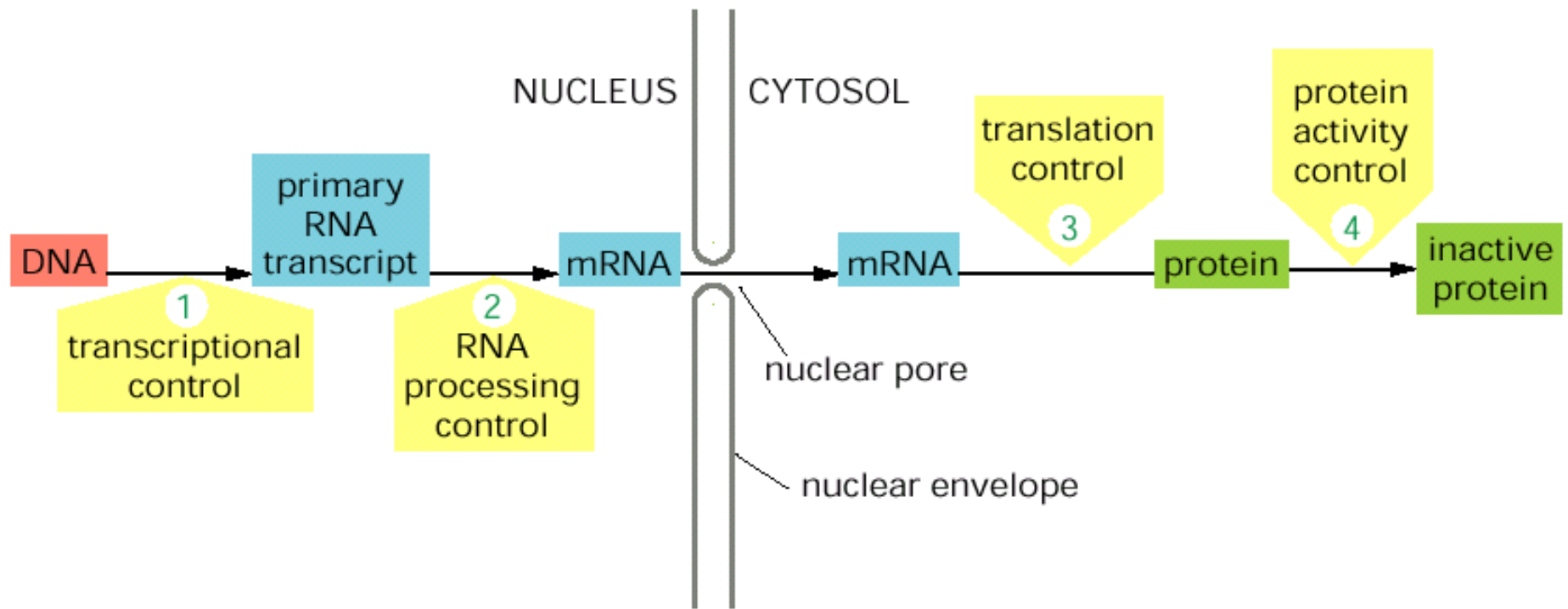
# Posttranslační modifikace

- ubikvitinace – připojení malého proteinu ubiquitinu k upravovanému proteinu přes aminokyselinu lysin ( její volný  $-NH_2$  konec). Připojování ubiquitinu na proteiny slouží jako molekulární hodiny, které určují stáří proteinu. Proteiny s mnoha navázanými ubiquitiny jsou degradovány v cytoplasmě pomocí proteazomu. Kromě této funkce, specifické navázání několika molekul ubiquitinu slouží k regulaci funkce některých proteinů
- sumoylace - připojení proteinu SUMO1, regulace funkce proteinů.
- proteolýza – odštěpení části molekuly proteinu – vede často k aktivaci nebo deaktivaci funkce proteinu.

# Posttranslační modifikace

- acetylace – acetylace koncové  $\text{-NH}_2$  lysinu snižuje jeho bazicitu a zeslabuje tak iontové interakce
- hydroxylace – hydroxylace prolinu nebo lysinu v kolagenu, slouží ke stabilizování specifické konformace molekuly kolagenu (trojitá šroubovice).
- disulfidické můstky - oxidace dvou  $\text{-SH}$  skupiny cysteinu na  $\text{-S-S-}$
- vazba prostetických skupin – např. FAD, FMN, hem, nutné pro funkci některých enzymů

# Kontrola exprese genu



**Table 12.4****Antibiotic inhibitors of protein synthesis**

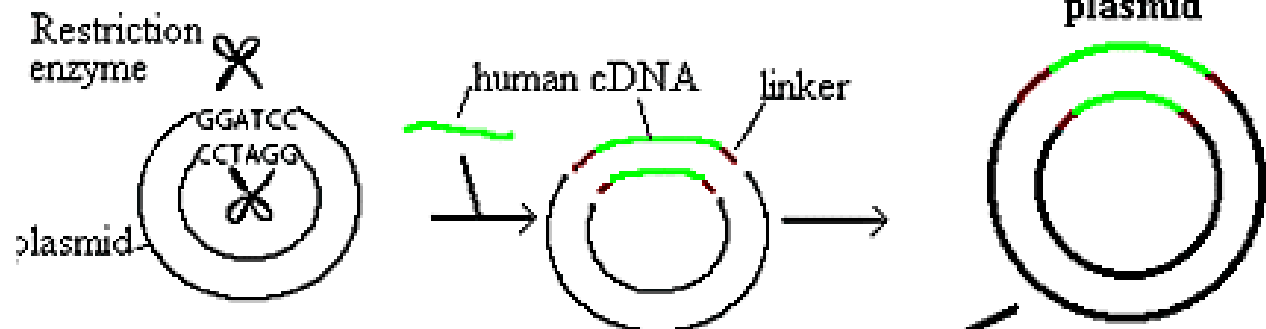
Antibiotic	Mode of Action
Puromycin	Causes early termination by mimicking the action of an aminoacyl-tRNA; acts on prokaryotes and eukaryotes
Streptomycin	Causes misreading of mRNA and inhibits initiation; acts on prokaryotes
Tetracycline	Binds to the A site of ribosomes and blocks entry of aminoacyl-tRNAs; acts on prokaryotes
Erythromycin	Binds to ribosome and inhibits translocation; acts on prokaryotes
Chloramphenicol	Binds to 50S subunit and inhibits peptidyl transferase; acts on prokaryotes
Cycloheximide	Inhibits translocation of eukaryotic peptidyl-tRNA
Linezolid	Blocks formation of 70S initiation complex in prokaryotes

**Table 12-4 Concepts in Biochemistry, 3/e**

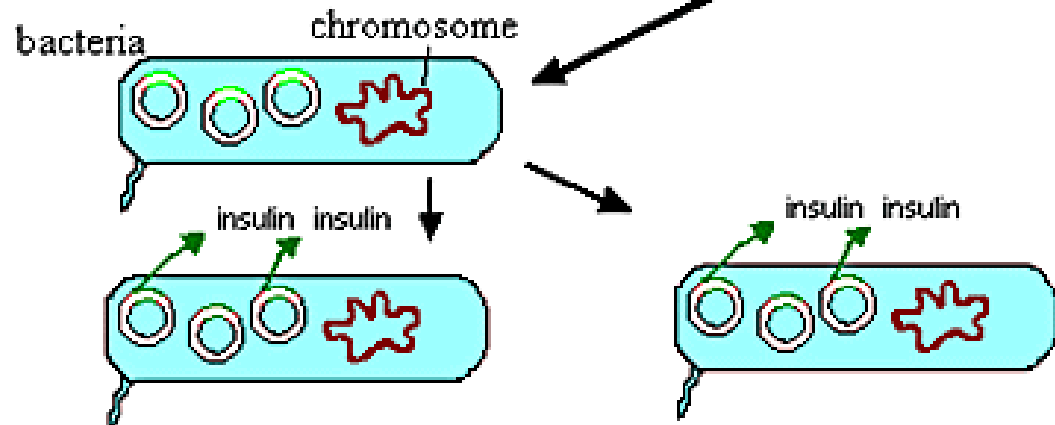
© 2006 John Wiley &amp; Sons

# Genetické inženýrství

## Transfer of the Insulin gene



## Cloning the Insulin Gene





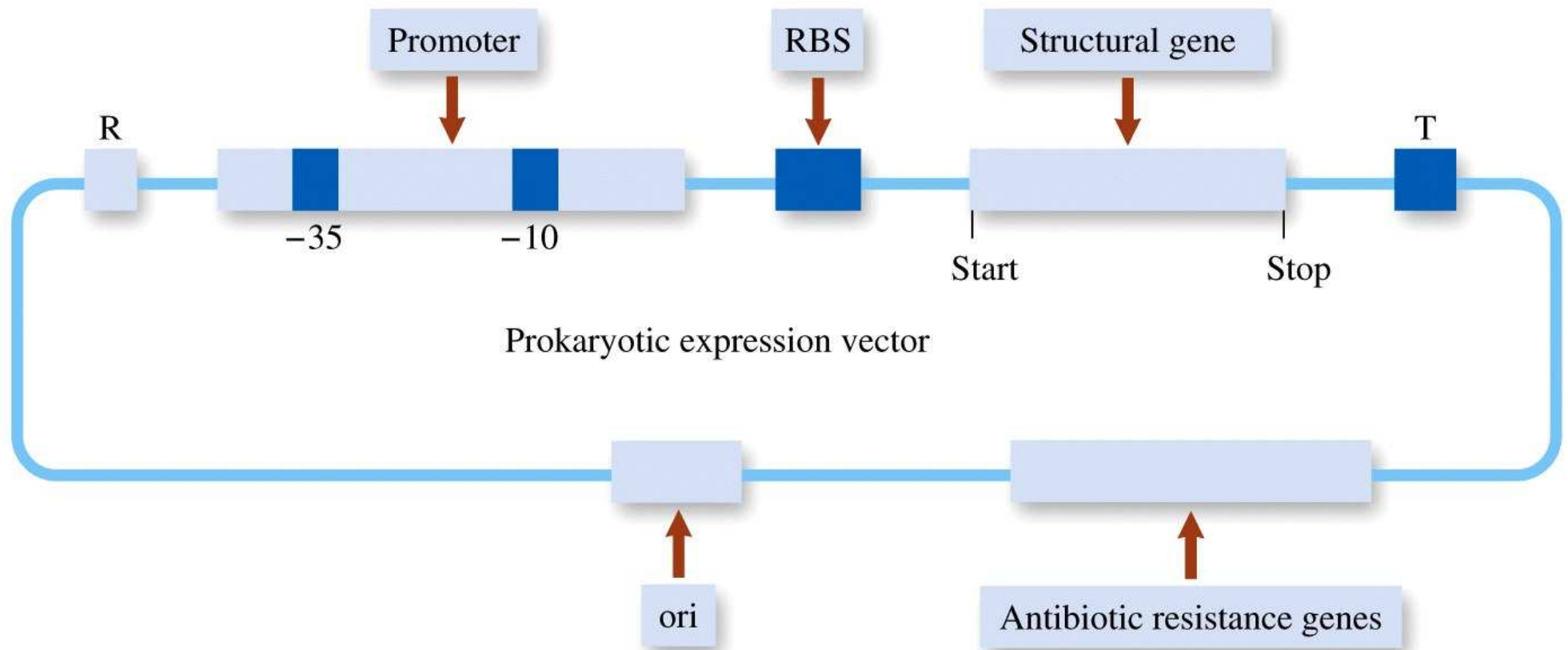
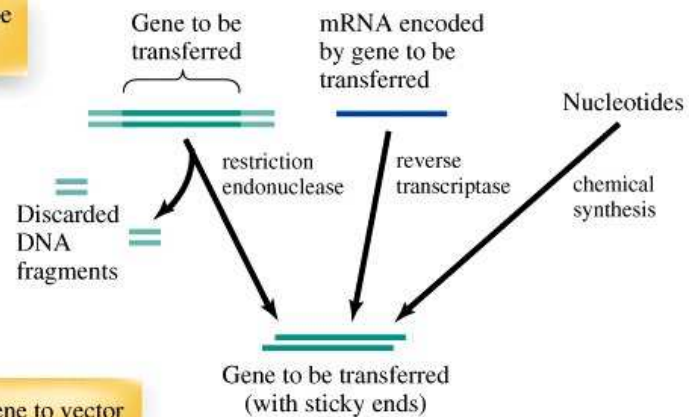
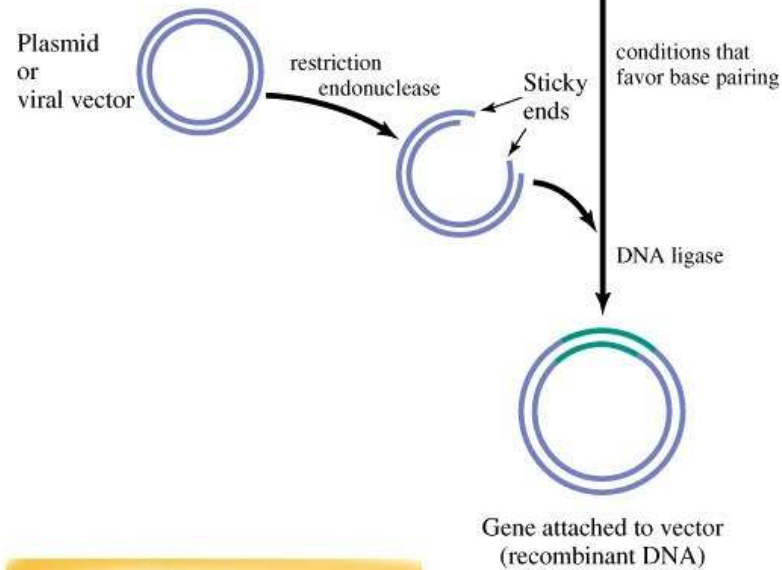


Figure 13-3 Concepts in Biochemistry, 3/e  
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Step 1. Obtain gene to be transferred.



Steps 2 and 3. Attach gene to vector



Steps 4 and 5. Introduce recombinant DNA into host cell.

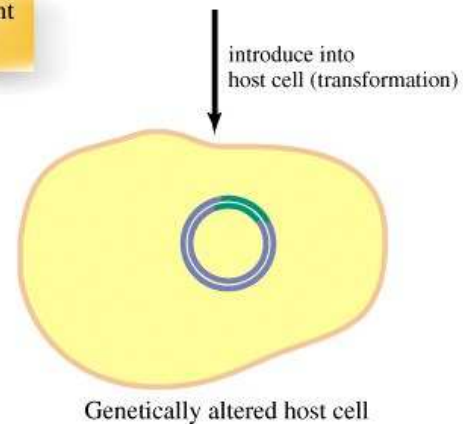


Figure 13-1 Concepts in Biochemistry, 3/e



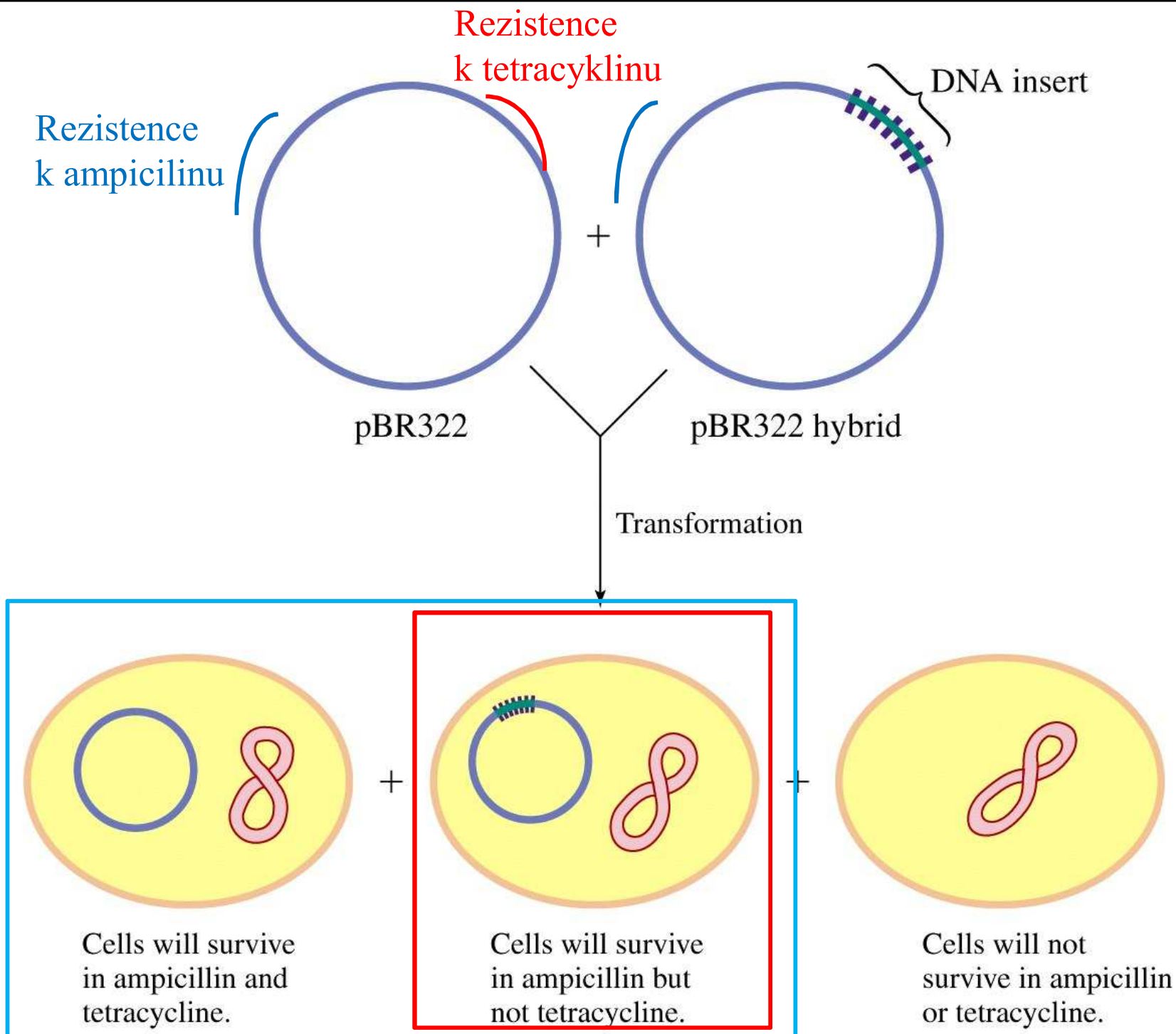


Figure 13-6 part 3 Concepts in Biochemistry, 3/e  
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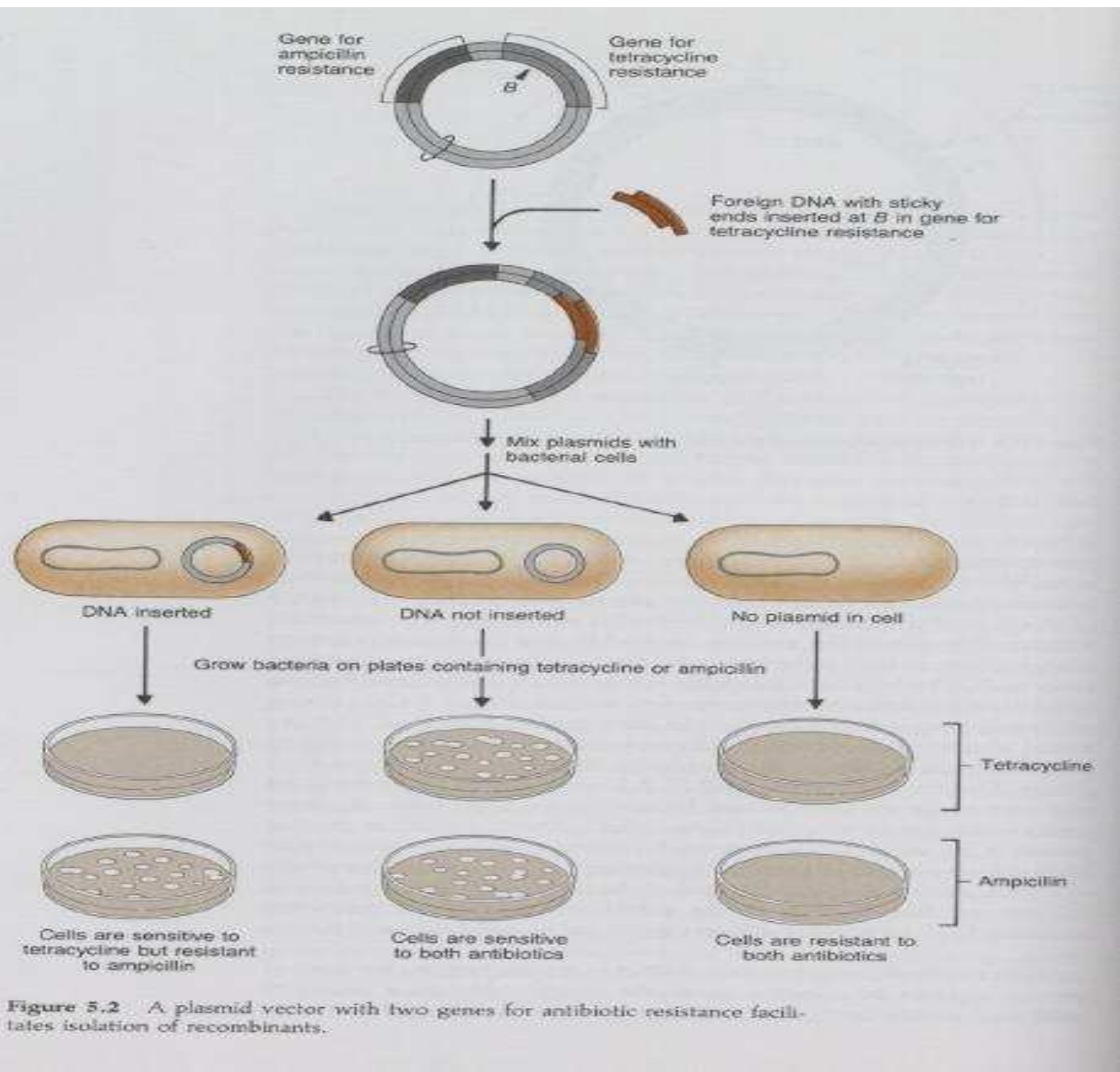
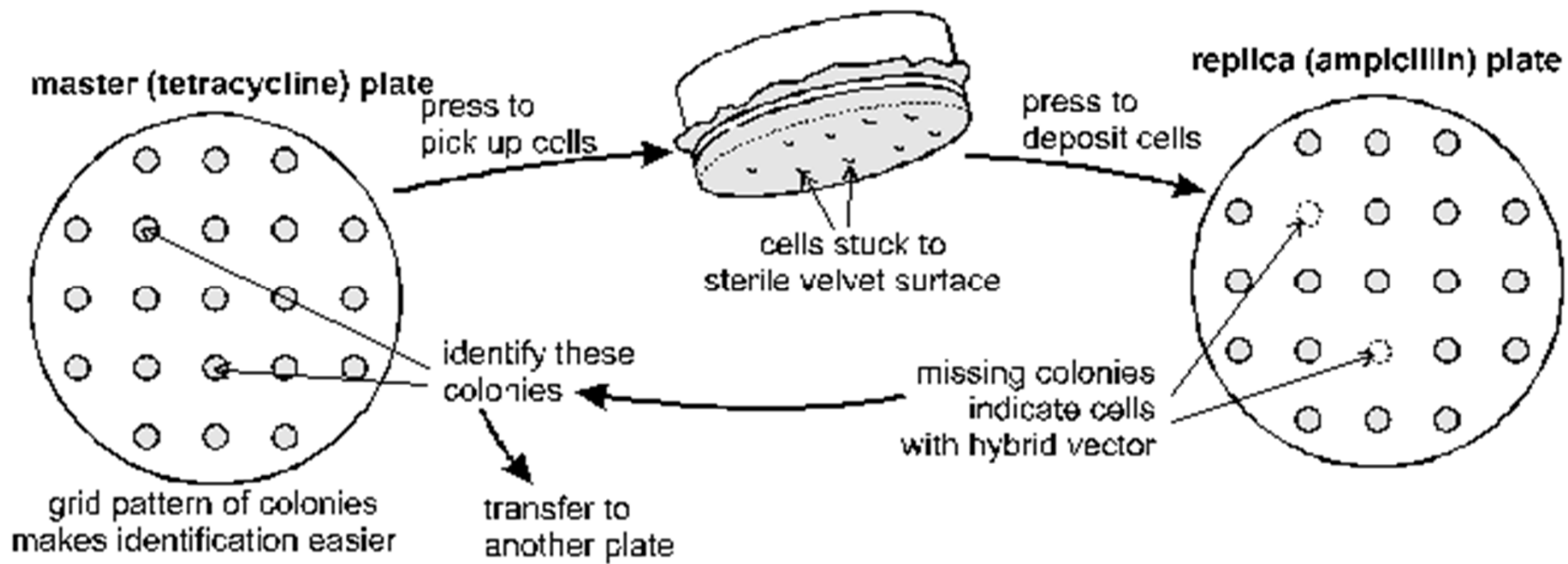


Figure 5.2 A plasmid vector with two genes for antibiotic resistance facilitates isolation of recombinants.

