

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**

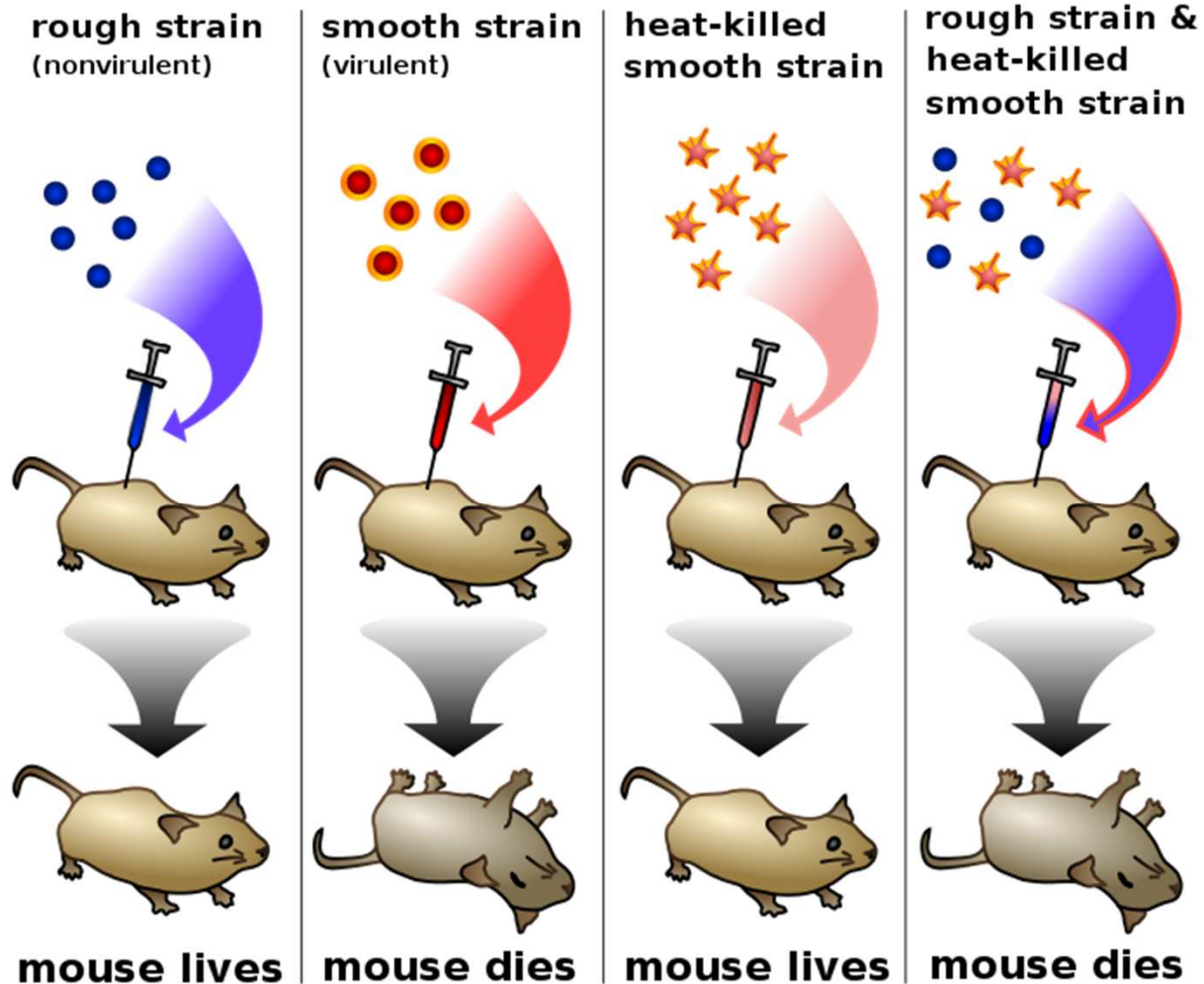
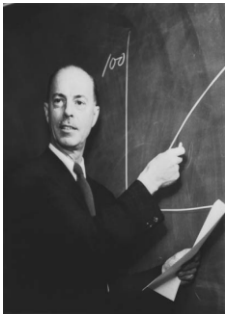
4. Leven (1909)

– **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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(živočišné tkáně – thymus a kvasinky) – nazývá izolovanou látku – **kyselina nukleová - DNA**

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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
- H_3PO_4

Funkce :

DNA – nositel genetické informace

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

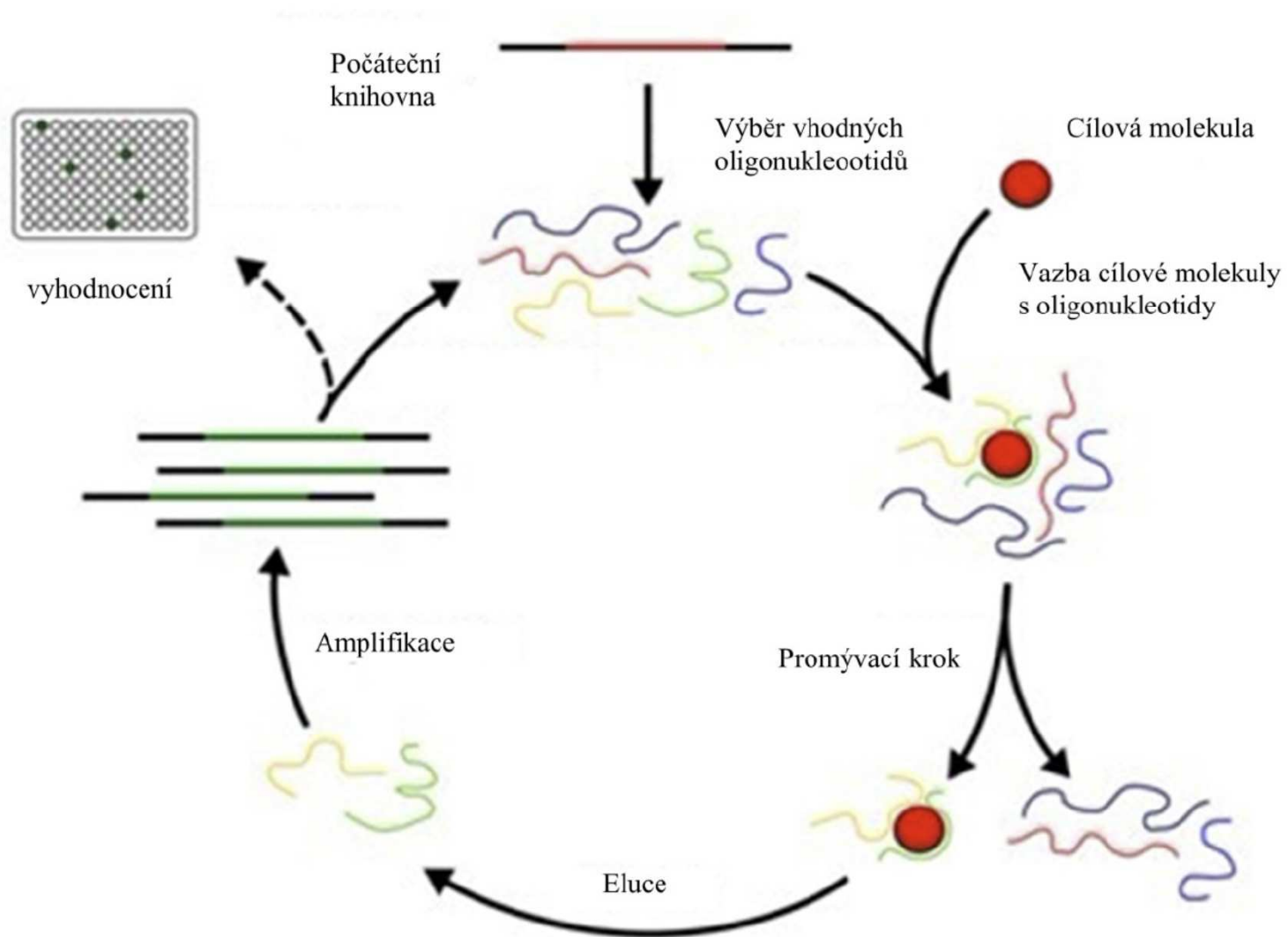
- DNAzymy

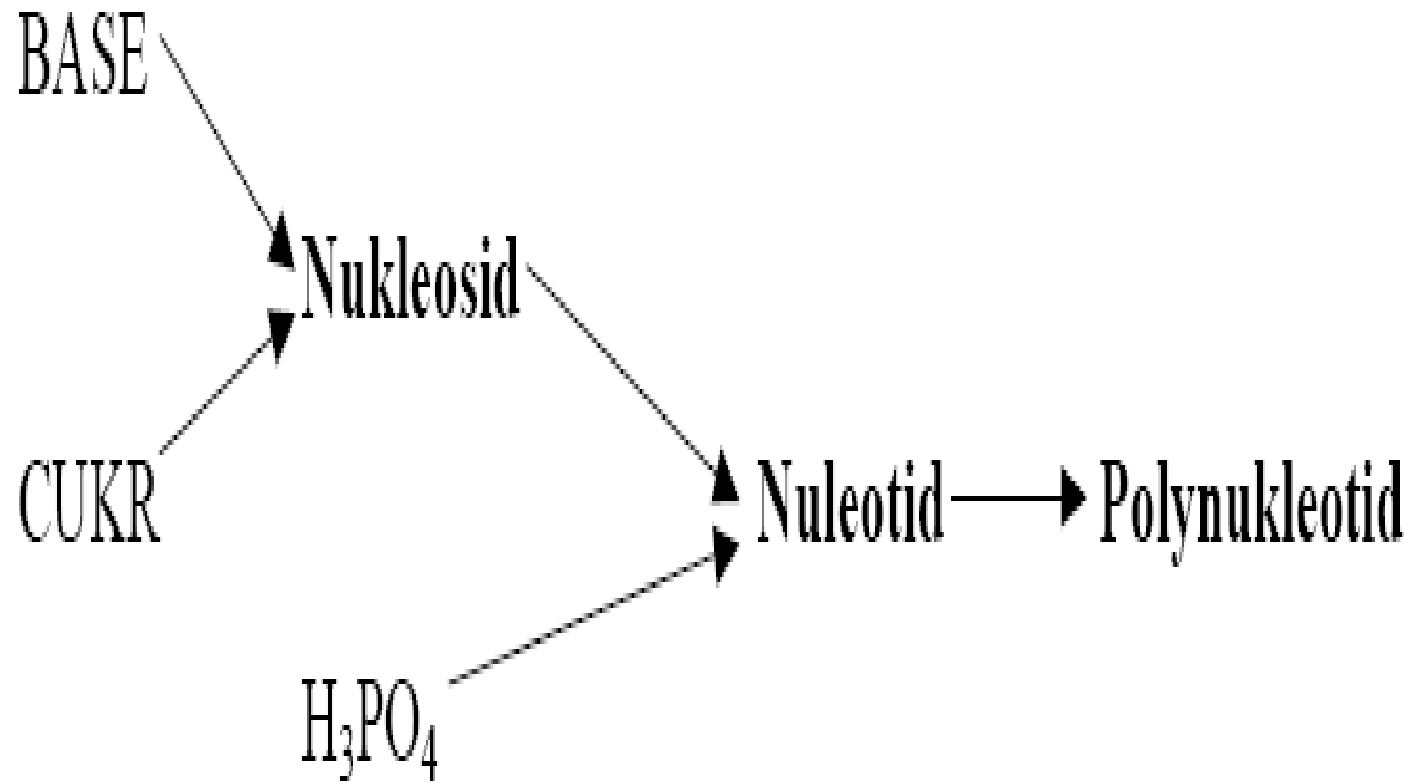
RNA – realizace genetické informace (u RNA virů i nositel genetické informace)

- Ribozymy - biokatalyzátory

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

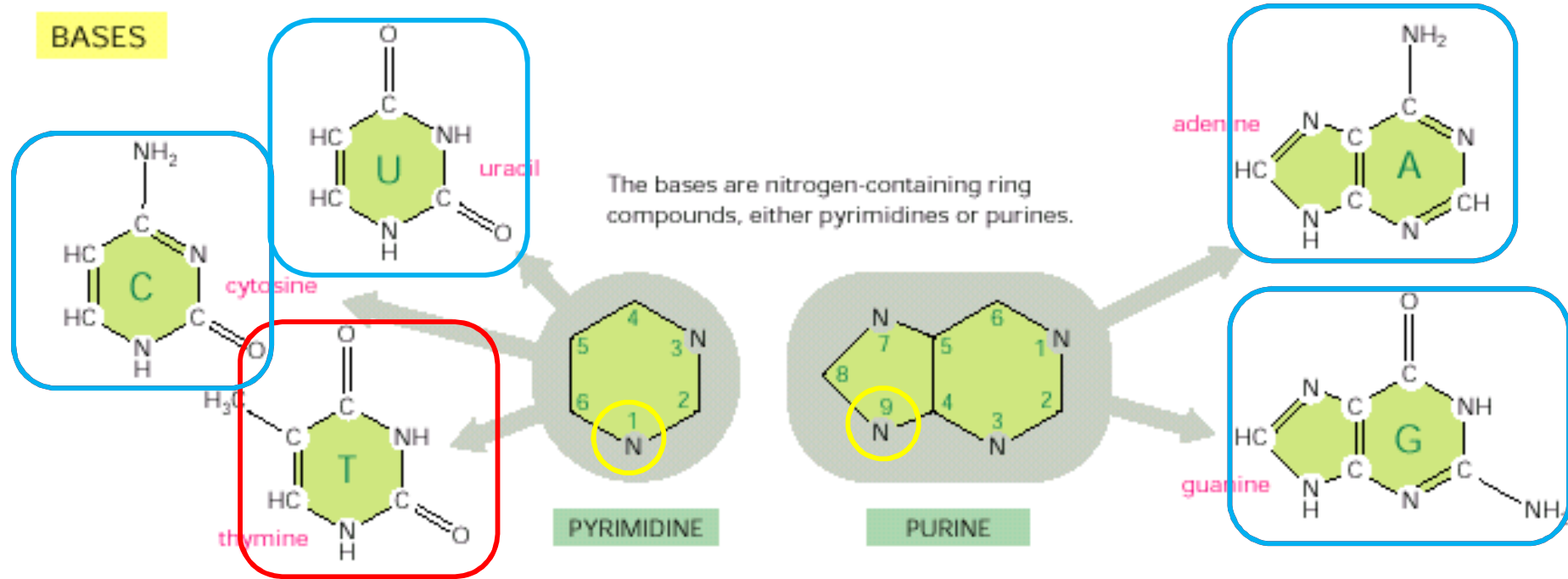
SELEX



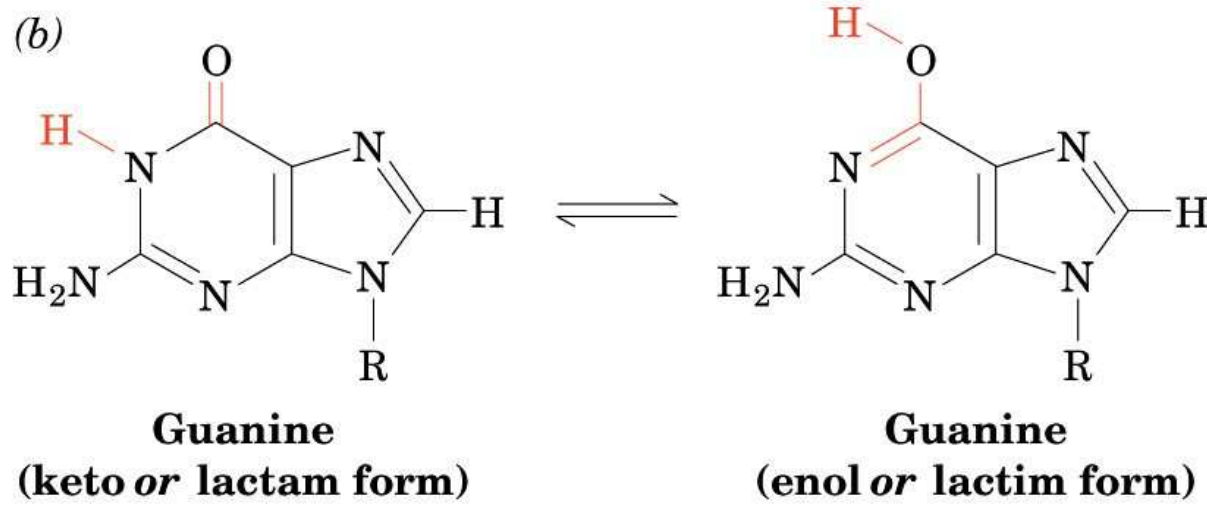
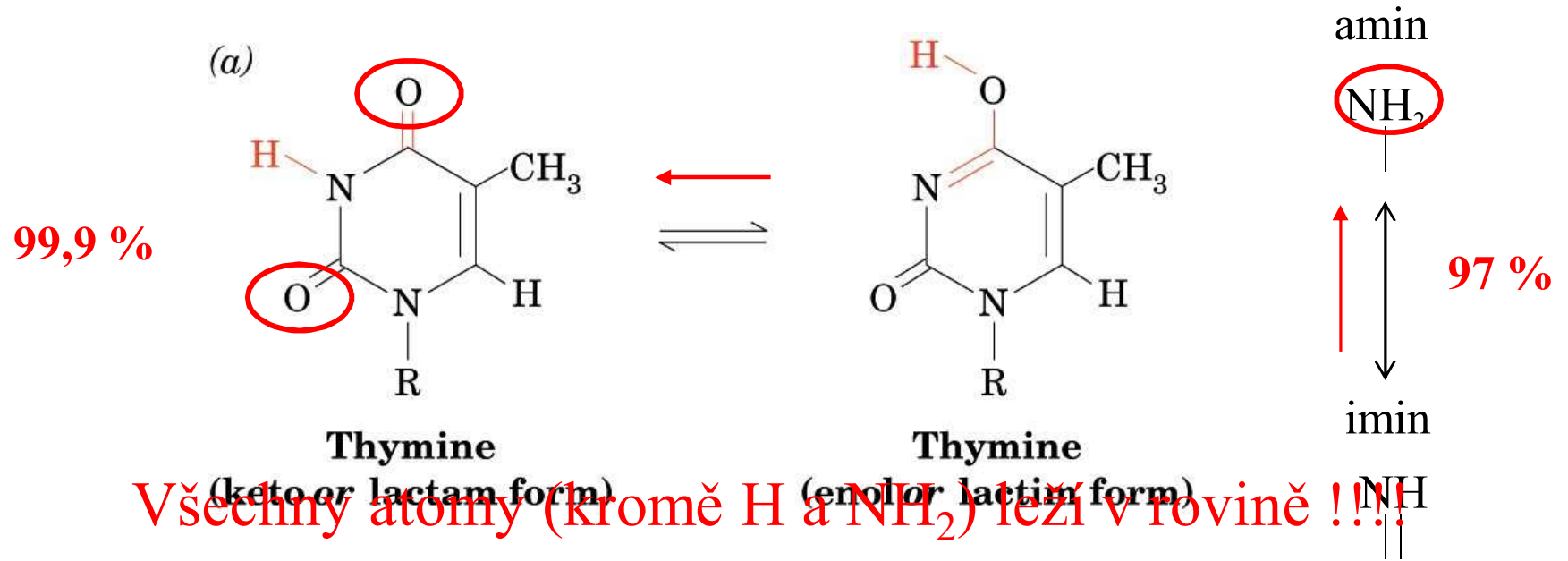


Báze

RNA



Báze -tautomerie

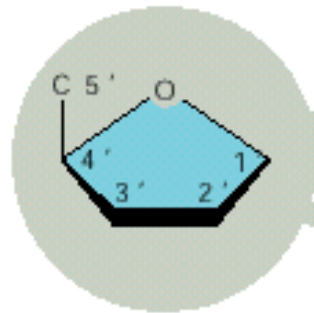


Monosacharidy

SUGARS

PENTOSE

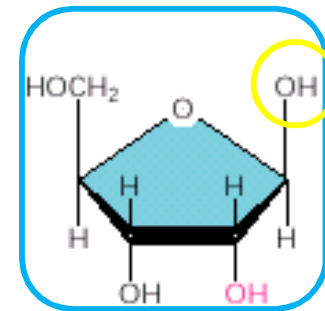
a five-carbon sugar



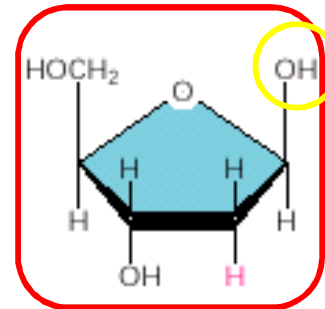
two kinds are used

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



β -D-ribose
used in ribonucleic acid

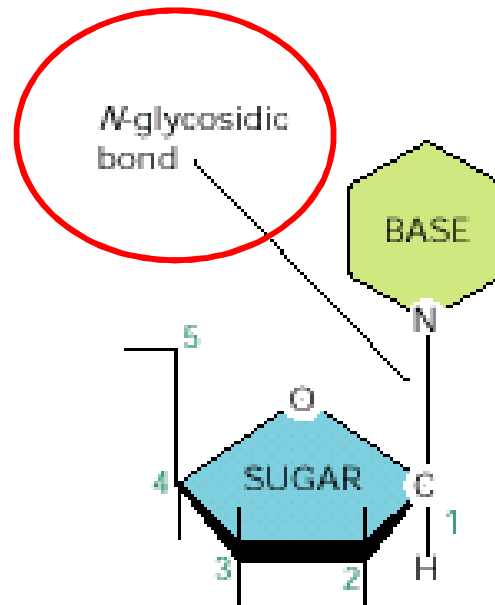


β -D-2-deoxyribose
used in deoxyribonucleic acid

DNA

Nukleosid

BASIC SUGAR LINKAGE

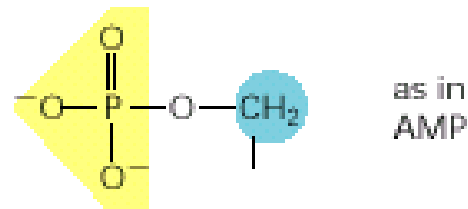


The base is linked to the same carbon (C1) used in sugar-sugar bonds.

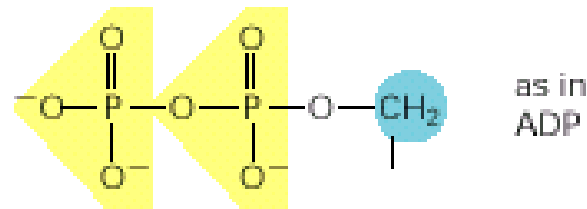
Kyselina fosforečná

PHOSPHATES

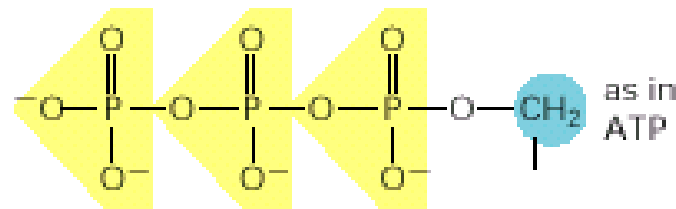
The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.



Ester



Anhydrid



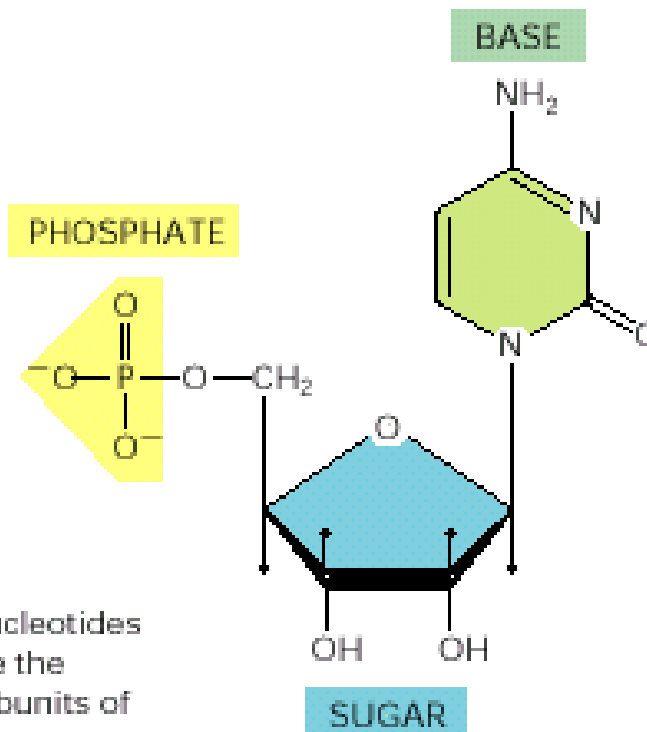
Anhydrid

The phosphate makes a nucleotide negatively charged.

Nukleotid

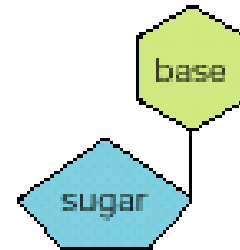
NUCLEOTIDES

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

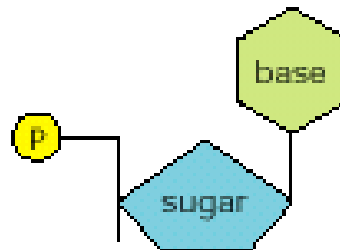


Nucleotides are the subunits of the nucleic acids.

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

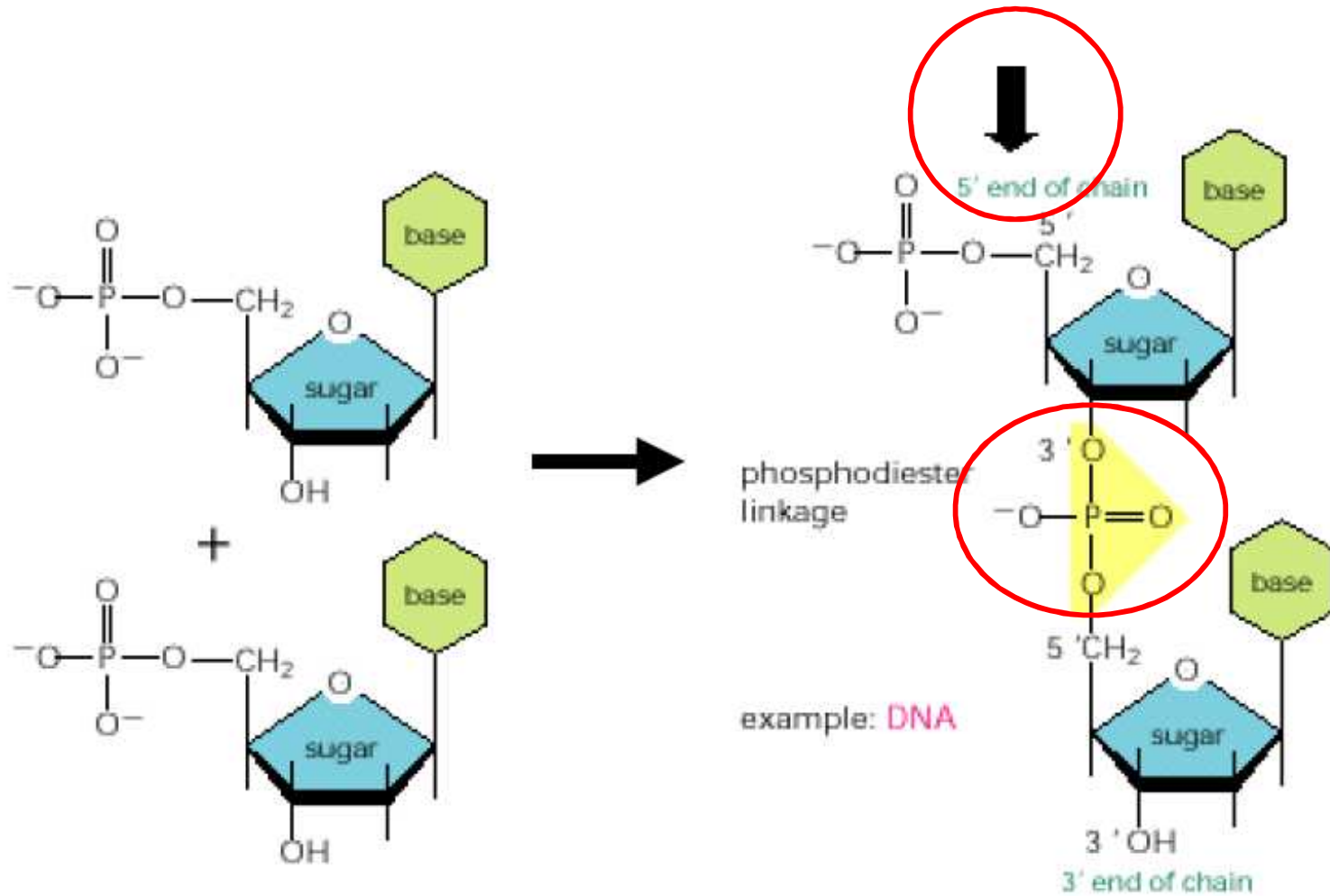
UDP = uridine diphosphate

ATP = adenosine triphosphate

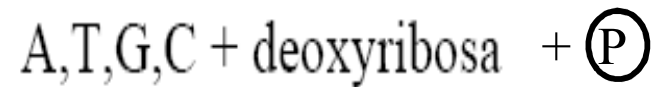
Funkce nukleotidů

- 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

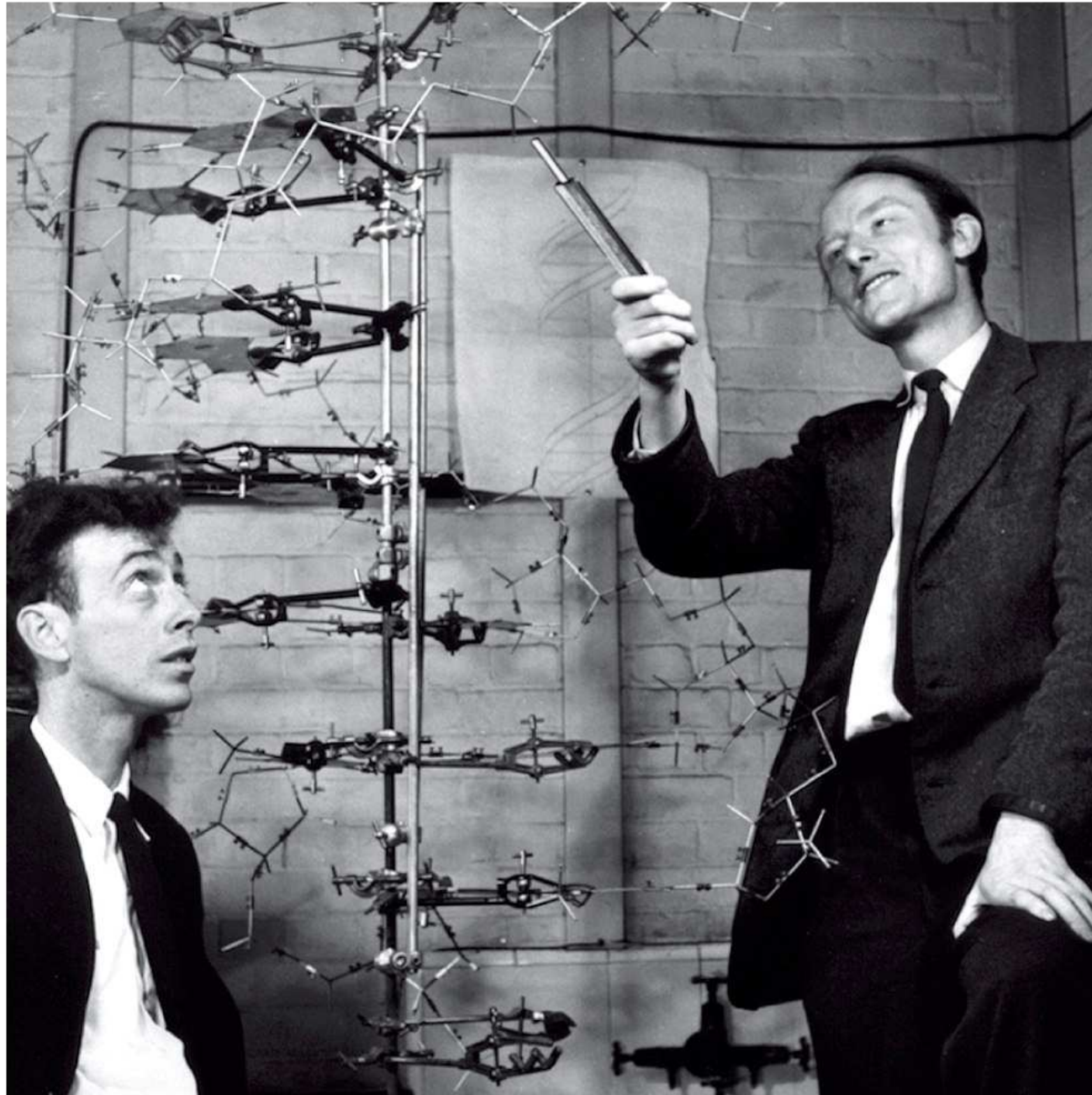
- Chragaffovy pravidla – poměr bazí v DNA