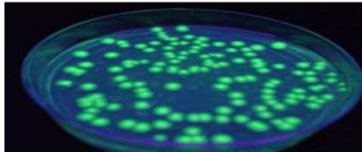


# Inklusní tělíska



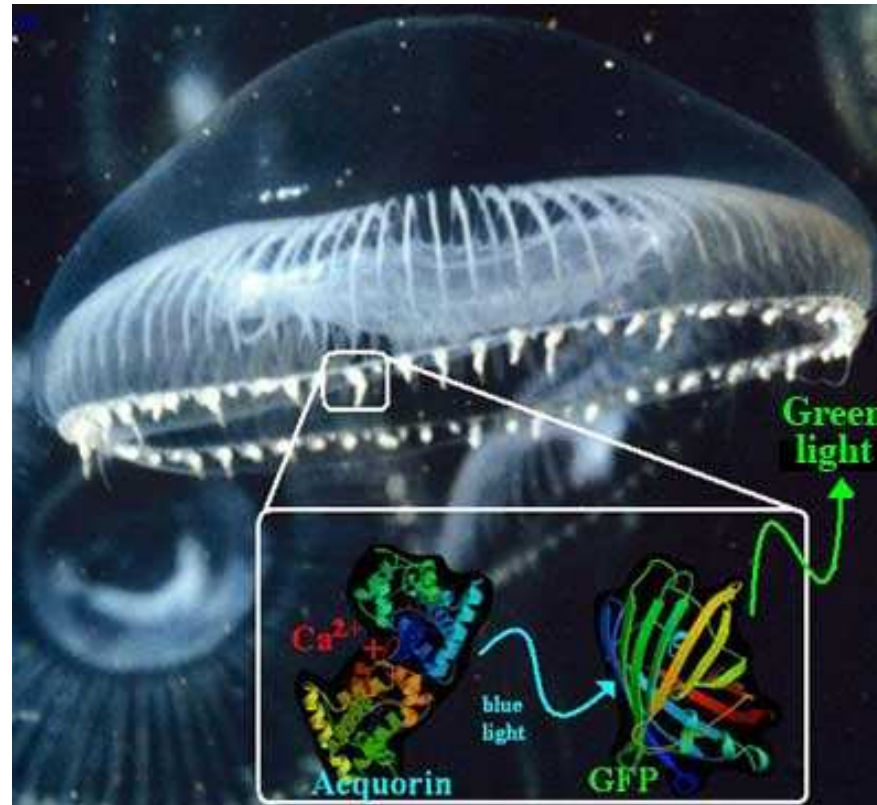
Courtesy of Teruhiko Beppu, Nihon University, Japan

# Využití AC pro purifikaci rekombinantních proteinů

řízní kotva	imobilizovaný ligand	podmínky vazby	podmínky eluce
Glutathion S-transferasa <b>GST</b>	redukovaný glutathion	Neutrální pH, nedenaturující prostředí, glutathion musí být redukovaný a GST musí být aktivní	volný redukovaný glutathion
Histidinová kotva <b>His-tag</b>	Chelatovaný nikl nebo kobalt	Neutrální pH bez redukčních a oxidačních látek	>200 mM Imidazol, nízké pH, silné chelatační činidlo
Maltose Binding Protein <b>MBP</b>	Amylosa	Neutrální pH, nedenaturující prostředí; přídavek NaCl k snížení nespecifické sorbce	maltosa
Protein A	IgG	Neutrální pH, nedenaturující prostředí	změna pH, iontové síly
Green Fluorescent Protein <b>GFP</b>	Anti-GFP antibody 	Neutrální pH, nedenaturující prostředí	nízké pH, iontová síla

# Green Fluorescent Protein

## *Aequorea victoria*



# Green Fluorescent Protein

## NC 2008

*Osamu Shimomura*

*Martin Chalfie*

*Roger Tsien*



izoloval GFP z medúzy



jak GFP navázat na jiné proteiny

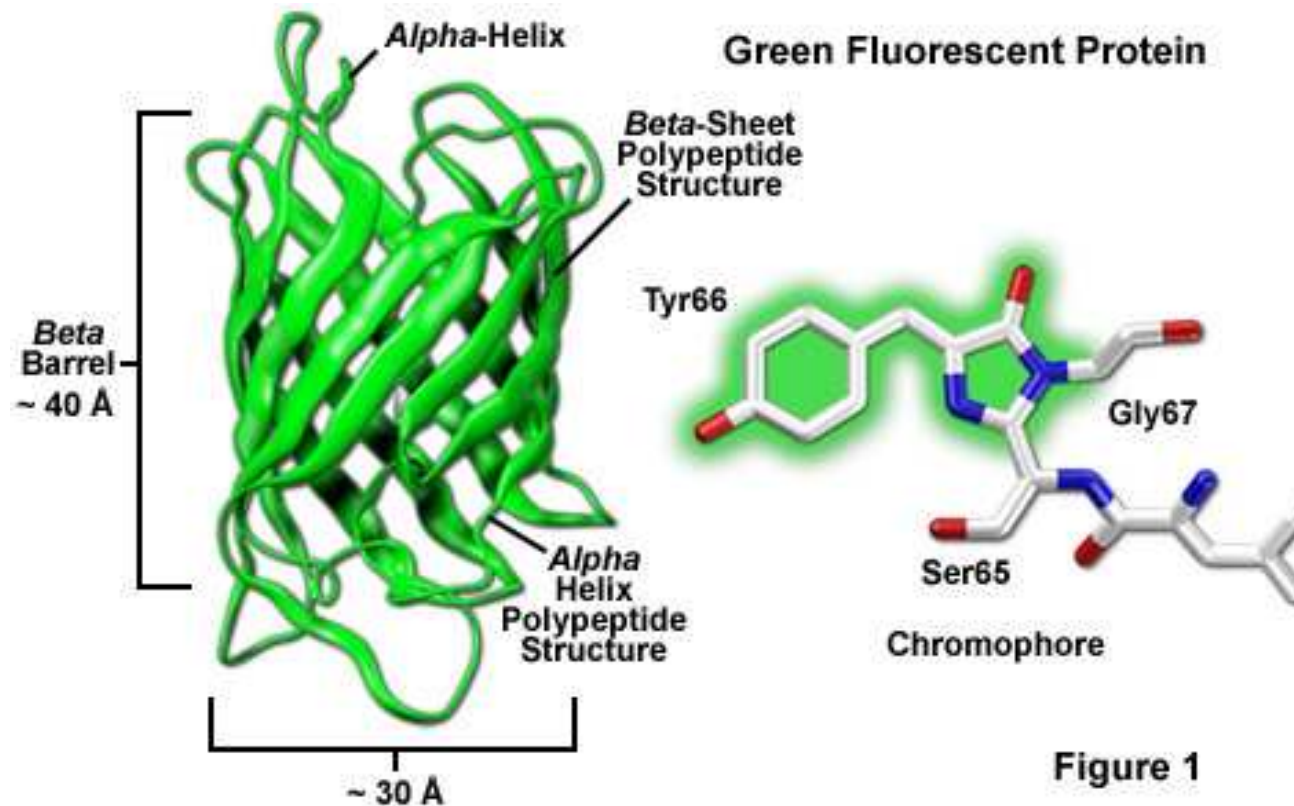


vysvětlil fluorescenci

# Green Fluorescent Protein

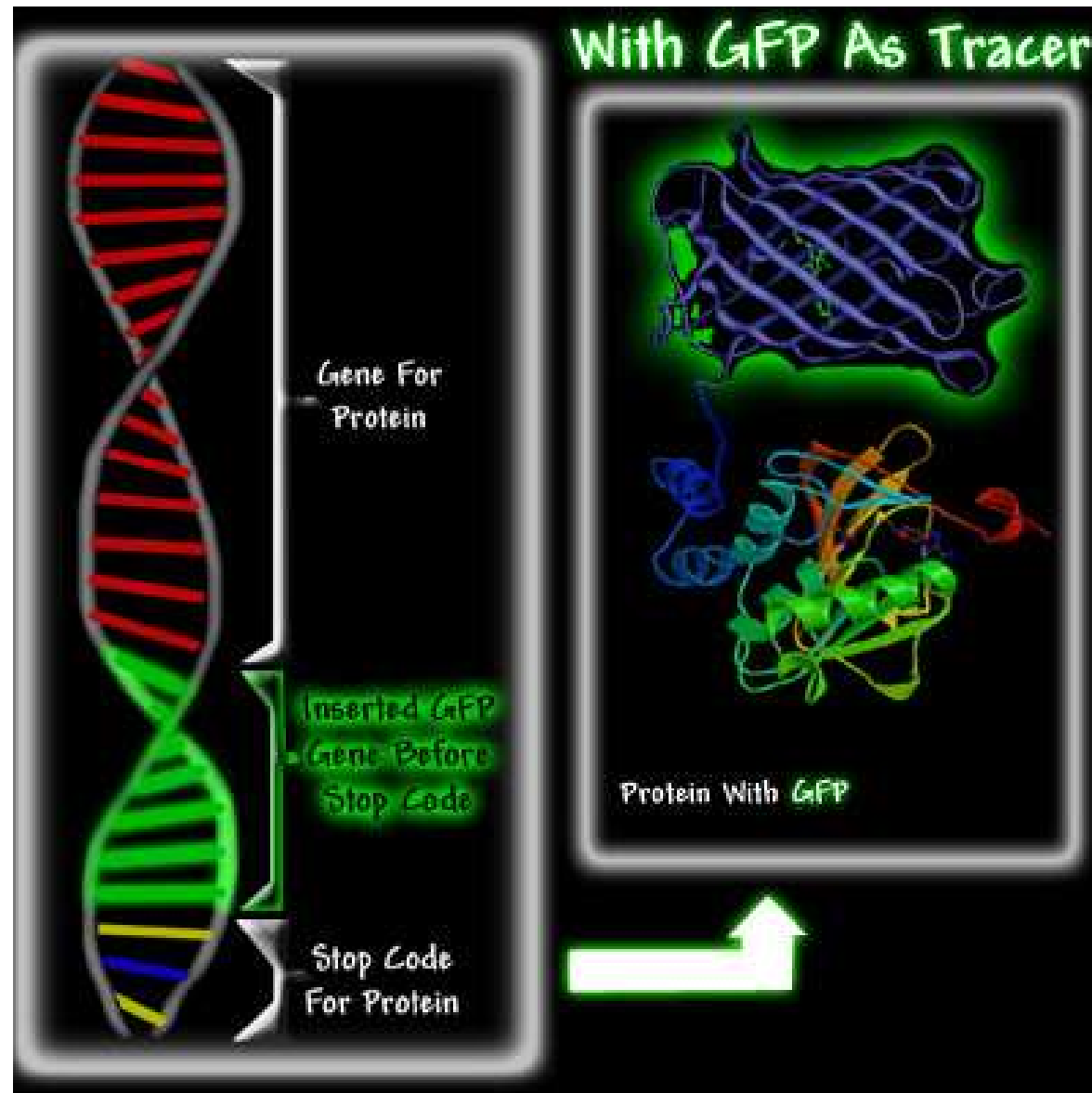
## GFP AMK sekvence – 238 AMK

MSKGEELFTGVVPLVELDGDVNGQKFSVSGEGEGDATYGKLTNFICT  
TGKLPVPWPTLVTTFSYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTI  
FYKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKMEYNYNS  
HNVYIMGDKPKNGIKVNFKIRHNIKDGSVQLADHYQQNTPIGDGPVLLP  
DNHYLSTQSALS KDPNEKRDHMILLEFVTAARITHGMDELYK





# Green Fluorescent Protein

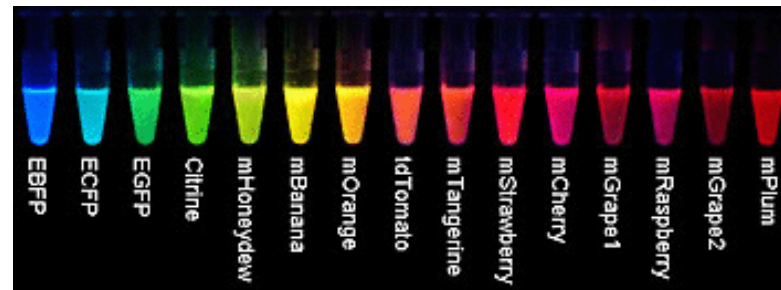


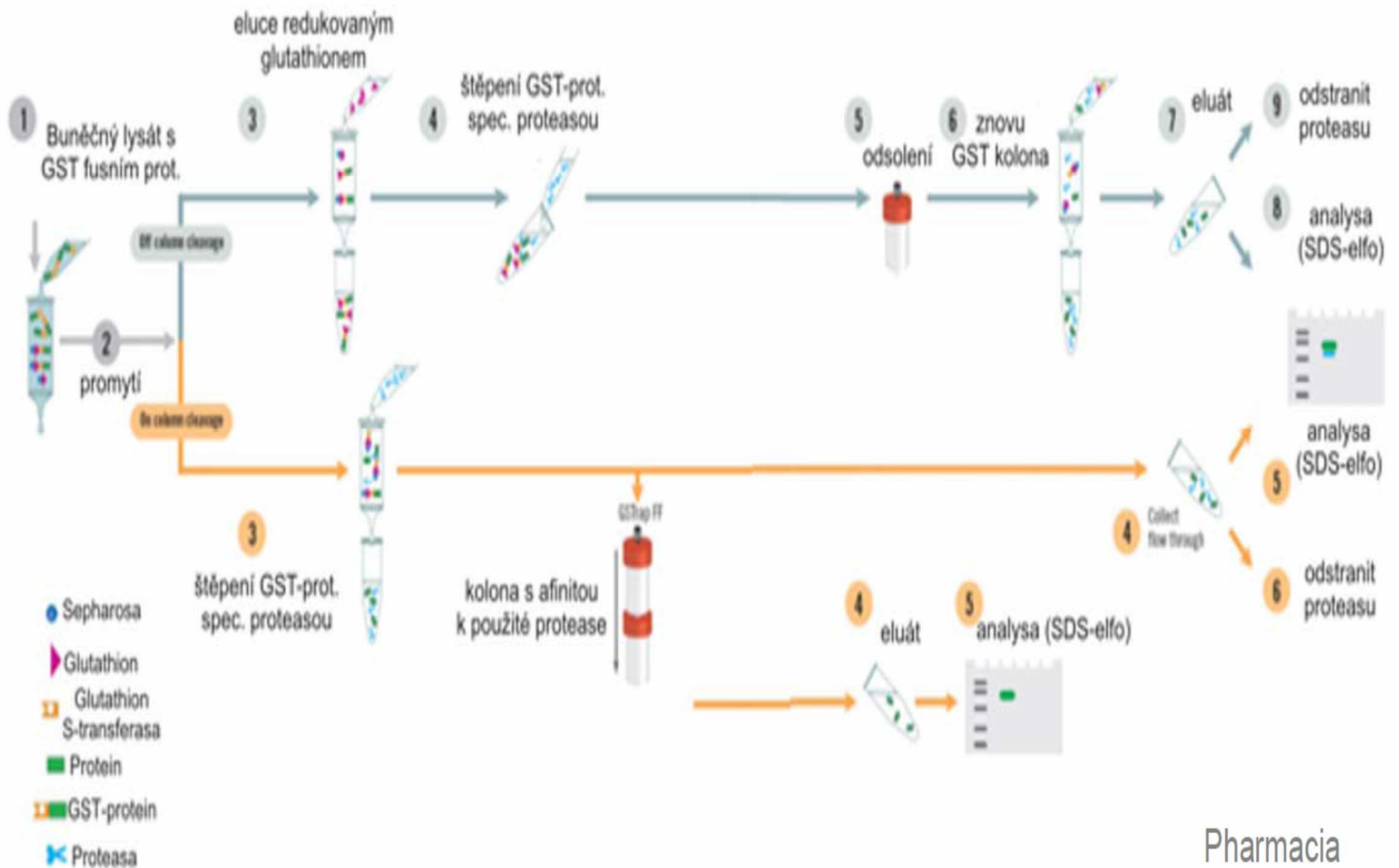
Reportérový gen

# Green Fluorescent Protein



# Green Fluorescent Protein



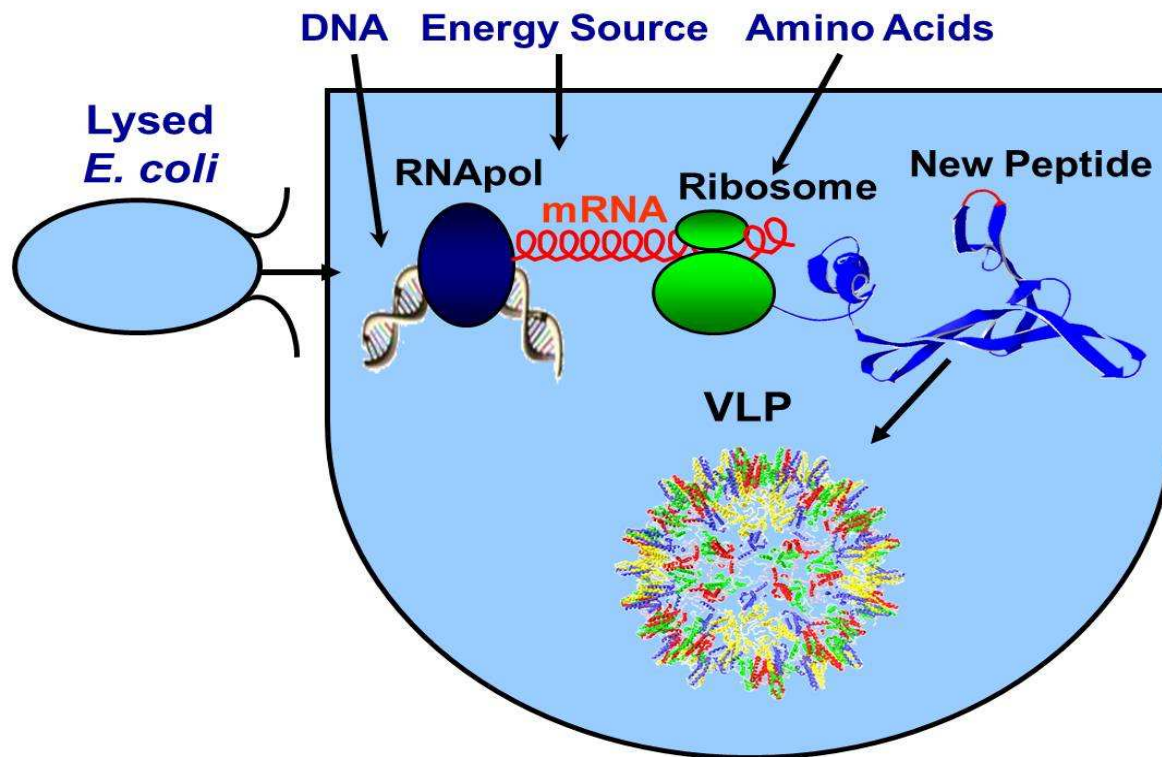


Pharmacia

**Table 13.1****Recombinant proteins and their use**

Protein	Use
Human insulin	Treatment of diabetes
Human somatotropin (growth hormone)	Treatment of dwarfism
Bovine somatotropin (BST)	Enhances milk production in dairy cattle
Porcine somatotropin	Enhances growth in pigs
Pulmozyme (DNase)	Treatment of cystic fibrosis
Tissue plasminogen activator (TPA)	Treatment of heart attack, stroke victims; dissolves blood clots
Erythropoietin	Stimulates erythrocyte production in anemia
Interferons	Antiviral agent; treatment of cancers
Atrial natriuretic factor	Reduces high blood pressure
Leptin	Treatment of obesity
Hepatitis B vaccine	Treatment of hepatitis
Herceptin	Monoclonal antibody to treat metastatic breast cancer
Superoxide dismutase	Destroys reactive oxygen species; treatment of arthritis

# Cell-free protein synthesis

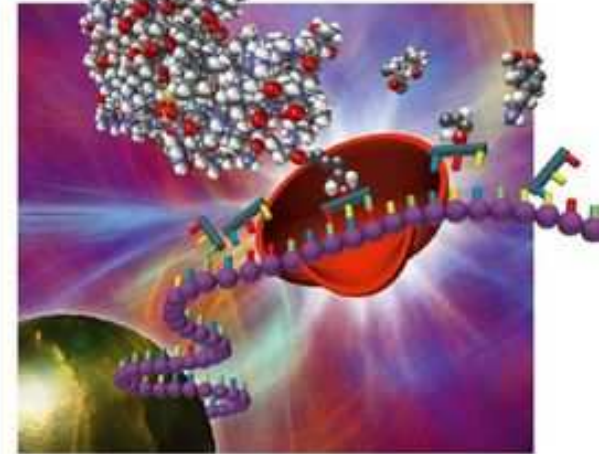


Edited by Alexander S. Spirin  
and James R. Swartz

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## Cell-free Protein Synthesis

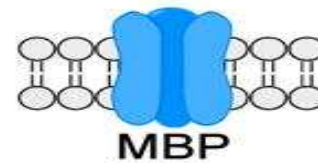
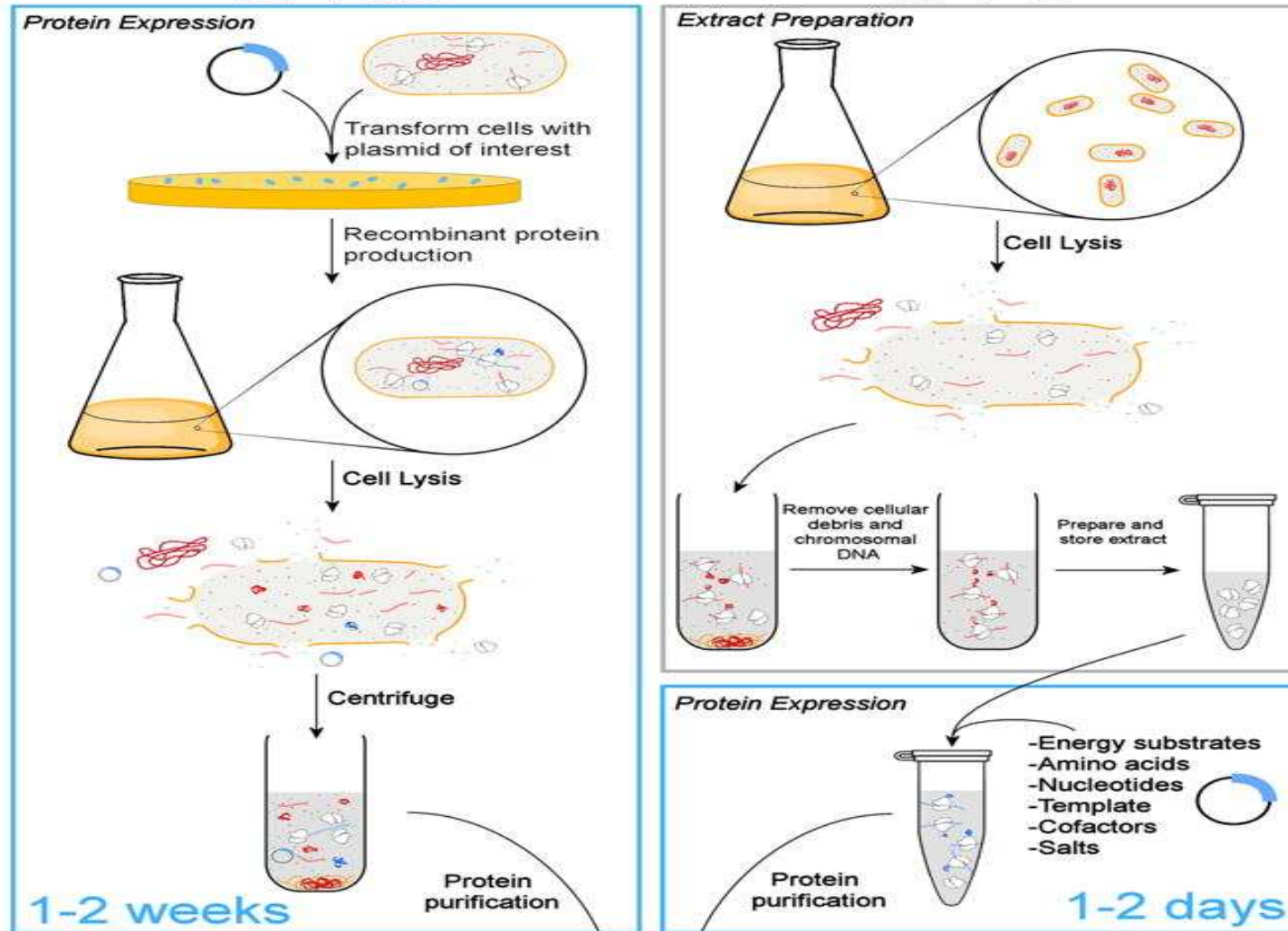
Methods and Protocols