- Donohue – báze v tautomerních ketoformách

- Franklinová –RTG difrakční analýza

Watson

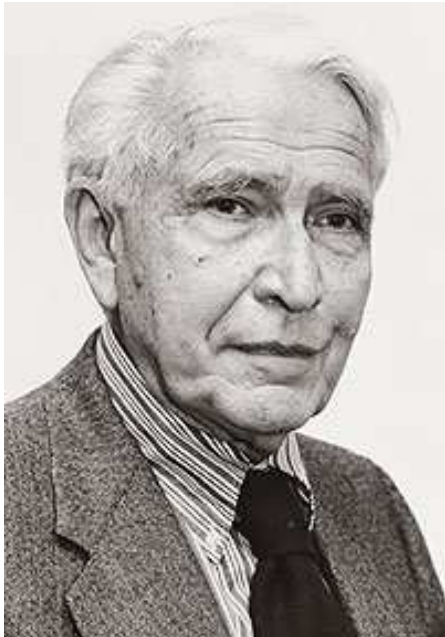


Crick

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

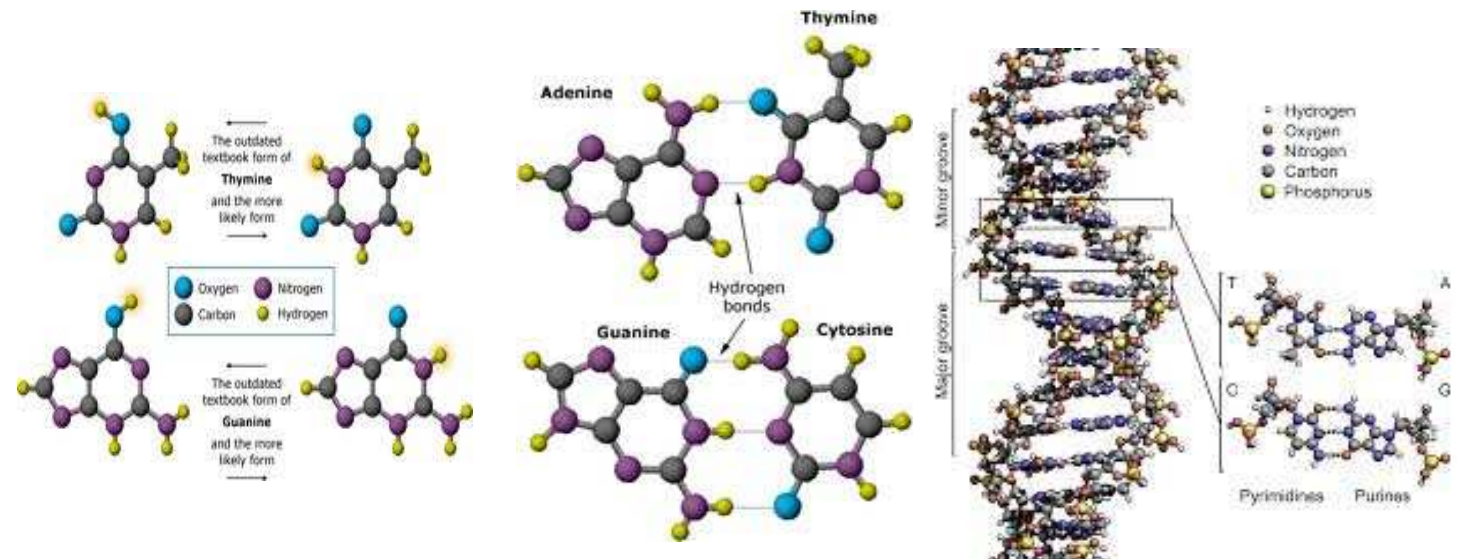
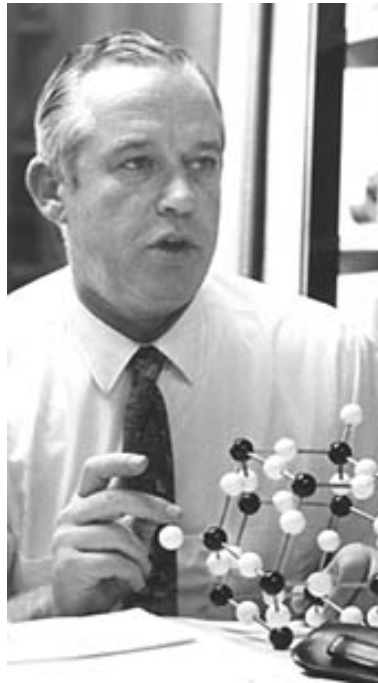
nezávisí na stáří

nezávisí na nutričních faktorech

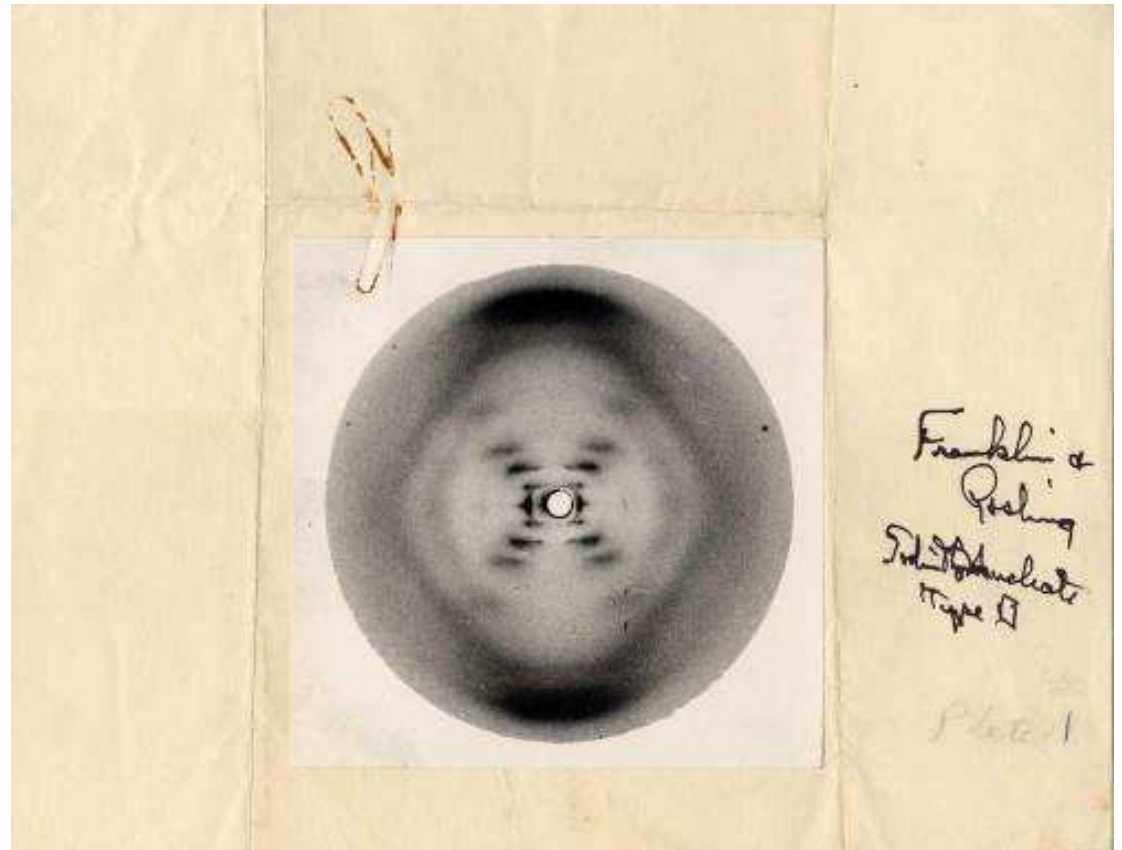
nezávisí na životním prostředí

Jerry Donohue

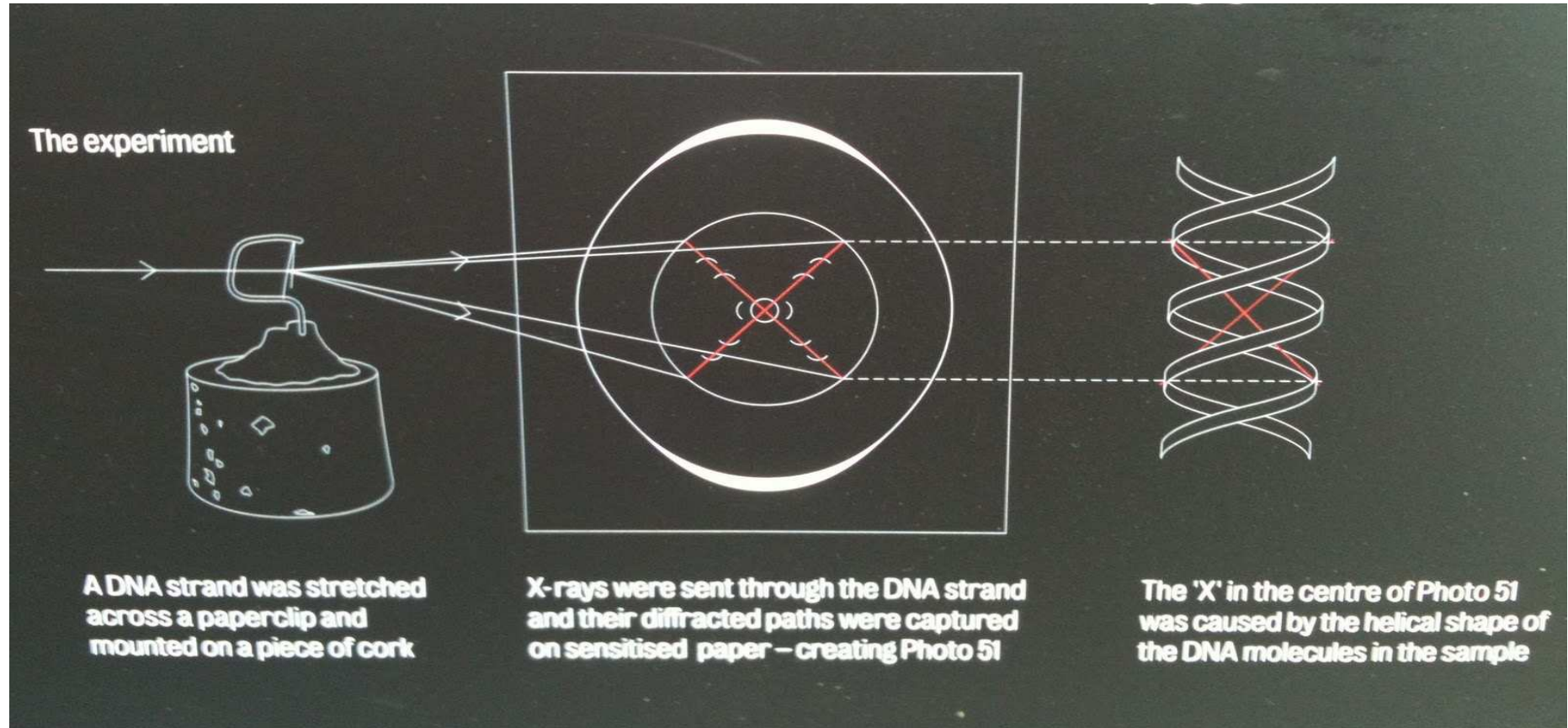
tautomerní formy bází



Rosalind Franklin



Obrázek 51



Nobelova cena 1962



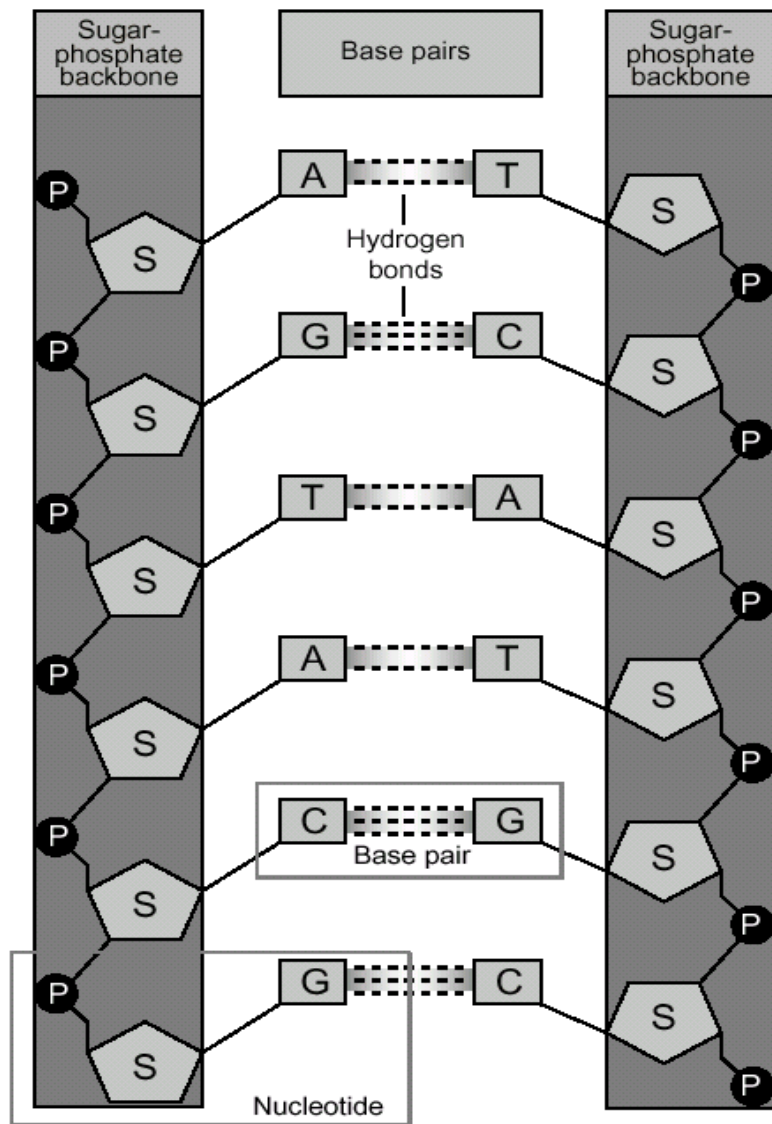
**Francis Harry
Compton Crick
(1916-2004)**



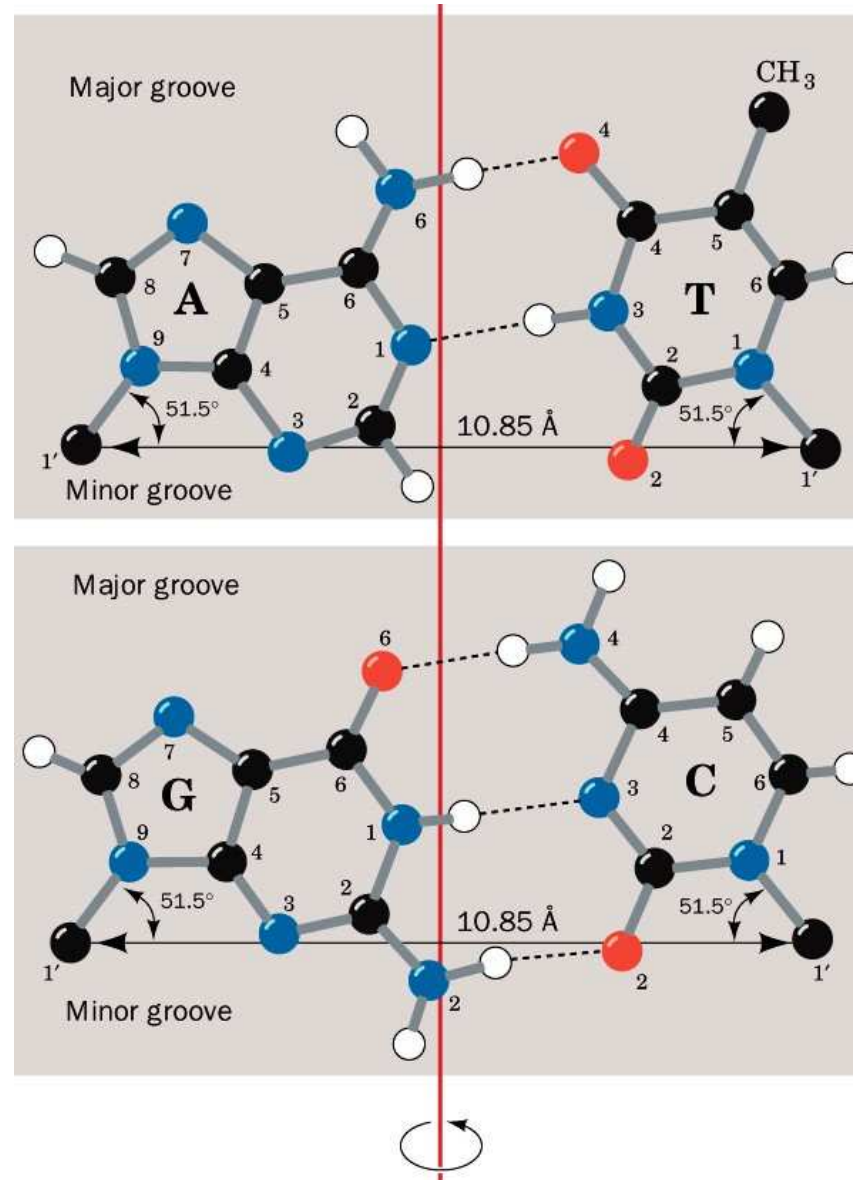
**James Dewey
Watson
(1928 -)**



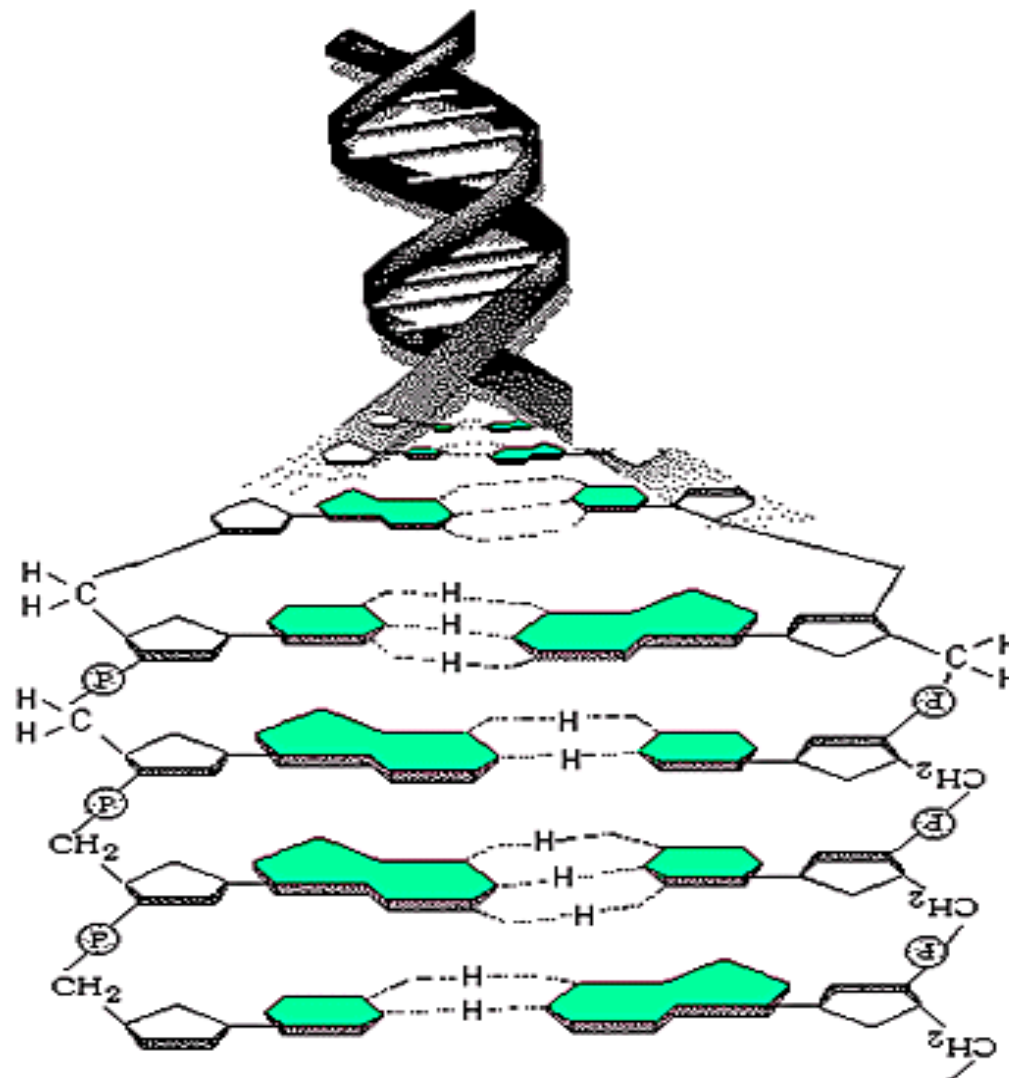
**Maurice Hugh
Frederick Wilkins
(1916-2004)**



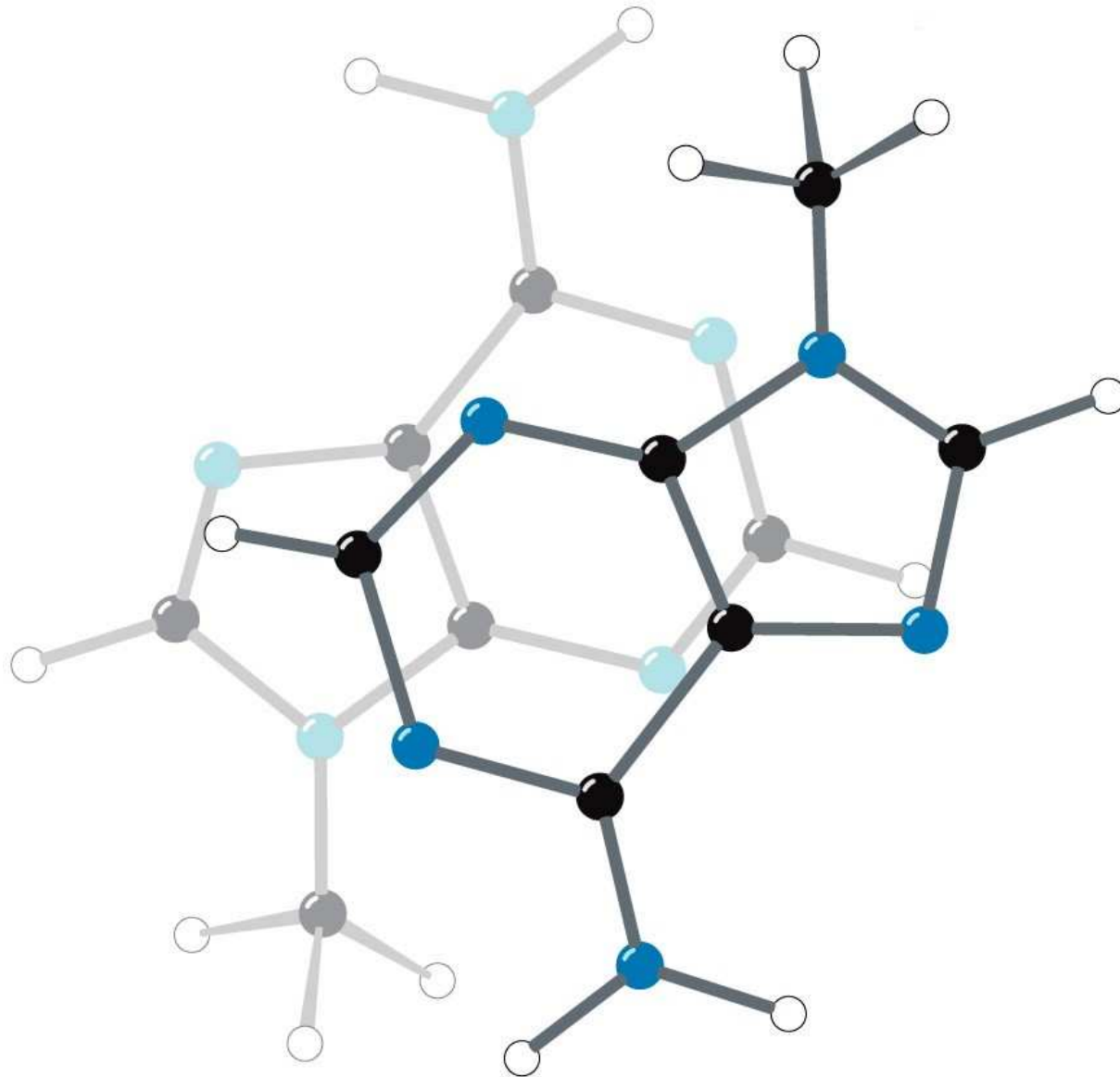
Párování basí – H můstky



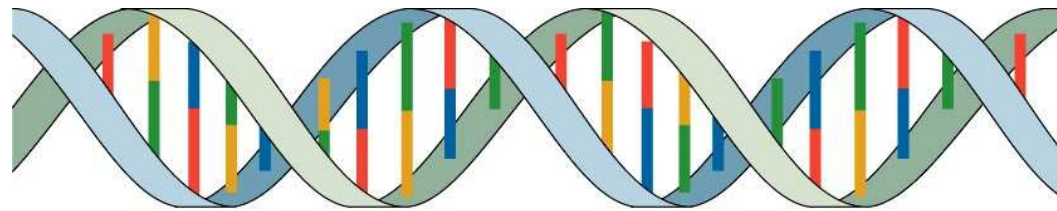
Stabilizující vazby v DNA



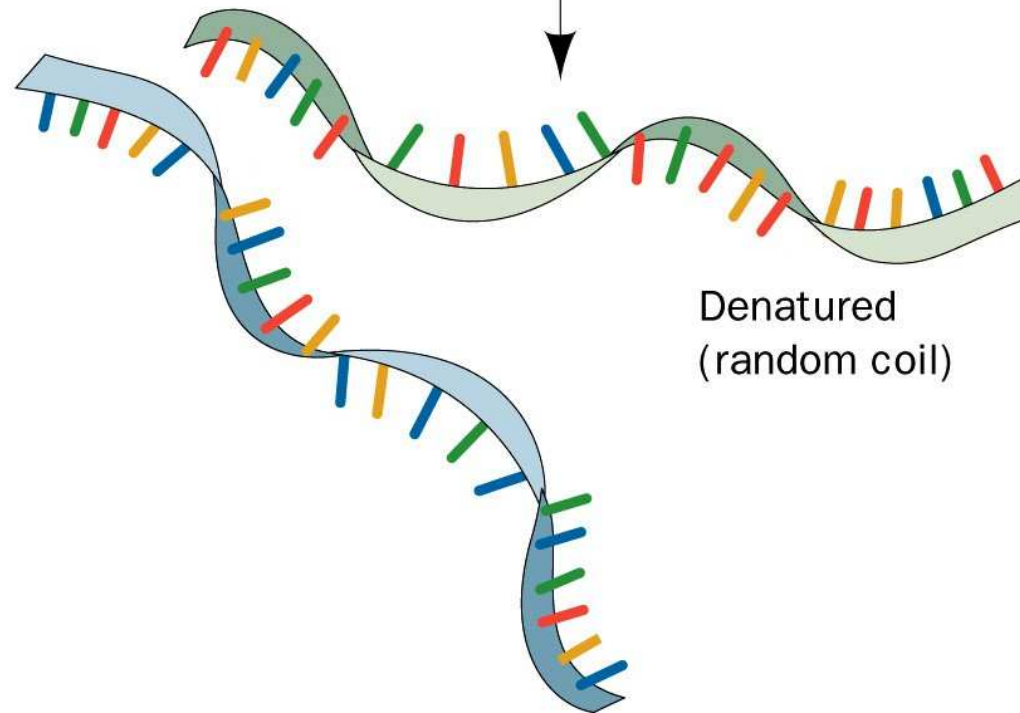
Stohování bází



Denaturace - renaturace

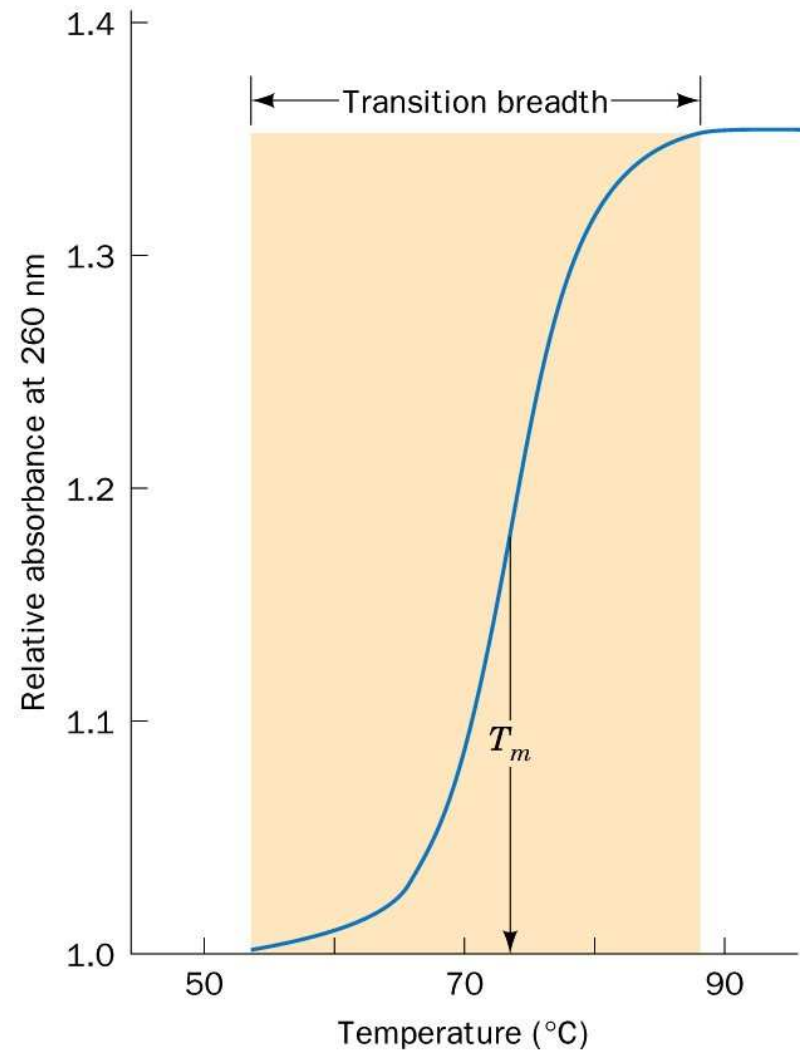
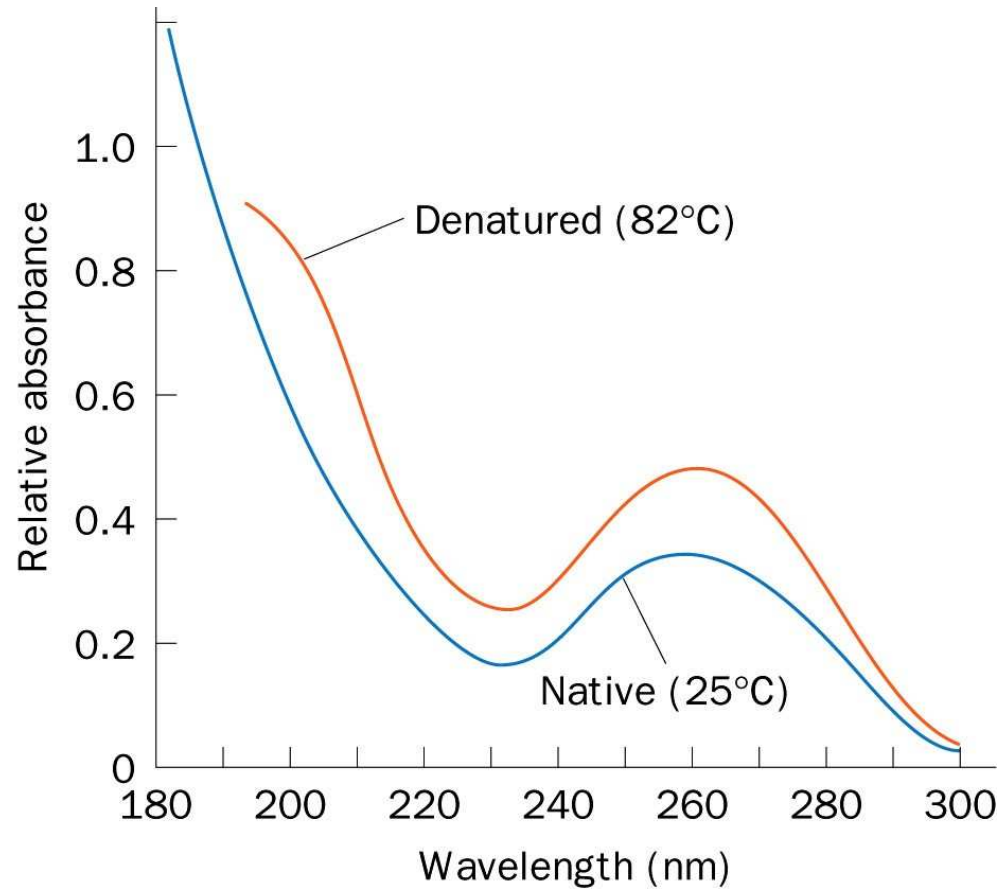


Native (double helix)



Denatured
(random coil)

Denaturace - renaturace



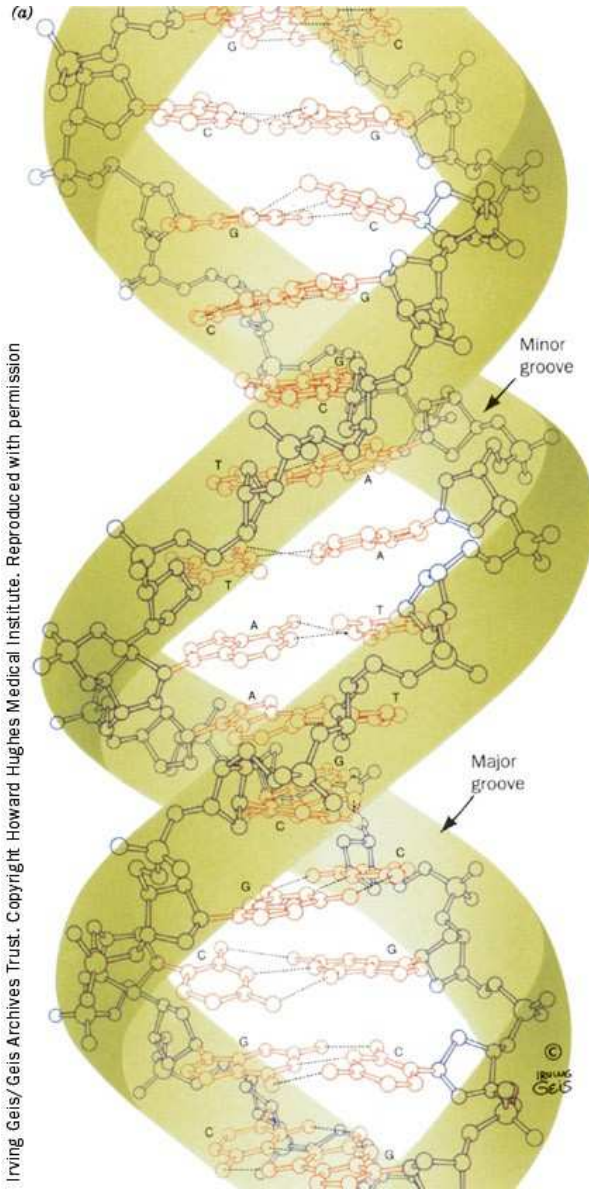
Formy DNA

• B - DNA - nativní 92 % H₂O, Na⁺
pravotočivá - 10 párů bází na závit

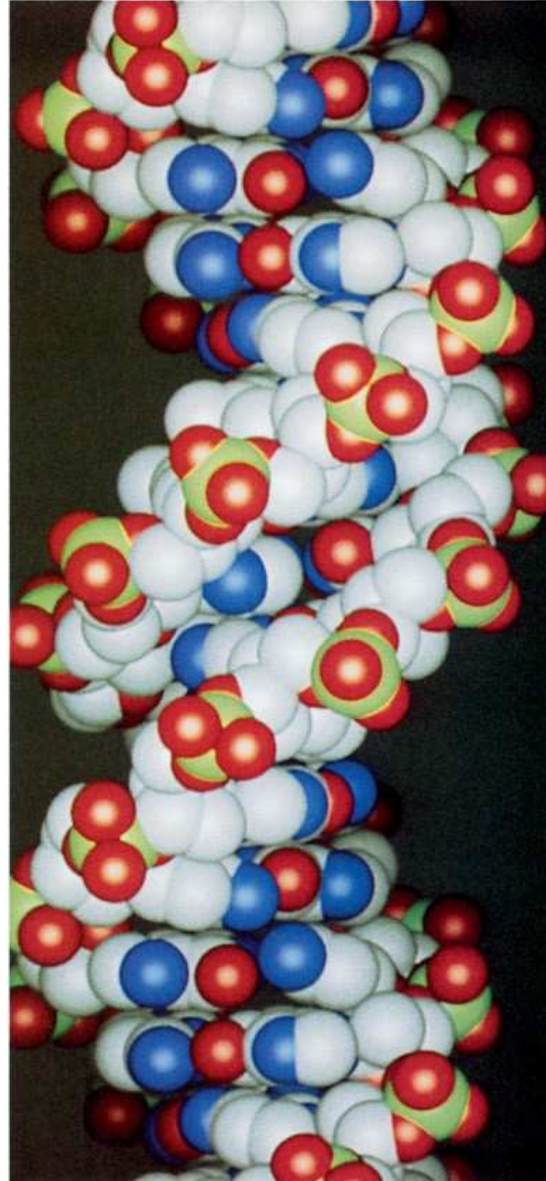
• A - DNA - 75 % H₂O, rovina bází 20°
pravotočivá - 11 párů bází na závit

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

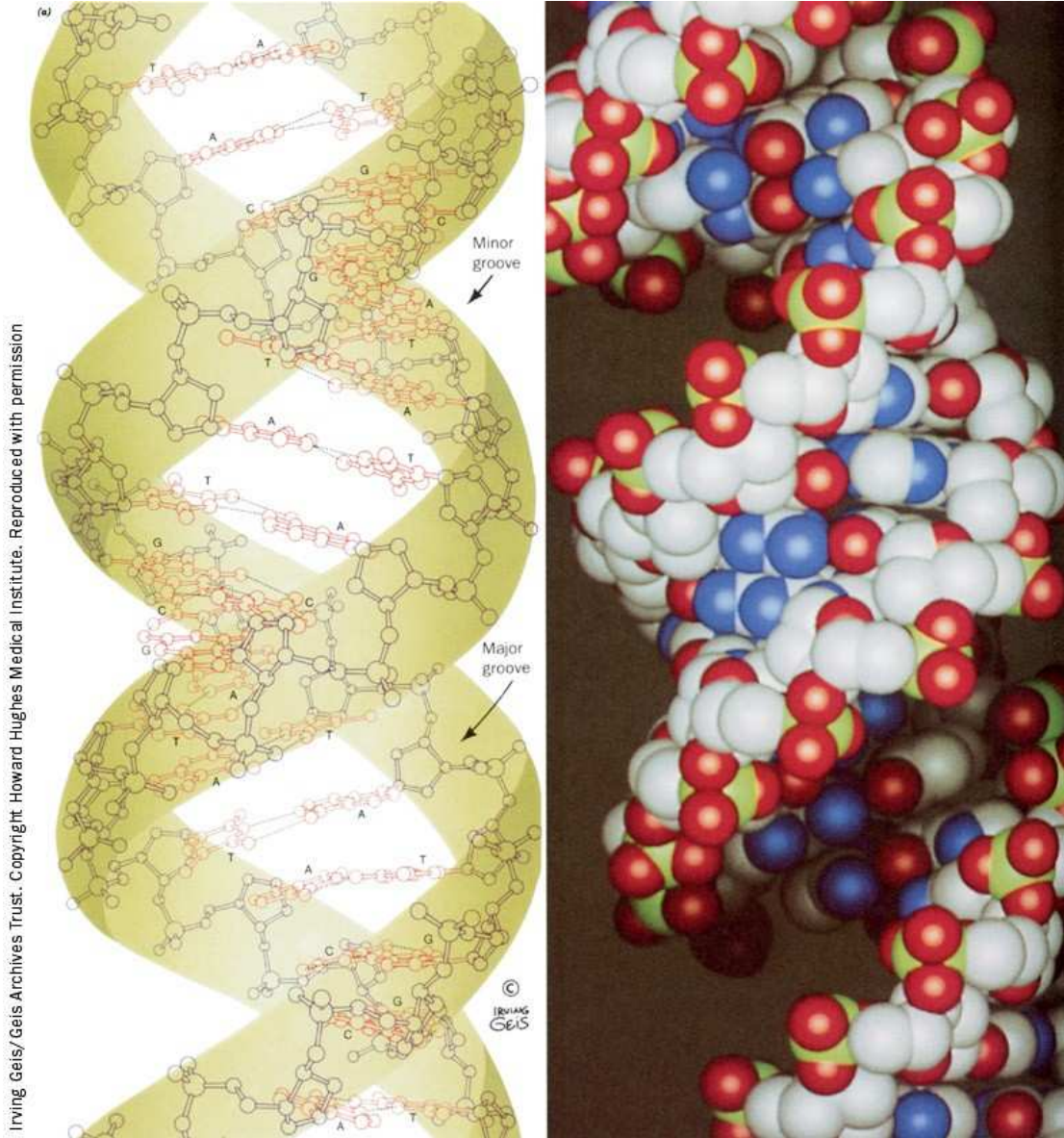
B DNA



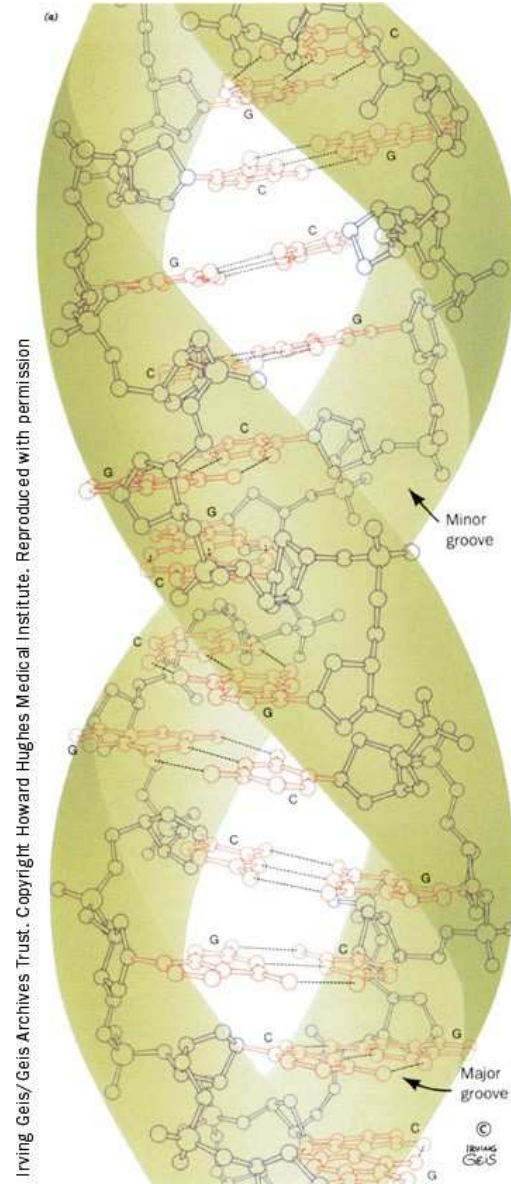
Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with permission



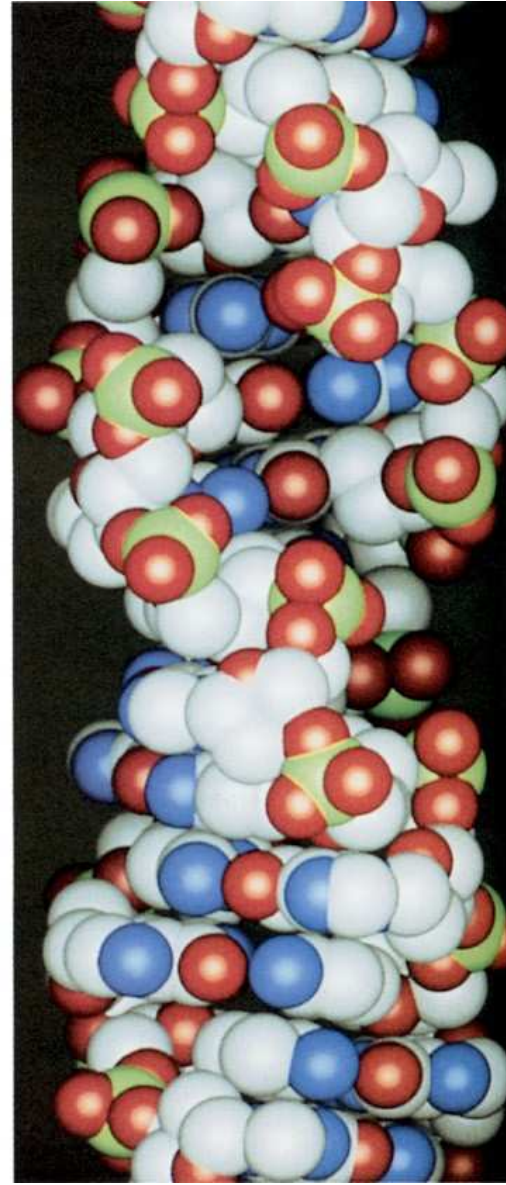
A DNA



Z DNA

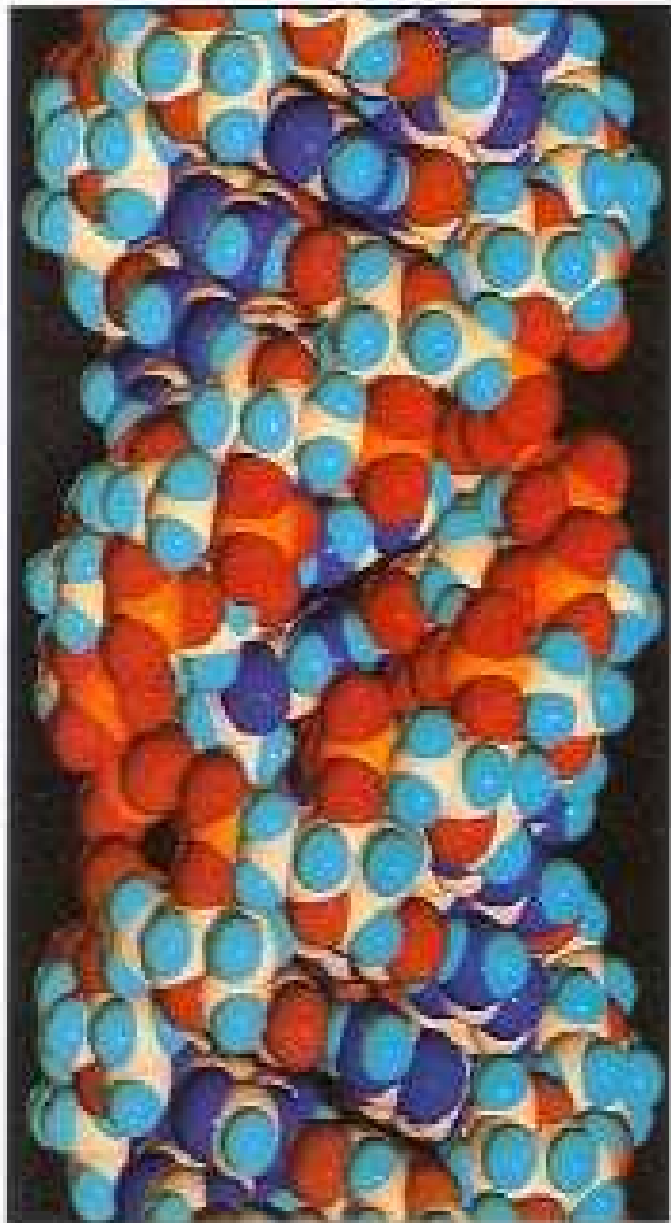


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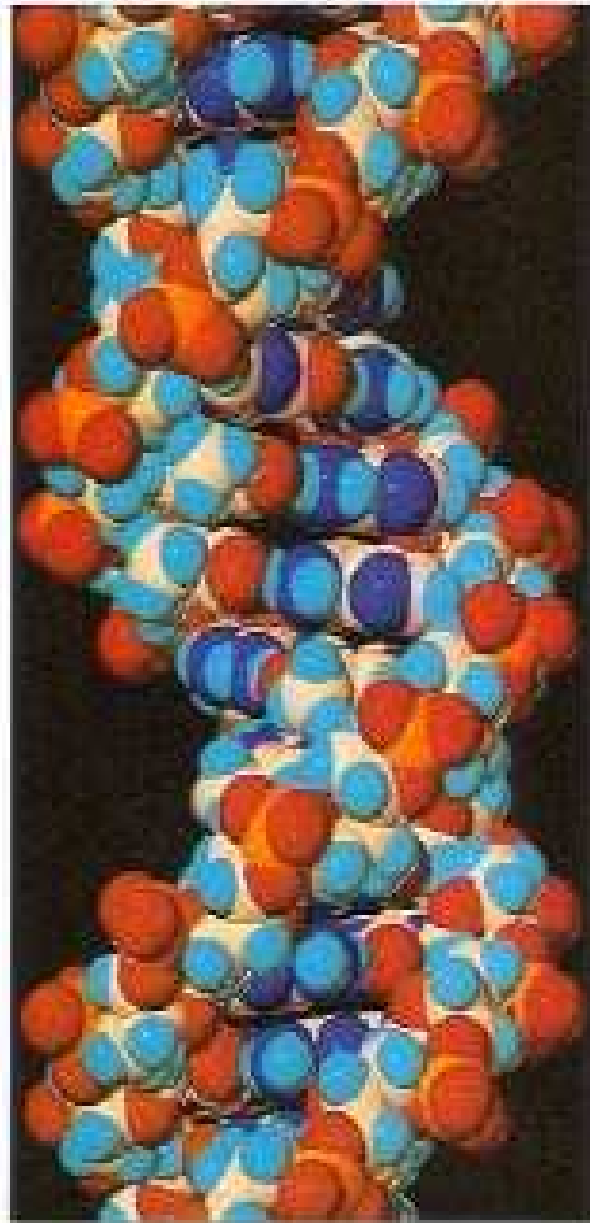


Základní číselné charakteristiky tří nejznámějších helikálních forem DNA

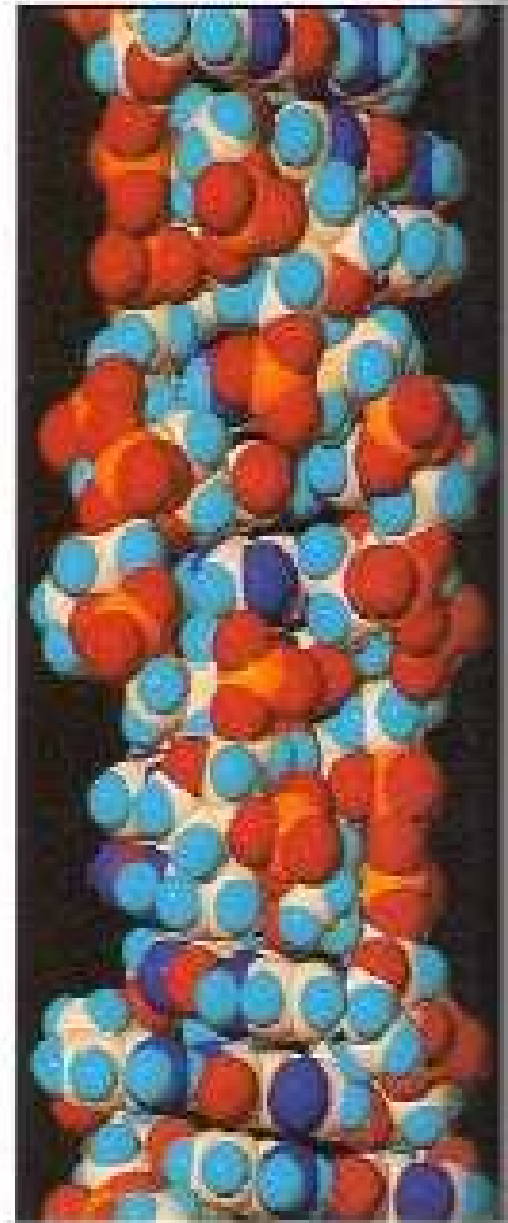
Atribut	A-DNA	B-DNA	Z-DNA
Točivá tendence šroubovice (chiralita) ^[1]	pravotočivá	pravotočivá	levotočivá
Opakování ^[2]	po každém páru	po každém páru	po každých dvou párech
Otočení po každém opakování ^[1]	32,7°	34,3°	
Průměrný počet párů na jedno otočení šroubovice ^[1]	11	10,5	12
Sklon páru k ose ^[2]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