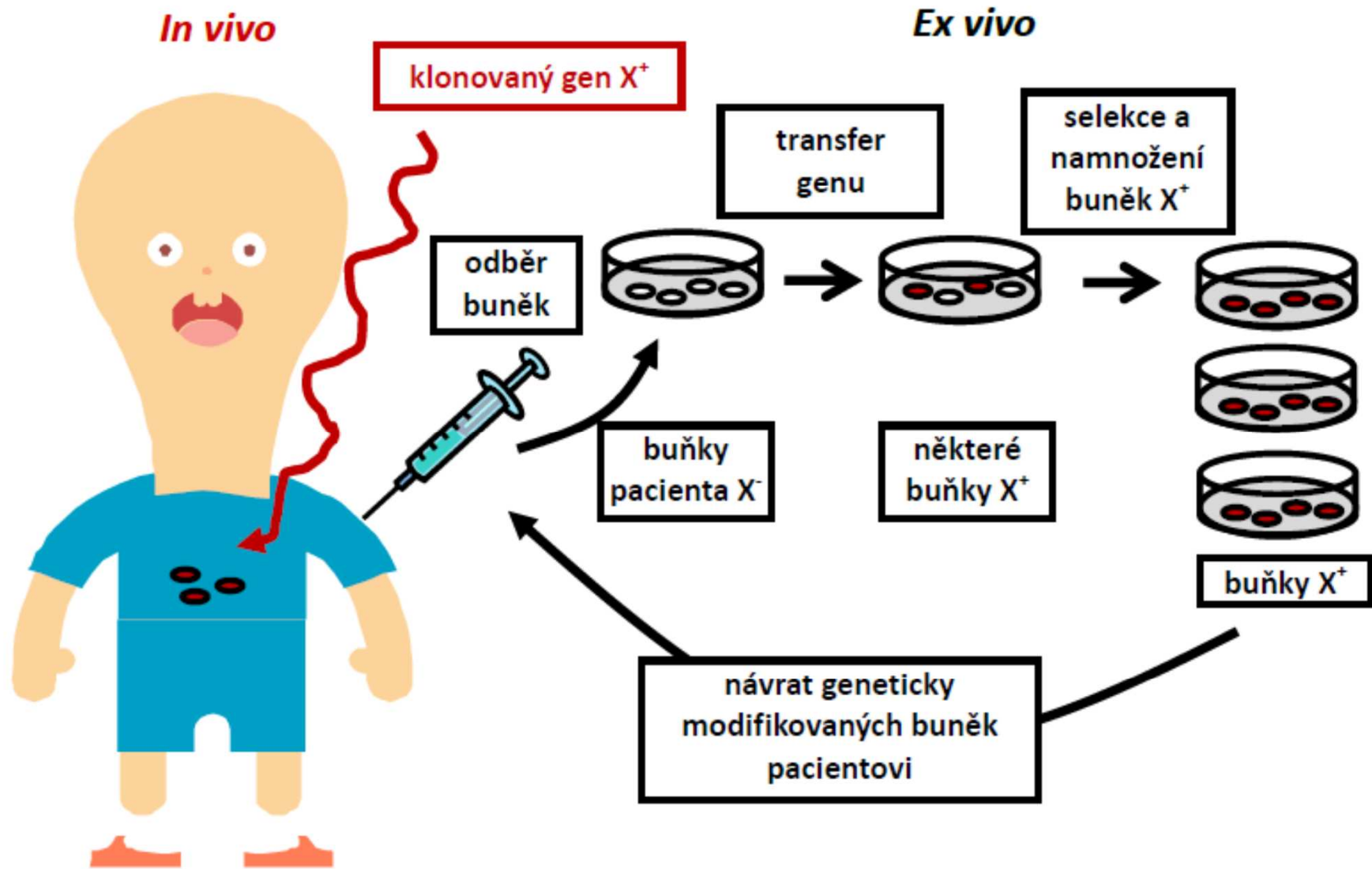
# Cell-free protein synthesis

*In vivo*

*CFPS*

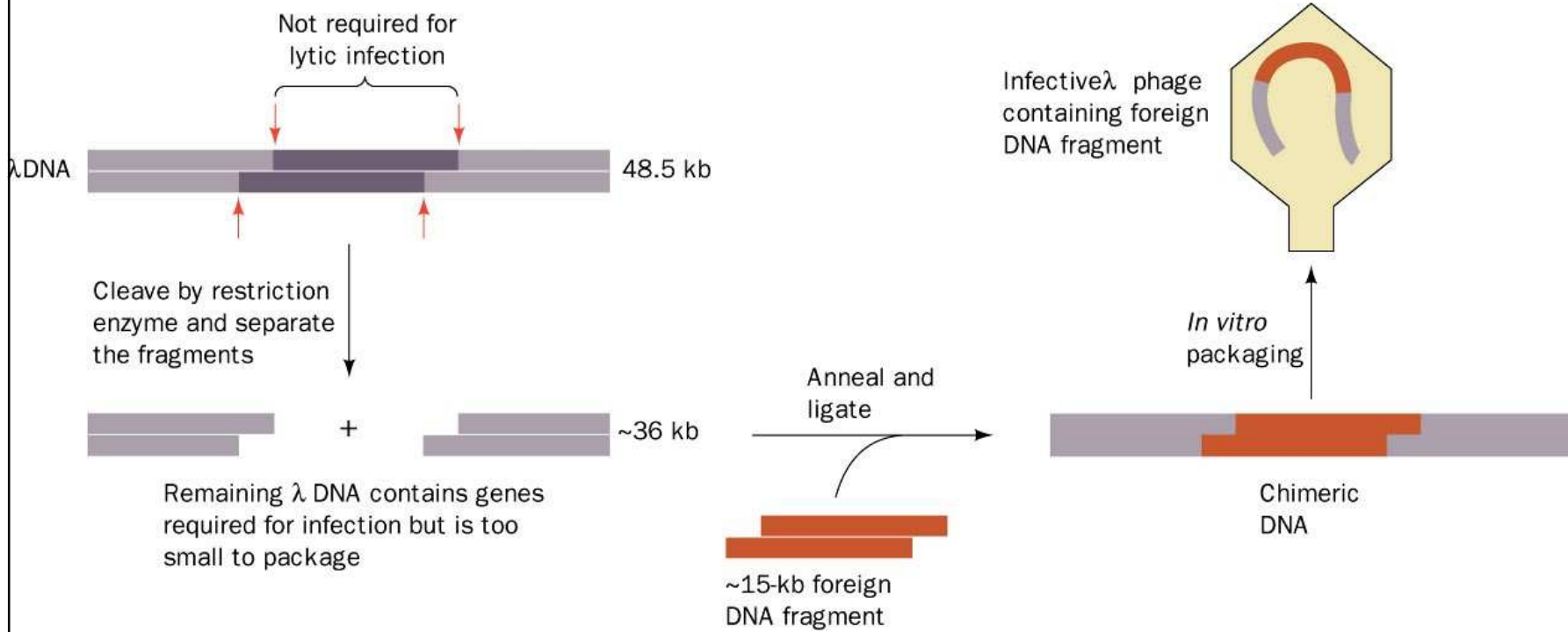


# Genová terapie





# Genová terapie



# Genová terapie

- Klasická genová terapie
  - zajistit produkci látky, která chybí
  - aktivovat buňky imunitního systému ve snaze pomoci odstranit nemocné buňky
- Neklasická genová terapie
  - inhibice exprese genu asociovaných s patogenezí
  - korekce genetického defektu a obnovení normální genové exprese

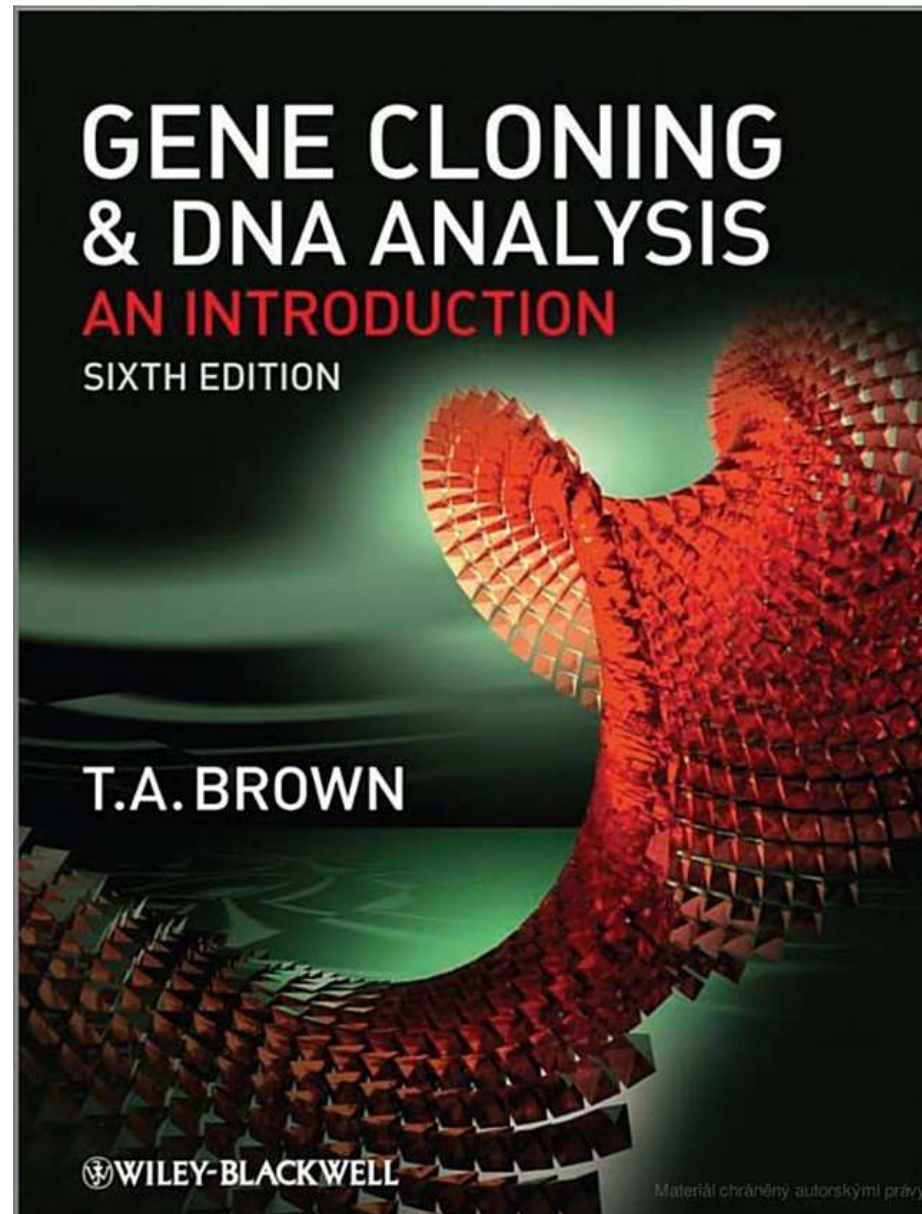
# Člověk: možní kandidáti na genovou terapii

- nemoci, které jsou způsobené defektem v jediném genu: ADA deficiency, cystická fibrosa, hemophilia, familiální hypercholesterolemie, alpha-1 antitrypsin deficiency
- nemoci, které vznikají nesprávnou interakcí několika genů: diabetes, hypertenze

**Table 13.2****Human gene therapy projects currently in or preparing for clinical trials**

Disease	Defective Protein, Gene, or Inserted DNA
Lesch-Nyhan syndrome	Hypoxanthine–guanine phosphoribosyl transferase (Section 19.5)
Amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease)	Superoxide dismutase
Adrenoleukodystrophy (ALD)	Very long chain fatty acid synthetase transporting protein
Severe combined immunodeficiency (SCID)	Adenosine deaminase
$\beta$ -Thalassemia	$\beta$ -Globin, a polypeptide of hemoglobin
Familial hypercholesterolemia	Liver receptor for low density lipoprotein (LDL) (Section 18.5)
Hemophilia	Blood-clotting factors
Duchenne's muscular dystrophy	Dystrophin
AIDS	The gene to produce a ribozyme that cleaves HIV RNA
Inherited emphysema	$\alpha_1$ -Antitrypsin
Cystic fibrosis	A product that unclogs lung mucus is inhaled in a nasal spray
Cancer	E1A and p53 tumor suppressor genes

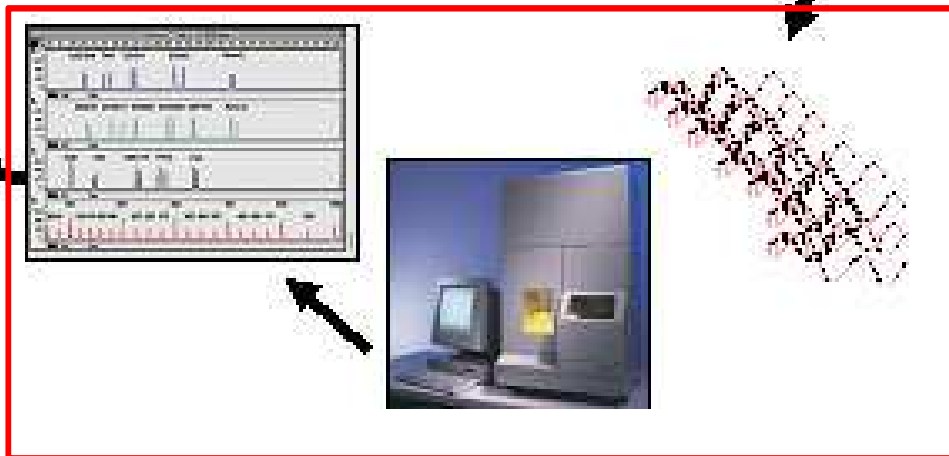
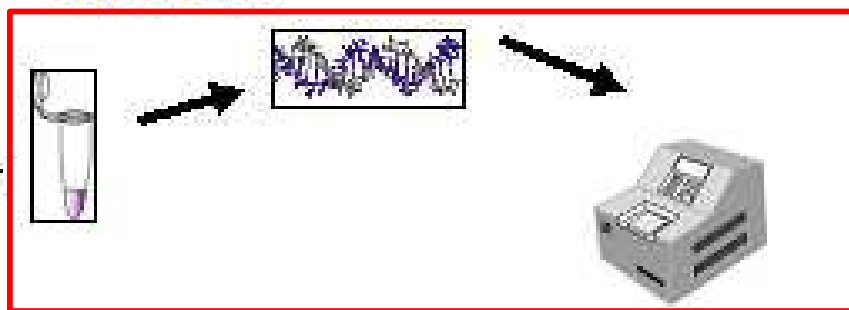
# Literatura



# Genetická daktyloskopie

The Process of DNA Typing  
via PCR

1 ng or less of starting material

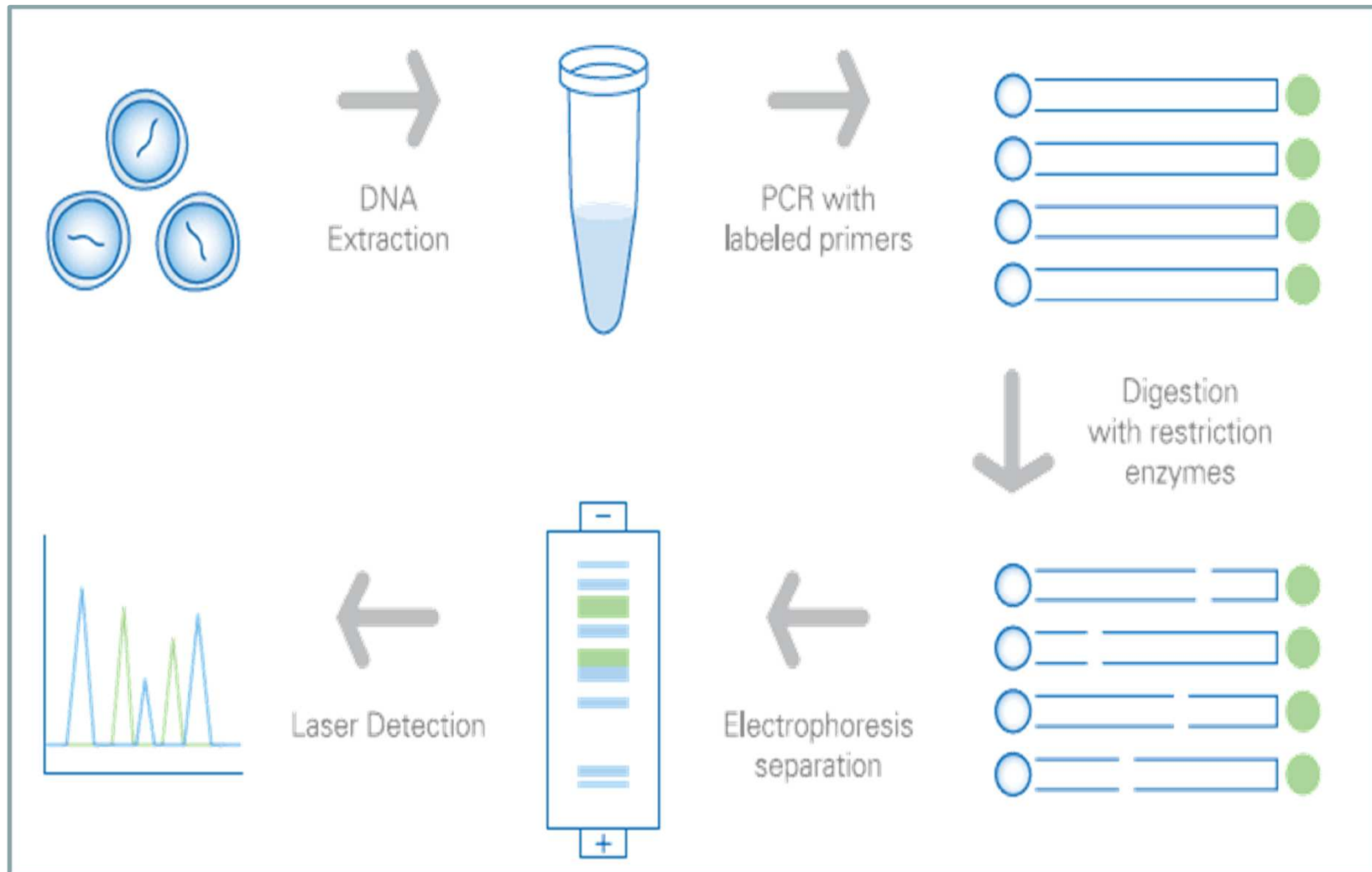


# Brief History of Forensic DNA Typing

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multilocus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

# Použití restrikčních enzymů - RFLP

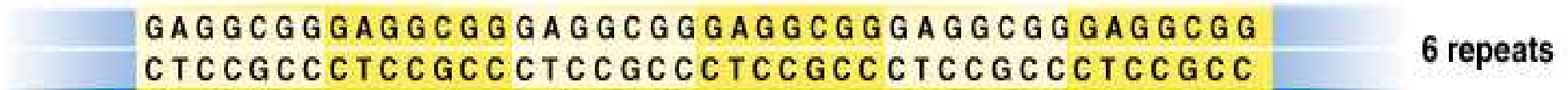
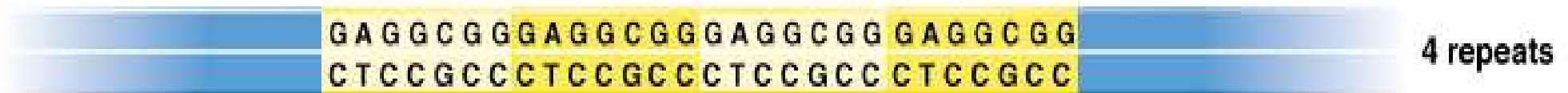
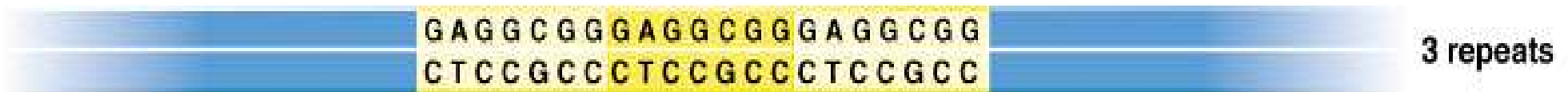
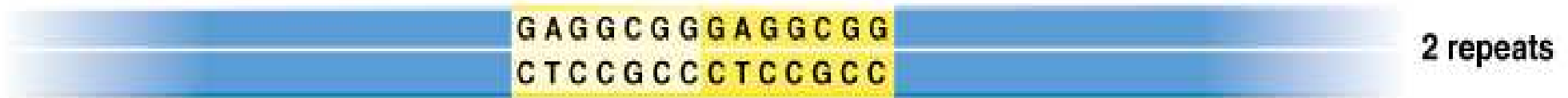
## Restriction Fragment Length Polymorphism





# Variable Number Tandem Repeats

9 – 100 pb

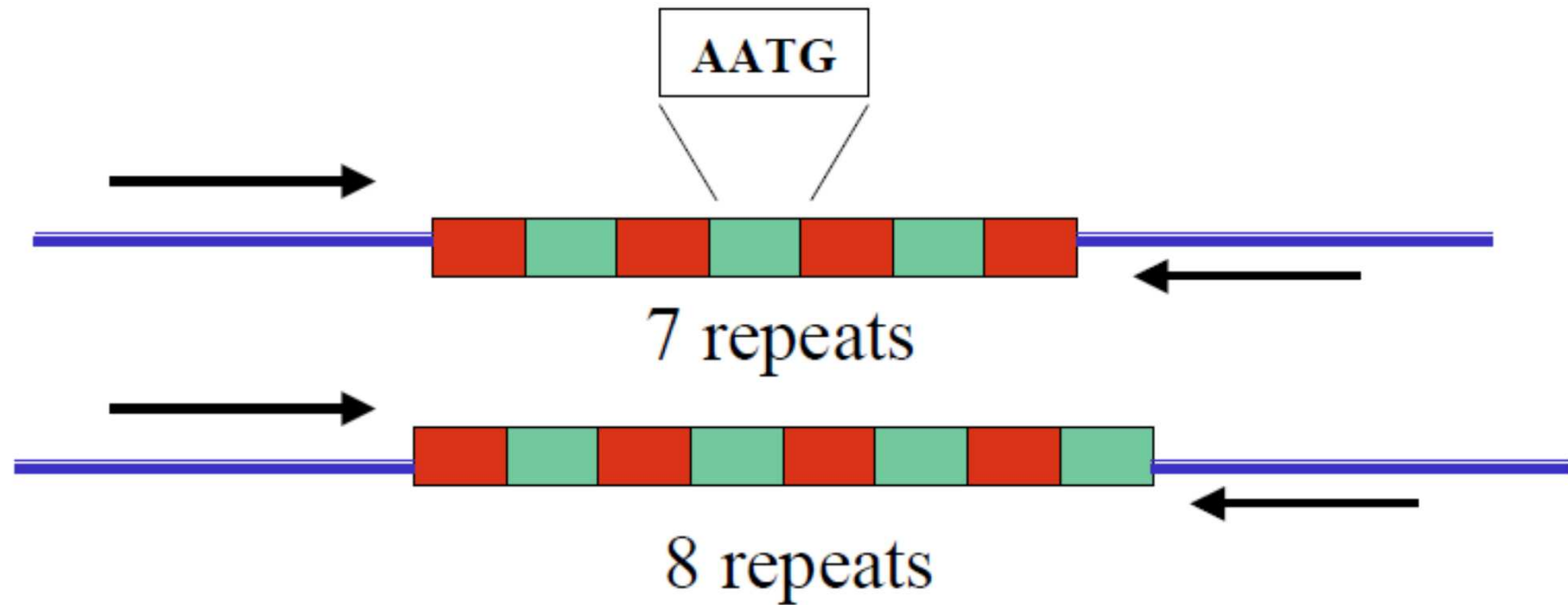


# Short tandem repeats

## 2 – 6 pb

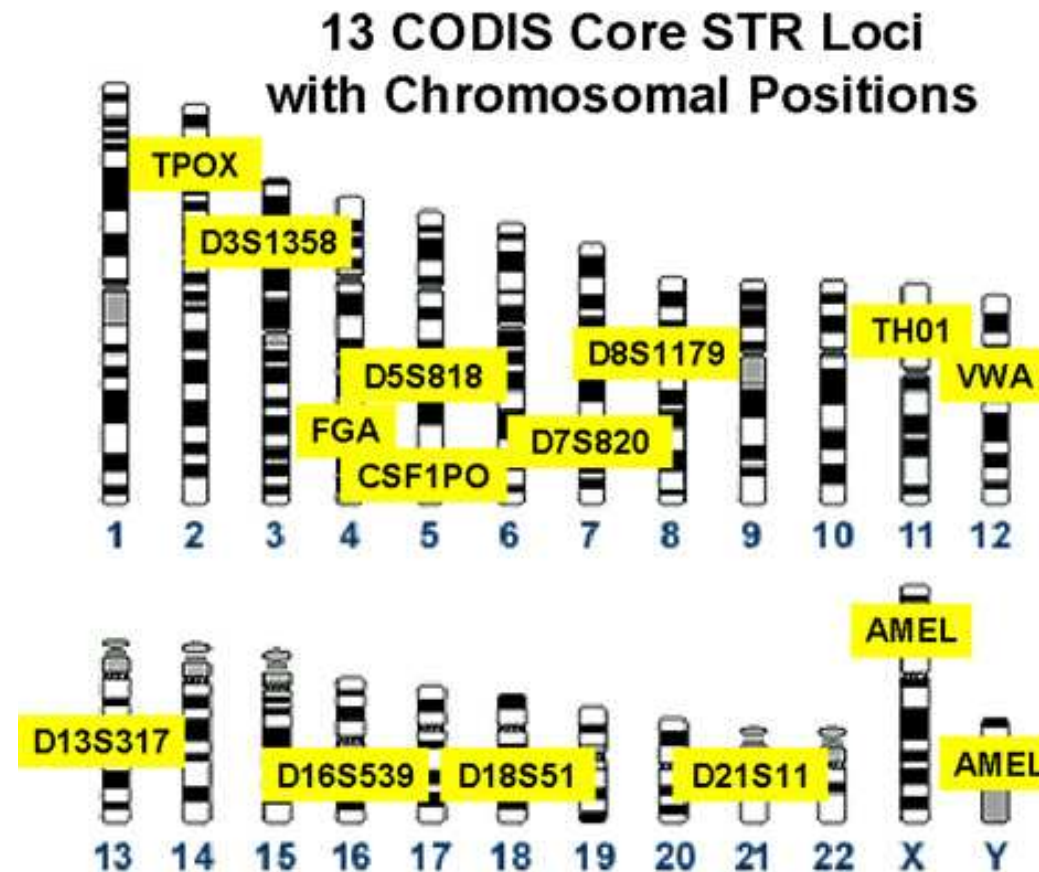
Repeat monomer	$P$ [%]	$REI$ [%]	$\alpha_B$	$\alpha_R$
A/T	59	1.75	1.0	0.2
C/G	41	0.12	1.1	0.4
AG/CT	27	0.27	2.6	0.24
AC/GT	20	0.66	2.6	
AT/TA	25	0.78	2.6	
CG/GC	10	0.02	2.6	
AAC/GTT	9	0.22	3.5	0.55
AAG/CTT	12	0.053	3.5	
AGG/CCT	11	0.077	3.5	