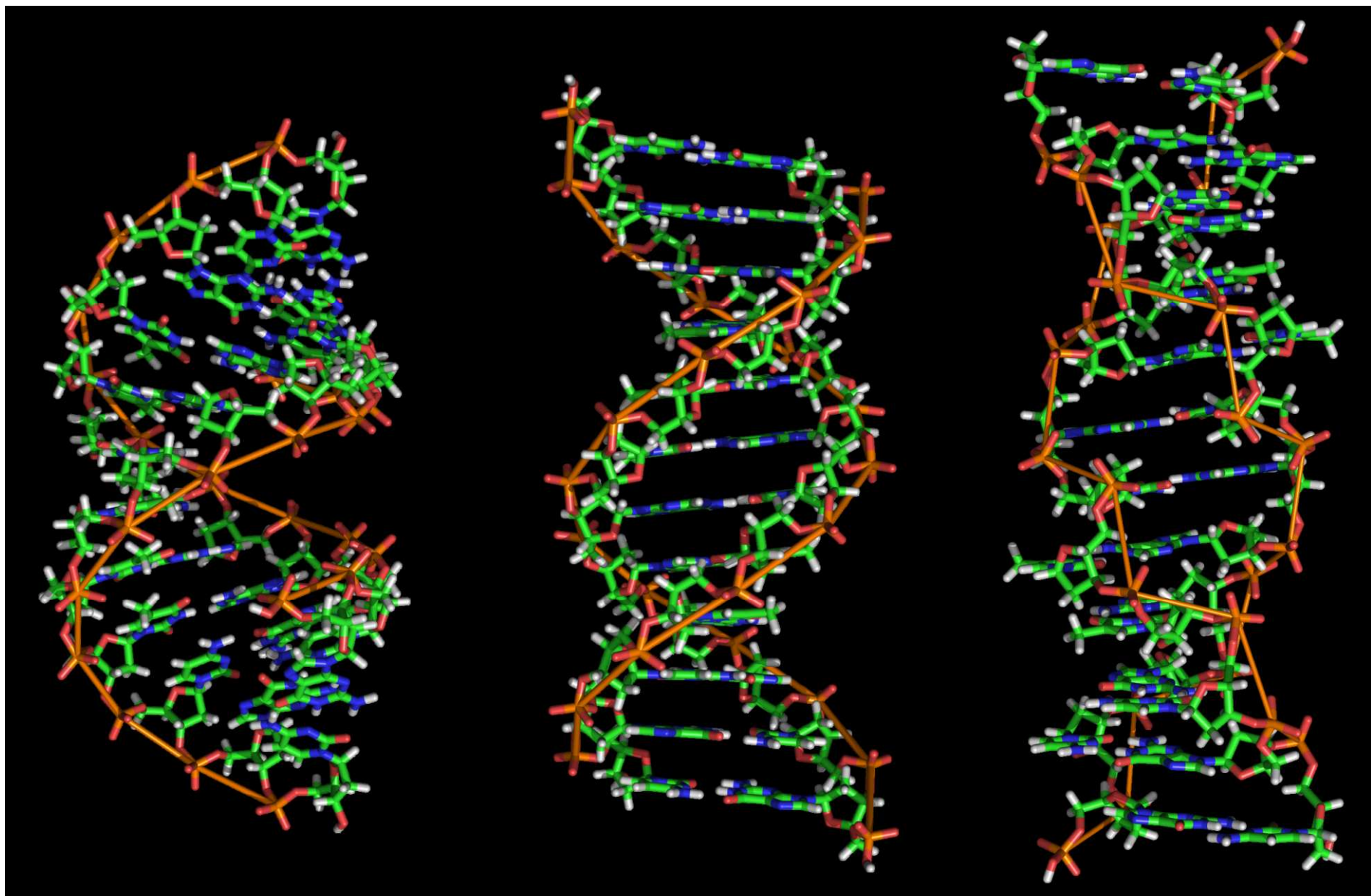
A-DNA



B-DNA



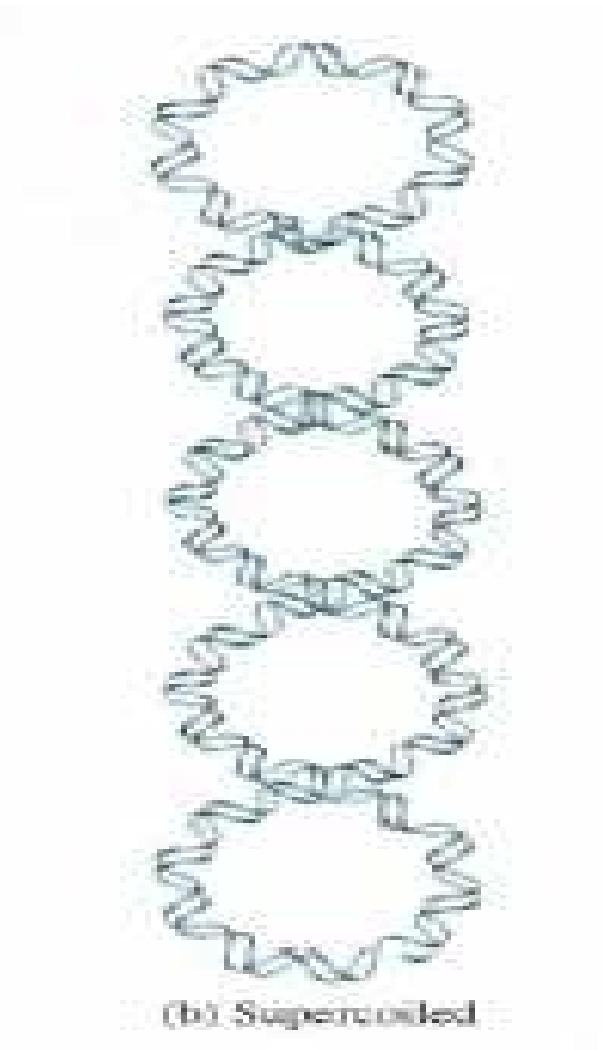
Z-DNA



Bakteriální DNA

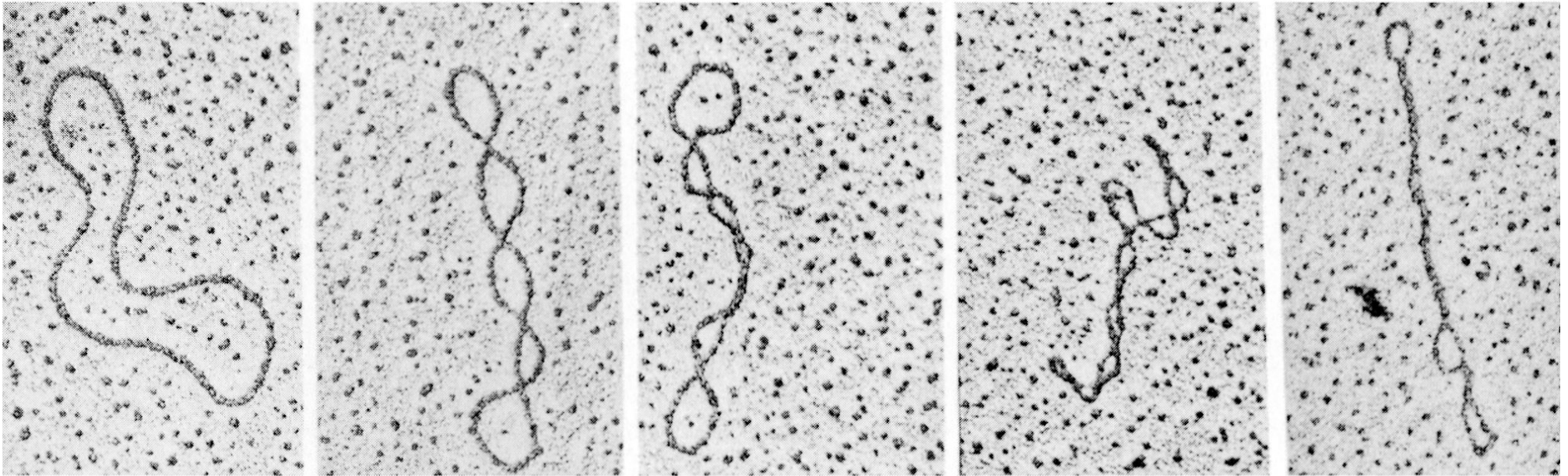


(a) Relaxed



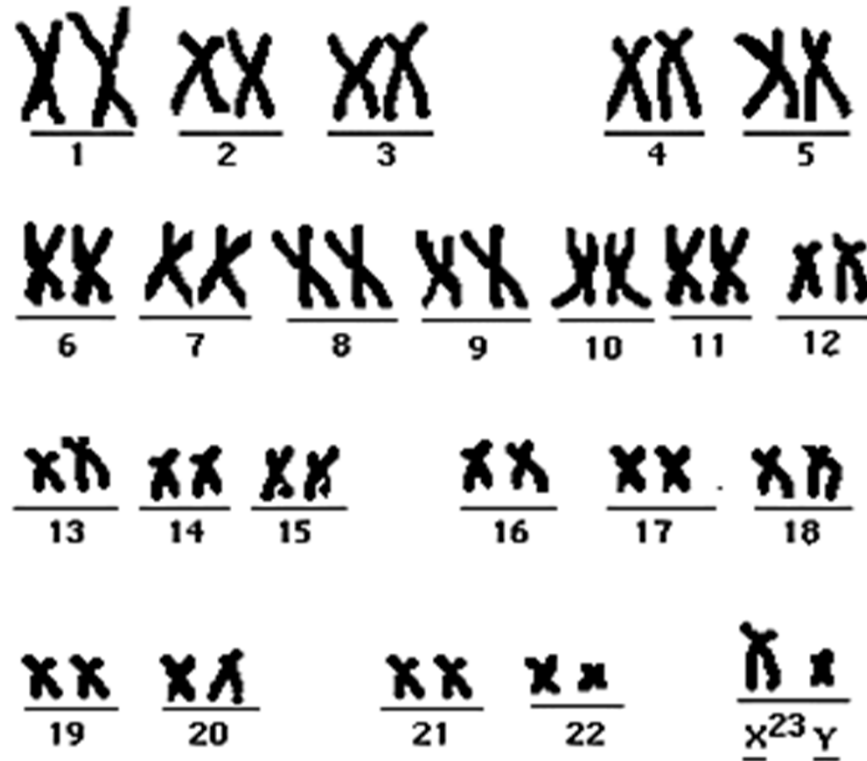
(b) Supercoiled

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

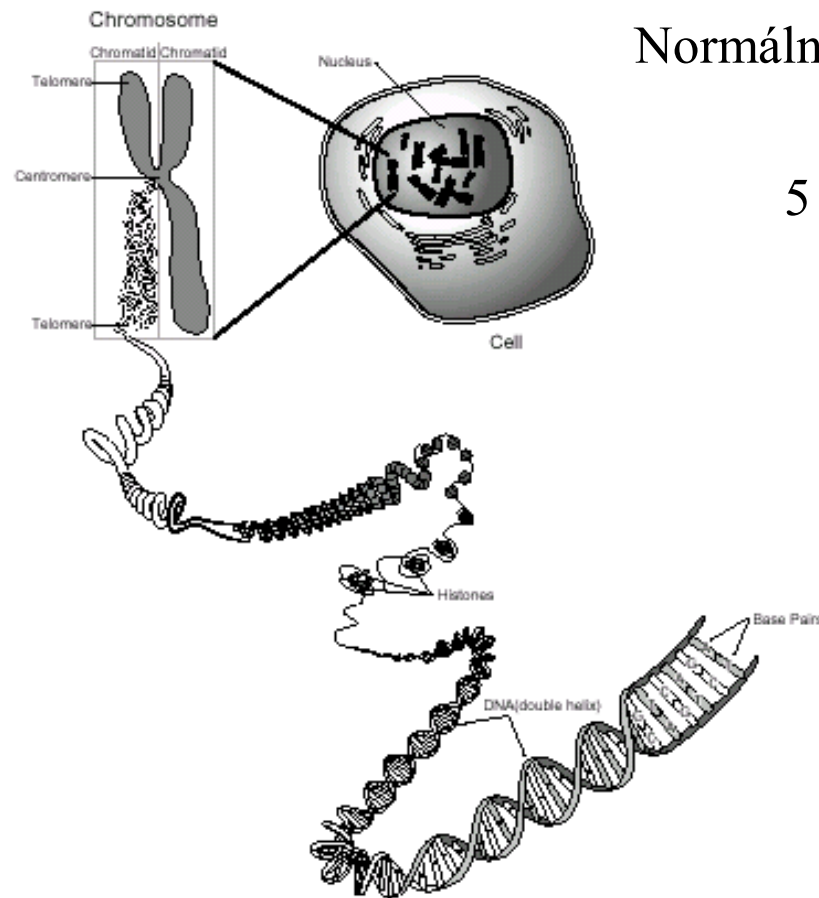


Jádro – mitotický chromozom

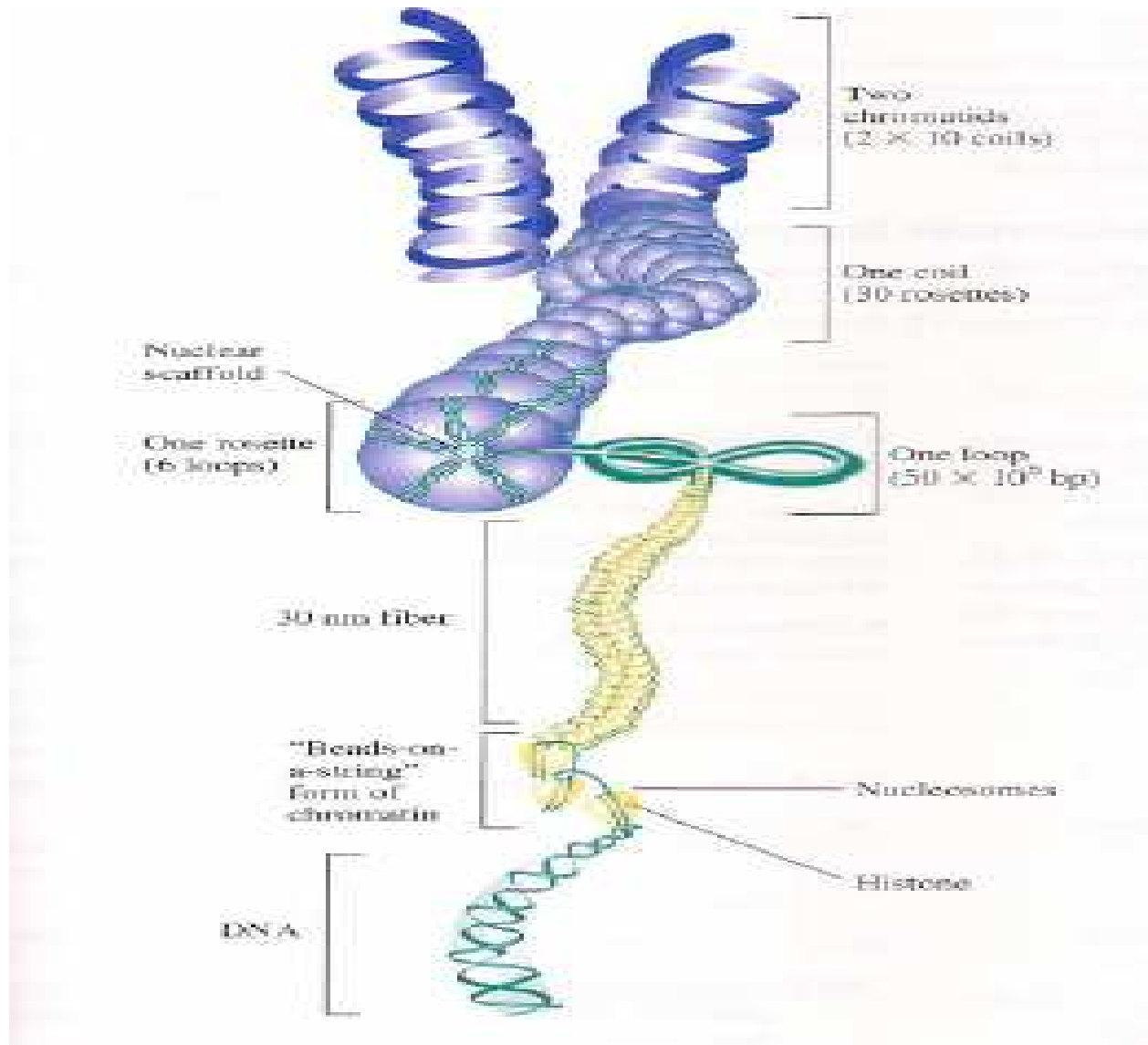
Metafáze - spiralizace

Normálně - despiralizace

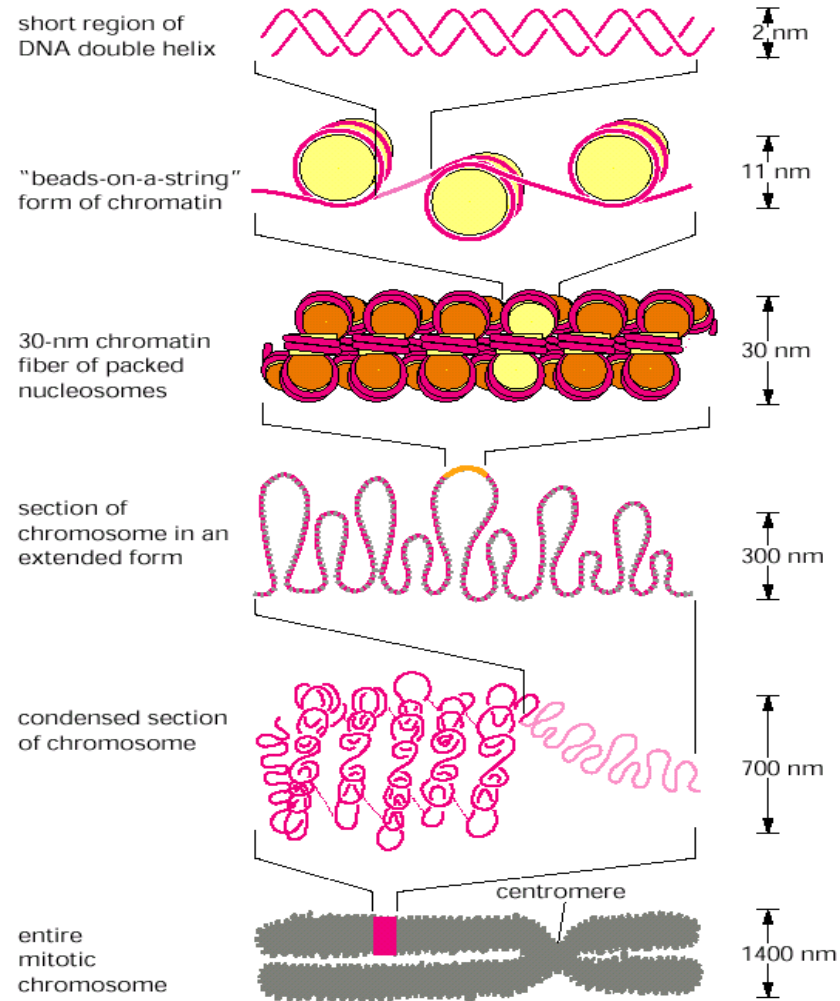
5 – 8 μm – DNA 2 metry



Chromozom



Chromatin



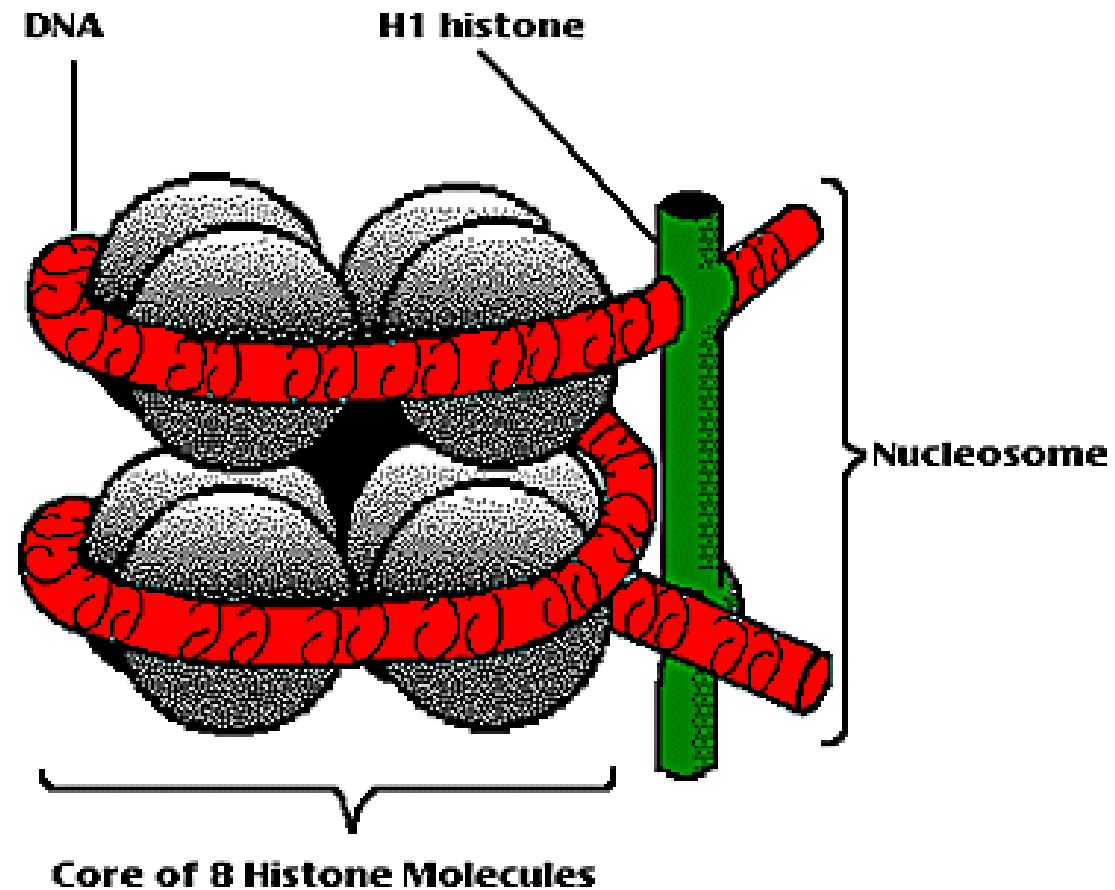
Nukleosom
(korálky)

(cívka)

(smyčka)

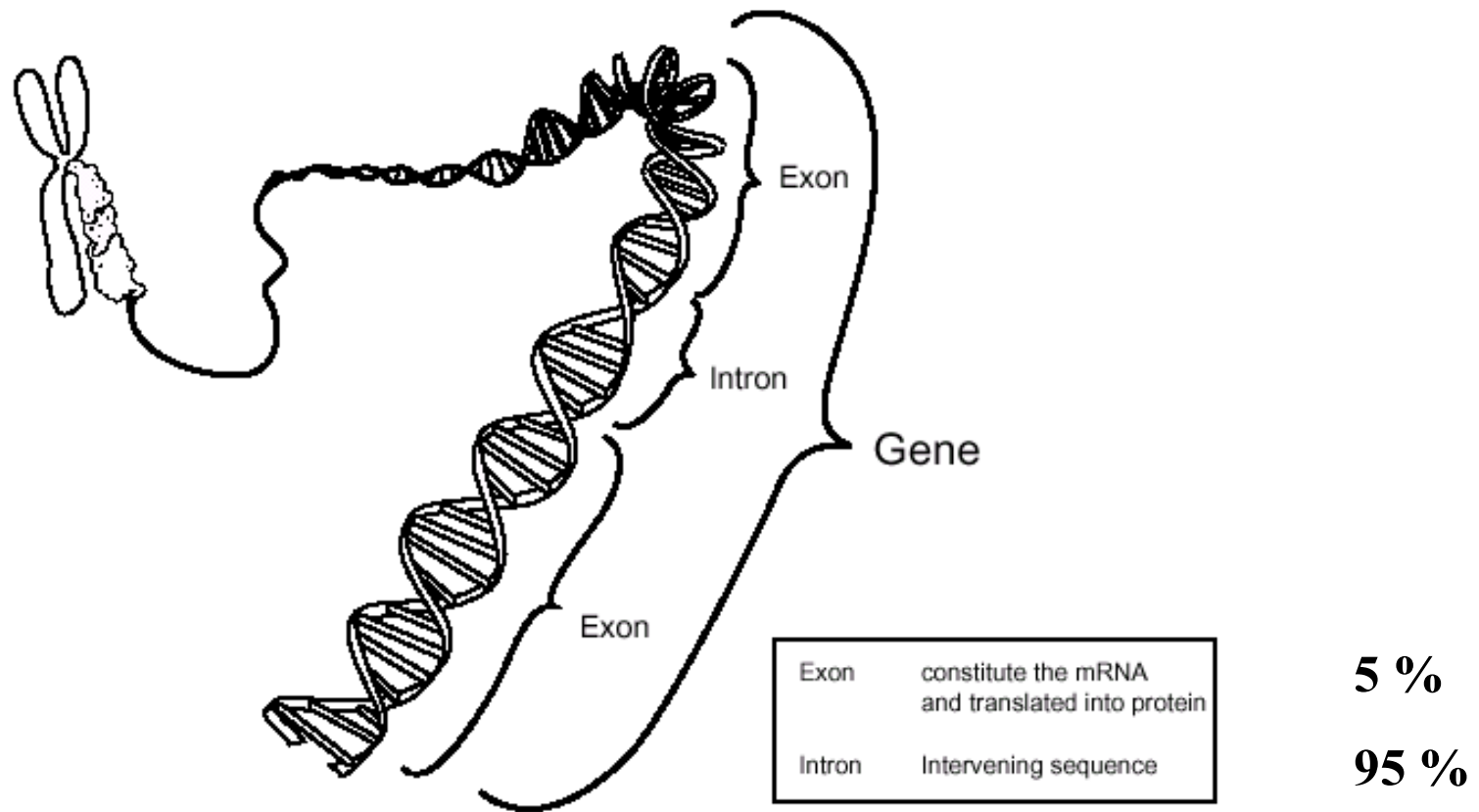
NET RESULT: EACH DNA MOLECULE HAS BEEN
PACKAGED INTO A MITOTIC CHROMOSOME THAT
IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

Nukleosom – DNA + 5 tříd histonů

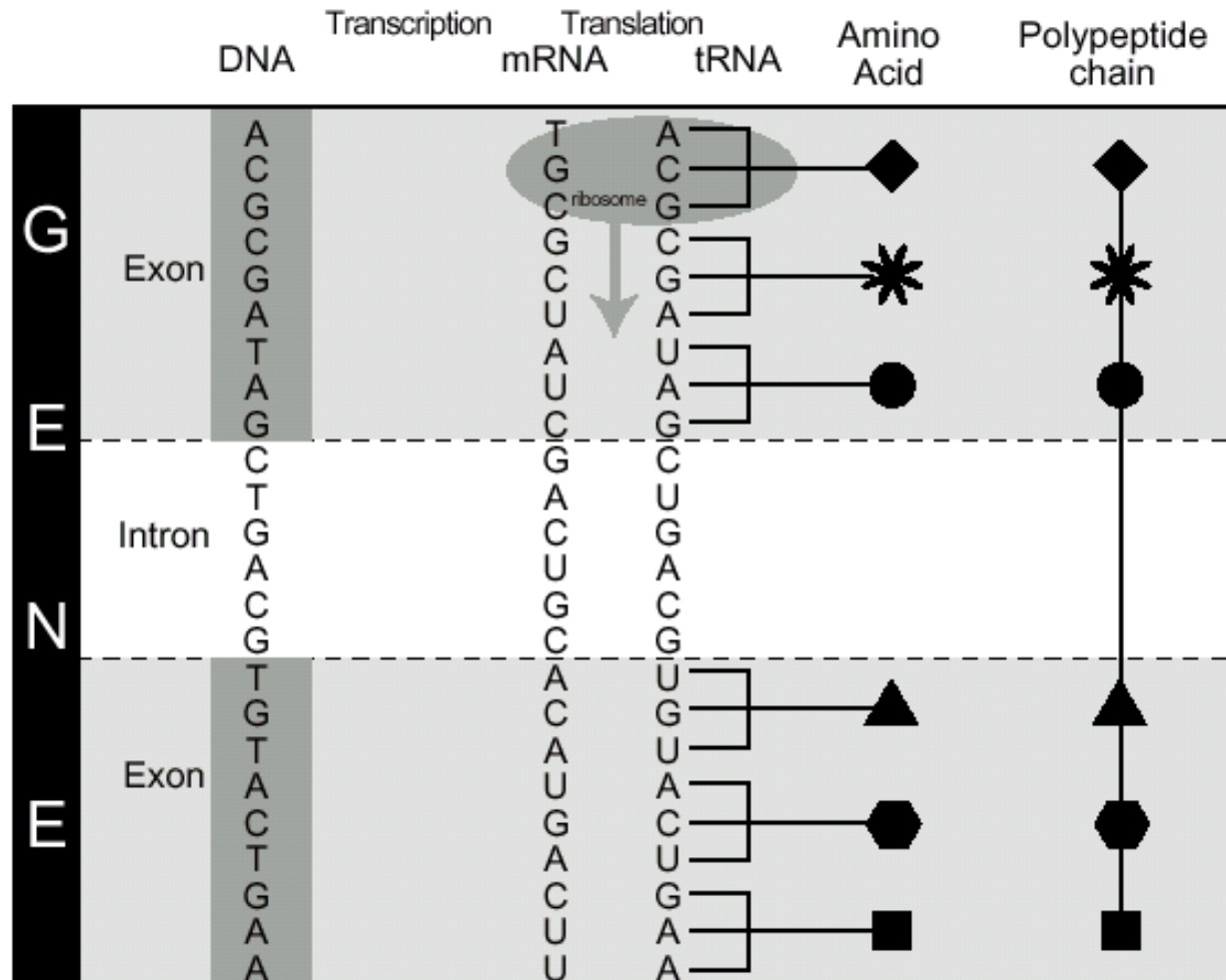


2 x H2A - 2 x H2B - 2 x H3 - 2 x H4

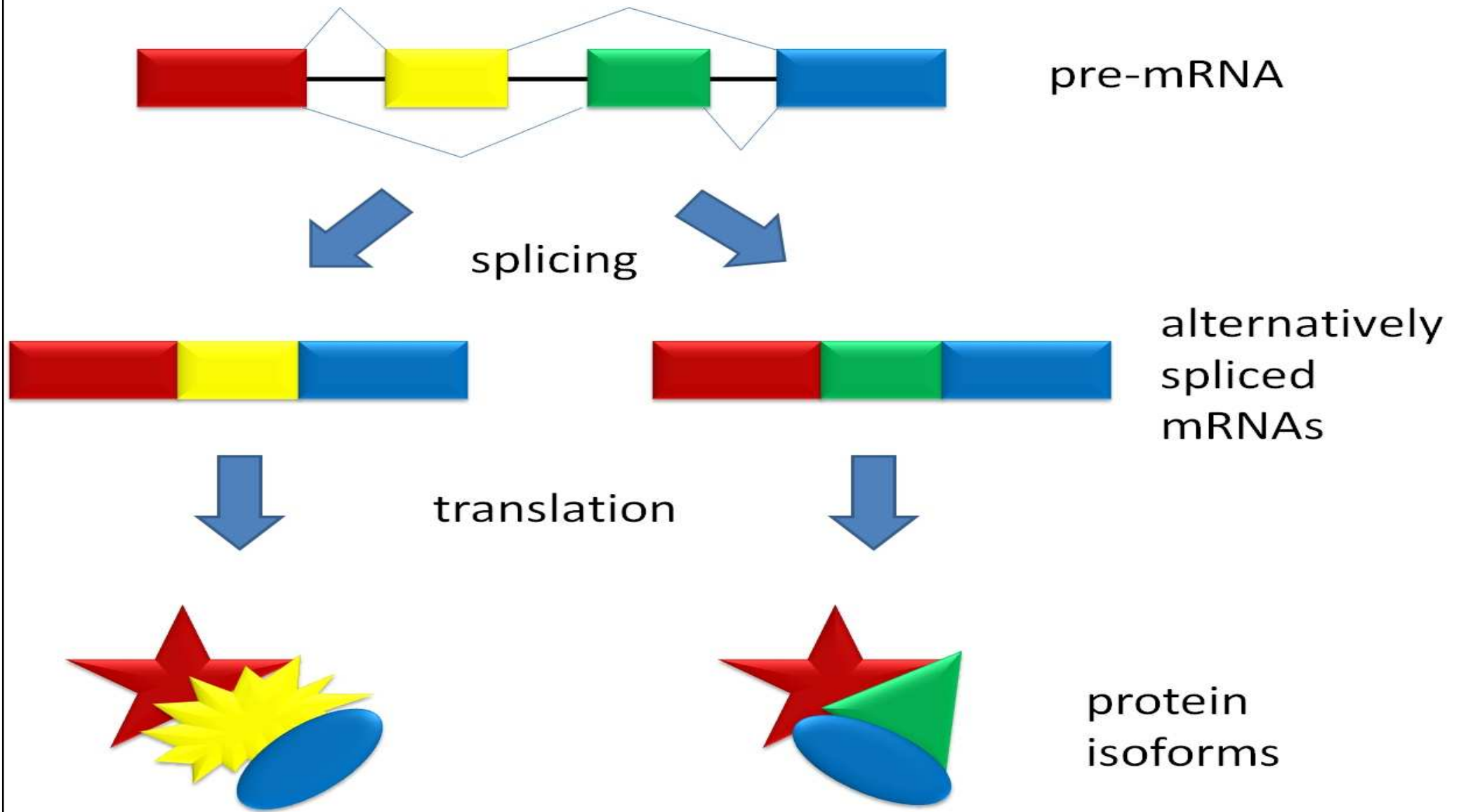
Exon a intron



Exon a intron



Význam intronů



Stanovení sekvence DNA

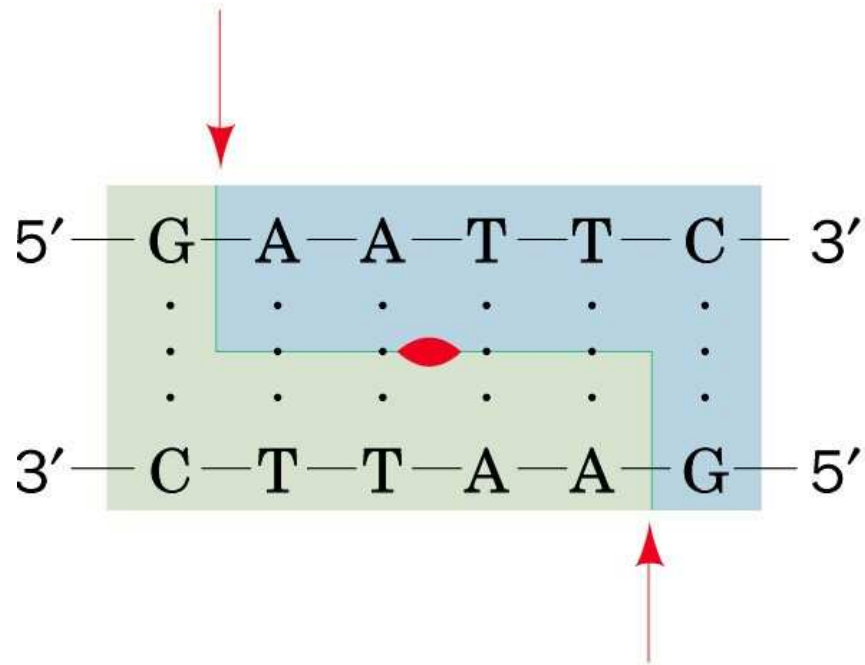
- Restrikční enzymy
- Chemické štěpení – Maxam Gilbertovo metoda
- Enzymová metoda
- Pyrosekvenování (enzymová metoda)

Restrikční enzymy

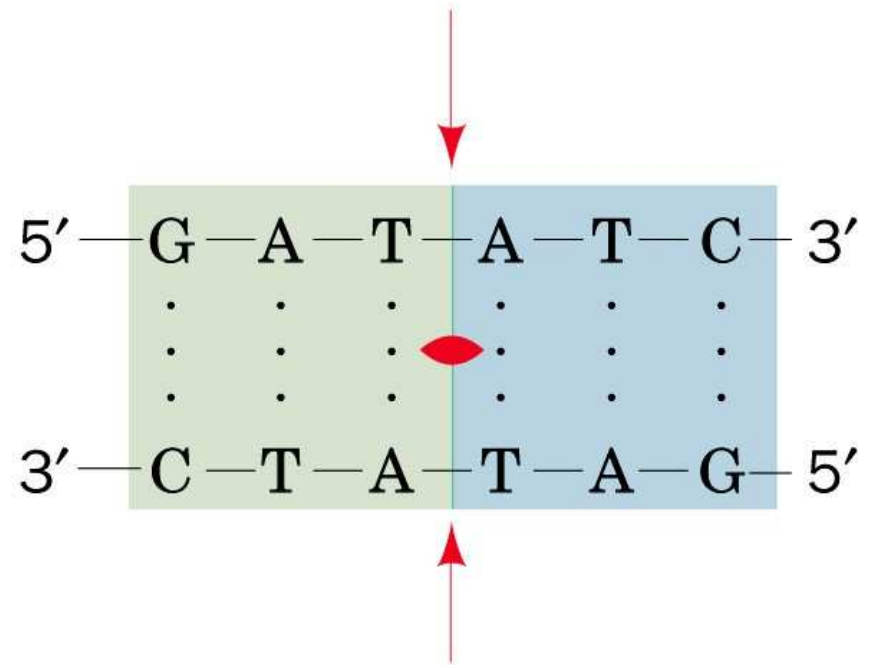
Enzyme	Recognition Sequence*	Microorganism
<i>AclI</i>	AGIC* ^T	<i>Arthrobacter luteus</i>
<i>BamHI</i>	G↓GATC* ^C	<i>Bacillus amyloliquefaciens</i> H
<i>BglI</i>	GCCNNNN↓NGCC	<i>Bacillus globigii</i>
<i>BglII</i>	A↓GATCT	<i>Bacillus globigii</i>
<i>EcoRI</i>	G↓AA* ^{TT} C	<i>Escherichia coli</i> RY13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

Restrikční enzymy

(a) *EcoRI*



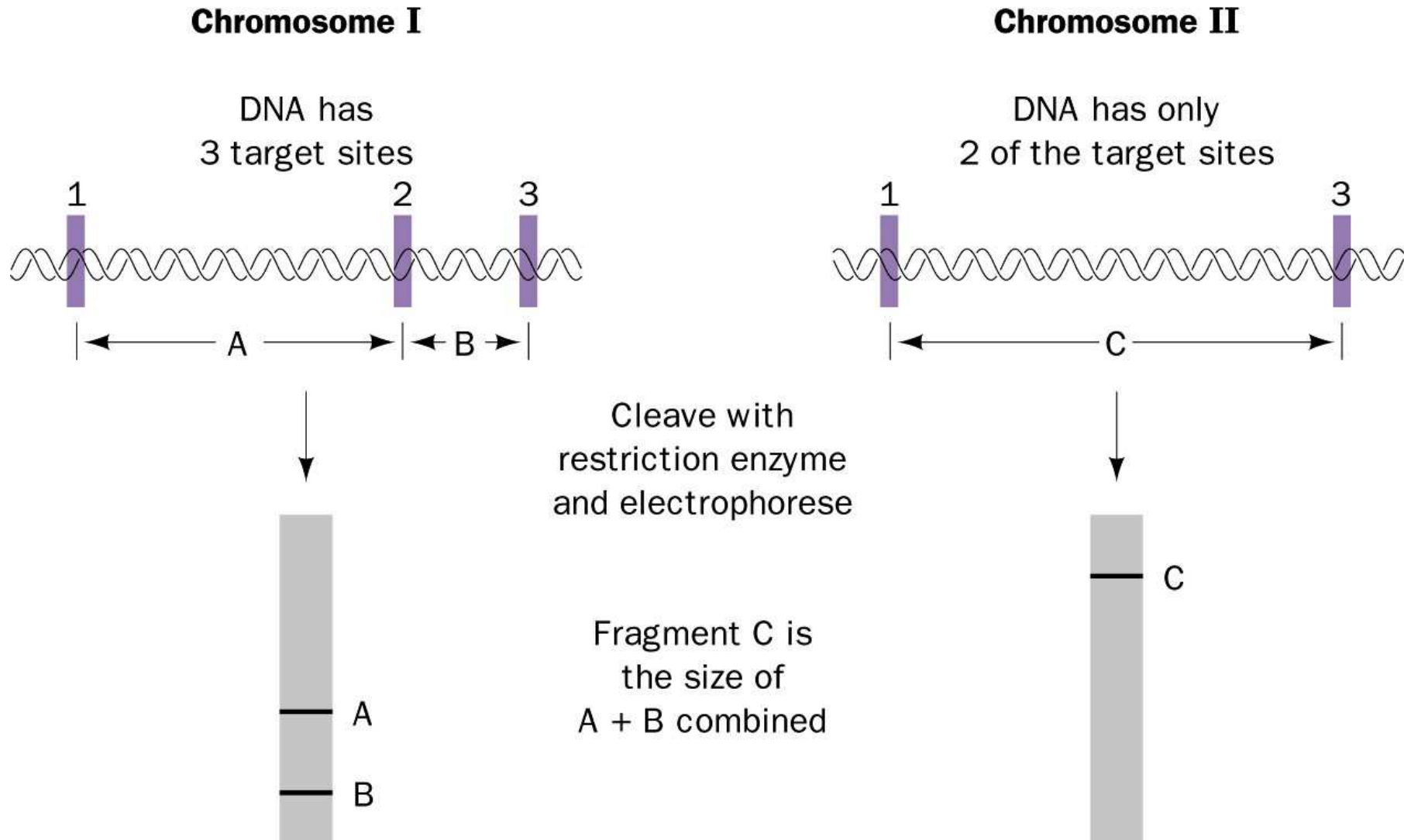
(b) *EcoRV*



↓ Cleavage site

● Twofold symmetry axis

Restrikční enzymy



Restrikční enzymy

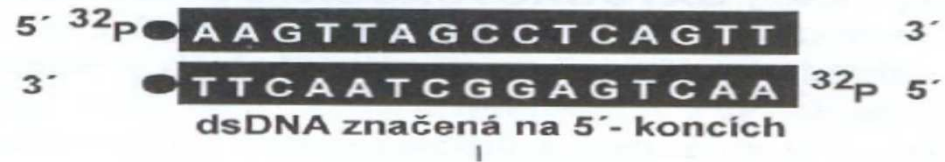


Maxam Gilbertova metoda

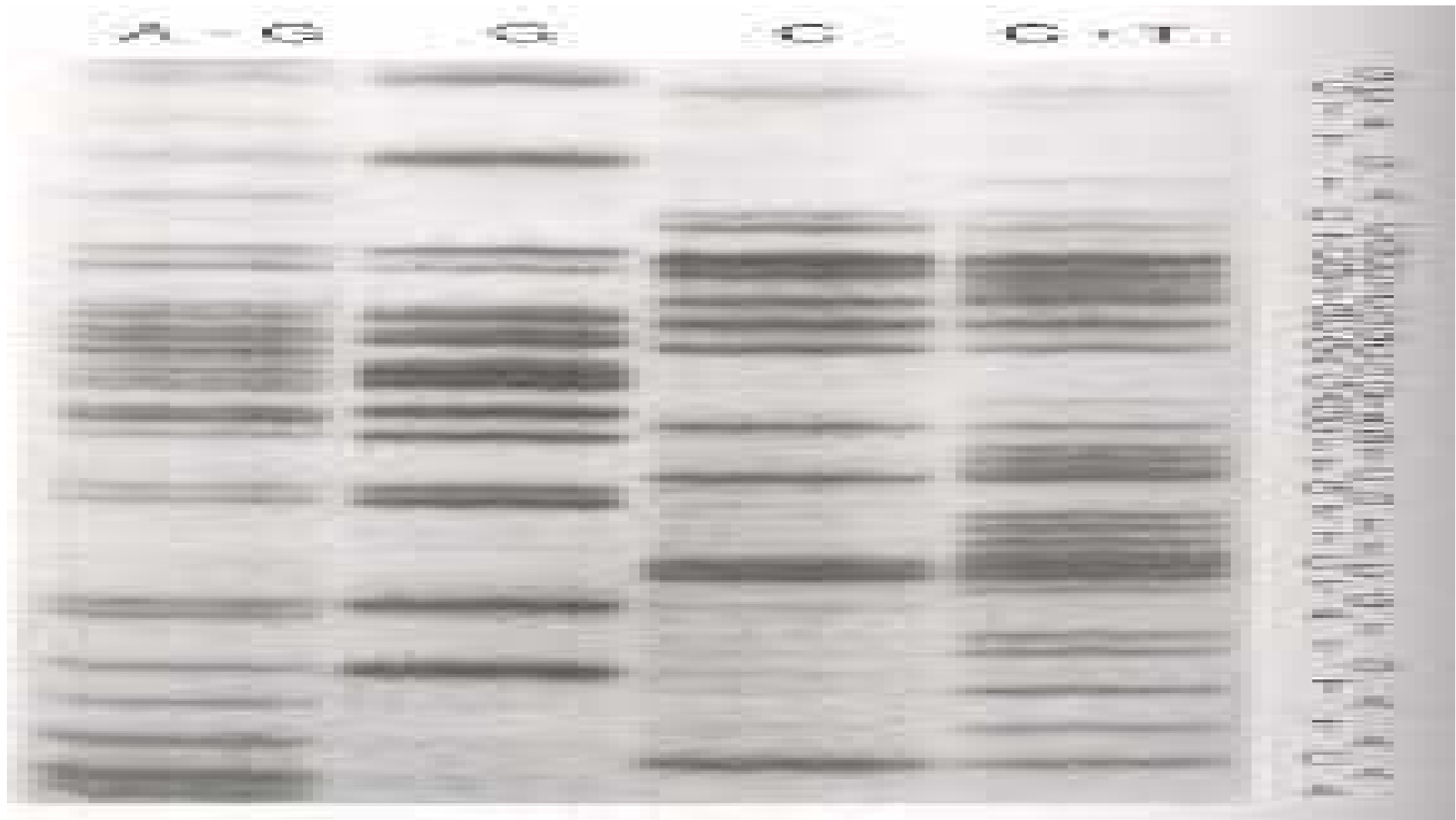
Maxam-Gilbertova metoda

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

Maxam Gilbertova metoda

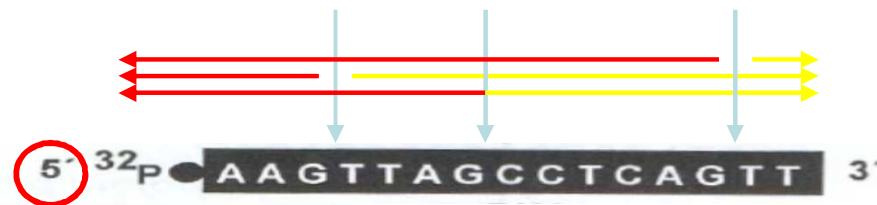


Maxam Gilbertova metoda



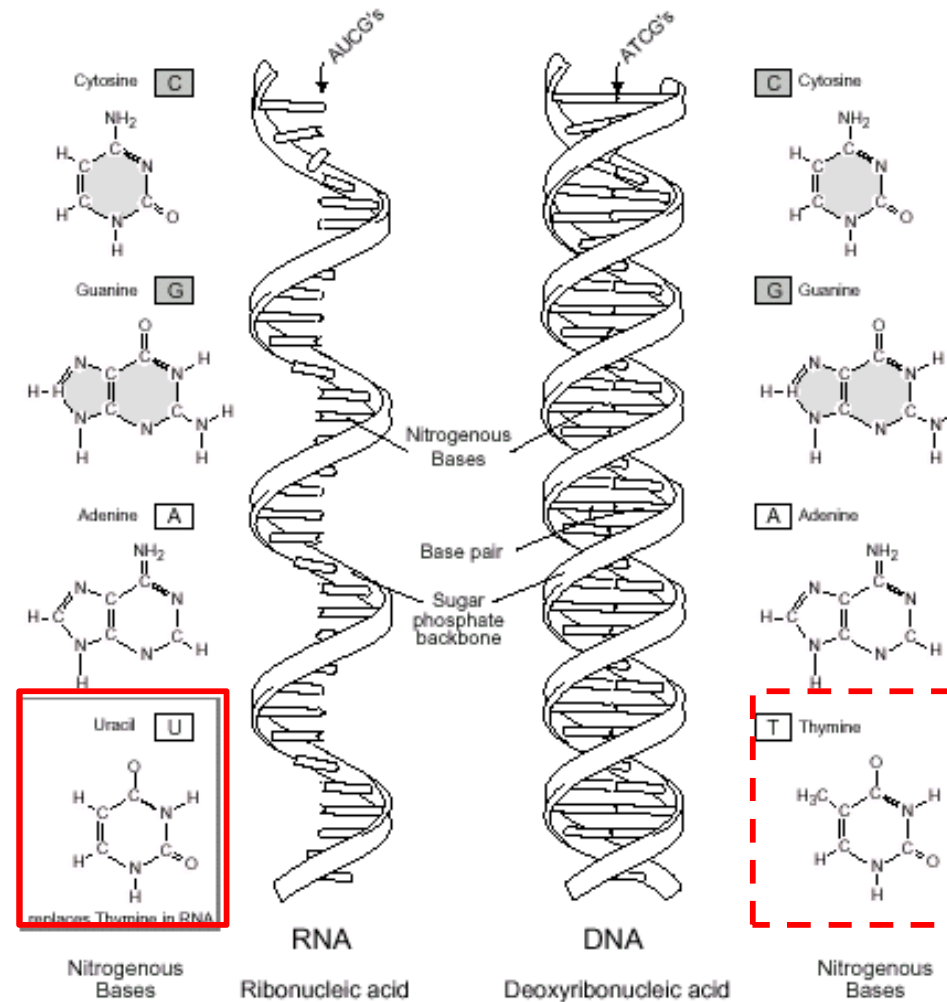
Maxam Gilbertova metoda

dimetylsulfát a piperidin



FUNKCE RNA versus DNA

Ribosa - Deoxyribosa



Jednovláknová - Dvouvláknová

Formy RNA

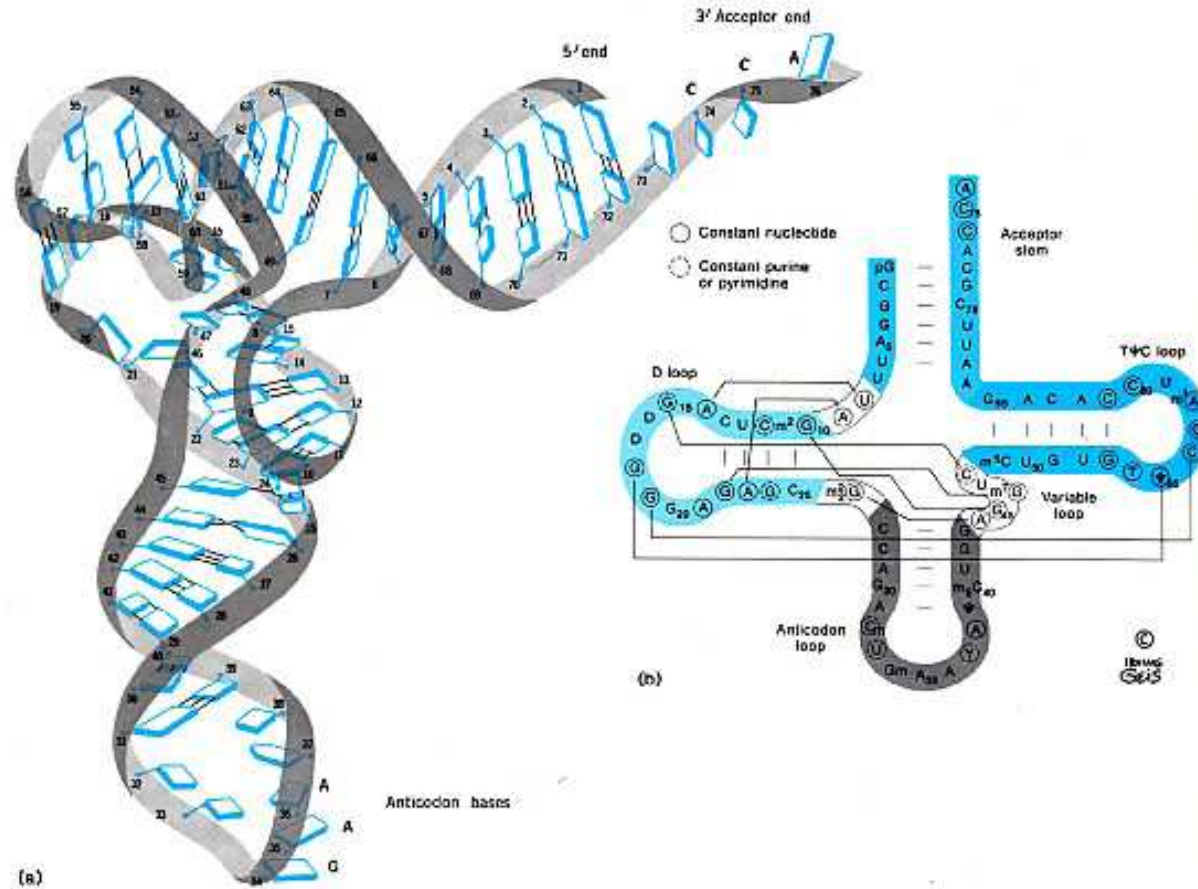
- mRNA – mediátorová, messenger,
informační – 5-10 %r