# Short tandem repeats



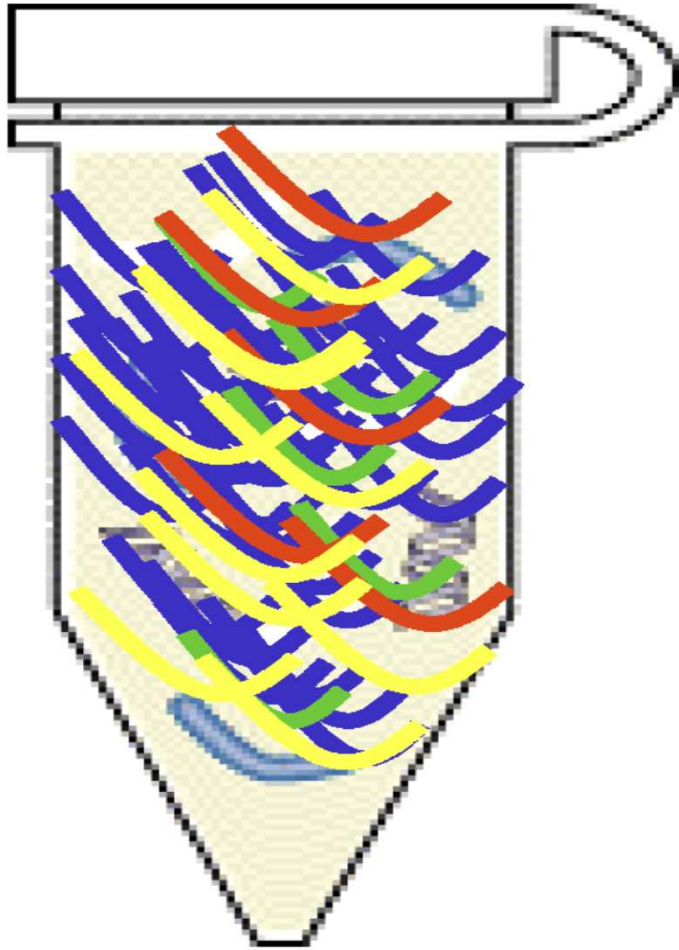
- Homozygot – obě alely stejné
- Heterozygot alely různě dlouhé

# Short tandem repeats



**Combined DNA Index System - 13  
UK DNA Database -10**

# Multiplex PCR

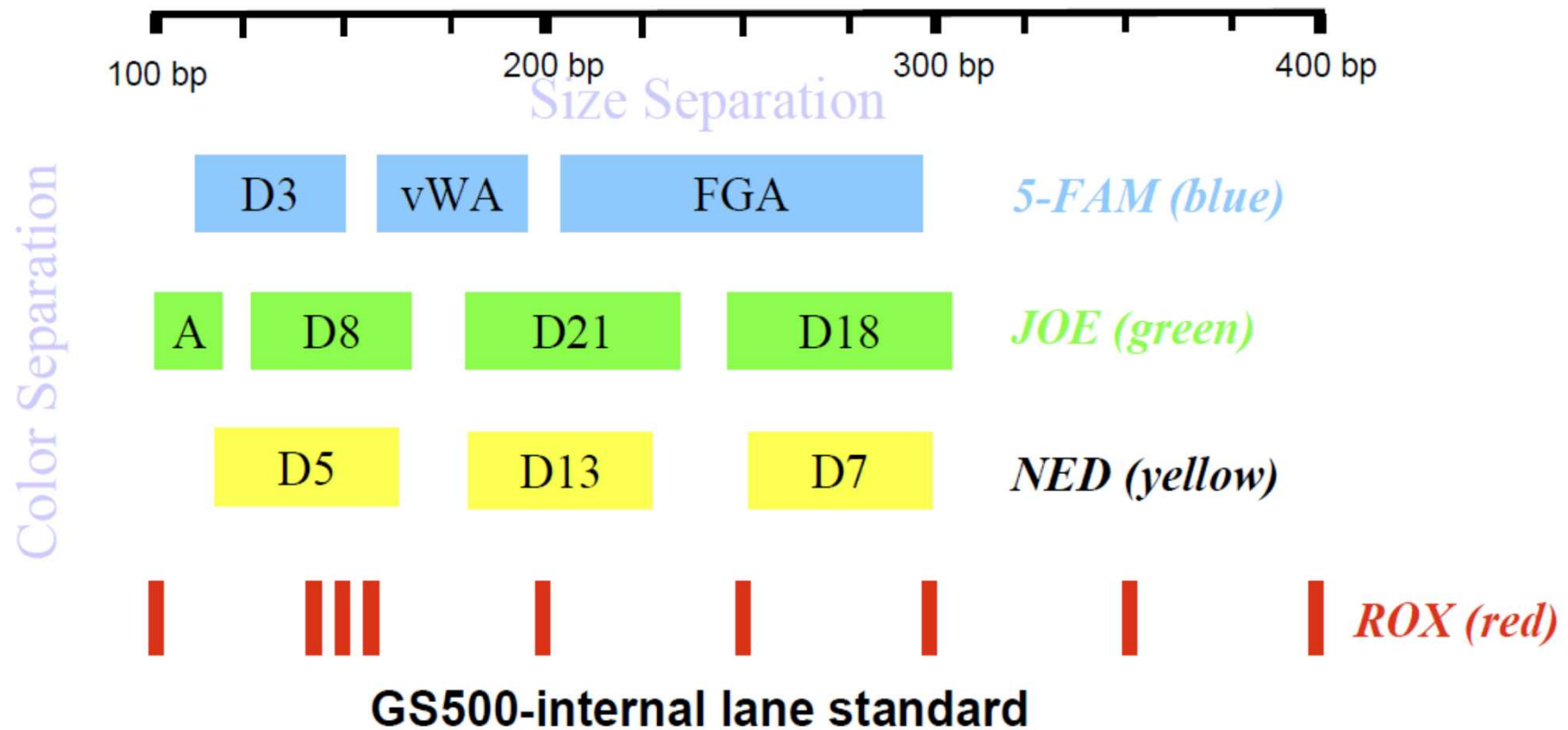


- 10 STR najednou
- Citlivost 1 ng DNA
- Možnost zpracovat směsi a degradované vzorky
- Různé fluorescenční barvičky pro překrývající se alely

# An Example Forensic STR Multiplex Kit

## AmpFISTR® Profiler Plus™

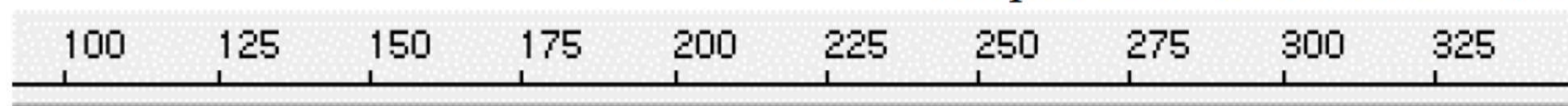
*Kit available from PE Biosystems (Foster City, CA)*



*9 STRs amplified along with sex-typing marker amelogenin in a single PCR reaction*

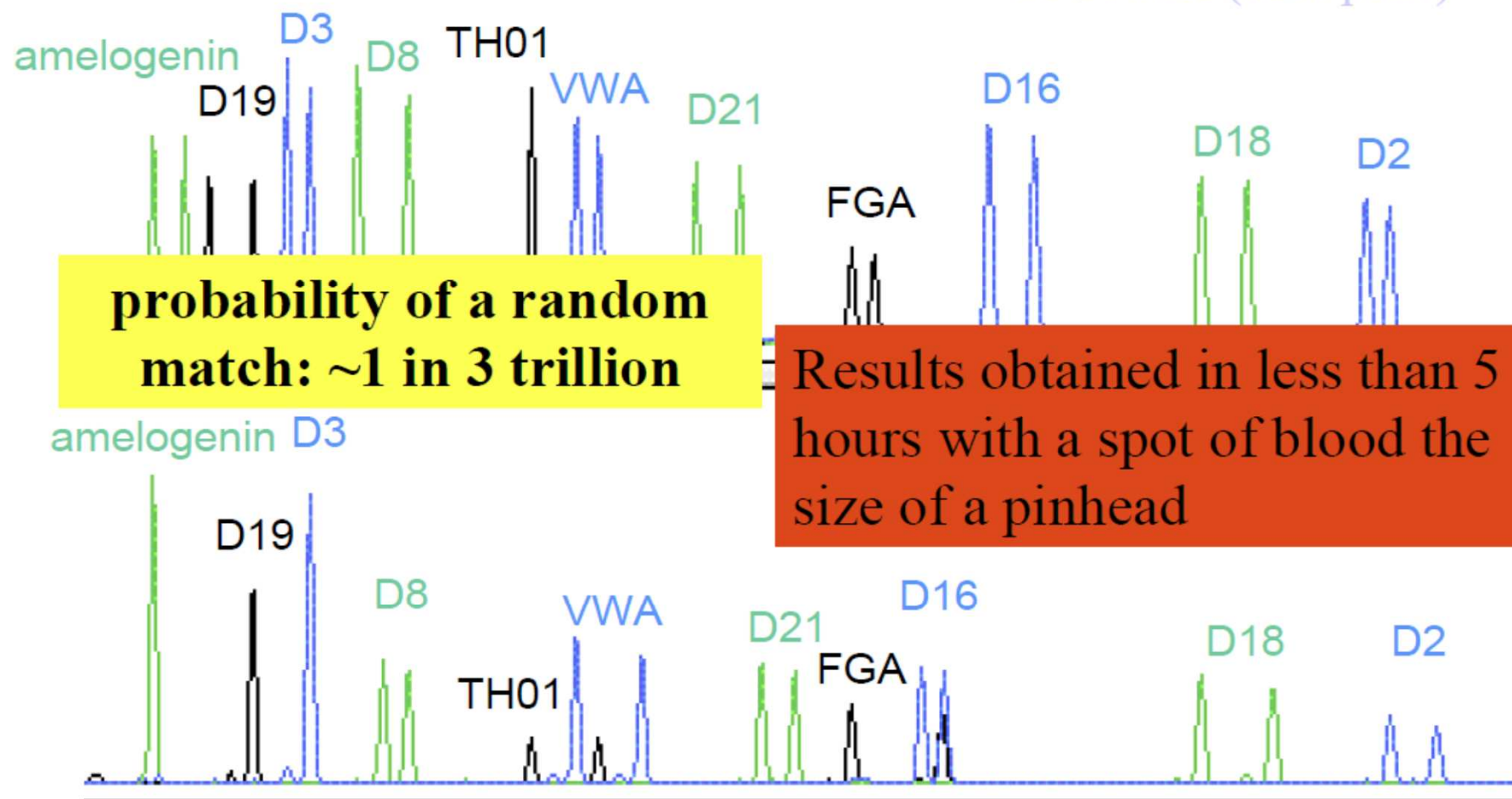
# Human Identity Testing with Multiplex STRs

AmpFISTR® SGM Plus™ kit



DNA Size (base pairs)

Two different individuals



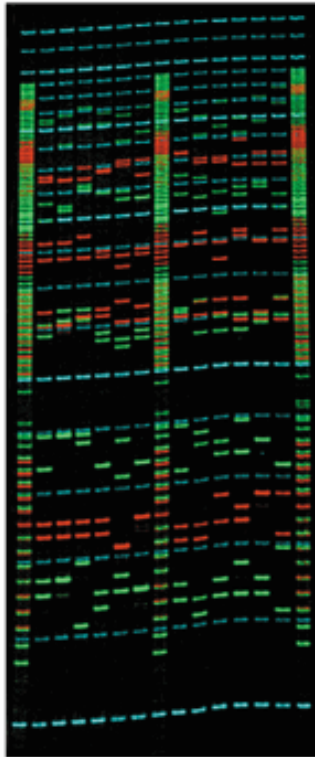
**probability of a random match: ~1 in 3 trillion**

Results obtained in less than 5 hours with a spot of blood the size of a pinhead

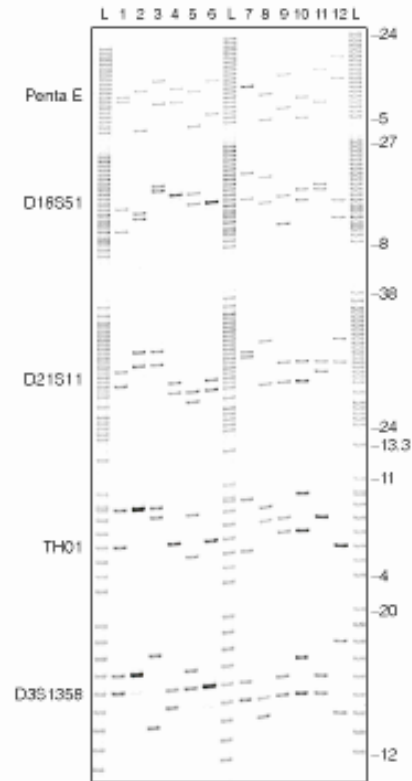
**Simultaneous Analysis of 10 STRs and Gender ID**

# Short tandem repeats

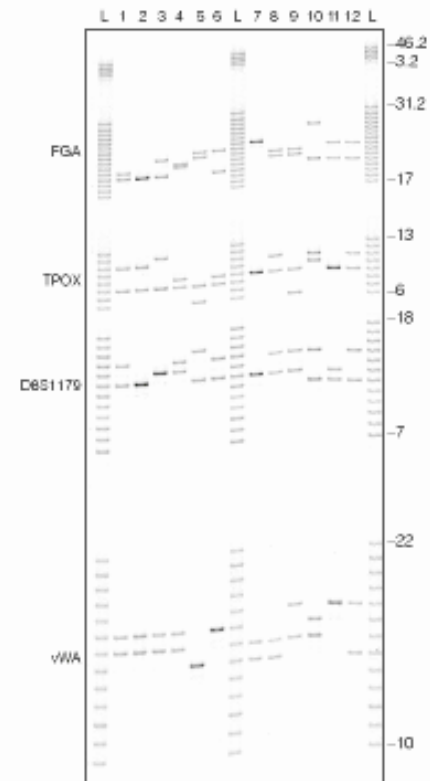
A.



B.



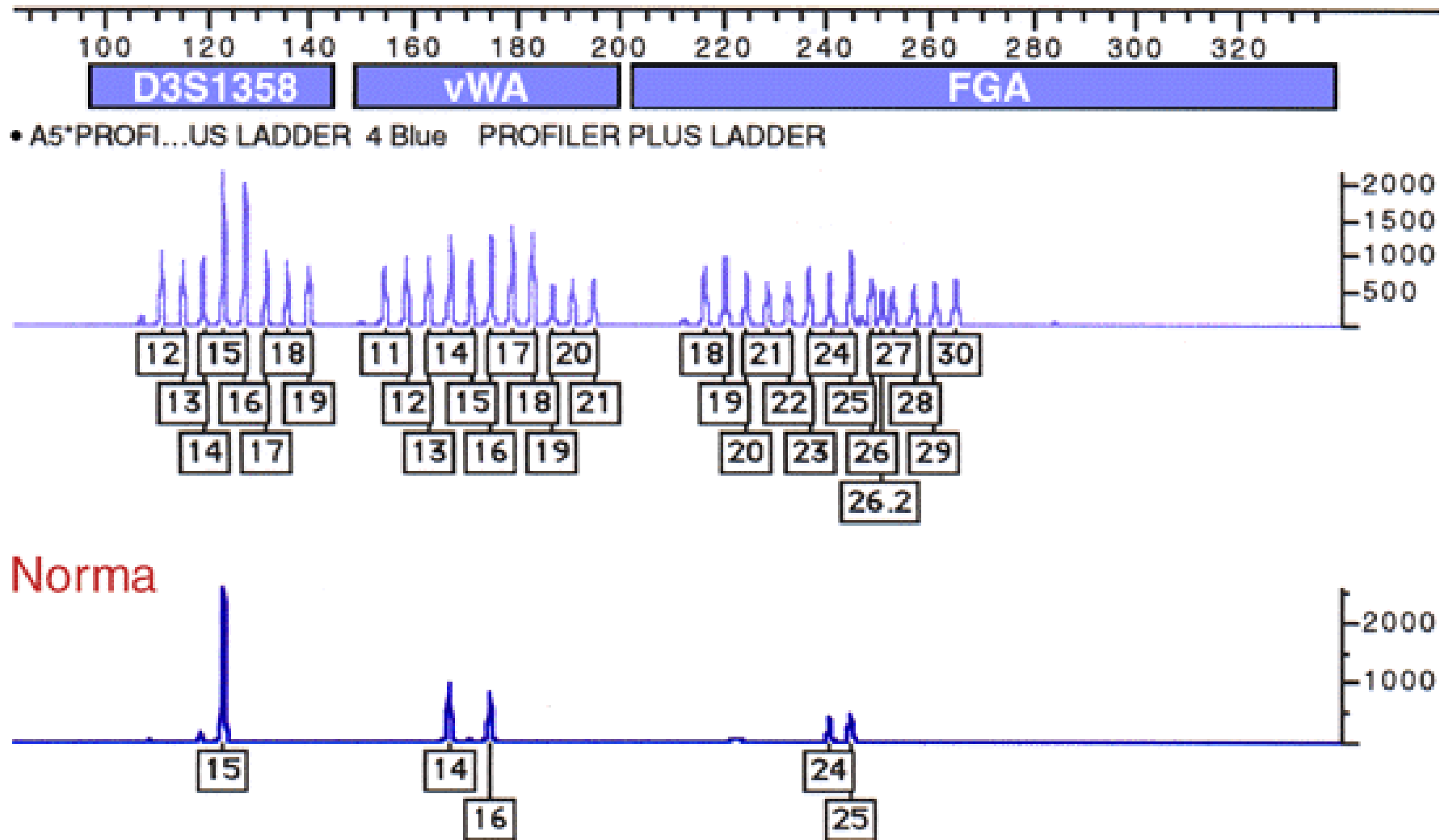
C.