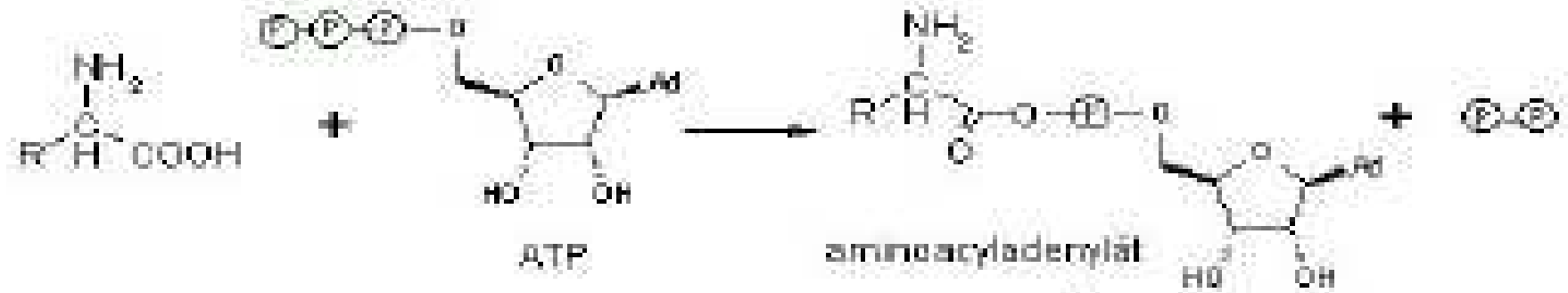
- rRNA – ribosomální – 80 %

- tRNA – transferová, přenosová – 10-15 %

60 tRNA

tRNA

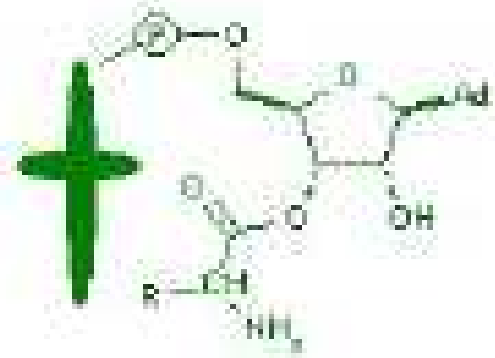




tRNA



aminoacyl-tRNA + AMP



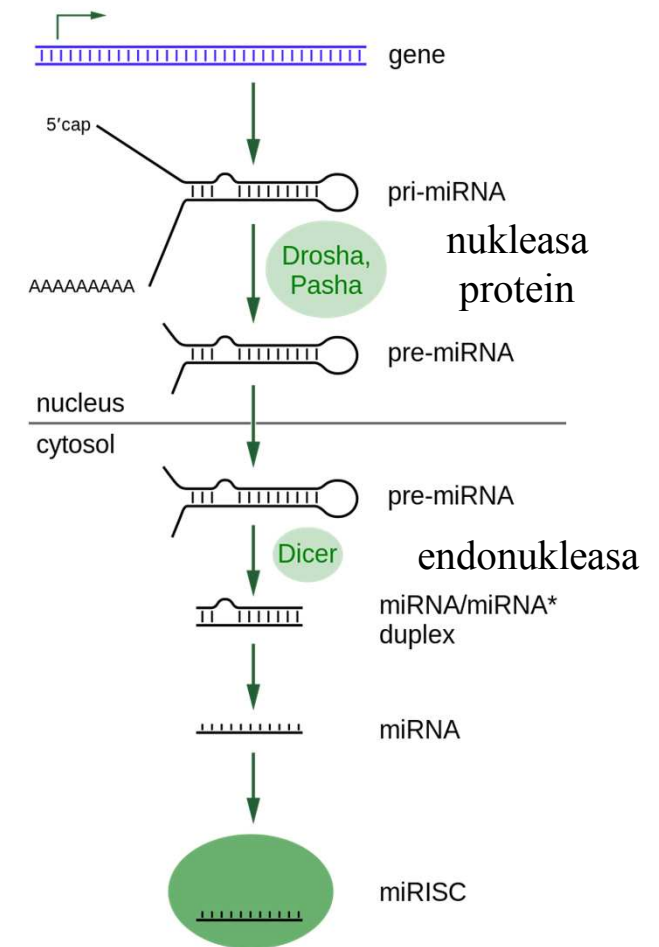
Formy RNA

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

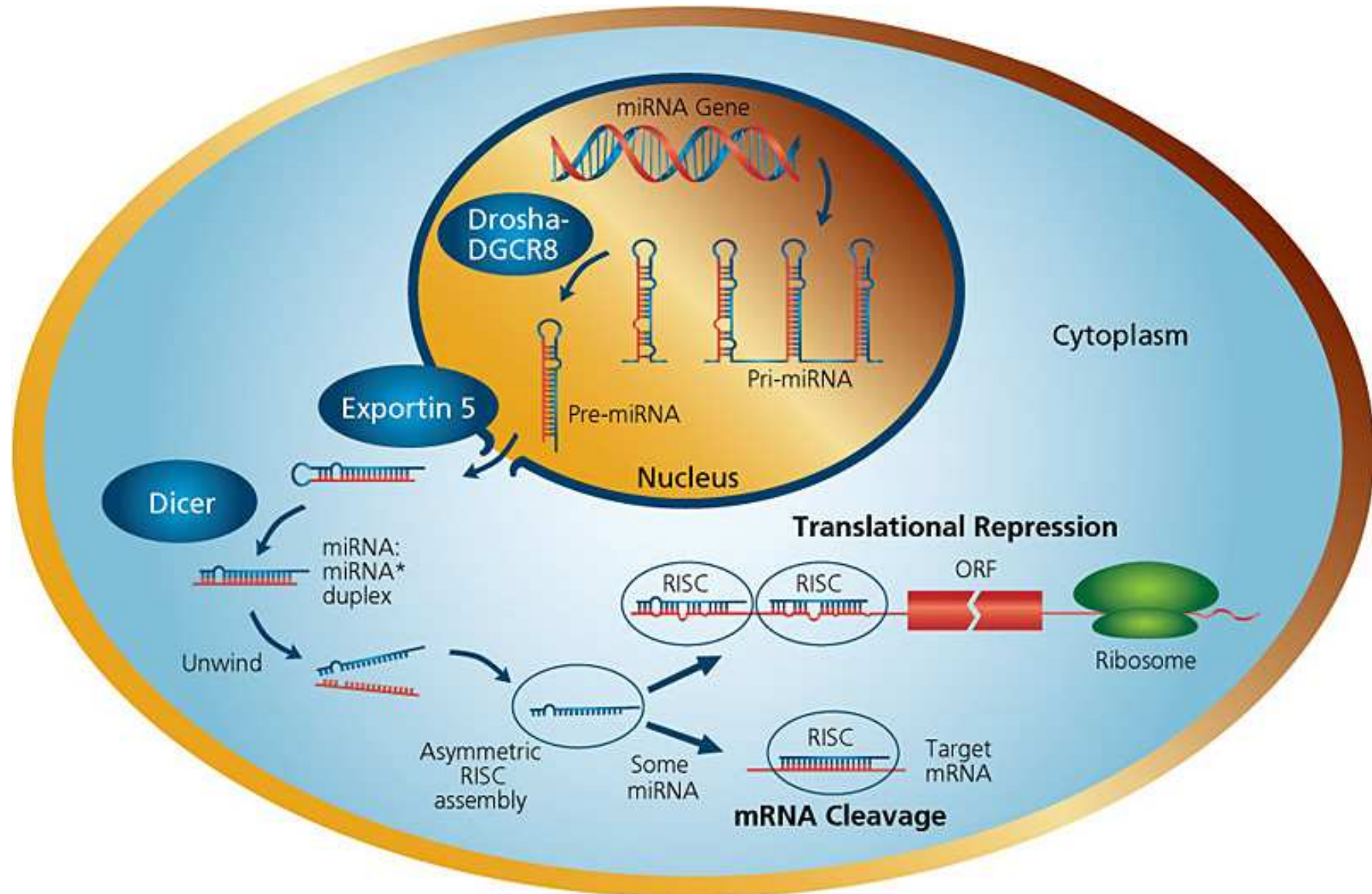
60 tRNA

1993 Victor Ambrose

miRNA - microRNA



Micro RNA



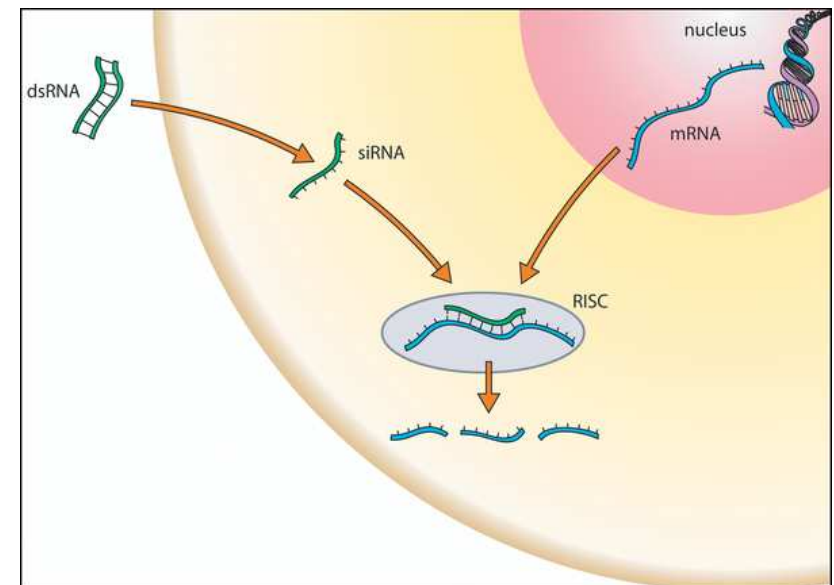
Formy RNA

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

60 tRNA

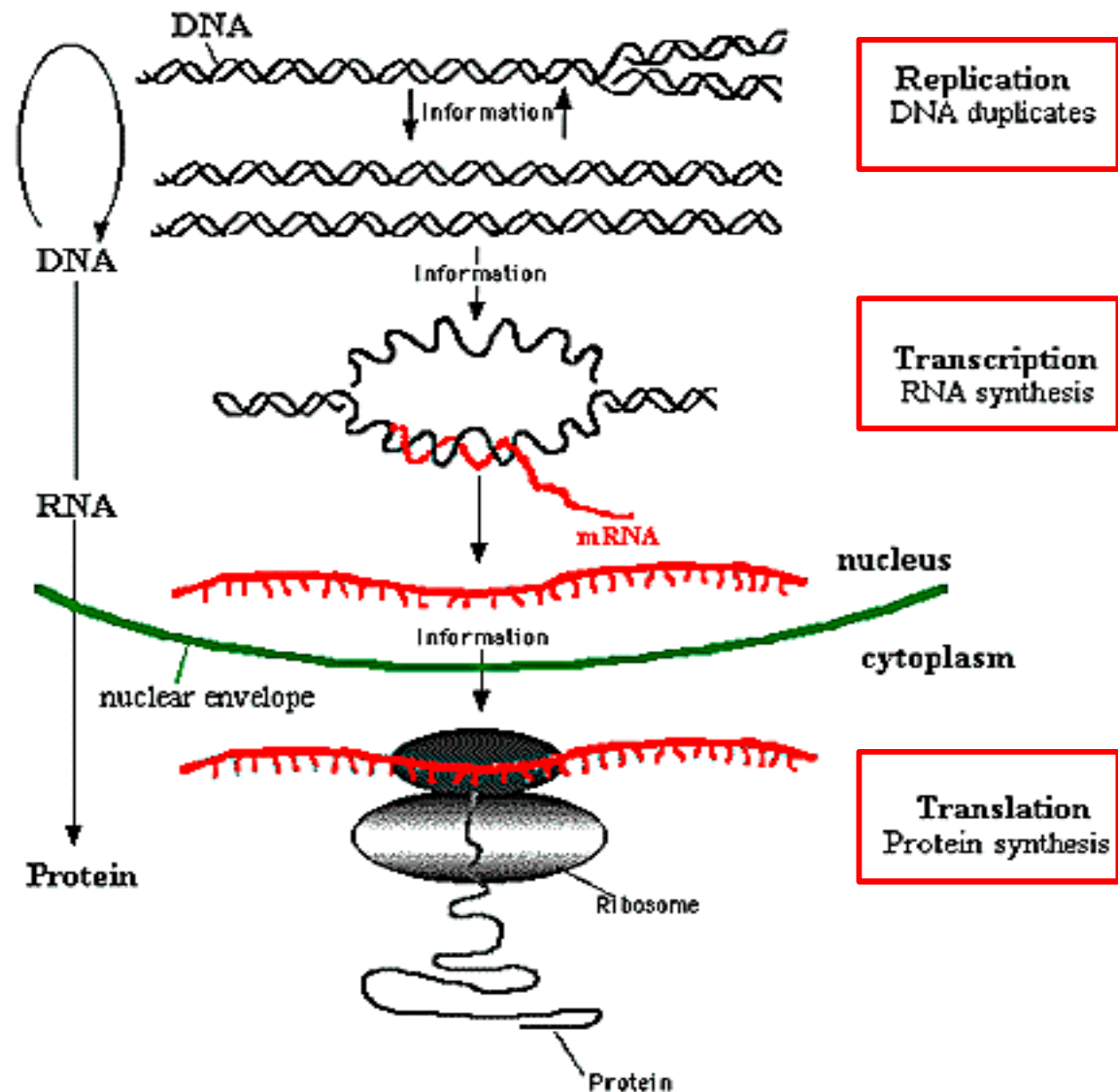
miRNA - microRNA

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

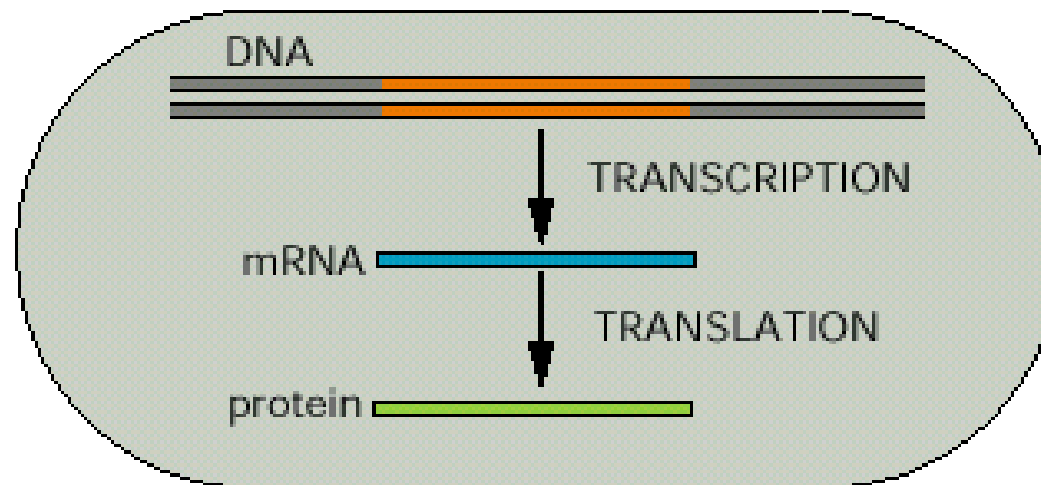
↓
Překlad

Polypeptide

—Arg—Gly—Ala—

Prokaryota

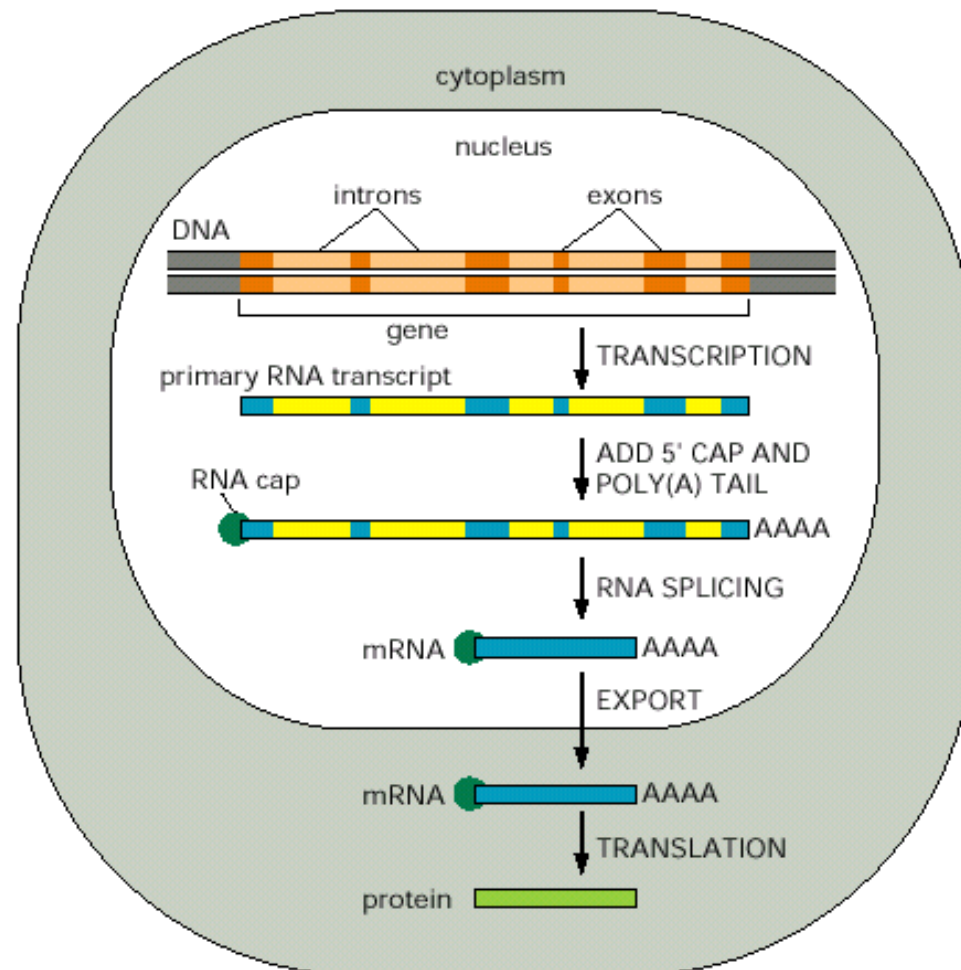
(B) PROCARYOTES



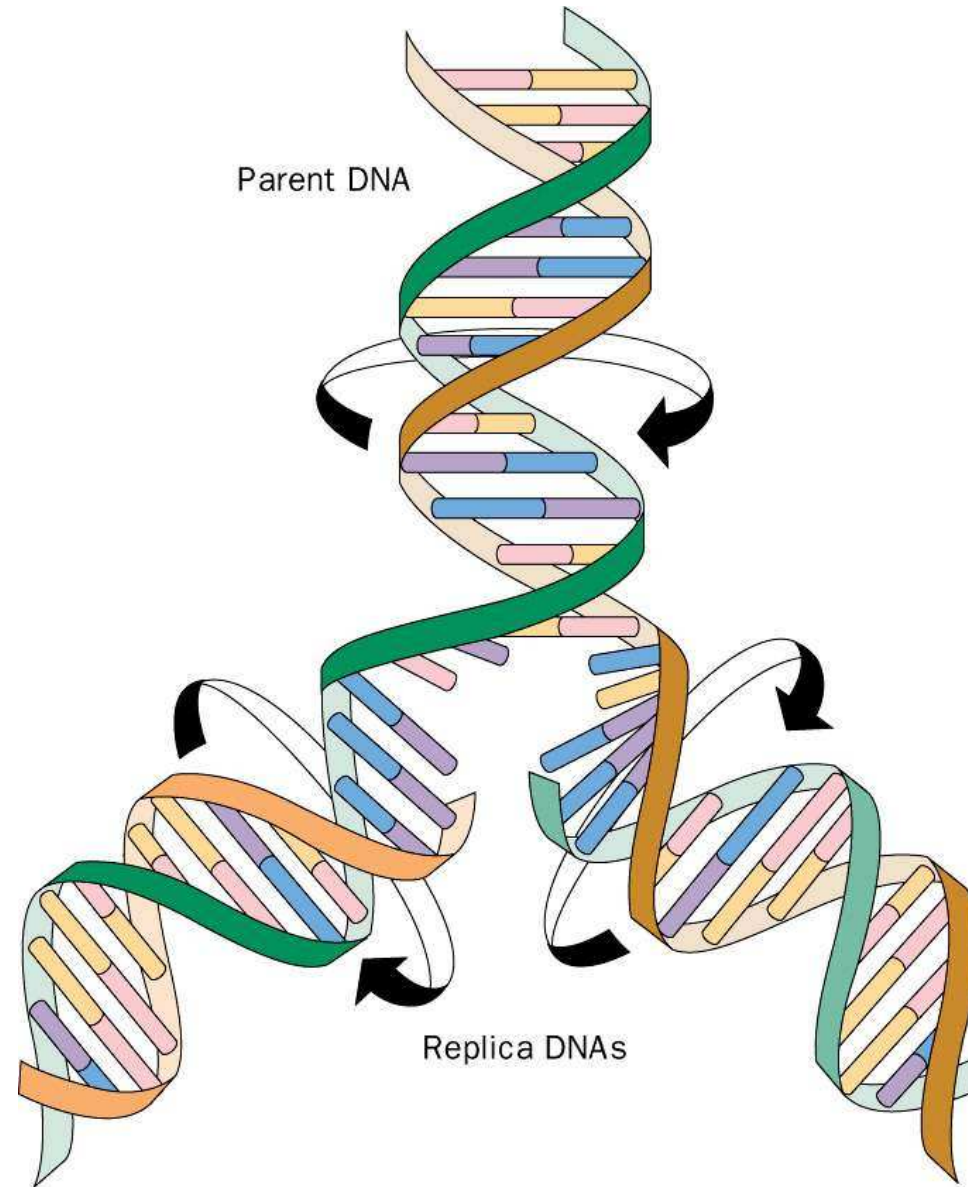
Eukaryota

(A) EUCARYOTES

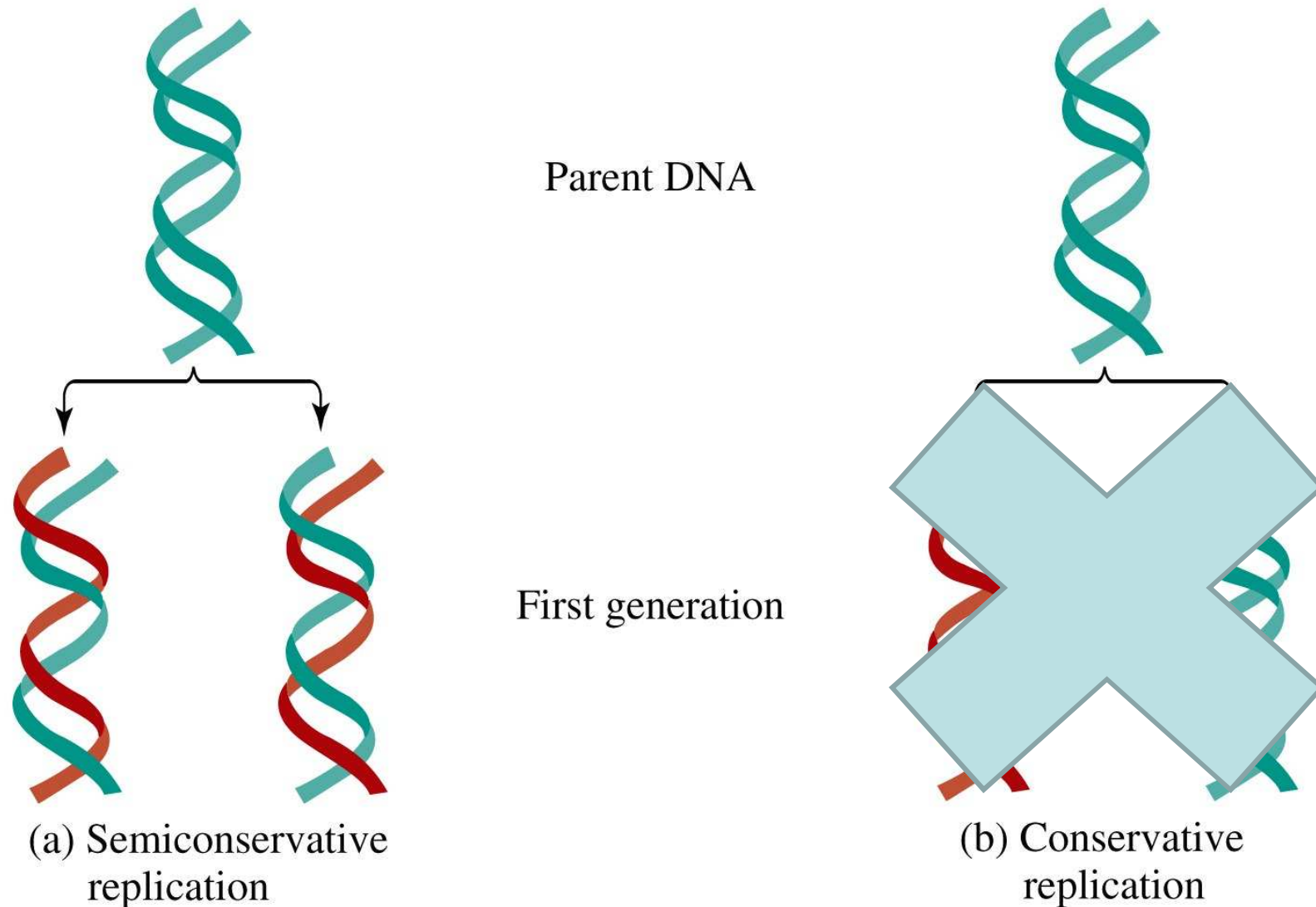
(B)



Replikace – zdvojení - DNA → DNA tvorby identických kopií DNA



Replikace DNA



Meselson a Stahlův experiment

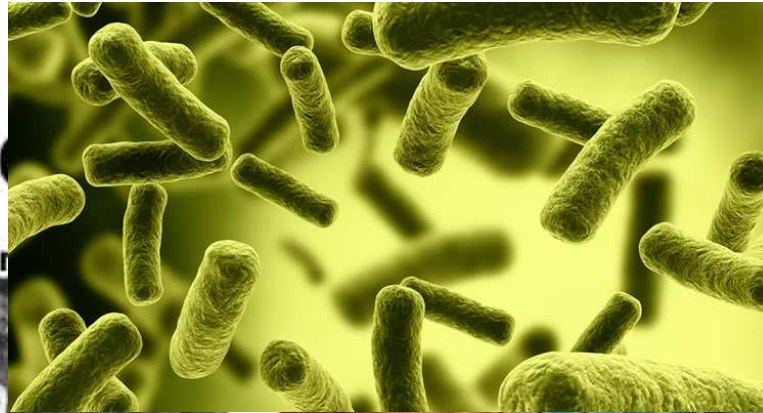
Mes

Matthew Meselson

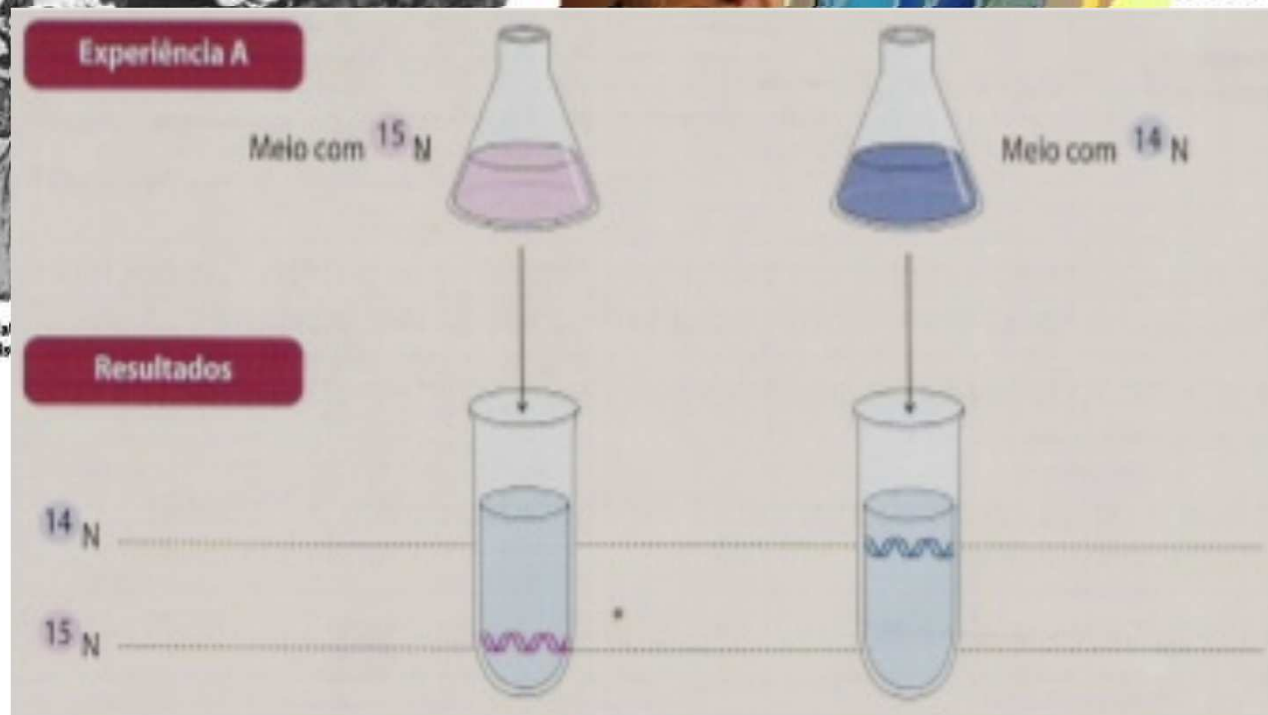
Fr



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



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

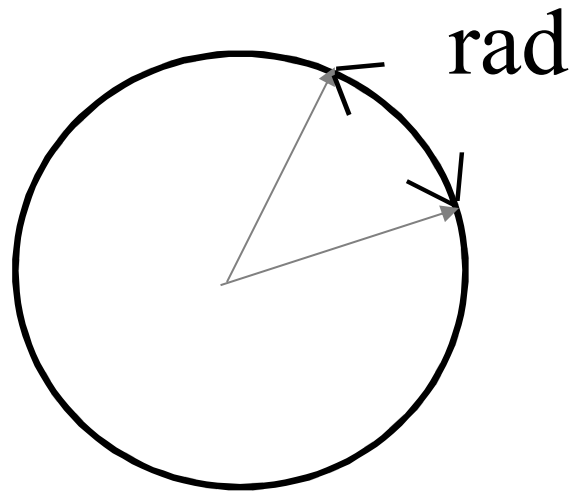
Otáčky \rightarrow g

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

ω - uhlová rychlost
(rad/s)

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

f – otáčky/min



Preparativní ultracentrifuga

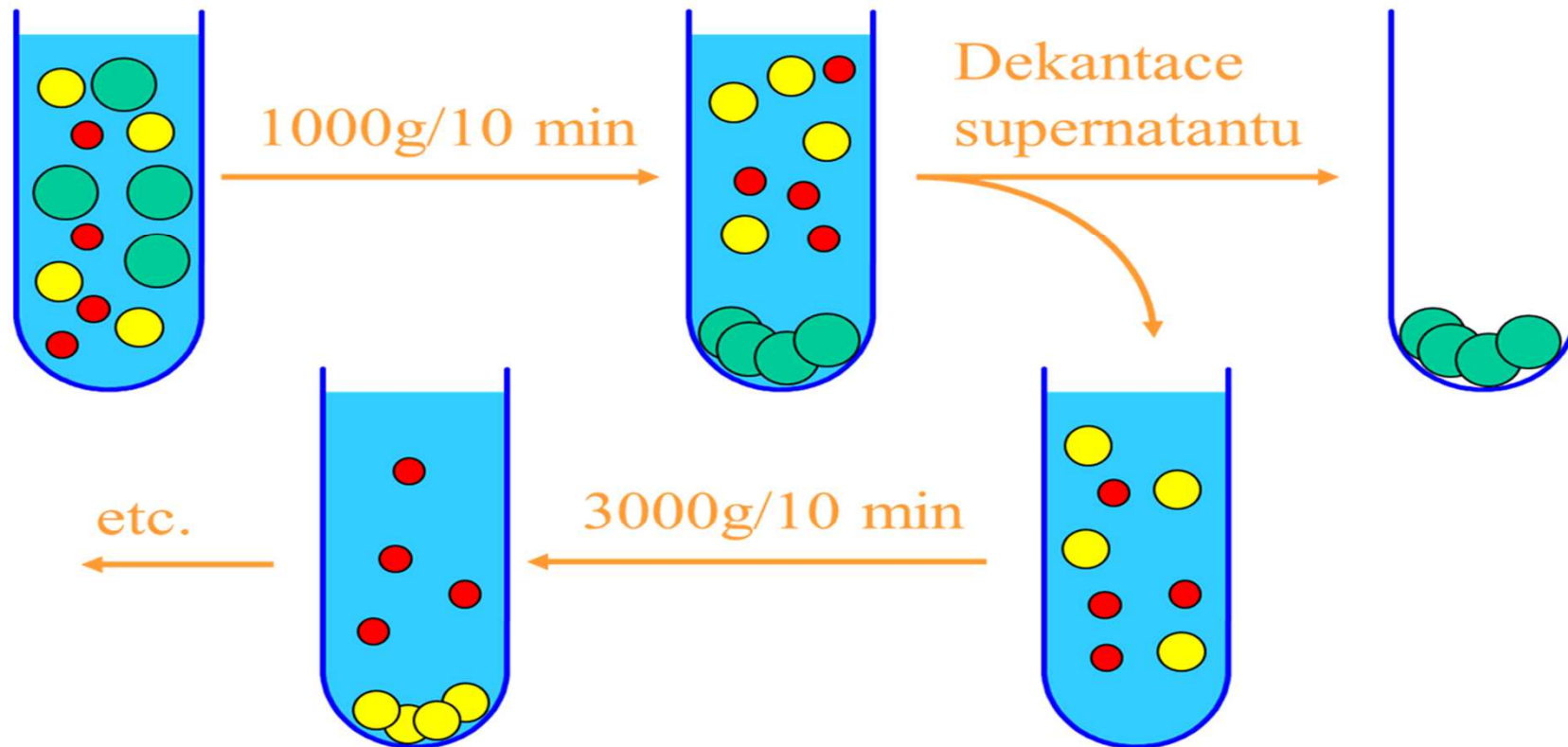


Rotory

- Úhlový – diferenciální centrifugace
- Výkyvné – zonální centrifugace

Diferenciální centrifugace

- opakovaná centrifugace se zvyšující se rychlostí otáček = gravitací



Úhlový rotor

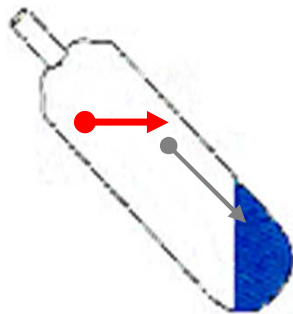
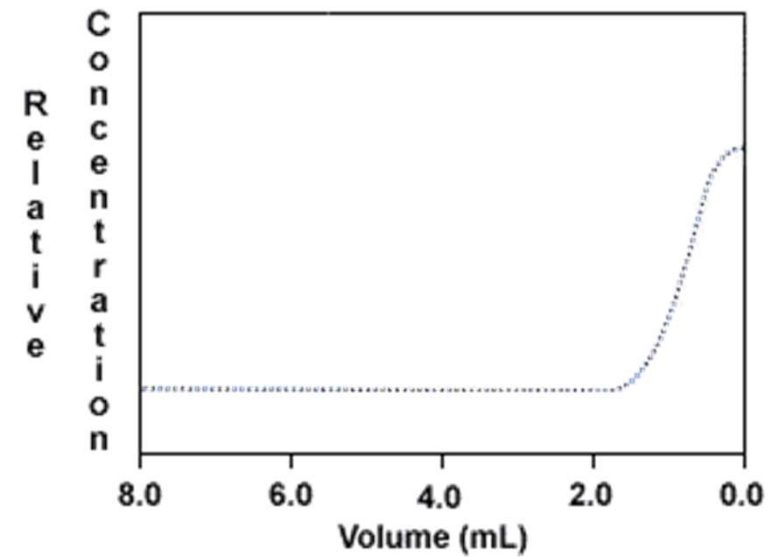
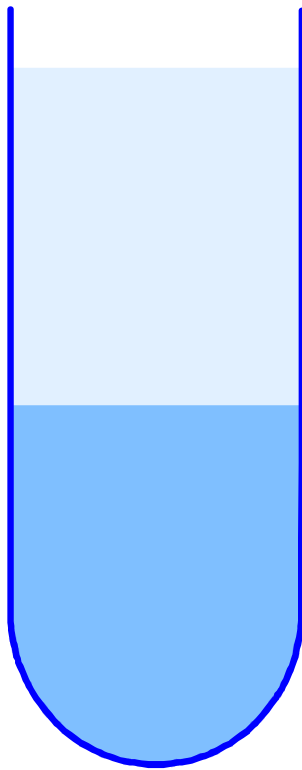