# Short tandem repeats



# Short tandem repeats



X - 106 Y - 112

# Vzorky biologického materiálu

- Krev
- Semeno
- Moč
- Sliny
- Vlasy
- Kostí
- Zuby
- Tkáně



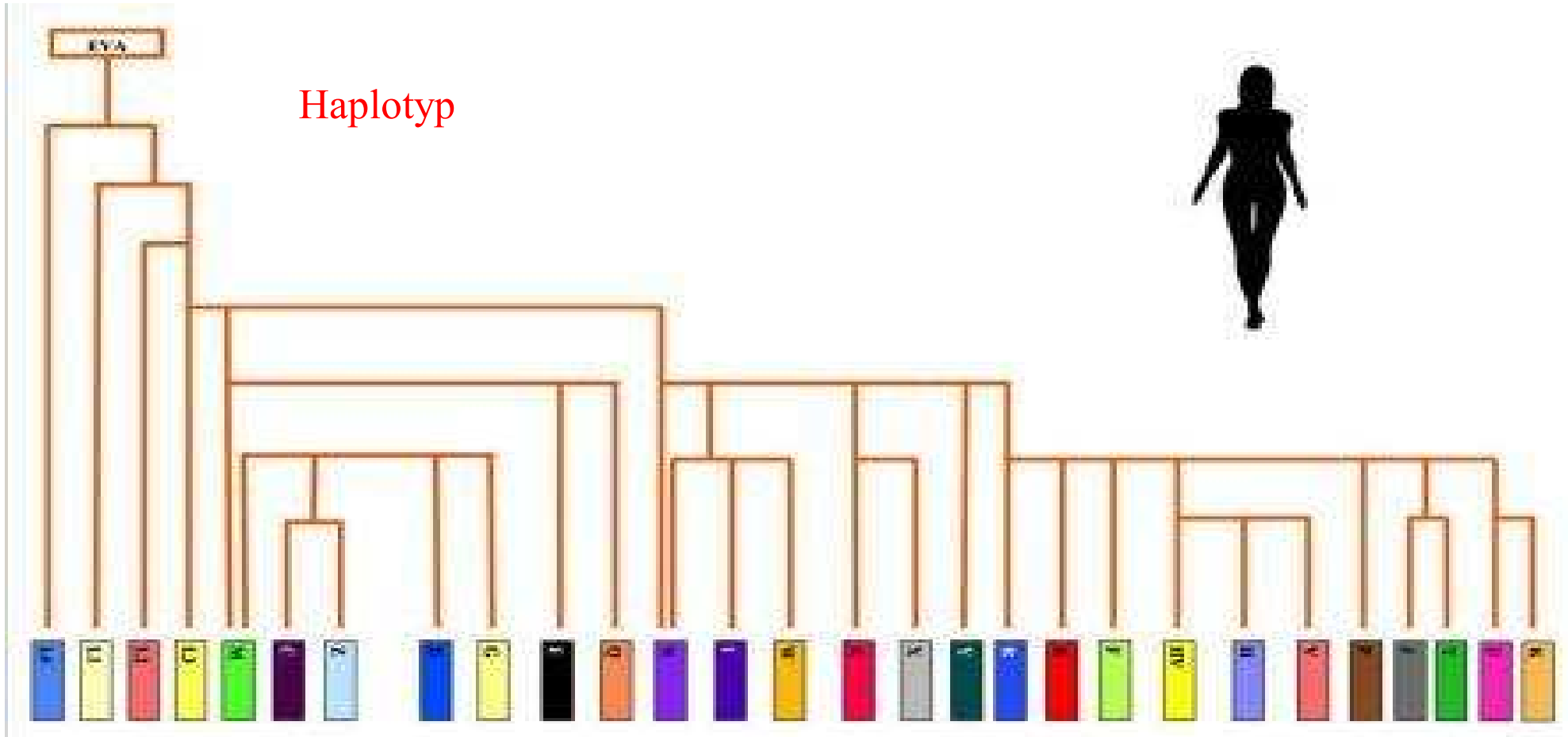
# Testy paternity

## Zjednodušené testy

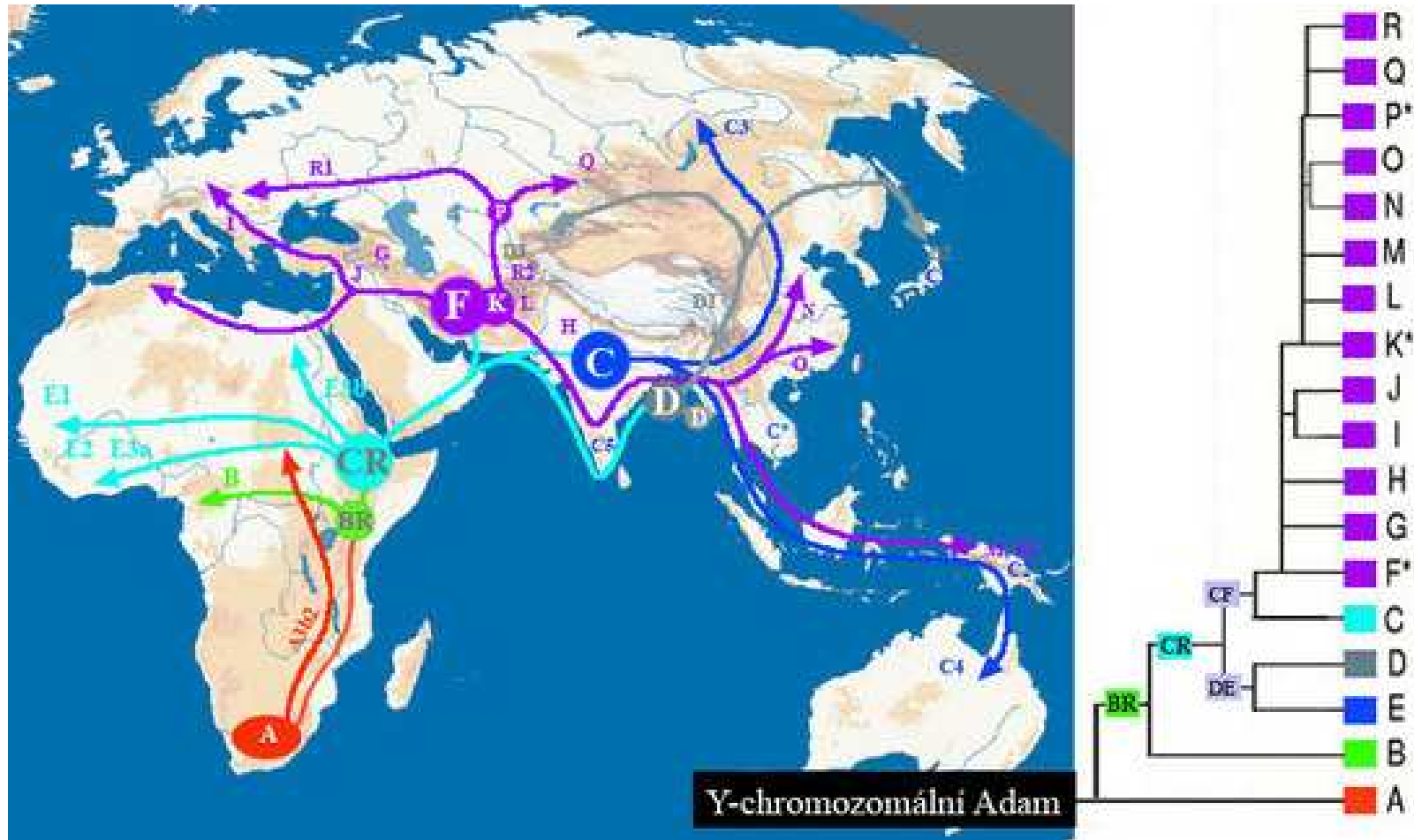
- STR na Y chromosomu – mužských potomků srovnání s otcem
- Mitochondriální DNA – dědí se po matce – matroklinní dědičnost

# Mitochondriální Eva před 200 000 let v Africe

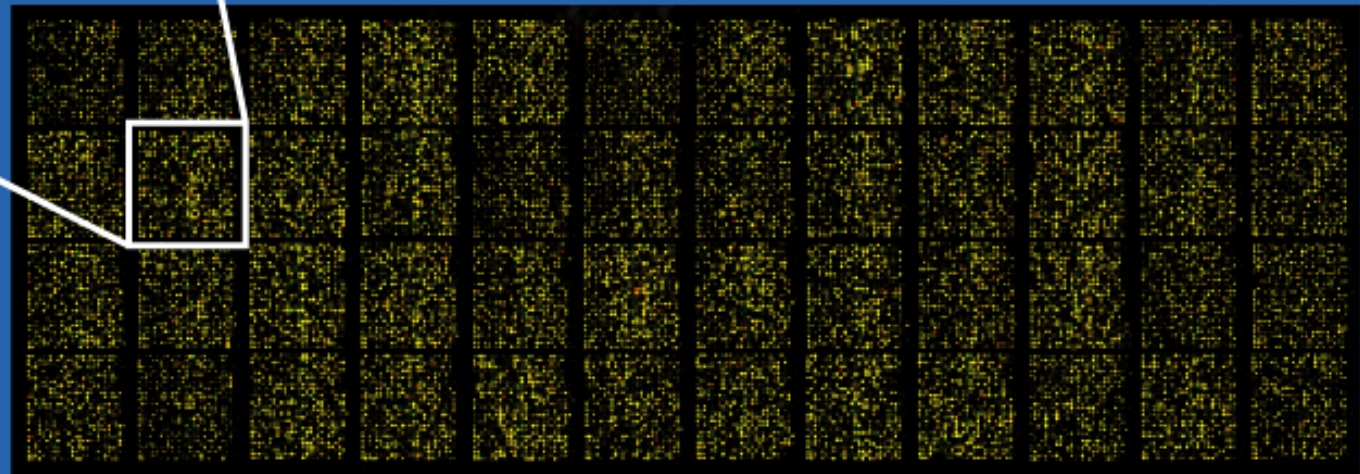
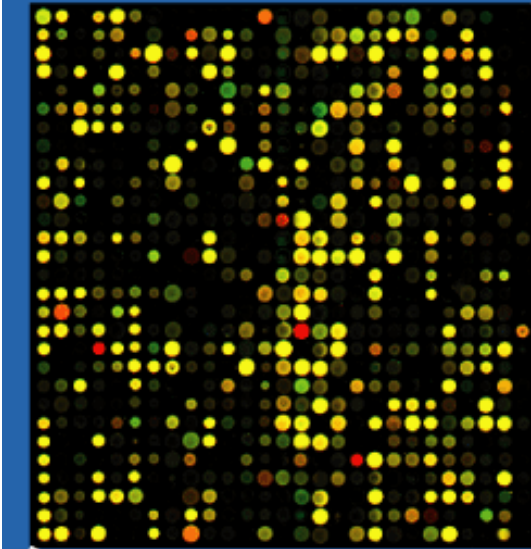
Haplotyp



# Y-chromozomální Adam před 110 000 let v Africe



# DNA chipy

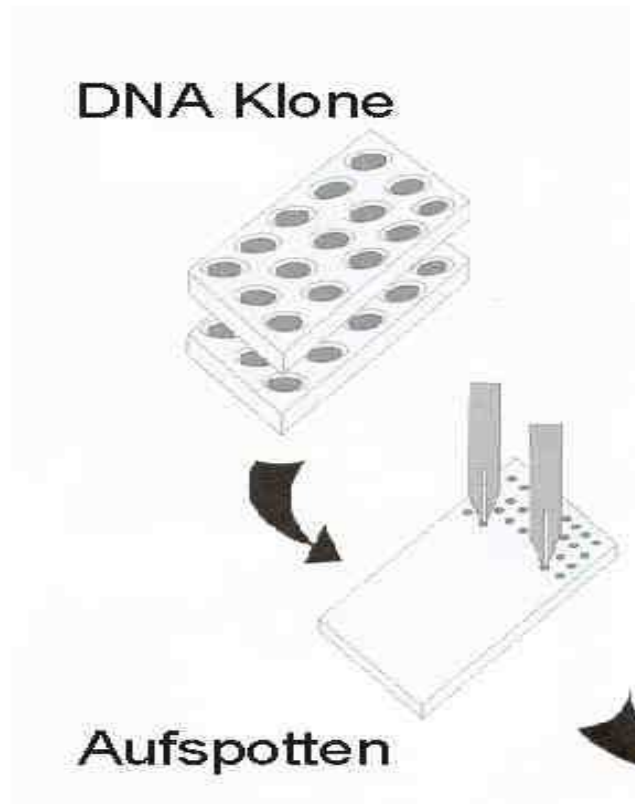




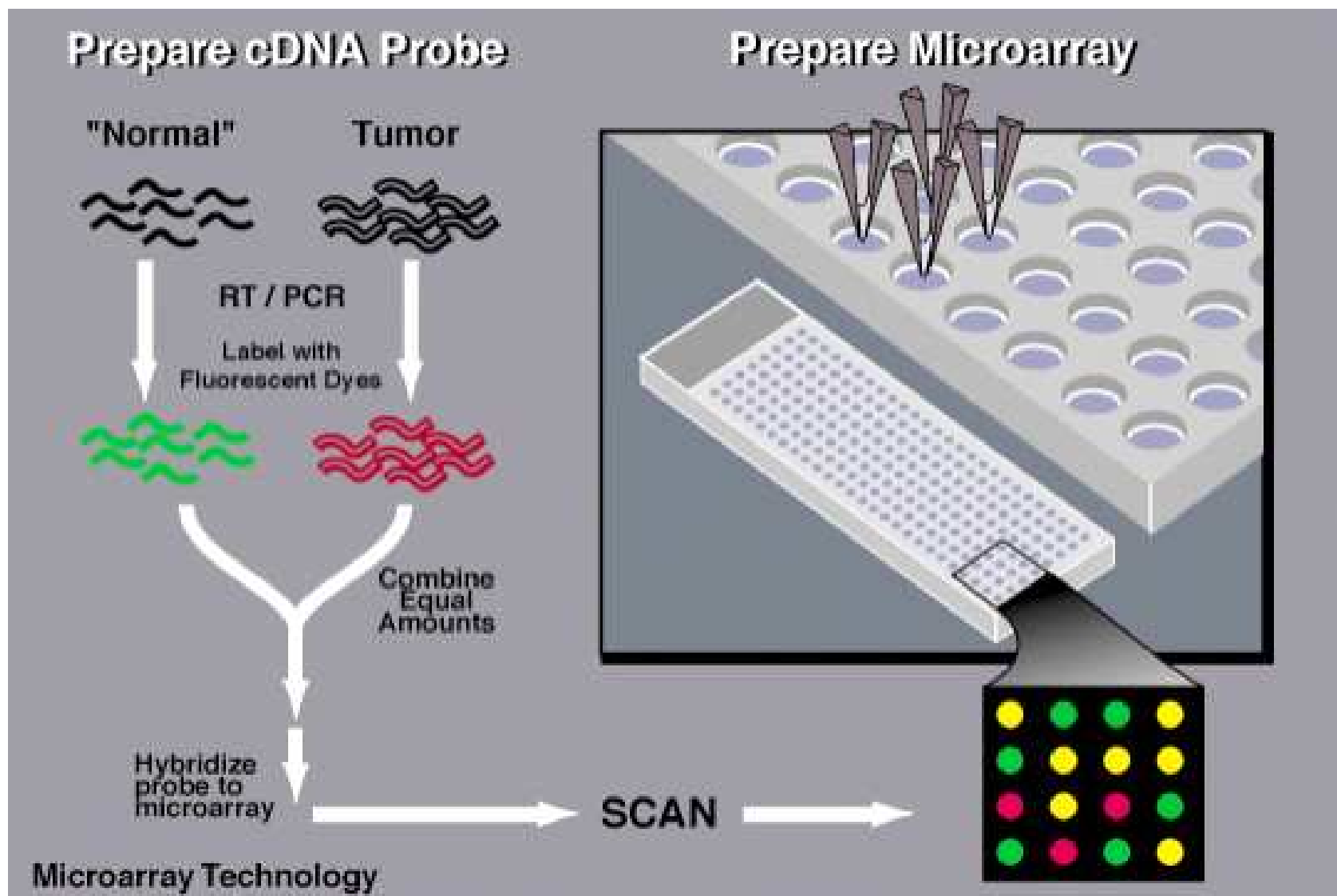
# DNA chipy

Fotolitografie

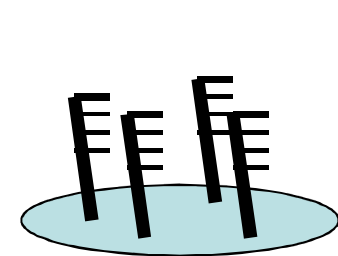
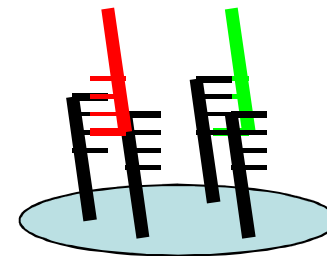
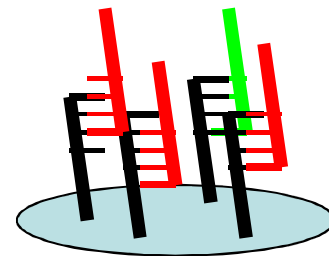
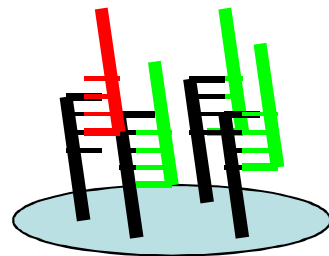
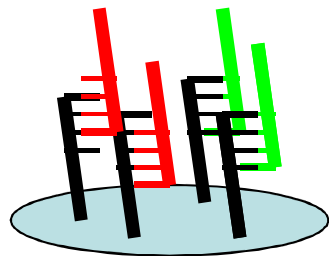
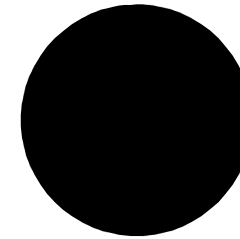
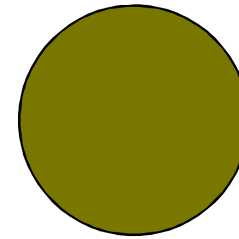
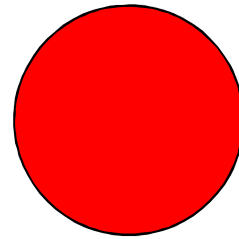
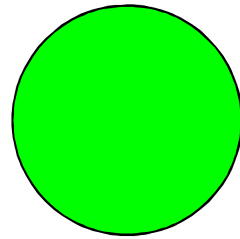
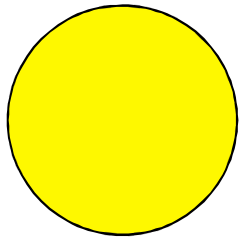
Kapkování



# DNA chipy



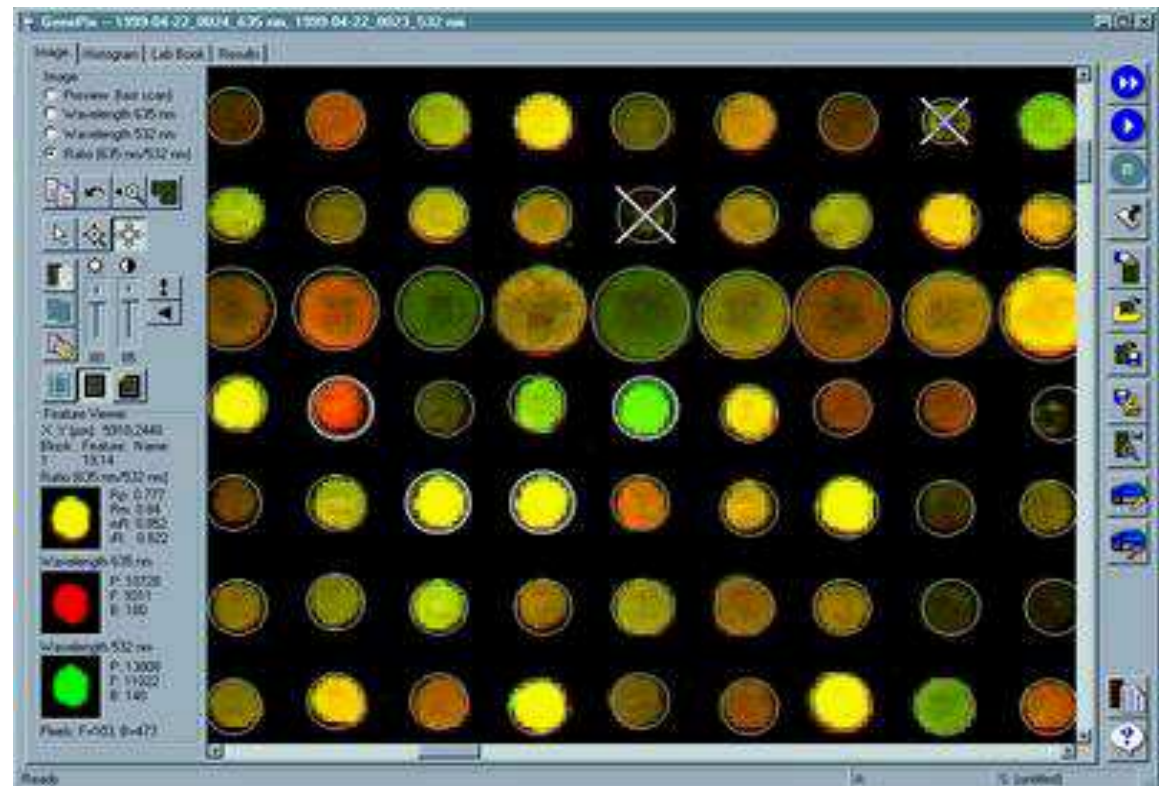
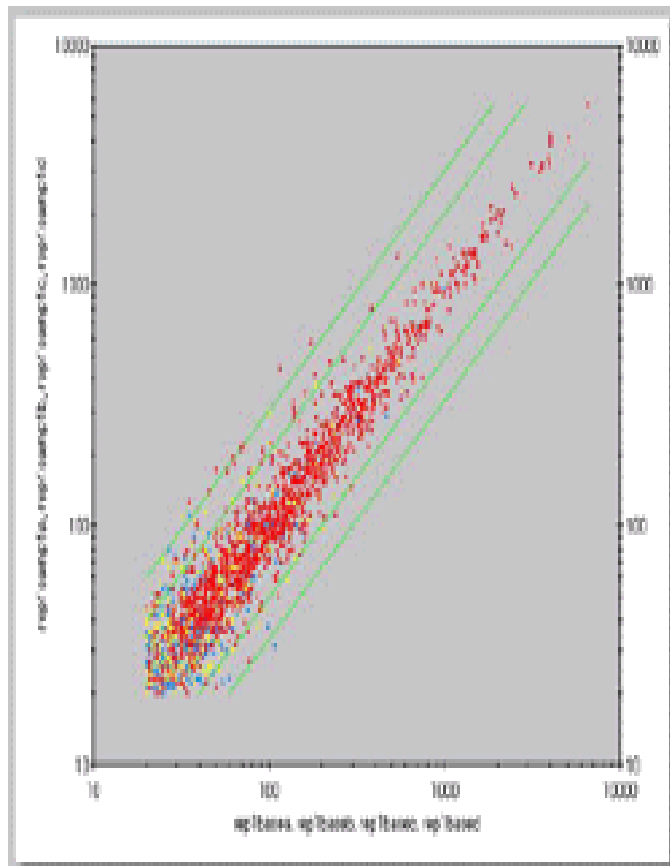
# DNA chipy - barva skvrn



# DNA chipy - vybavení



# DNA chip software

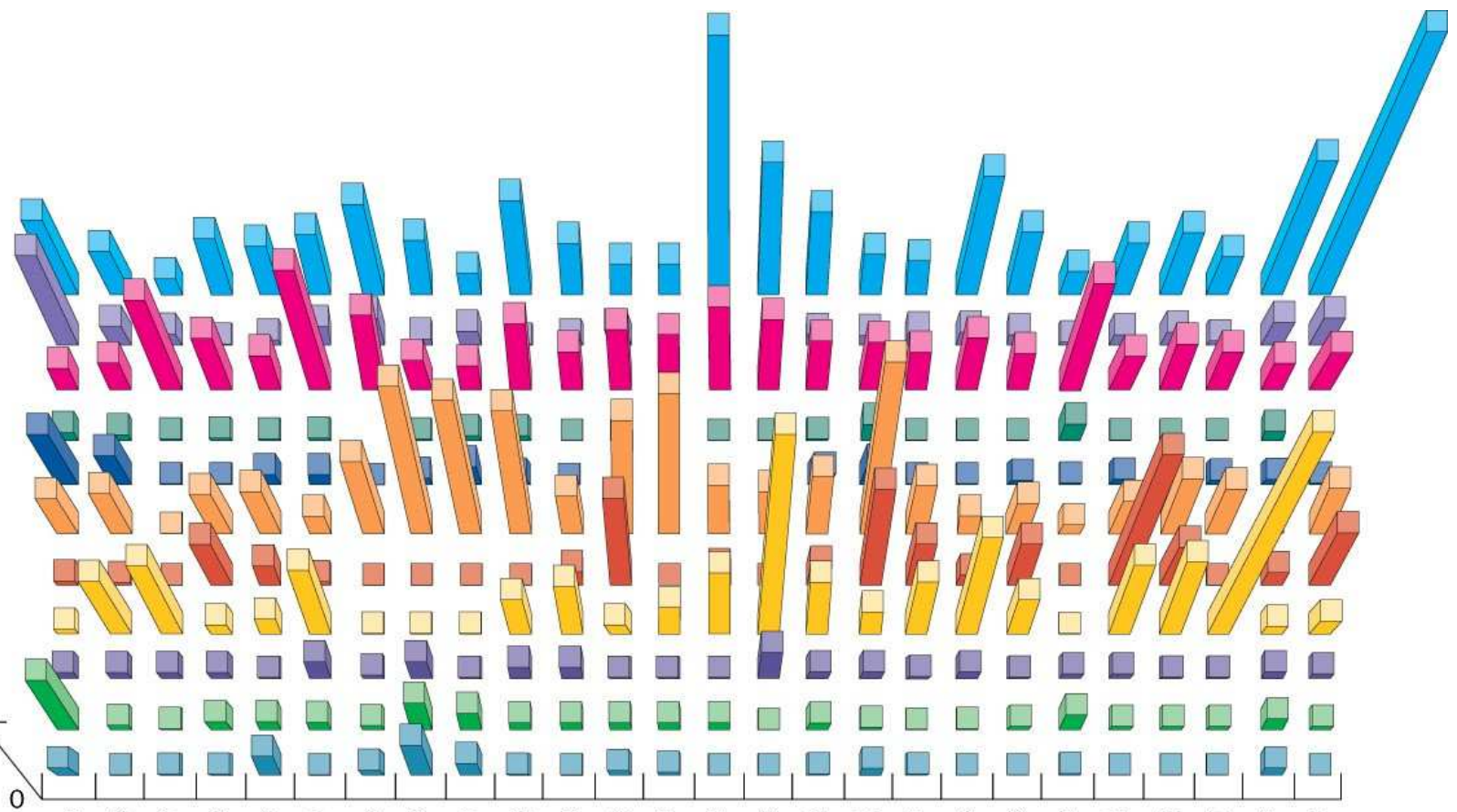


- Cyclin A
- Cyclin B
- Cyclin C
- Cyclin D1
- Cyclin D2
- Cyclin D3
- Cyclin E
- Cyclin F
- Cyclin G
- Cyclin H
- Cyclin I

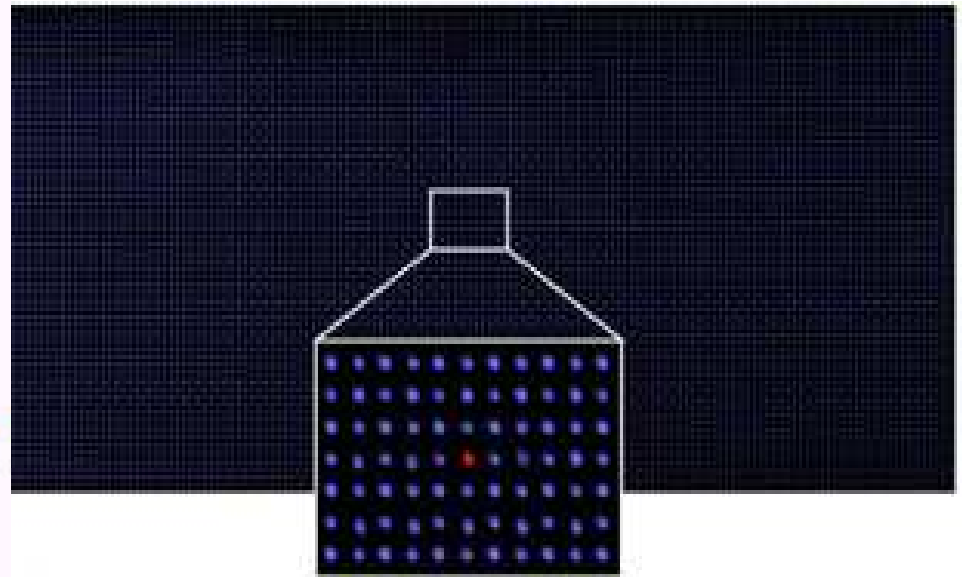
Expression  
(arbitrary units)

0  
100  
200

Testis  
Placenta  
Liver  
Small intestine  
Stomach  
Kidney  
Adipose  
Thymus  
Bone marrow  
Lung  
Trachea  
Lymph node  
Spleen  
Thyroid  
Pancreas  
Mammary gland  
Adrenal gland  
Pituitary gland  
Prostate  
Uterus  
Skeletal muscle  
Heart  
Aorta  
Skin  
Retina  
Brain

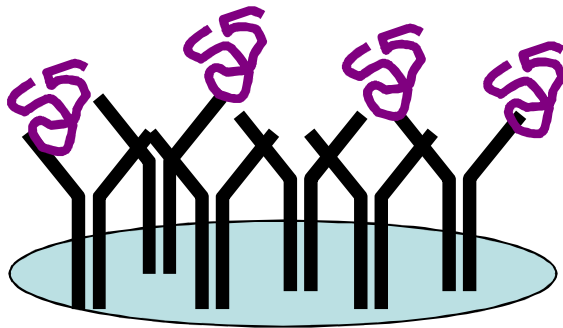


# Proteinové chipy

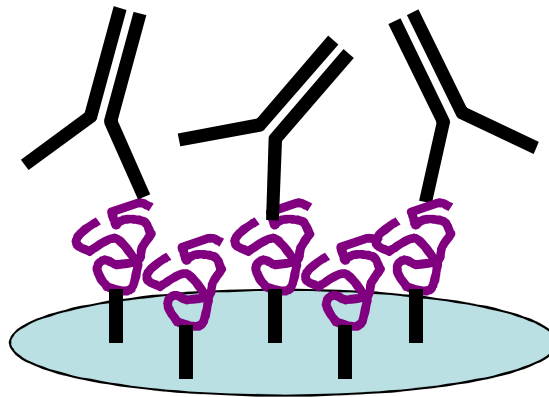


# Proteinové chipy – typy interakcí

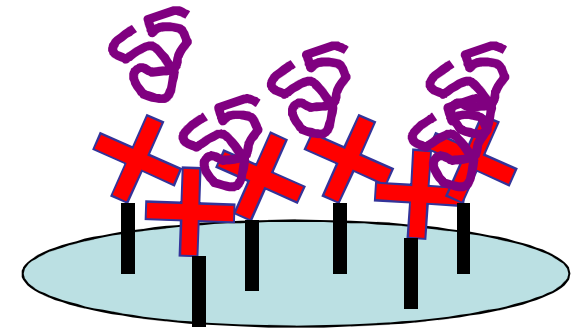
Protilátka



Antigen



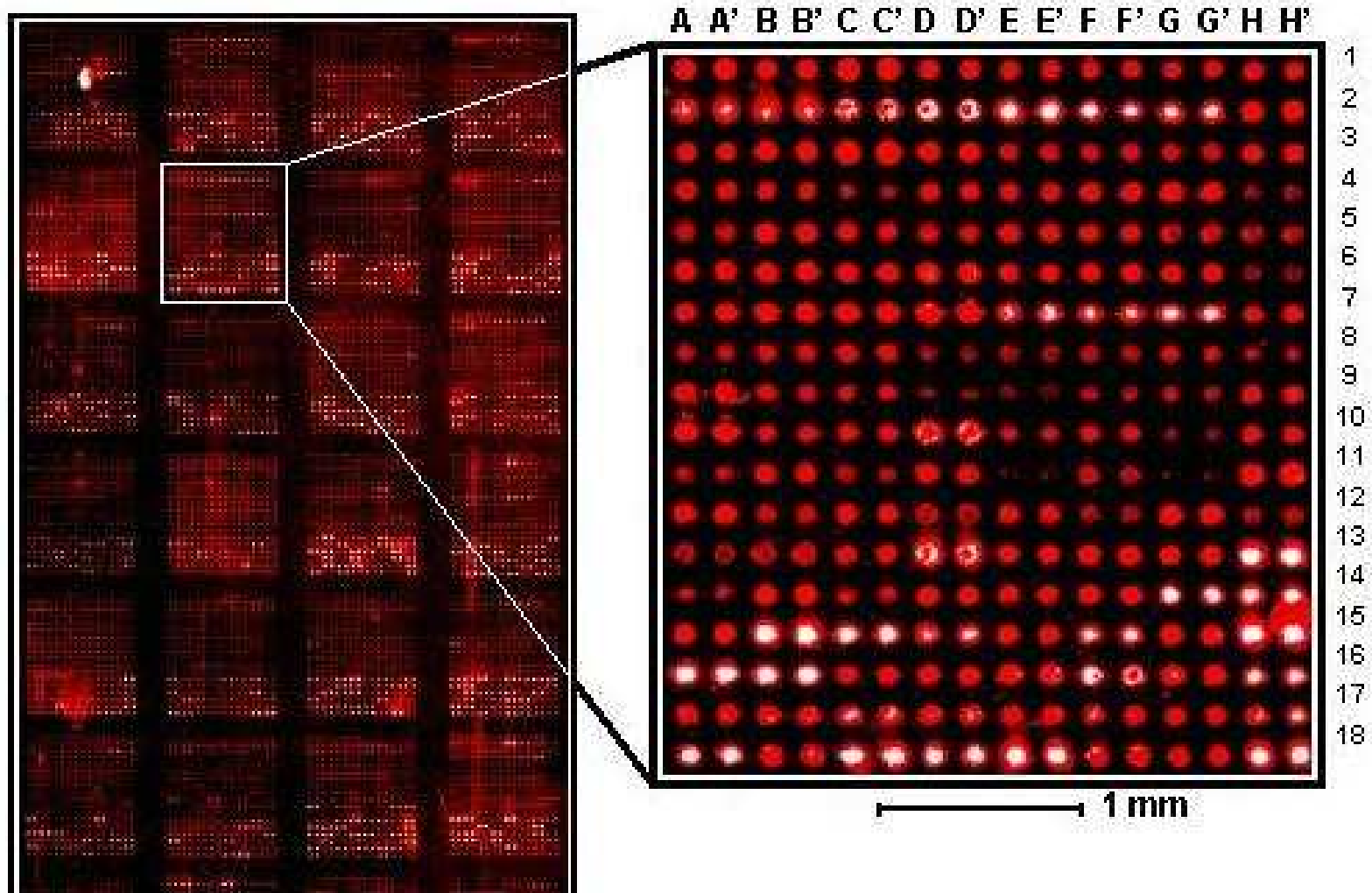
Ligand



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



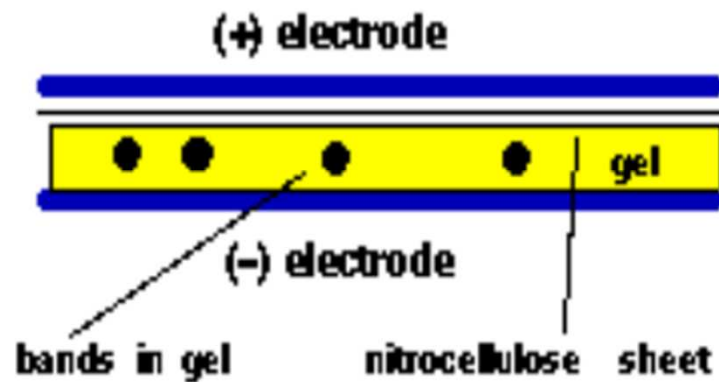
# Anti-GST Probe



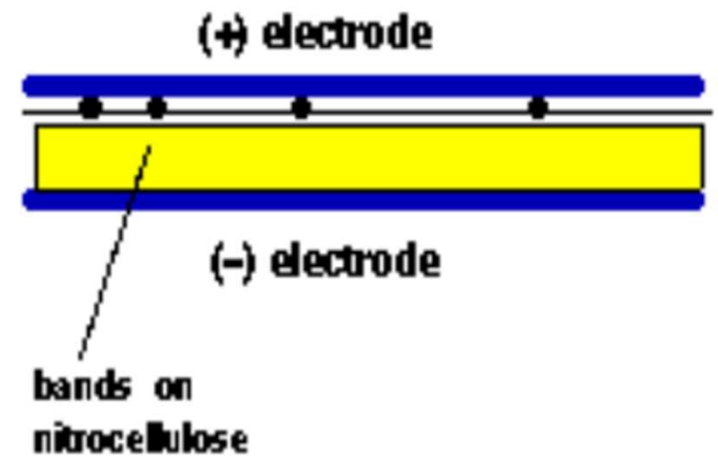
# Blotting

Side View:

Before Transfer:

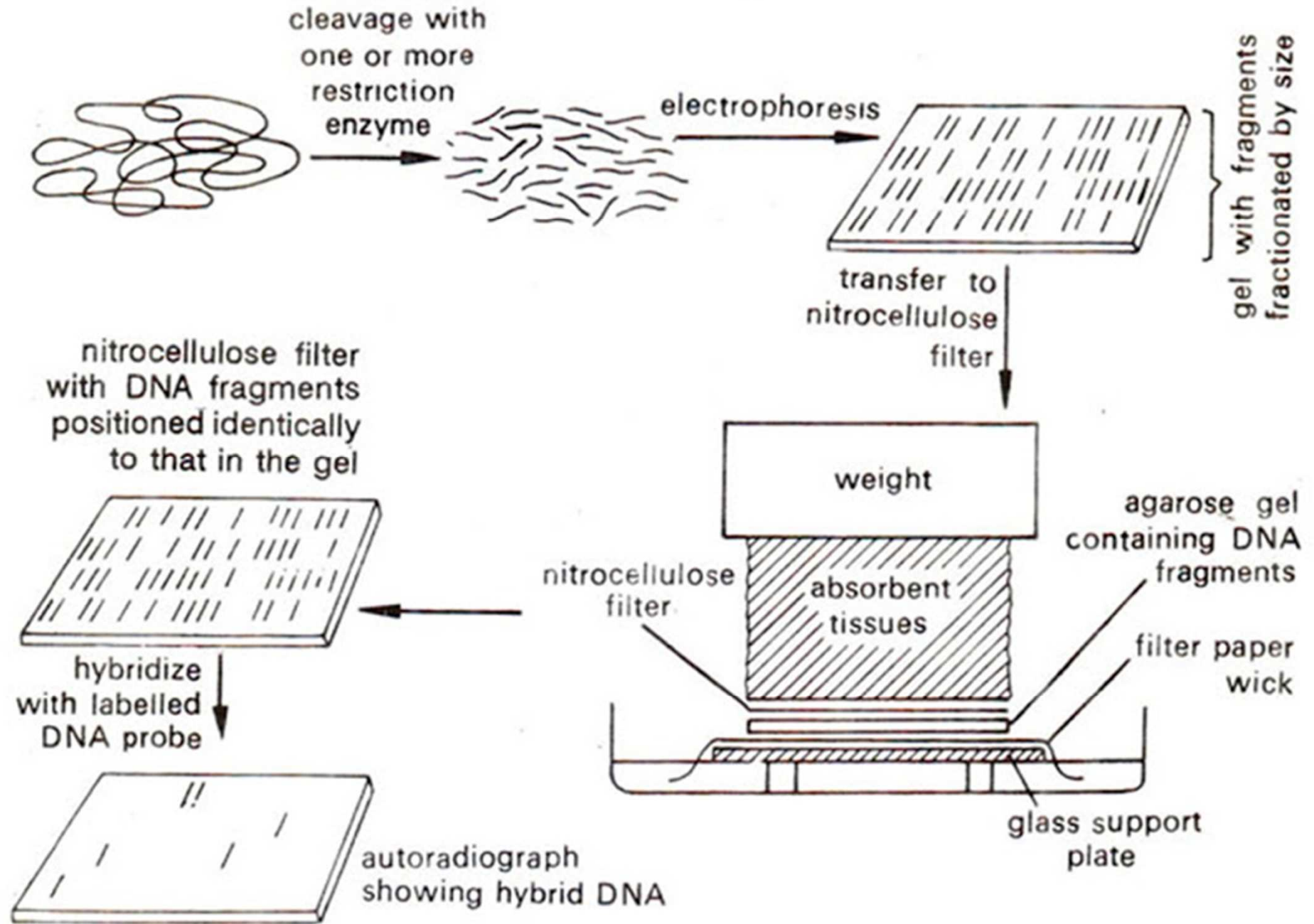


After Transfer:



Note: All the layers are pressed tightly together.

# Blotting NK



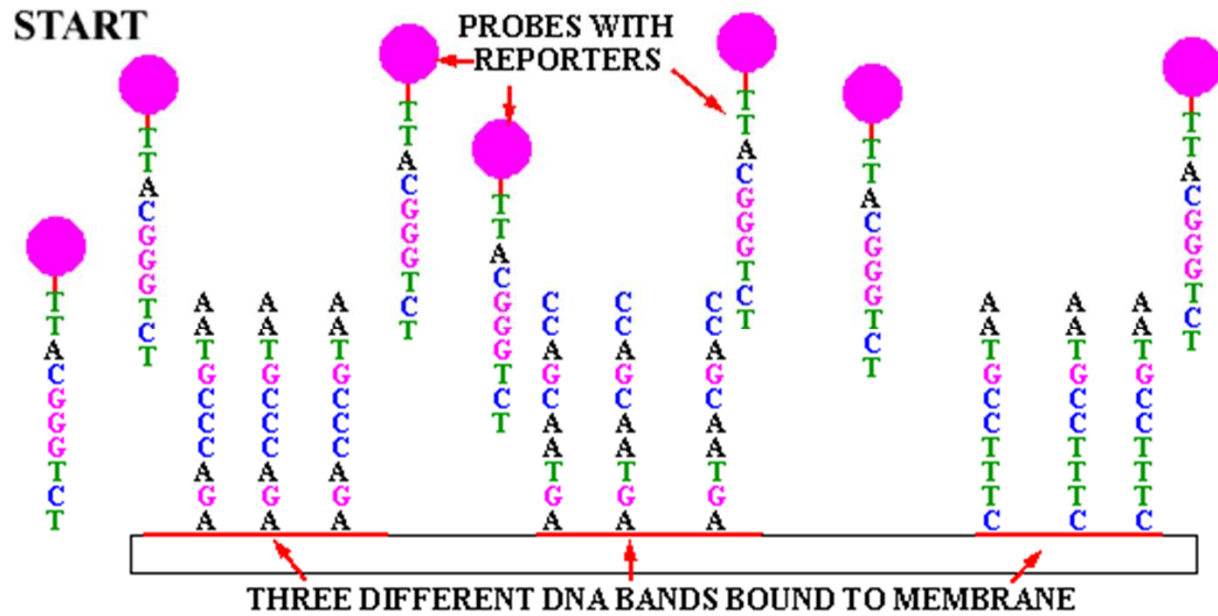
# Detekce

DNA

HYBRIDIZACE

- radioaktivní proba – vysoká senzitivita, Southern blot
- neradioaktivní proba – biotin – streptavidin, dioxigenin

# Detekce DNA



# 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

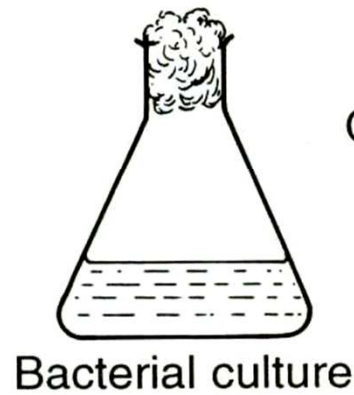
- na základě rozdílné rozpustnosti – extrakce, srážení
- na základě vlastností - chromatografie – polarita-adsorpční, náboj-ionexová
  - elektroforéza – velikost
- sedimentace - gradientová ultracentrifugace

# Postup

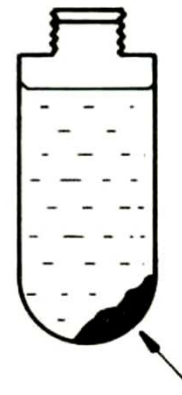
1. Rozbití buněk a membrán pro uvolnění NK
2. Inaktivace DNA- nebo RNA-degradujících enzymů (DNasy, RNasy).
3. Separace NK od dalších komponent uvolněných z buňky.
  - Extrakce/Precipitace
  - Chromatografie, elektroforéza
  - Ultracentrifugace

# Extrakce/Precipitace

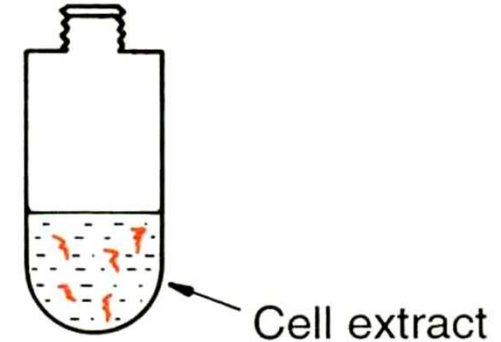
**1 A culture of bacteria is grown and then harvested**



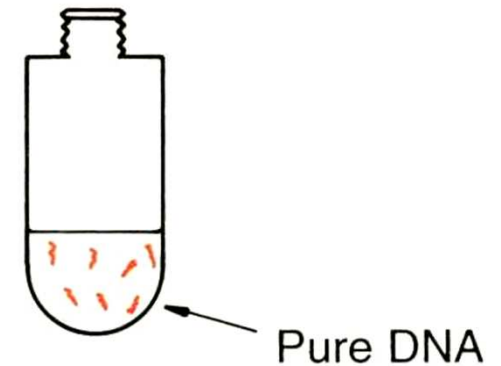
Centrifugation



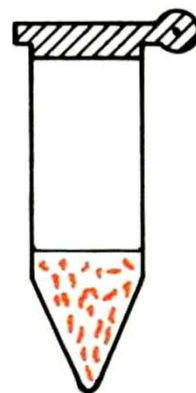
**2 The cells are removed and broken to give a cell extract**