Figure 4

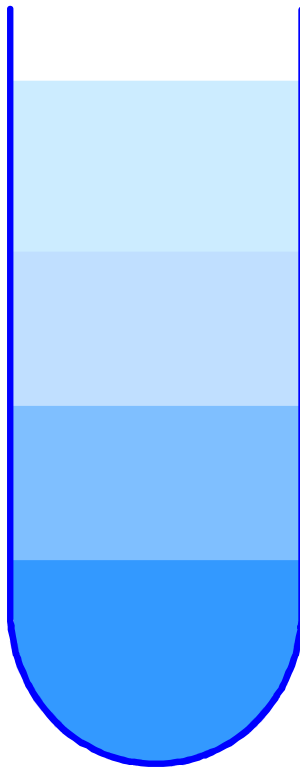


Gradientová centrifugace

Hustotní bariera



Diskontinuální

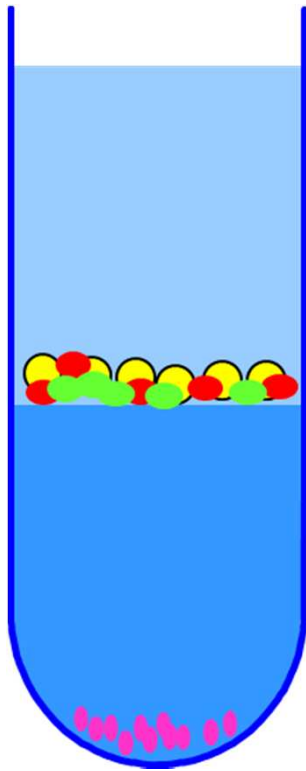


Kontinuální

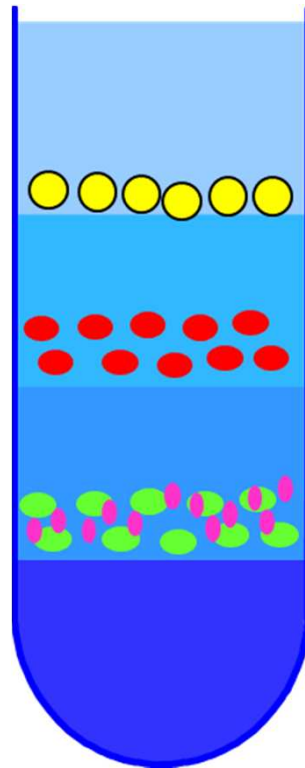


Gradientová centrifugace

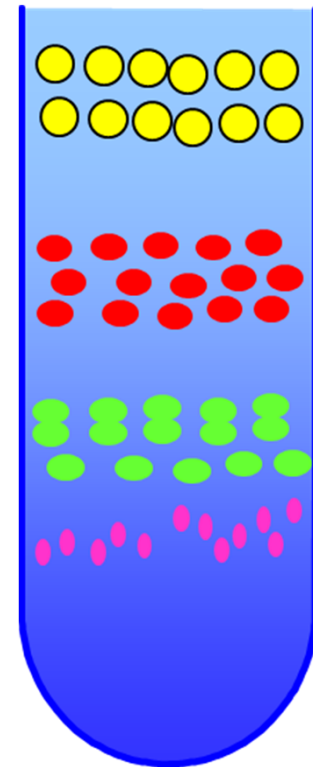
Hustotní bariera



Diskontinuální



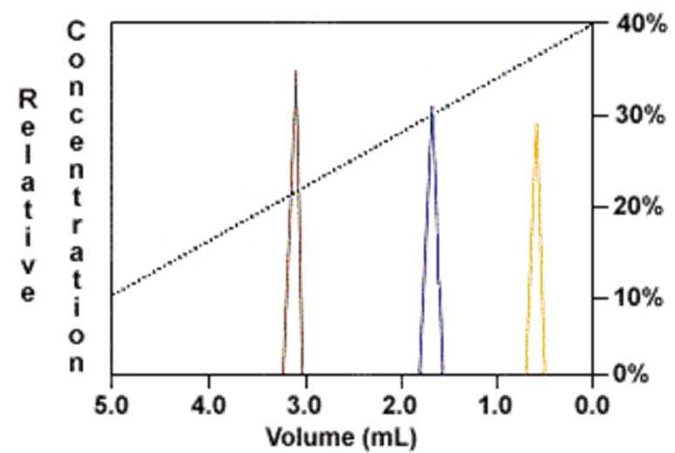
Kontinuální



Výkyvný rotor



Figure 2



Gradientová centrifugace média

- Sacharosa

- Glycerol

- Ficoll - dextran

- Percoll – SiO₂

Hypertonické prostředí

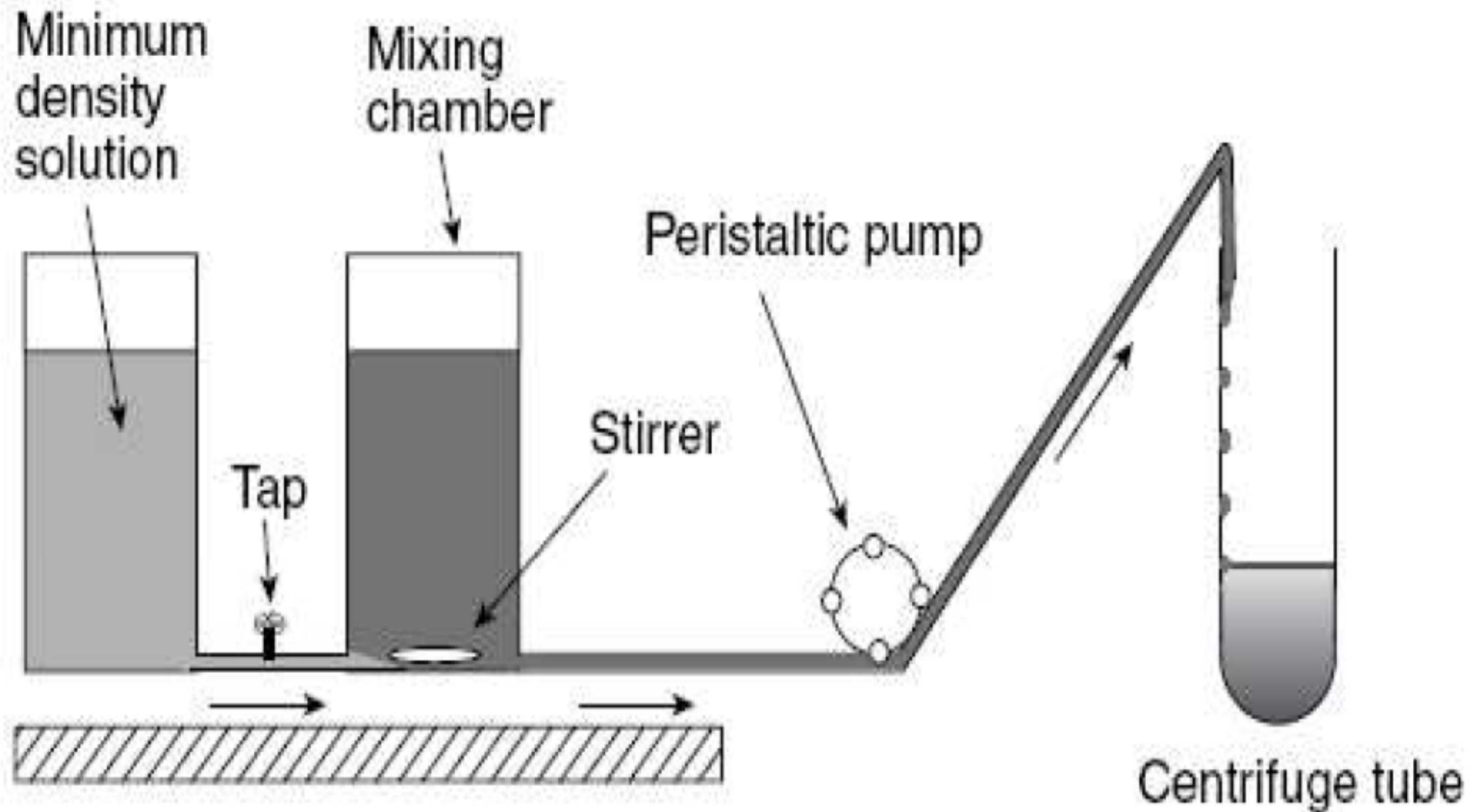
Nutno připravit gradient

- CsCl

- Cs₂SO₄

Gradient vzniká během centrifugace

Gradientová centrifugace



Gradientová centrifugace

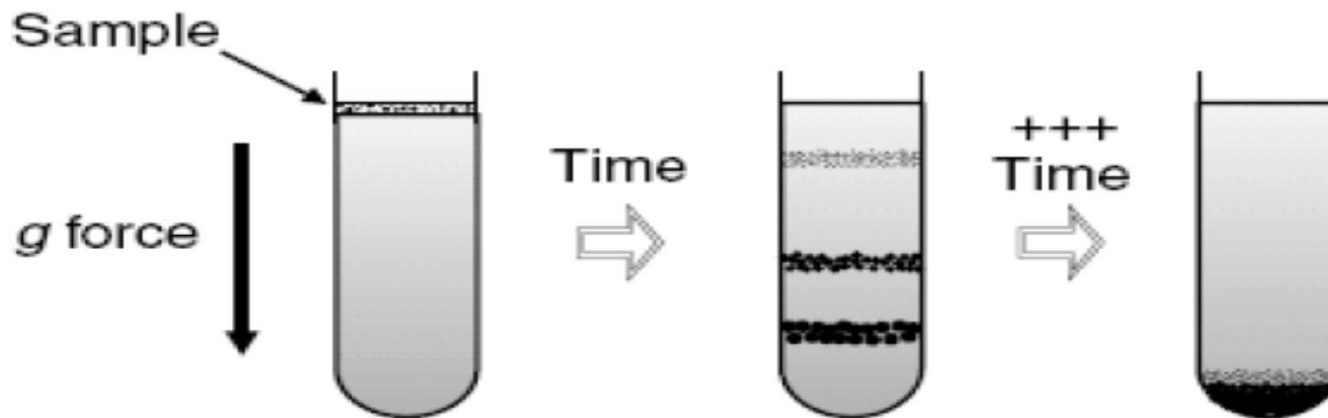
Metoda

Izopyknická

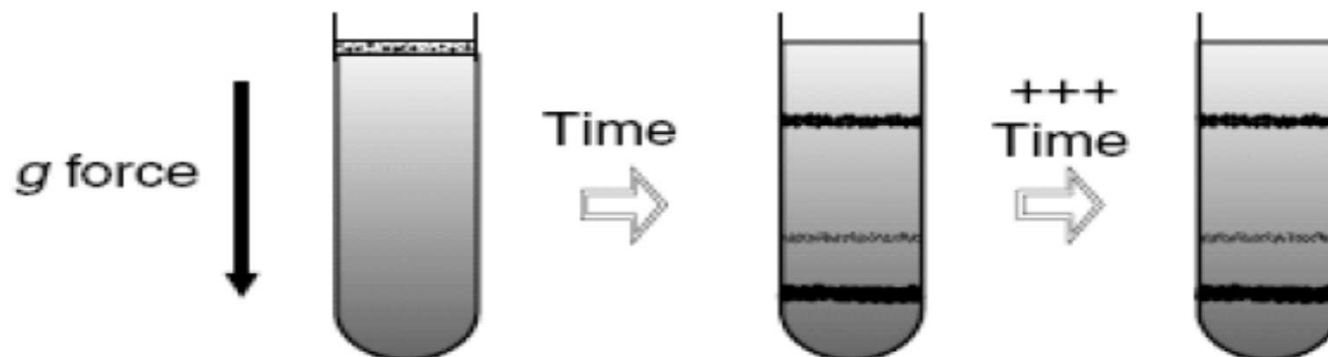
Nerovnovážná

Gradientová centrifugace

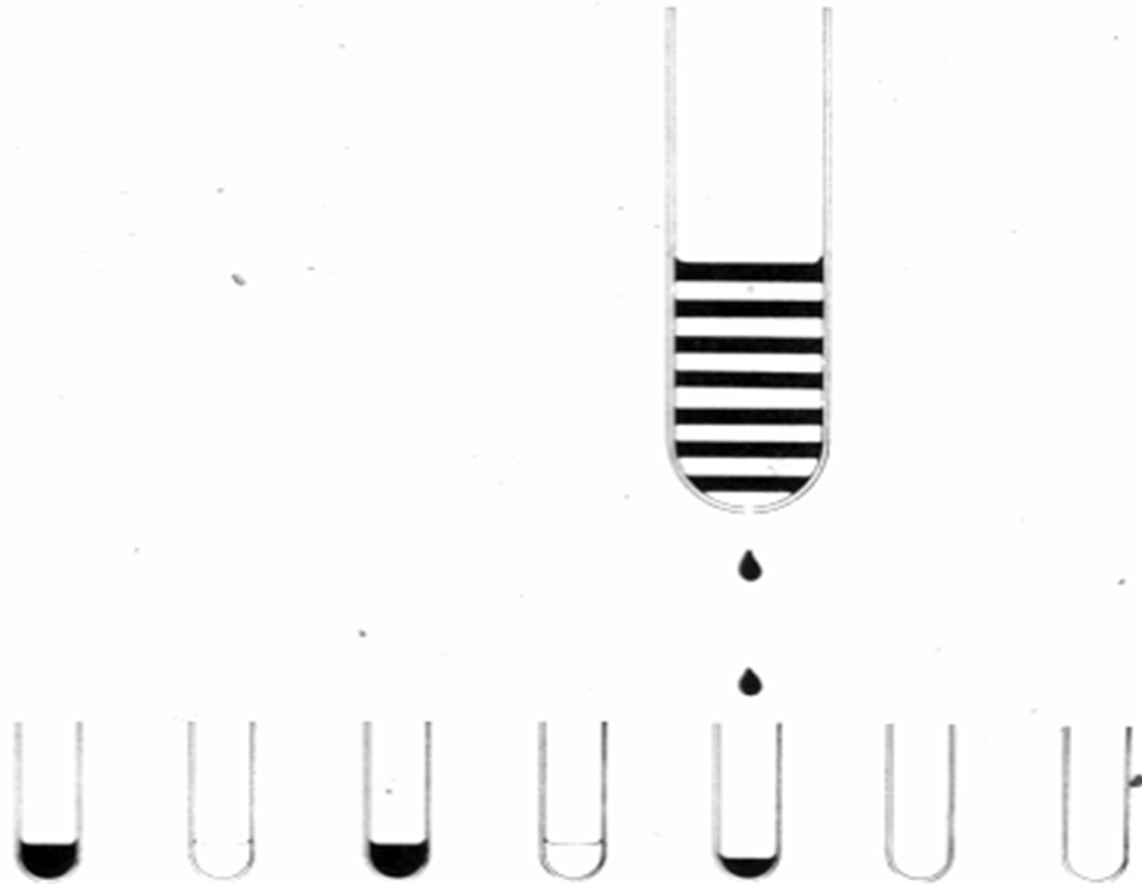
Rate-zonal centrifugation



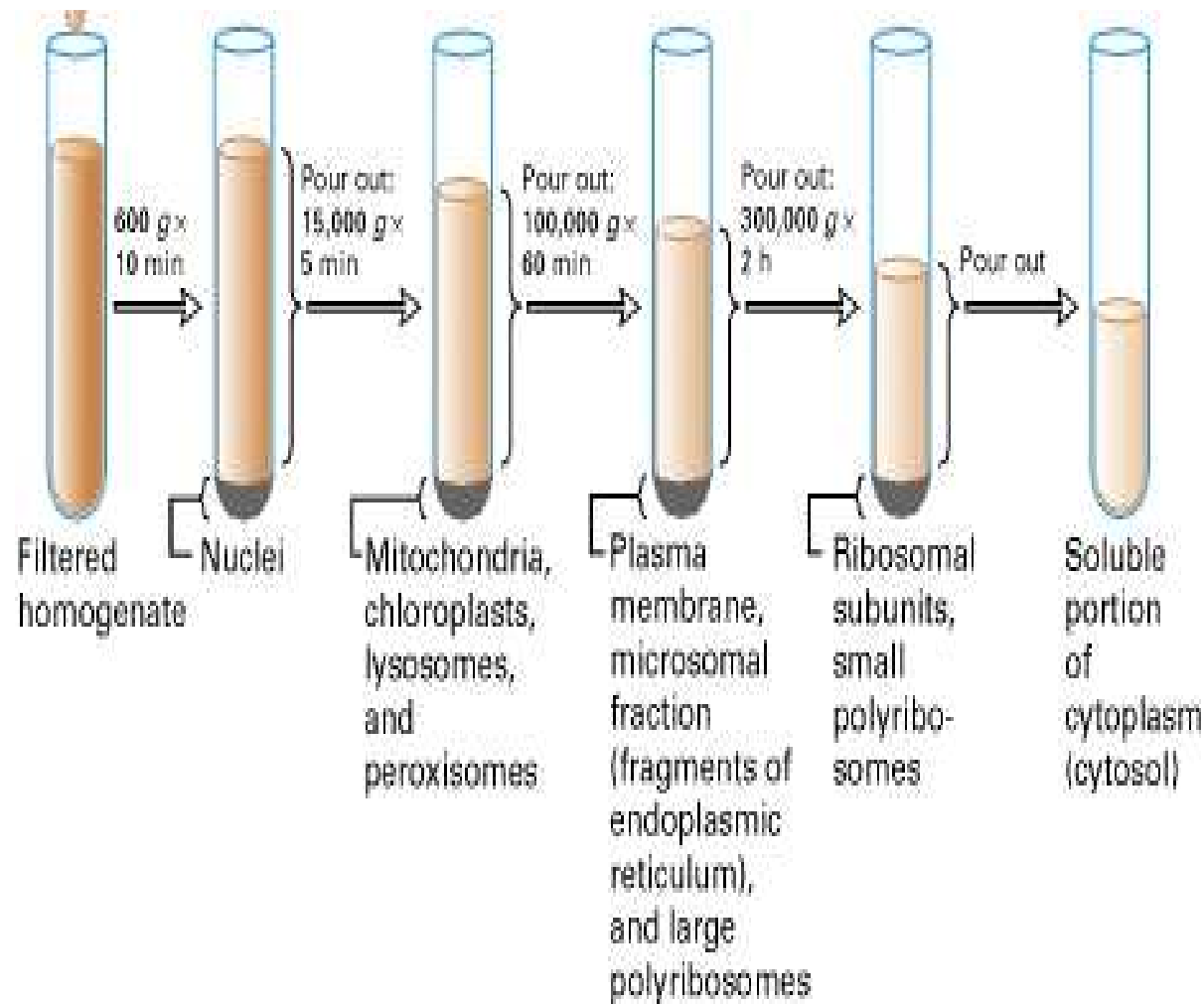
Isopycnic centrifugation



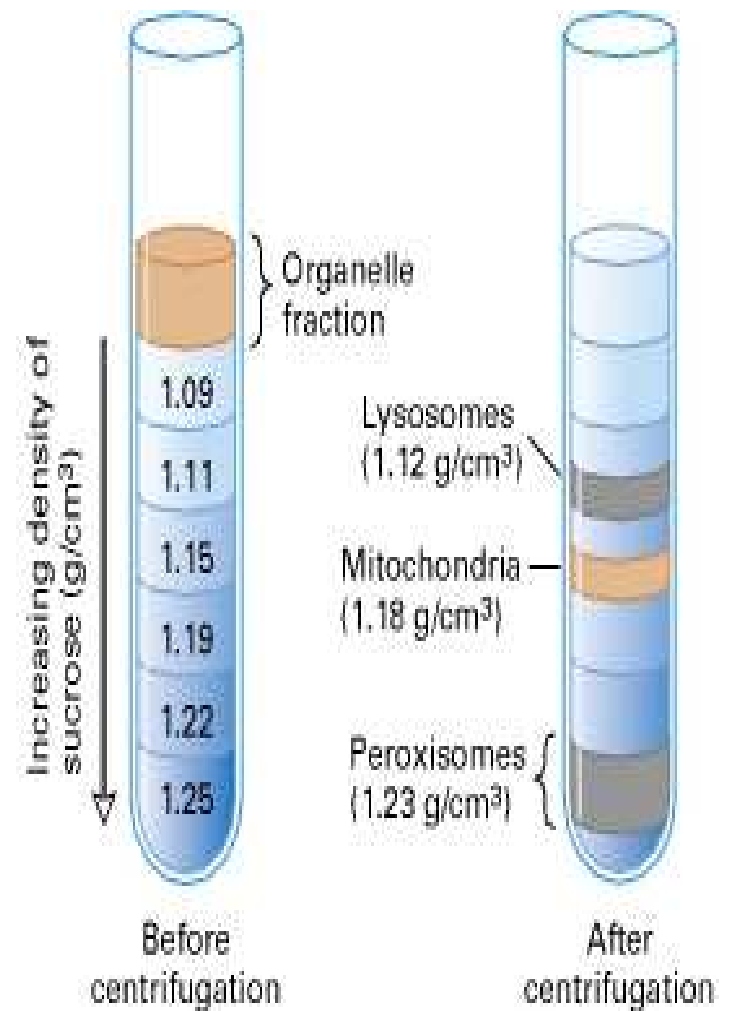
Gradientová centrifugace



Diferenciální versus gradientová centrifugace



4 x



1 x

Meselsonův a Stahlův experiment

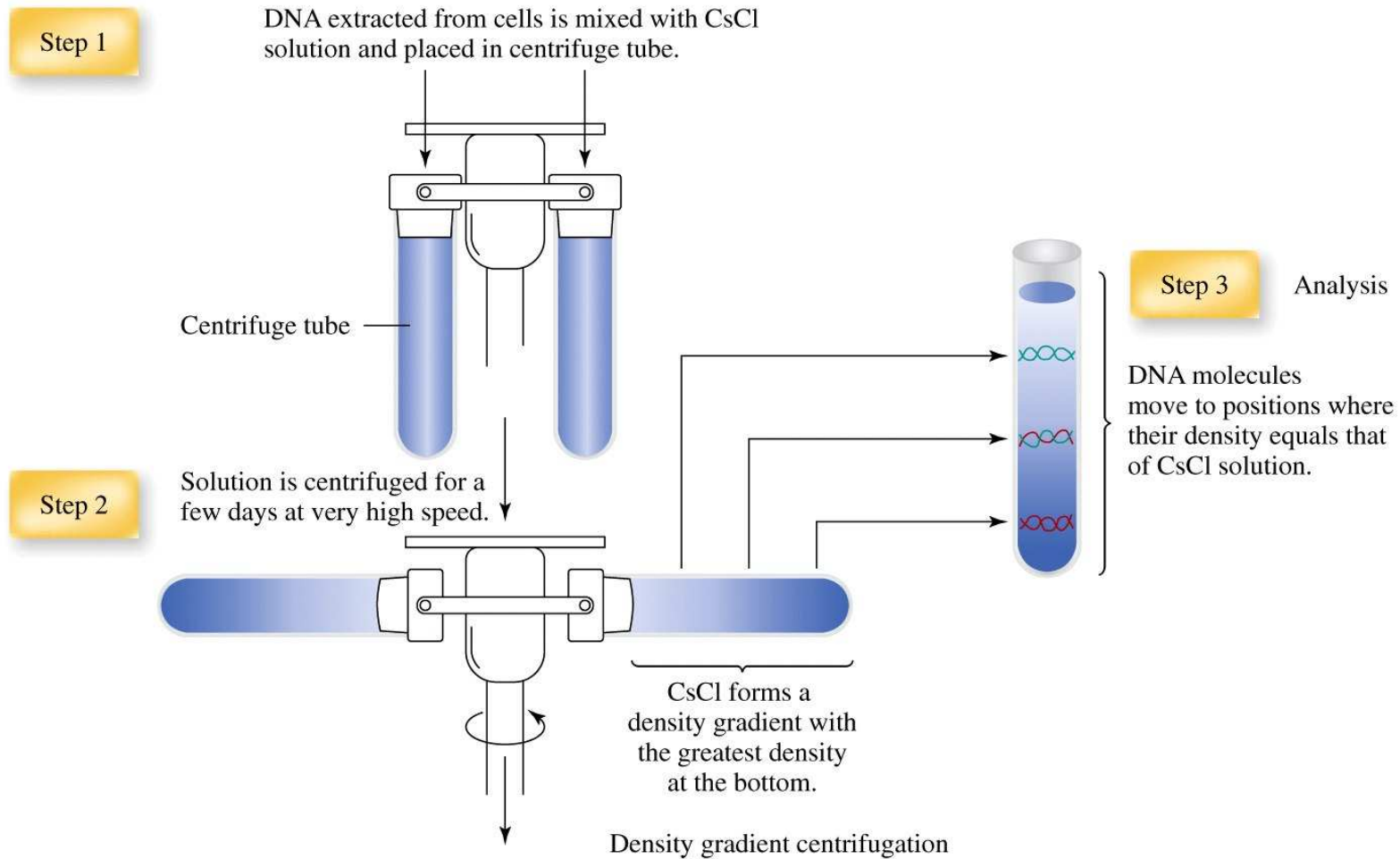


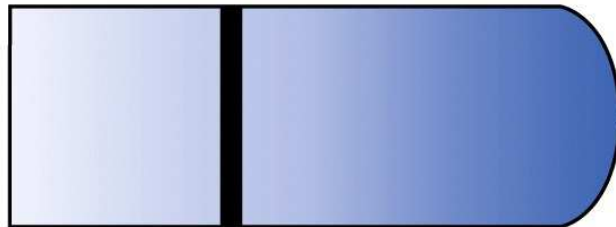
Figure 11-2a Concepts in Biochemistry, 3/e
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Meselsonův a Stahlův experiment

Direction of sedimentation



Heavy DNA



Light DNA



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

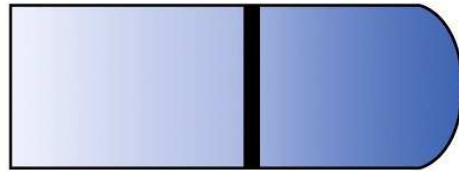


Normal ^{14}N -DNA
(with two light
strands)

Preliminary experiment

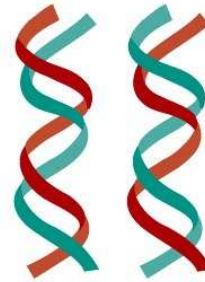
Meselson and Stahl experiment

Experimental results



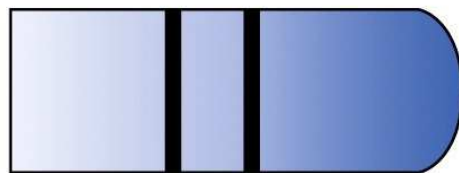
Hybrid DNA

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



Conclusions

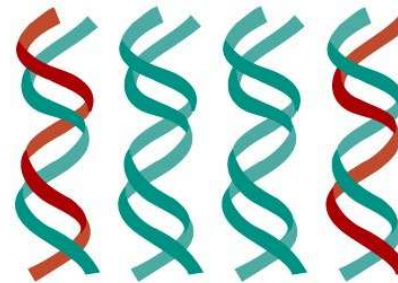
First generation:
Both DNAs contain one light and one heavy strand.



Light DNA

Hybrid DNA

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

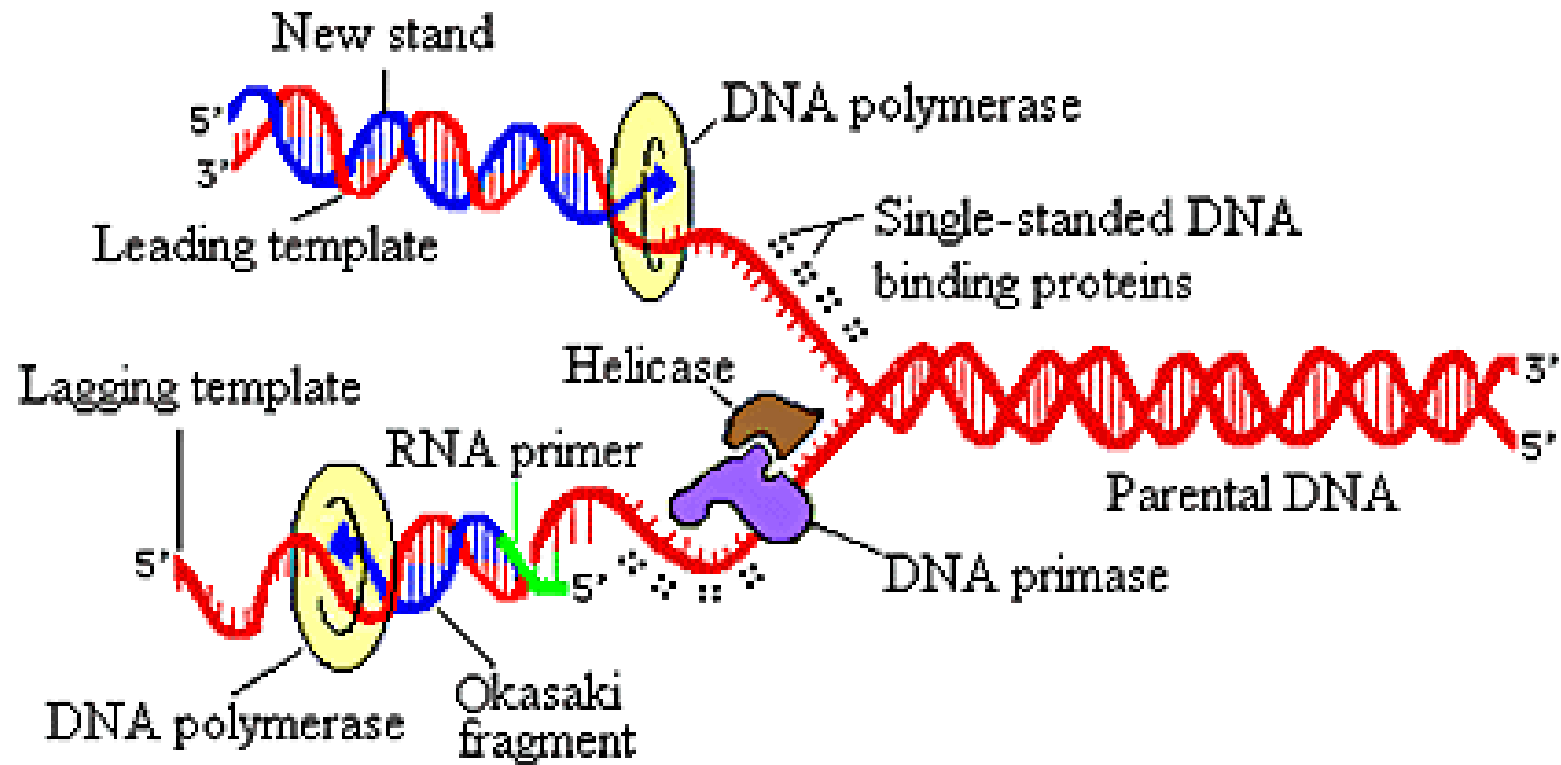


Second generation:
Two hybrid DNAs and two light DNAs are formed.

Actual experiment

Replikace

DNA + NTP + Mg²⁺ + 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
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Prokaryontní replikace

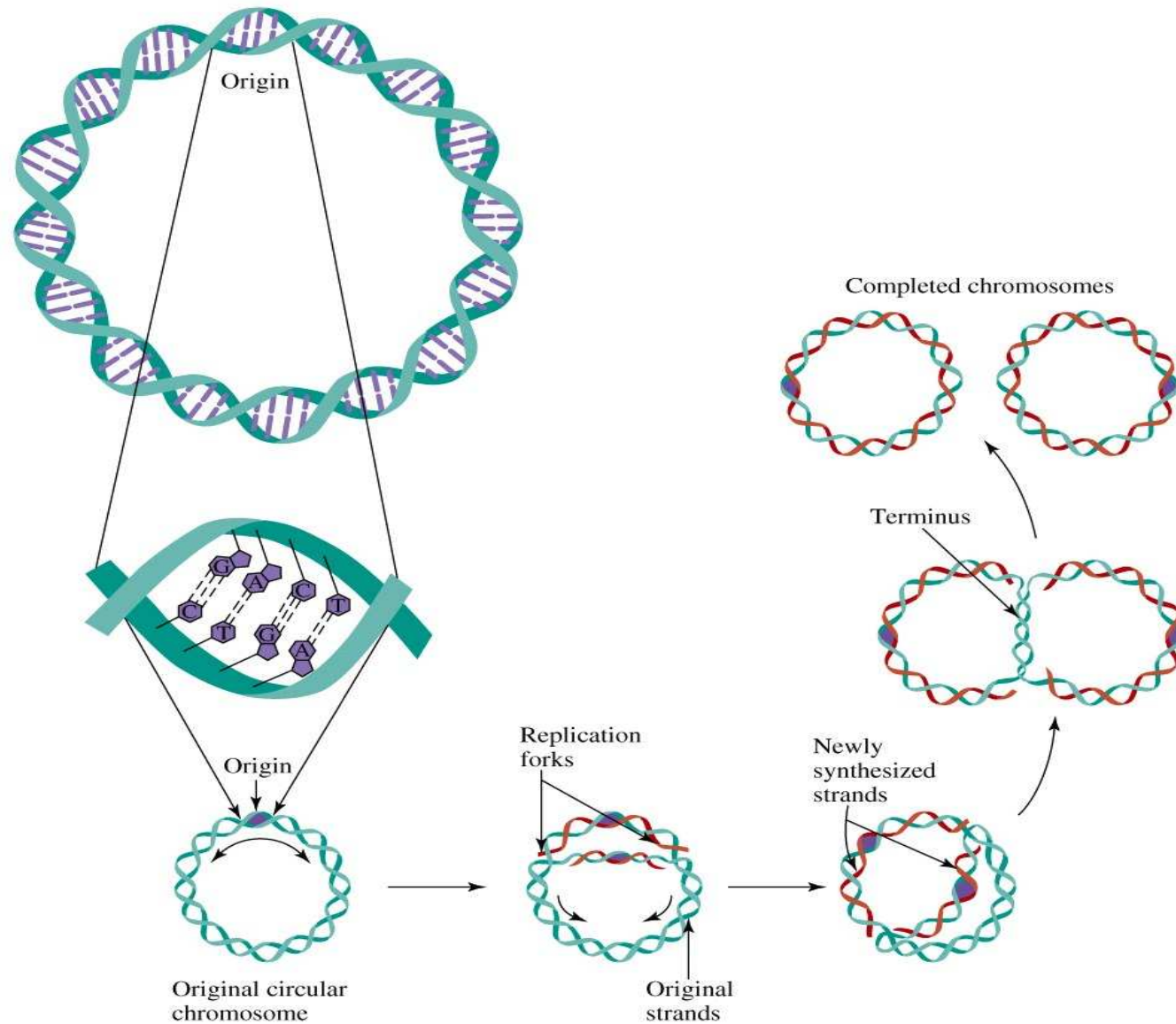
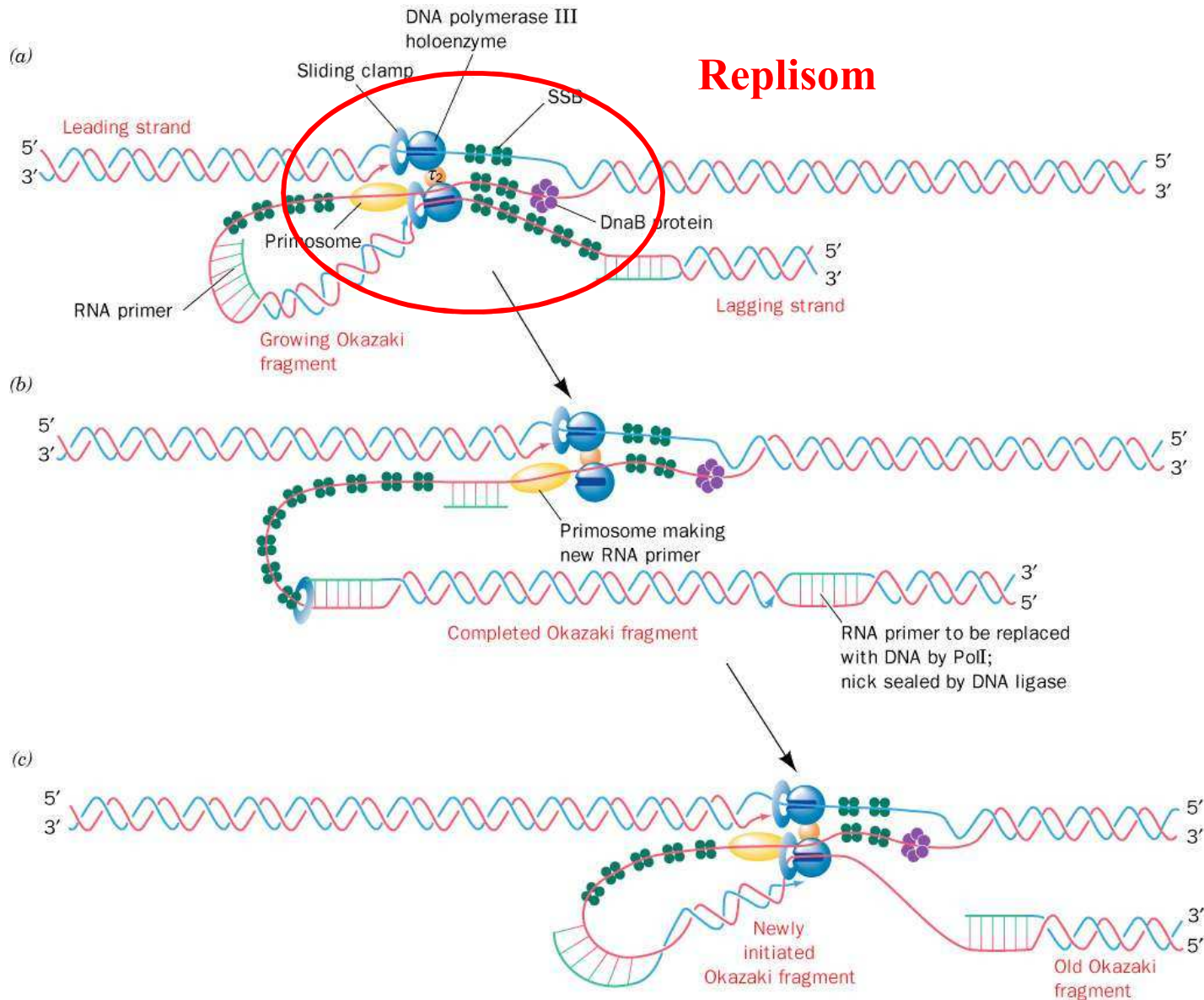


Figure 11-4 Concepts in Biochemistry, 3/e
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Prokaryontní replikace