Organic extraction



**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  
(pH 8)**

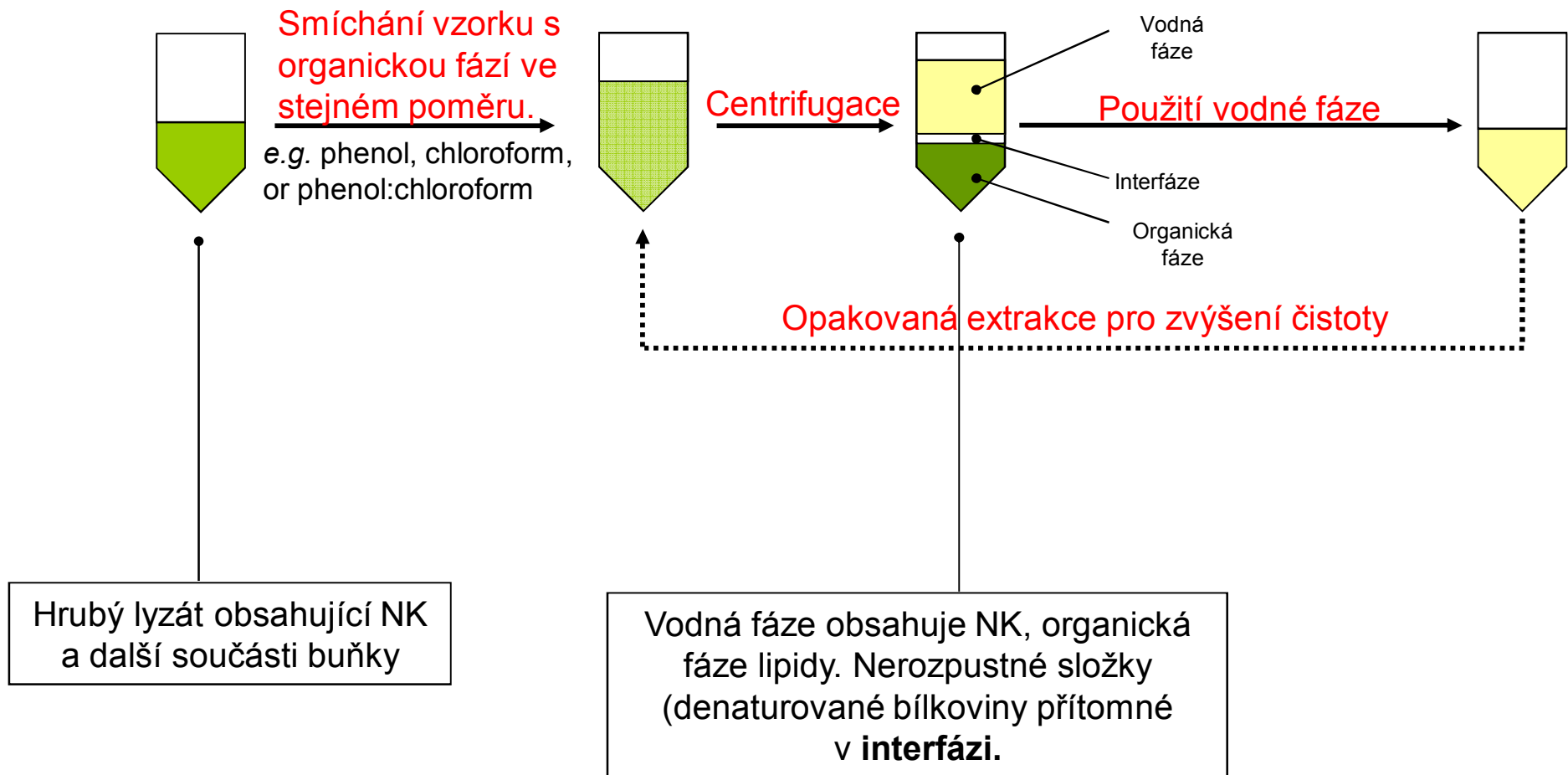
*4. Ethanolová precipitace*

*5. Působení RNAsy a proteinasy K*

*6. Opakování kroku 3 a 4.*

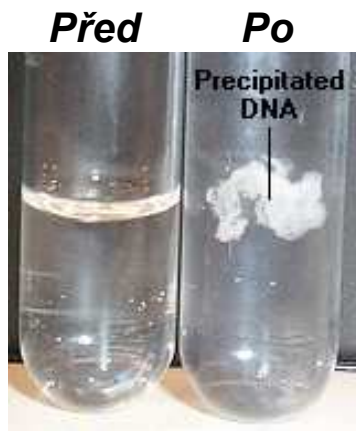
# Extrakce/Precipitace

## Krok 3: Organická extrakce



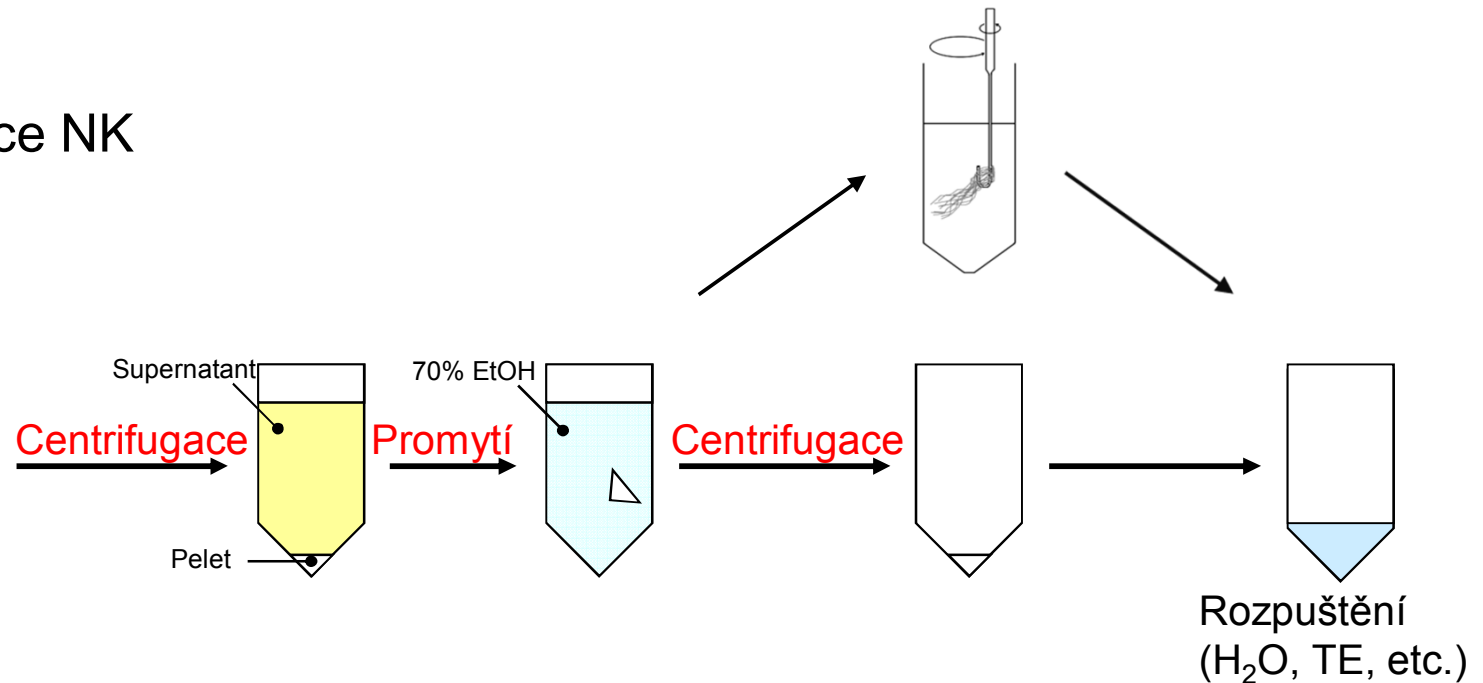
# Extrakce/Precipitace

## Krok 4: Precipitace NK



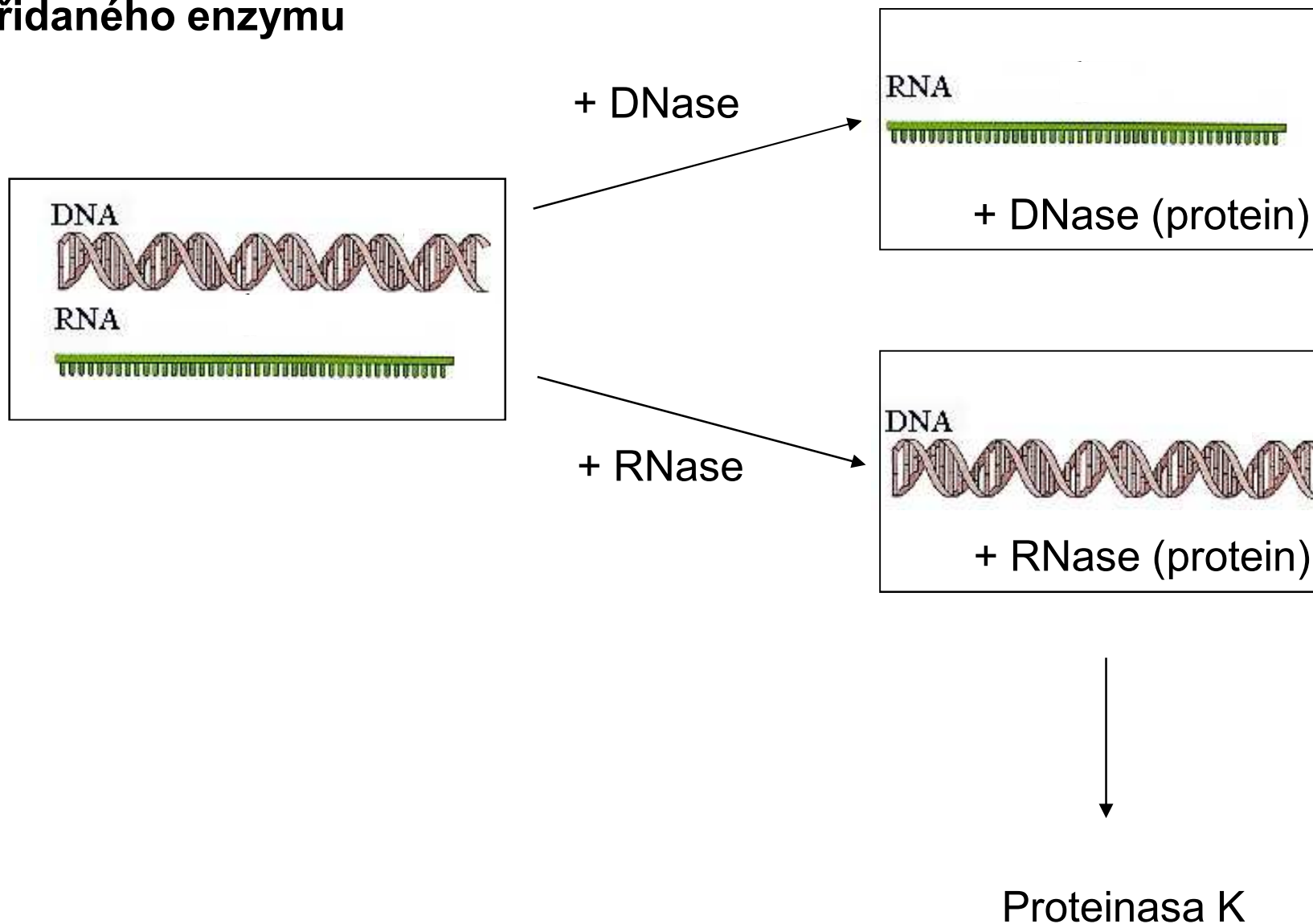
Přidání EtOH a soli

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



## Detail kroku 5

Použití nukleas pro odstranění nechtěné DNA nebo RNA  
a proteas pro odstranění zbývajících proteinů včetně  
přidaného enzymu

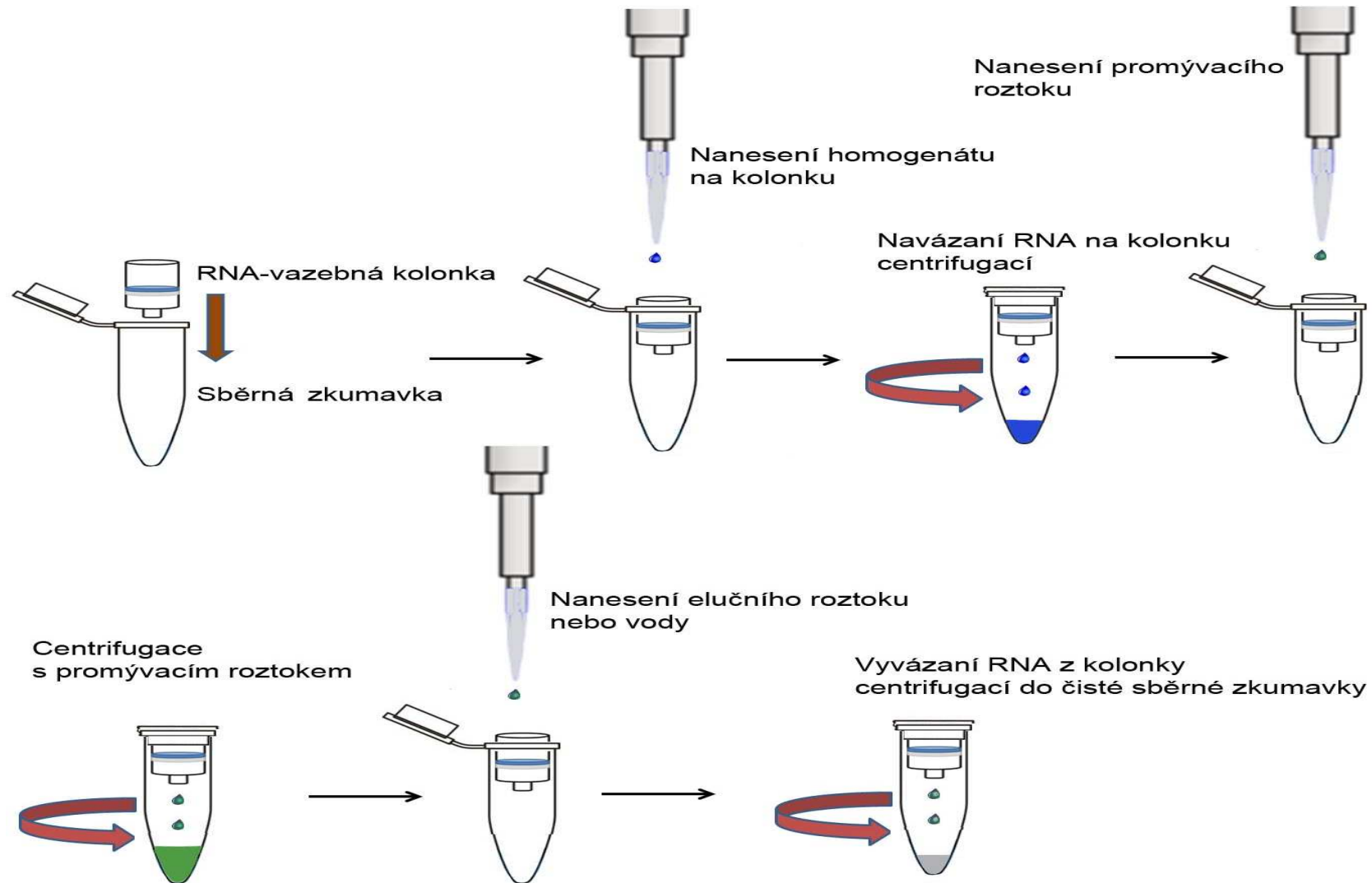




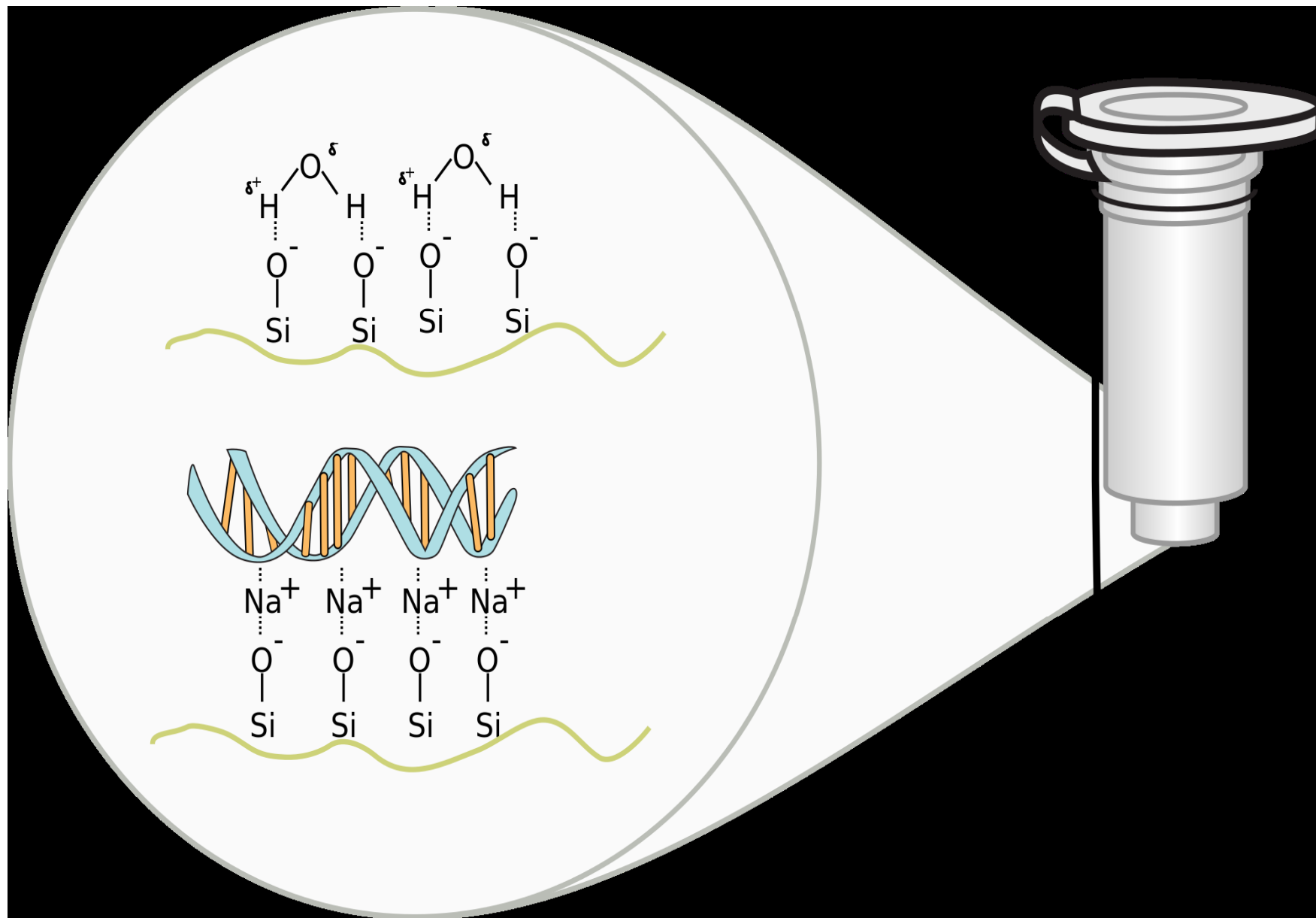
# Chromatografie

# Spin kolonky

**Princip izolace RNA pomocí centrifugačních RNA vazebných kolonek**

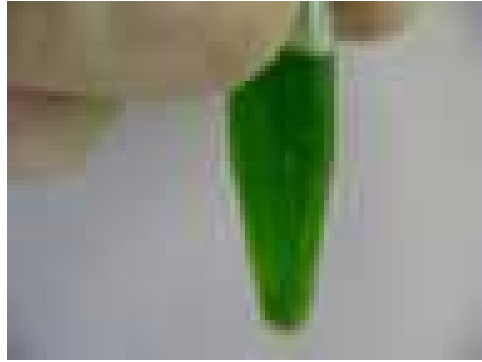


# Adsorpční chromatografie



# Adsorpční chromatografie

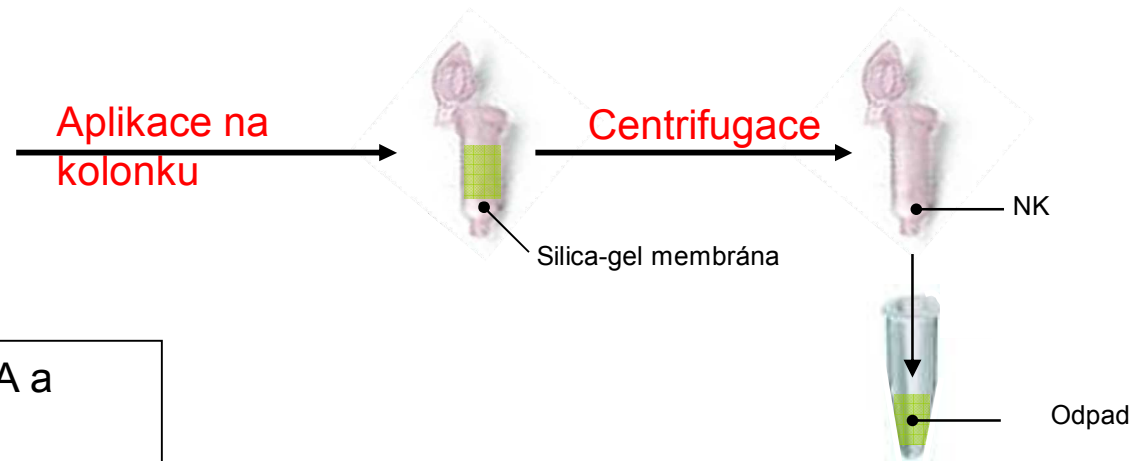
## Krok 1: Příprava lyzátu



Extrakční pufr pro vazbu DNA a RNA na silikagel:

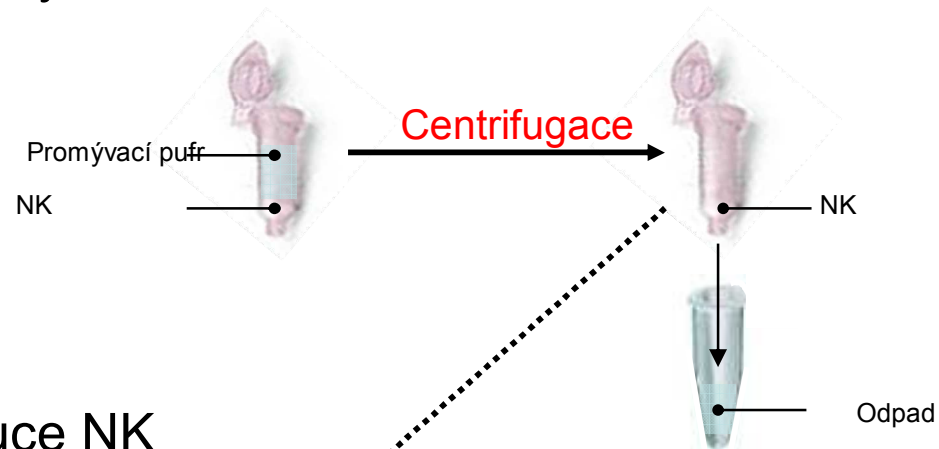
- nízké pH
- vysoká iontová síla
- chaotropní soli

## Krok 2: Adsorpce na silikagel

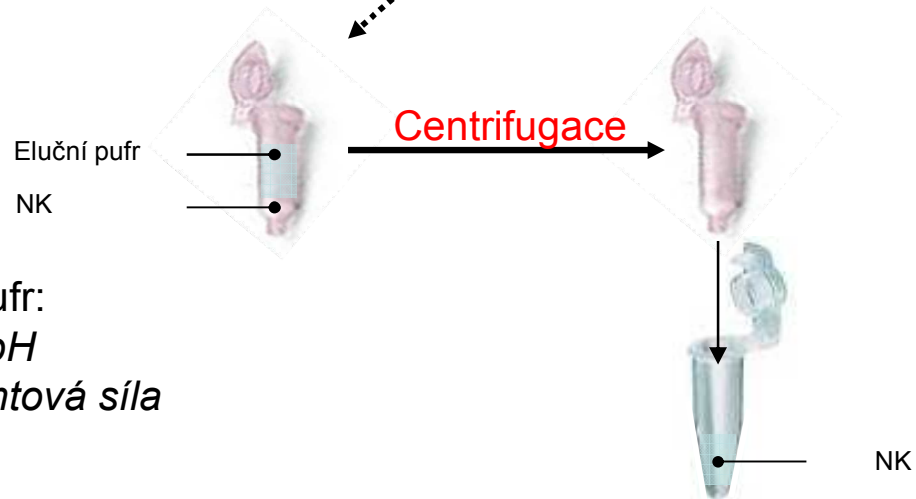


# Adsorpční chromatografie

## Krok 3: Vymytí kontaminant

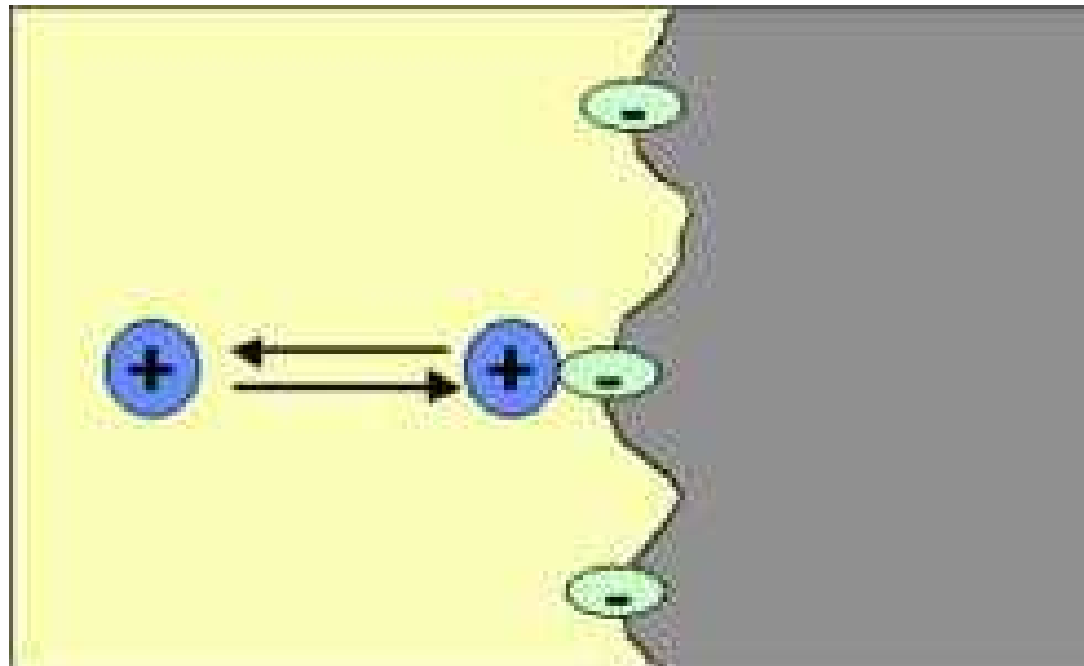


## Krok 4: Eluce NK



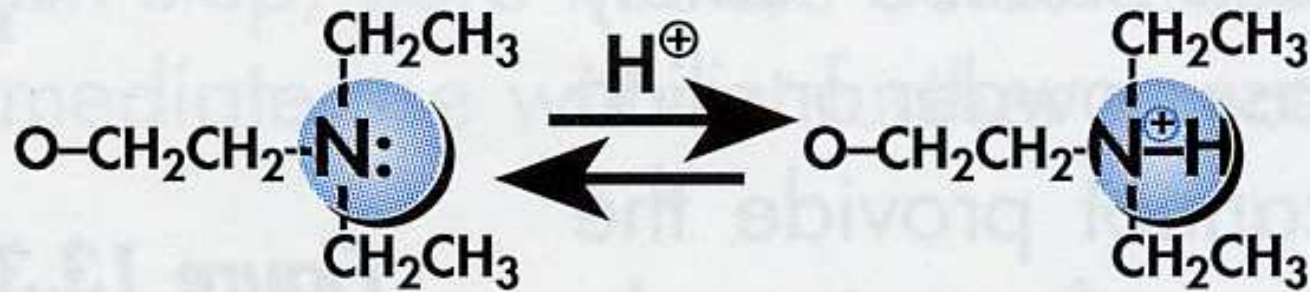
Eluční pufr:  
*Vysoké pH*  
*Nízká iontová síla*

# Ionexová chromatografie



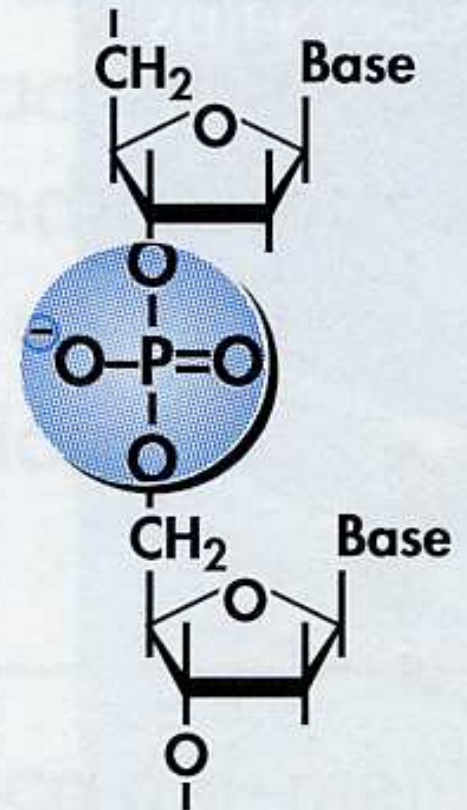
# Ionexová chromatografie

Vazba při nízkém pH nízké I



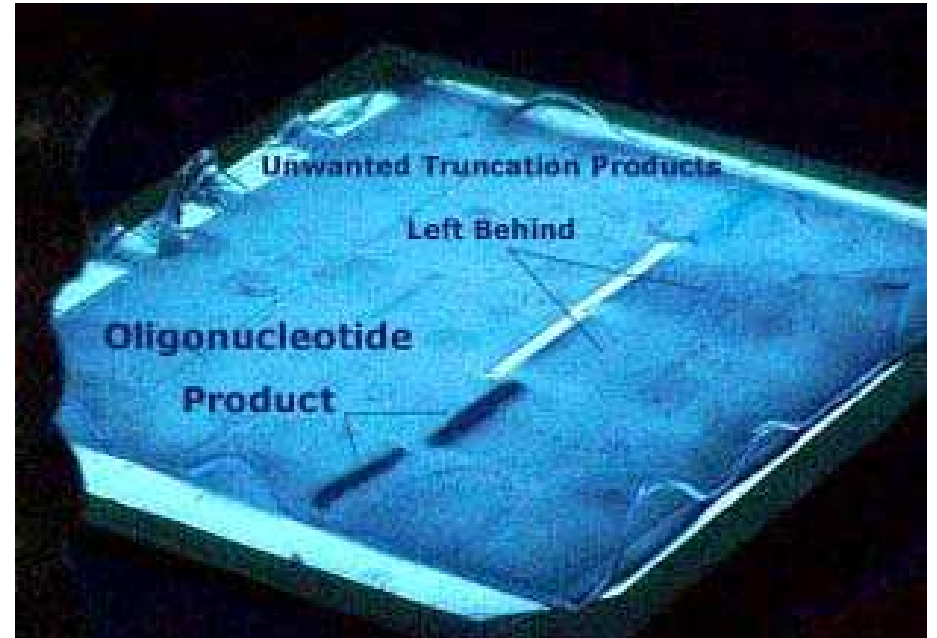
Eluce zvýšením pH nebo vysokou I

DEAE (diethylaminoethanol)



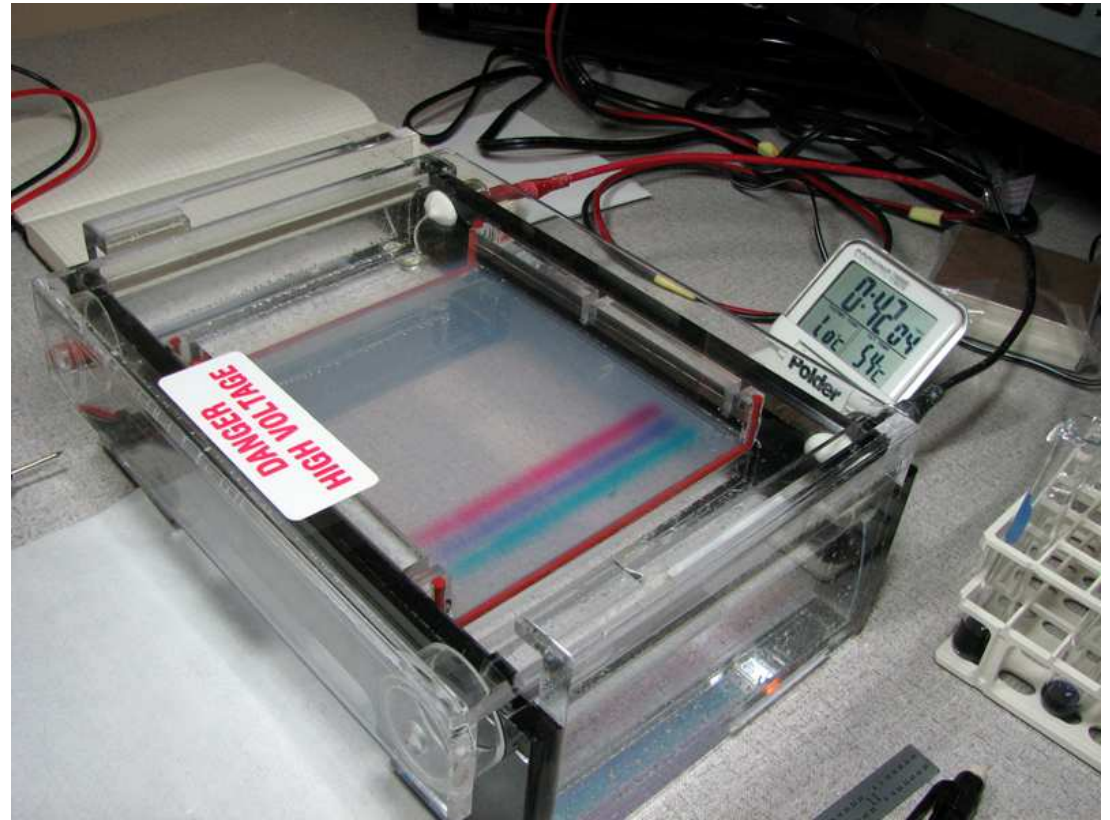
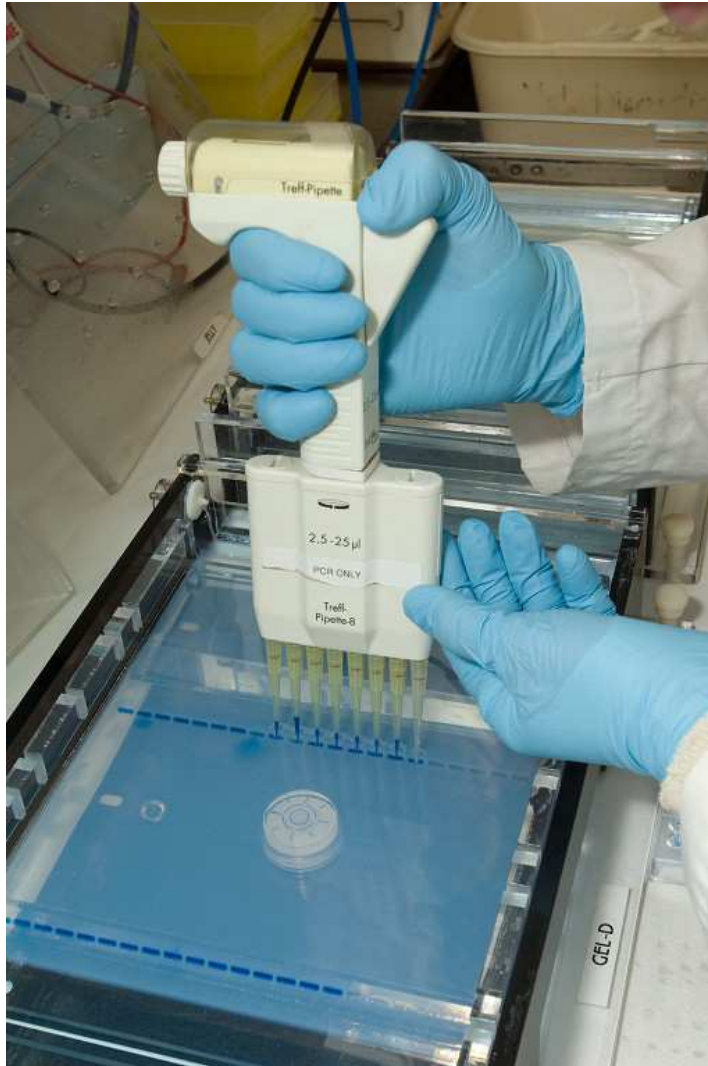
Chemical structure  
of DNA

# Preparativní elektroforéza

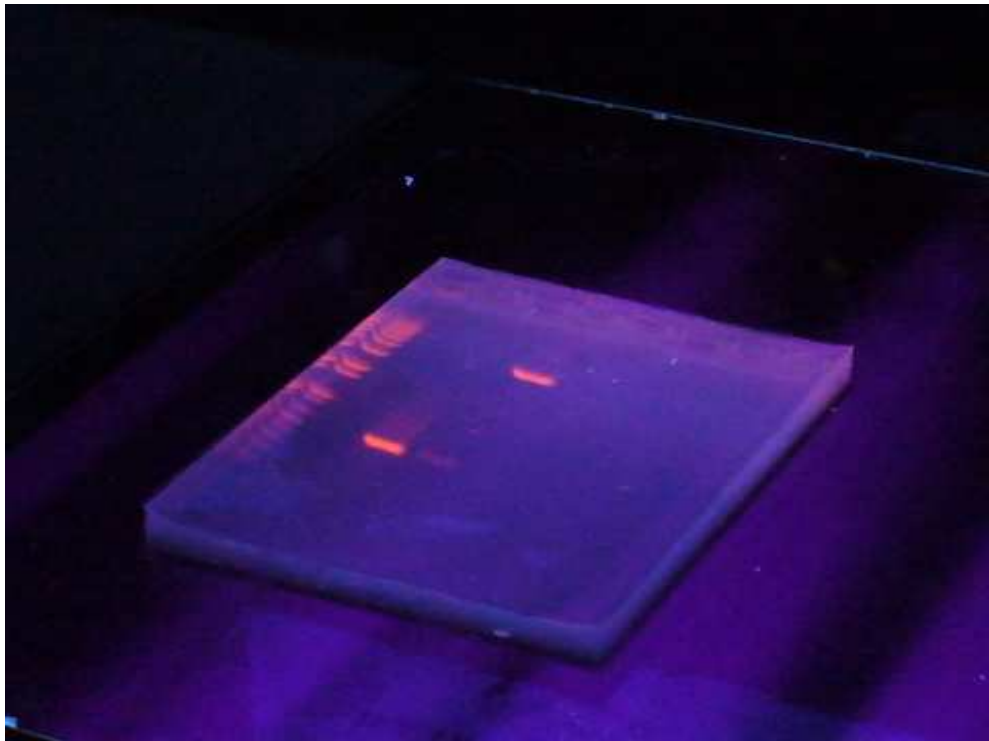




# Preparativní elektroforéza



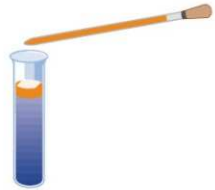
# Preparativní elektroforéza



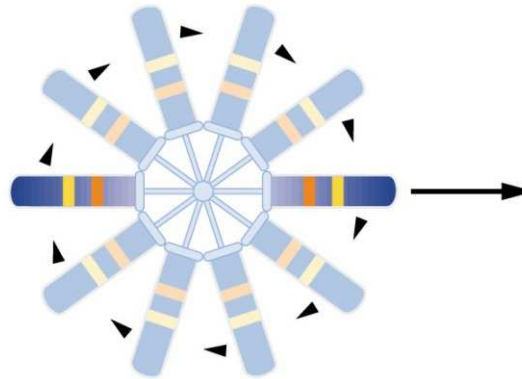
# Separation of Nucleic Acids by CsCl Gradient Centrifugation



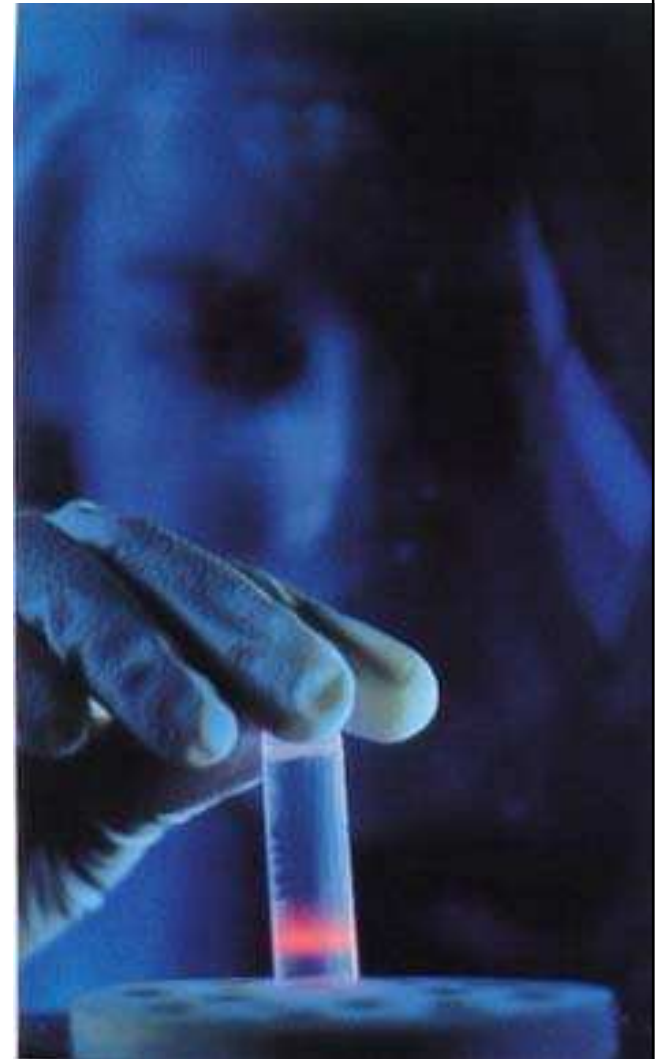
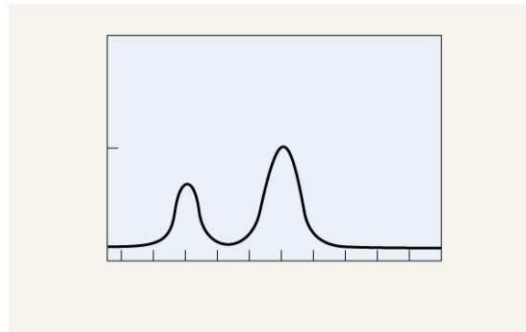
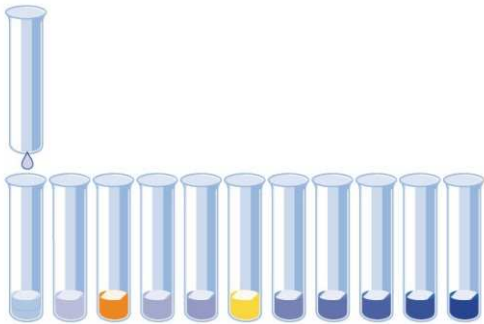
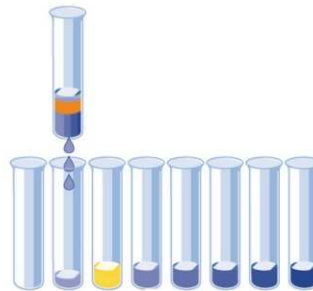
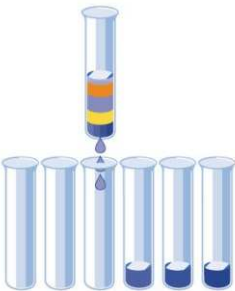
Sample of nucleic acid  
Experiment begins

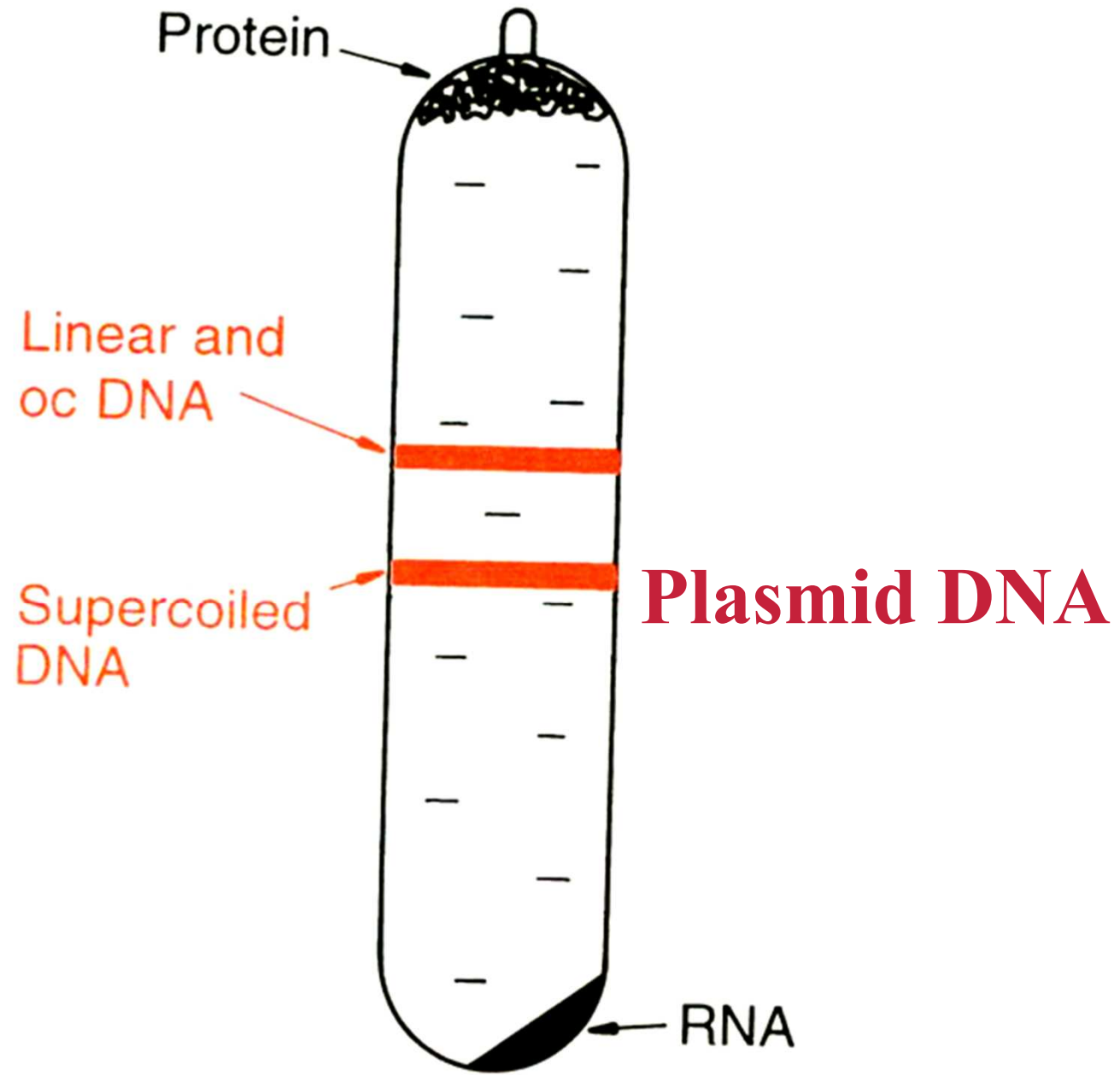


Sample loaded onto top of concentration gradient



Tubes placed in ultracentrifuge and rotated at high speed; Sample is separated into its two components





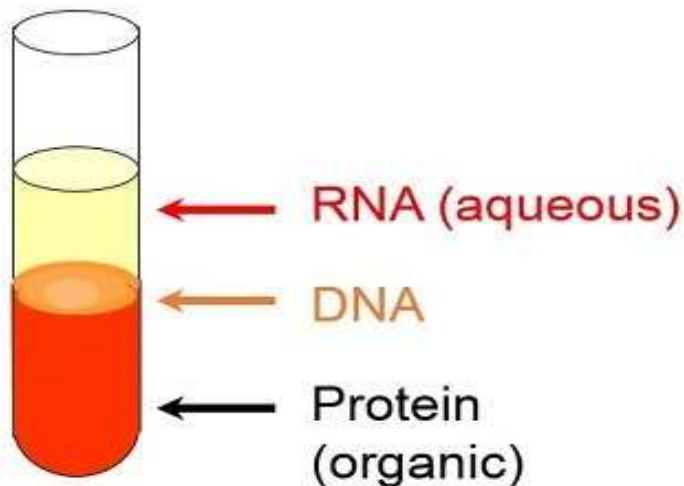
(a) An EtBr-CsCl density gradient

# Izolace RNA - speciální přístupy

- nutno použít inhibitory RNAsy
- extrakce guanidinium chloridem
- fenolová extrakce při  $\text{pH} < 4$  ( $\text{pH} 8$  pro DNA)

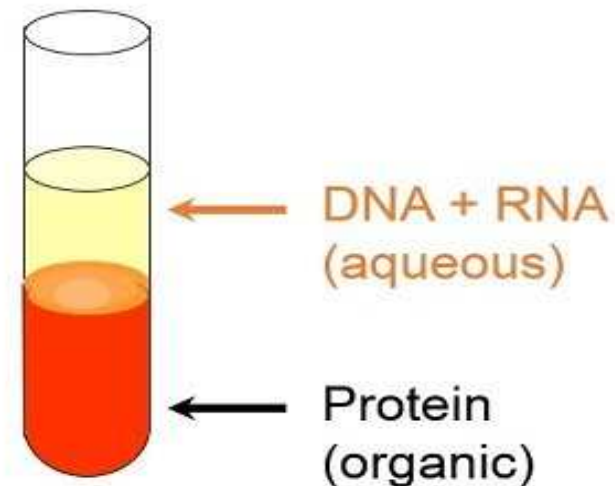
## Traditional Phenol Extraction

Phenol, pH 4



RNA

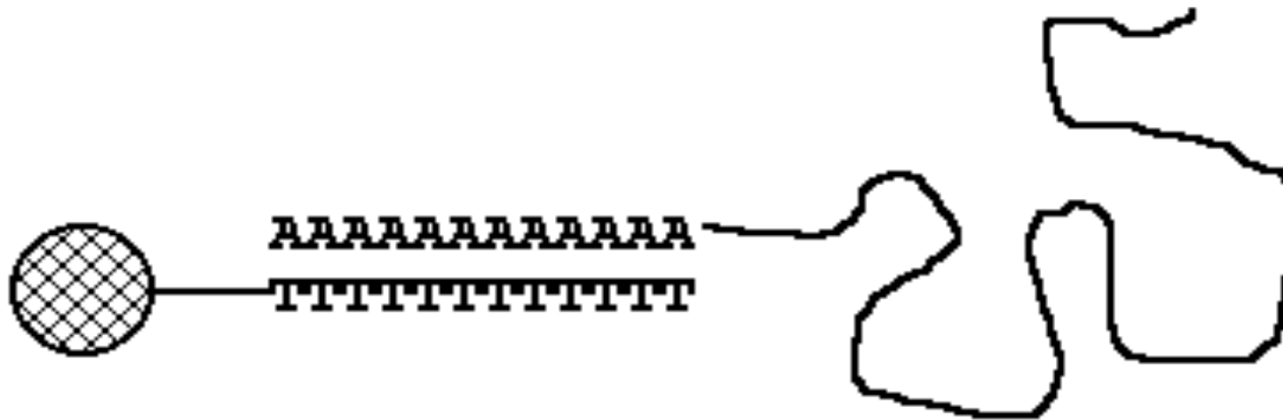
Phenol, pH 8



DNA

# Izolace RNA - speciální přístupy

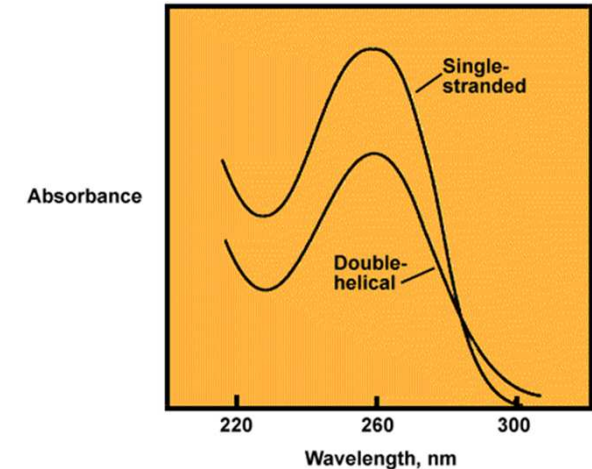
- působení RNase-free DNase
- selektivní precipitace rRNA, mRNA s LiCl
- oligo-dT afinitní chromatografie - mRNA



# Kontrola čistoty a kvantifikace NK

# Kontrola NK

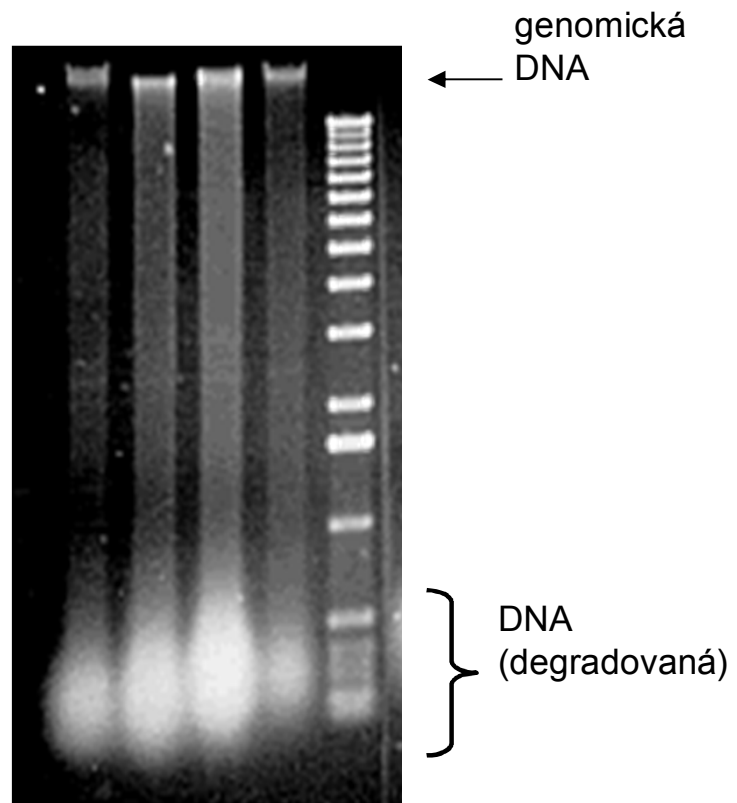
- spektrofotometricky
  - kvalita
  - kvantita
- gelová elektroforéza
  - kvalita



DNA	$A_{260}$	$1.0 \approx 50 \mu\text{g/ml}_{\text{ds}} \approx 33 \mu\text{g/ml}_{\text{ss}}$
	$A_{260}/A_{280}$	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