Replikace u E.coli

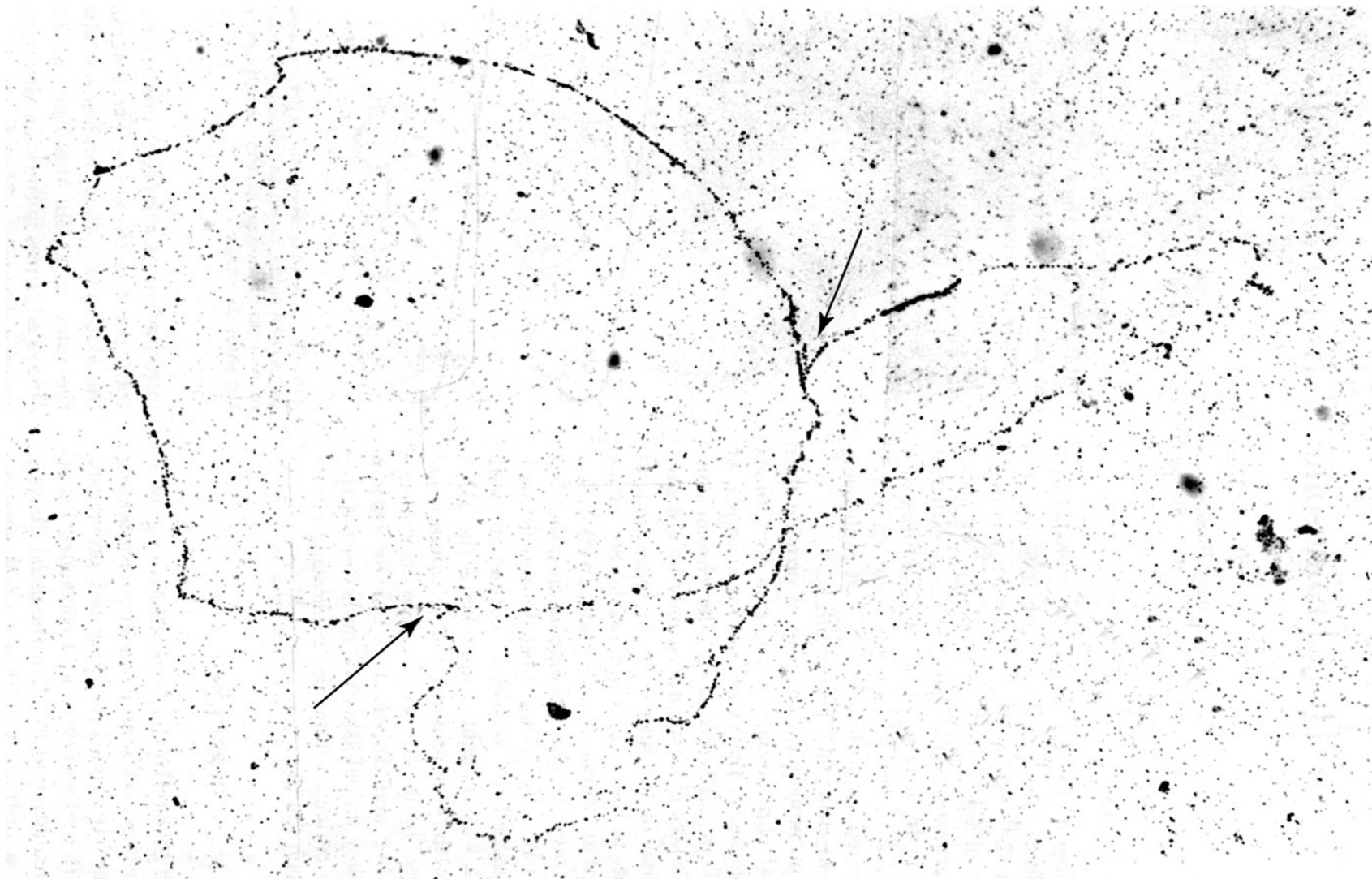


Figure 11-3 Concepts in Biochemistry, 3/e

Eukaryontní replikaci

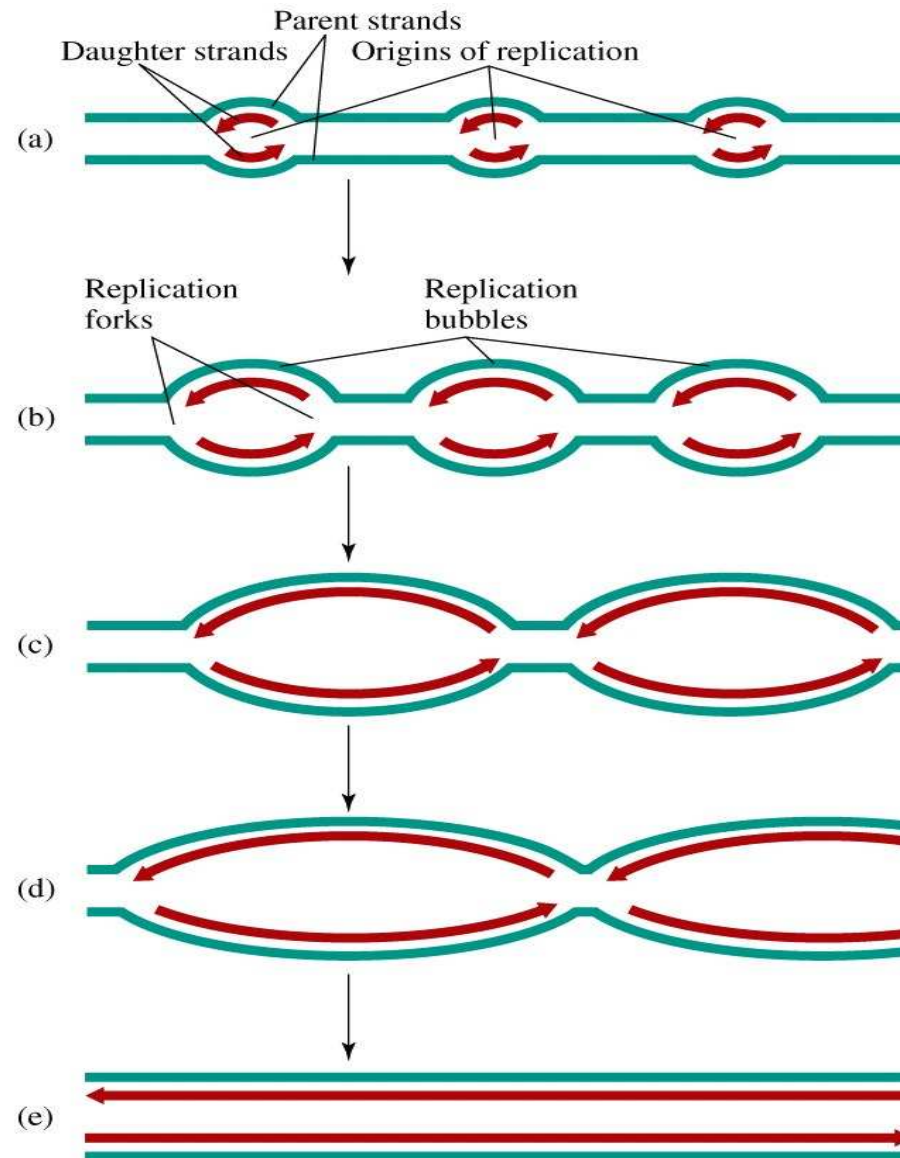
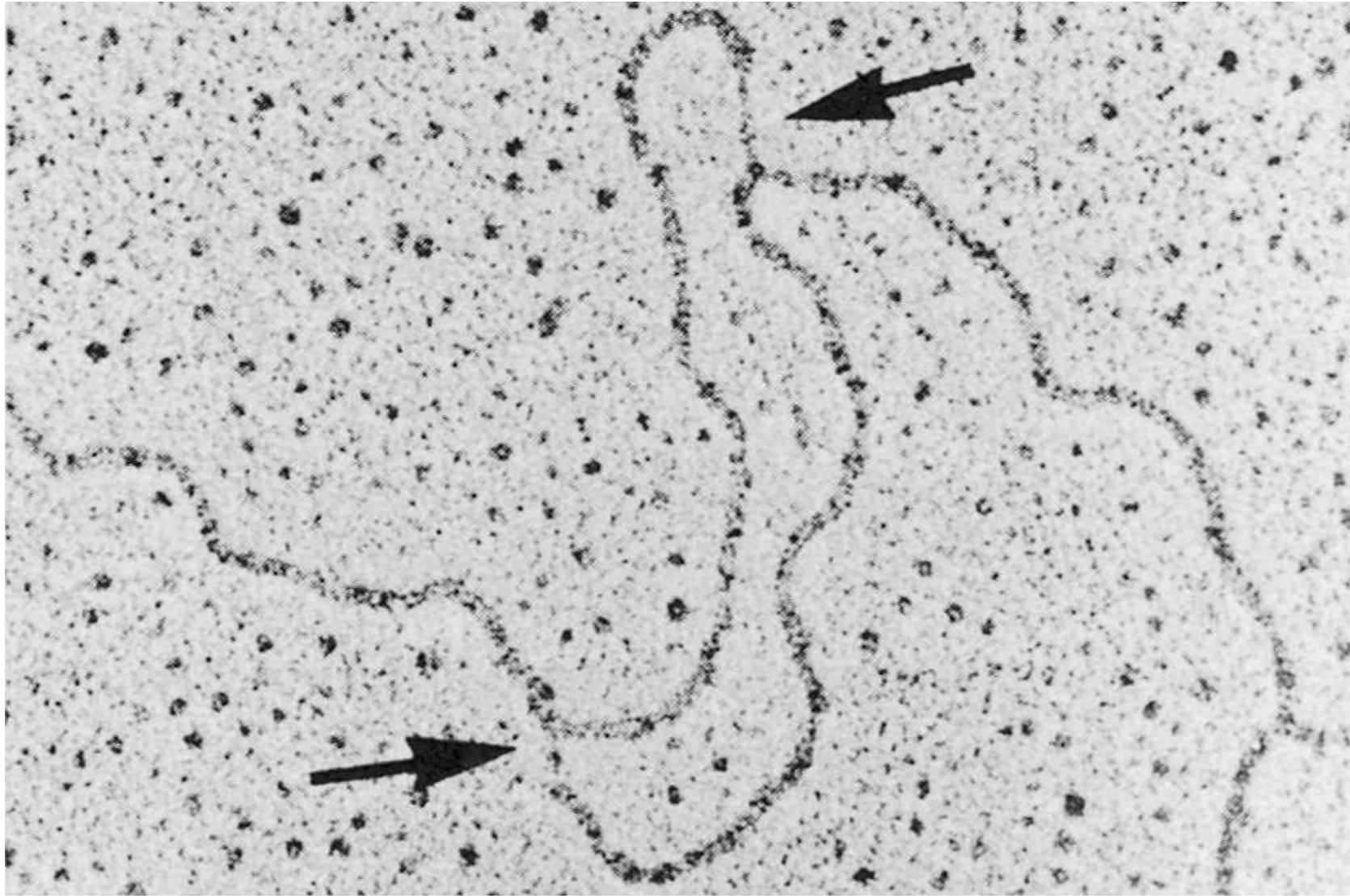


Figure 11-5 Concepts in Biochemistry, 3/e
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Replikace u drosofilý



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

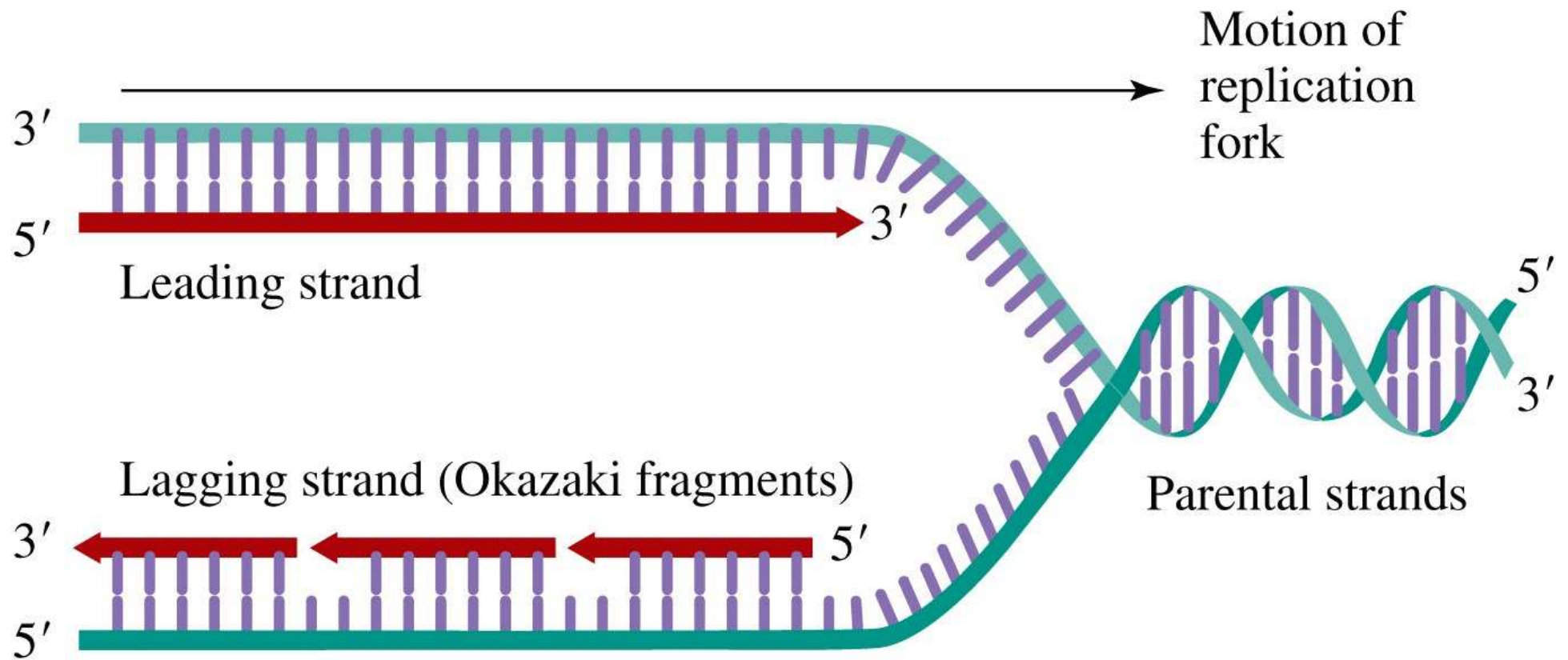


Figure 11-9 Concepts in Biochemistry, 3/e
 © 2006 John Wiley & Sons

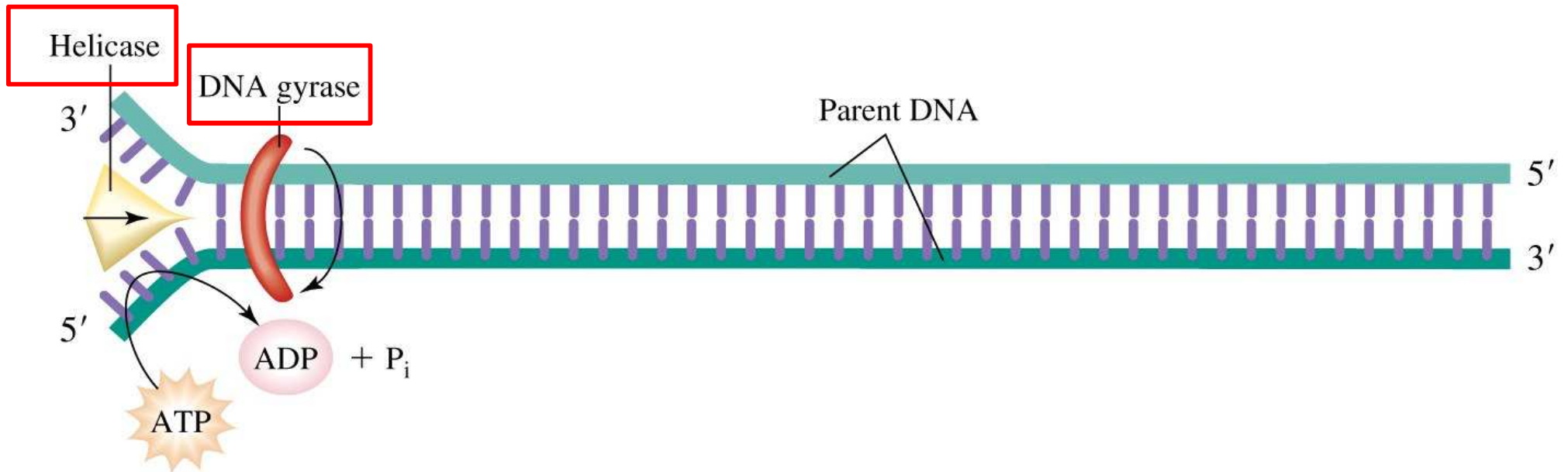


Figure 11-10a Concepts in Biochemistry, 3/e
© 2006 John Wiley & Sons

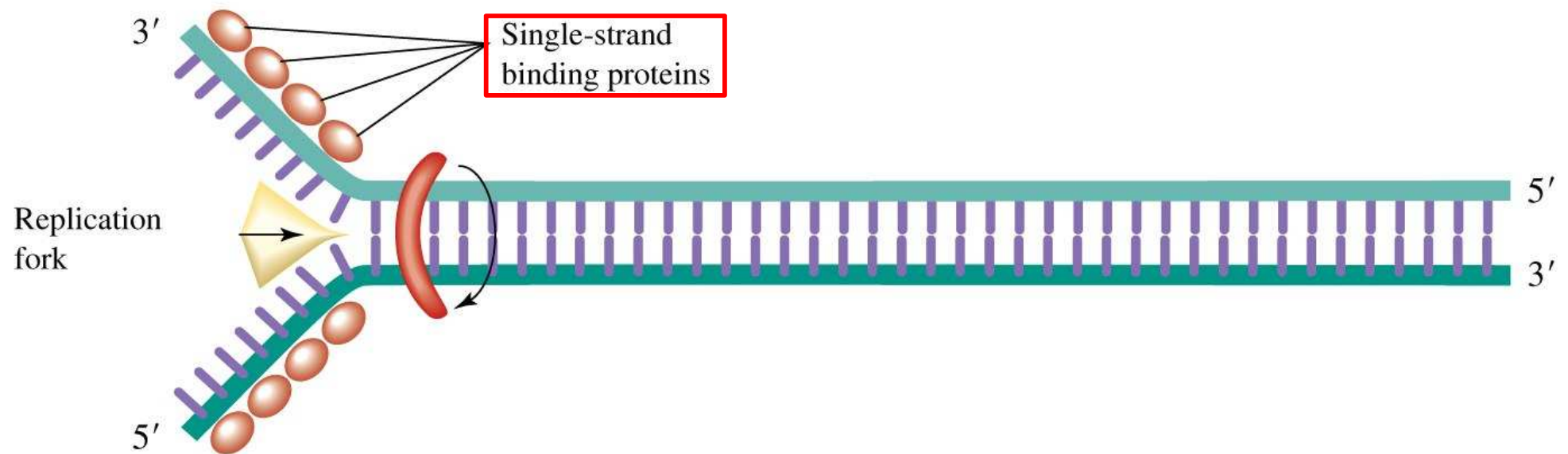


Figure 11-10b Concepts in Biochemistry, 3/e
© 2006 John Wiley & Sons

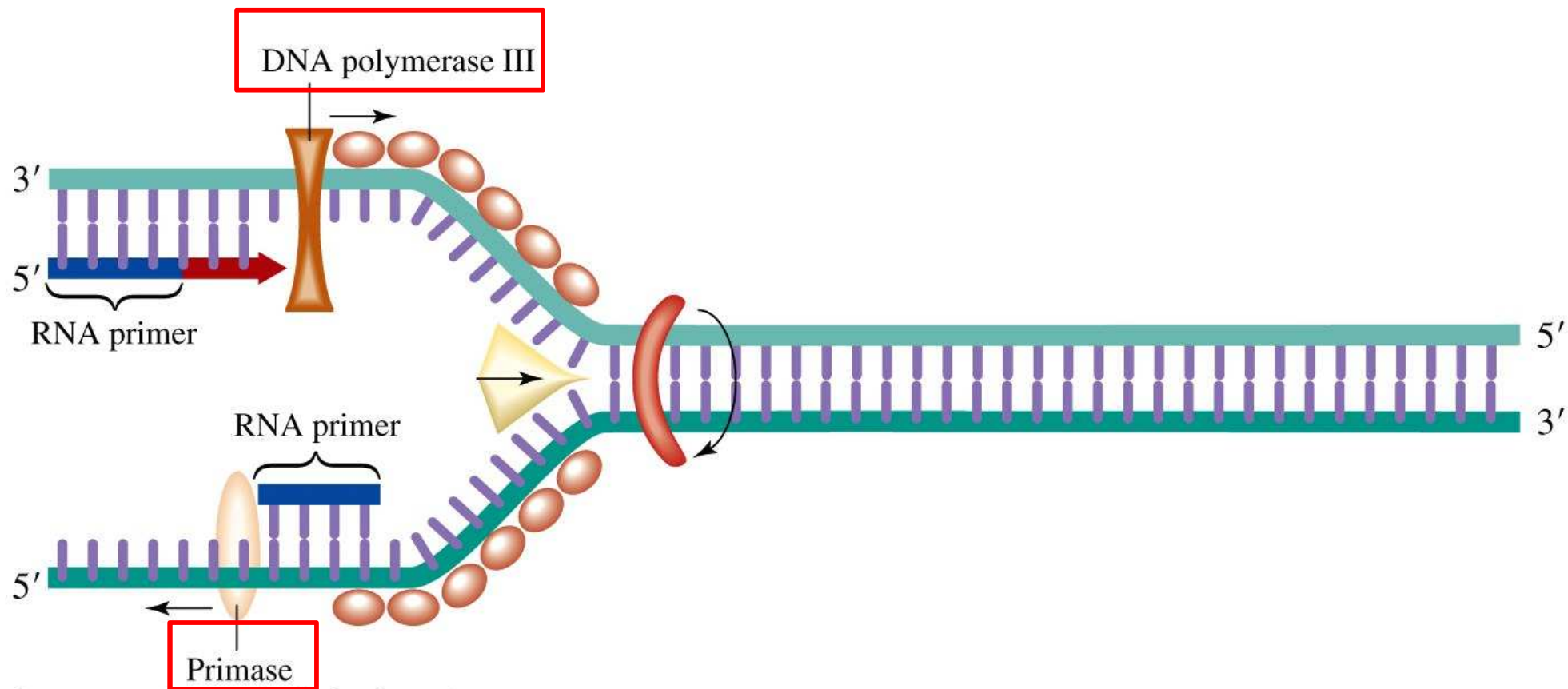
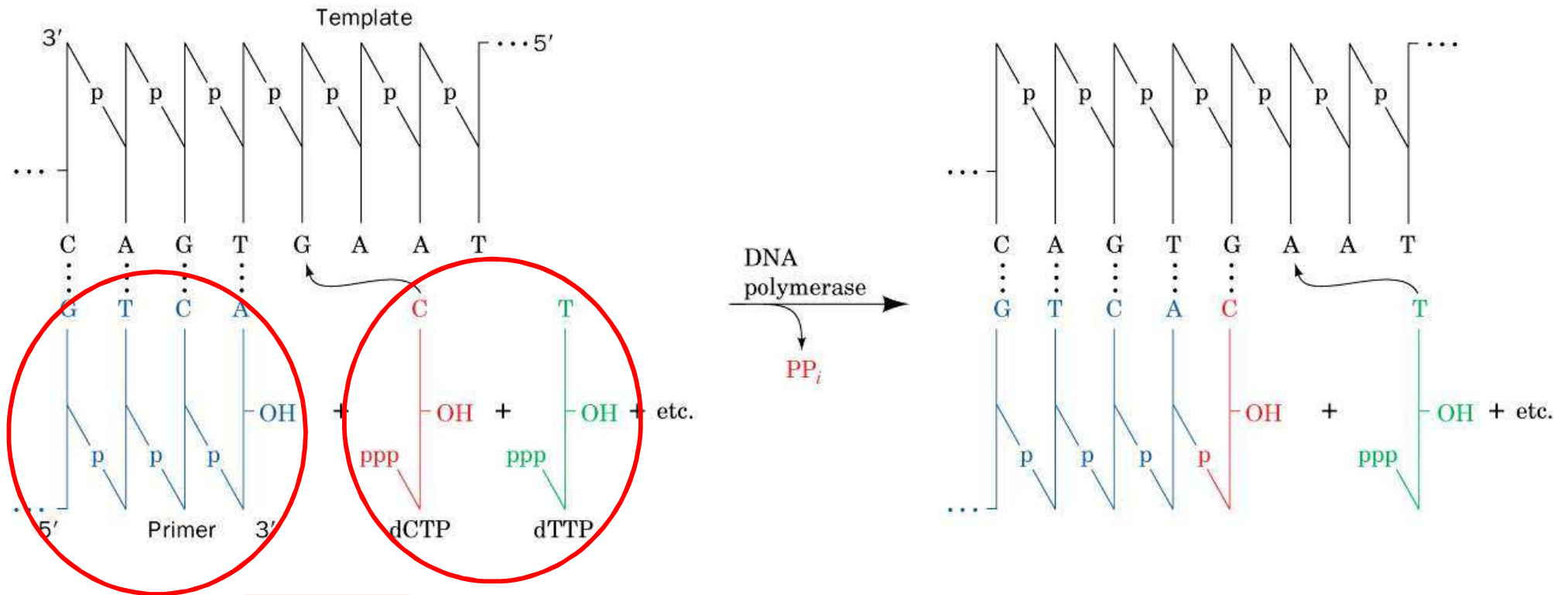


Figure 11-10c Concepts in Biochemistry, 3/e
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DNA polymeráza III



5' → 3'

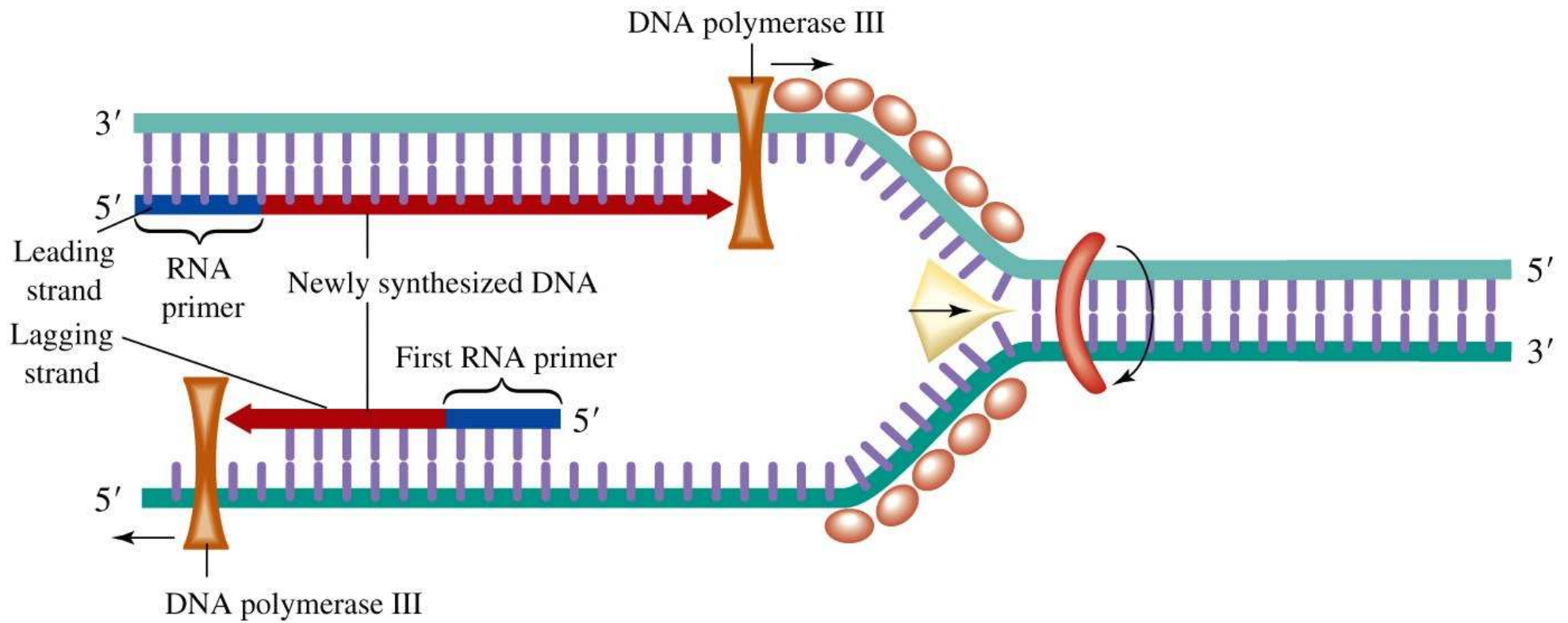


Figure 11-10d Concepts in Biochemistry, 3/e
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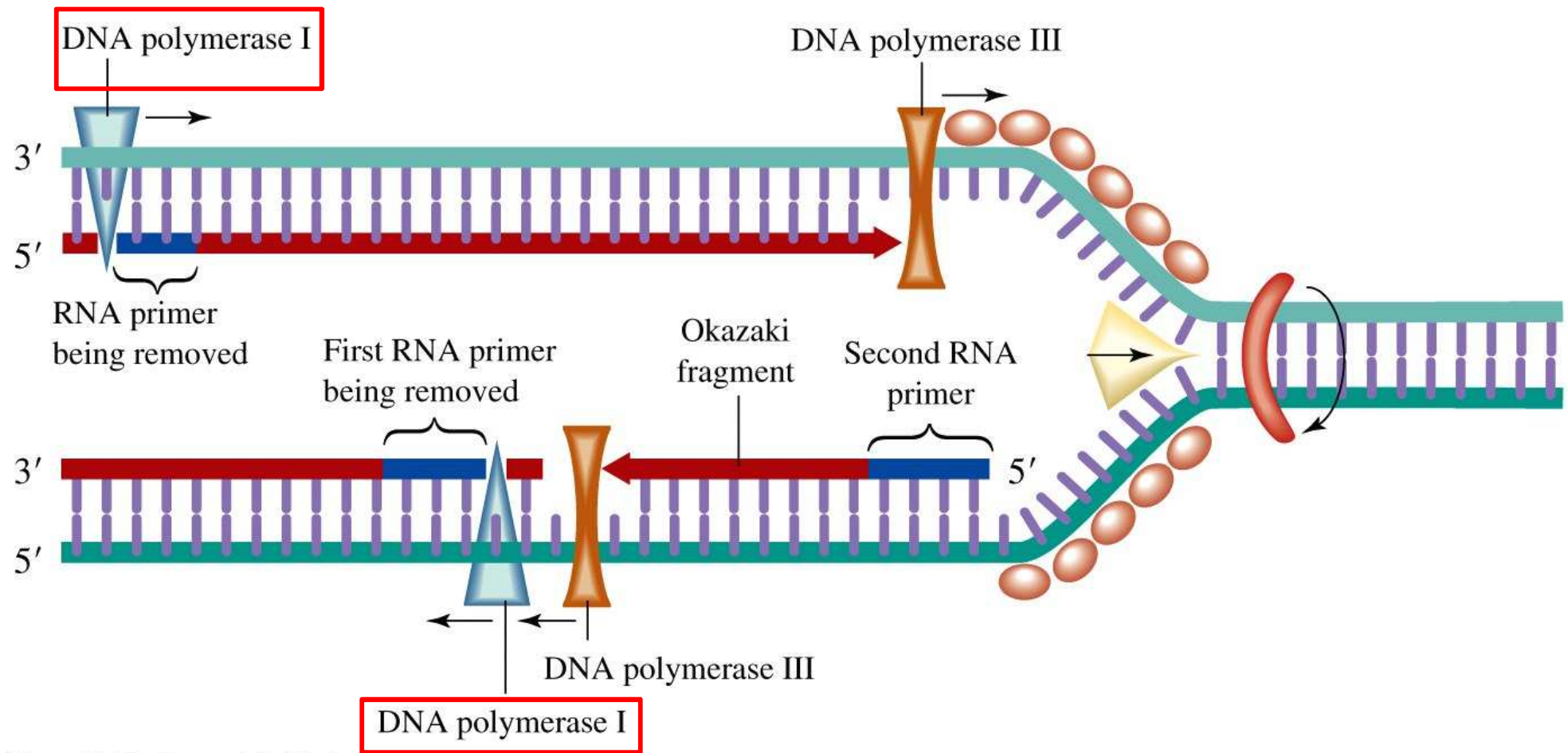


Figure 11-10e Concepts in Biochemistry, 3/e
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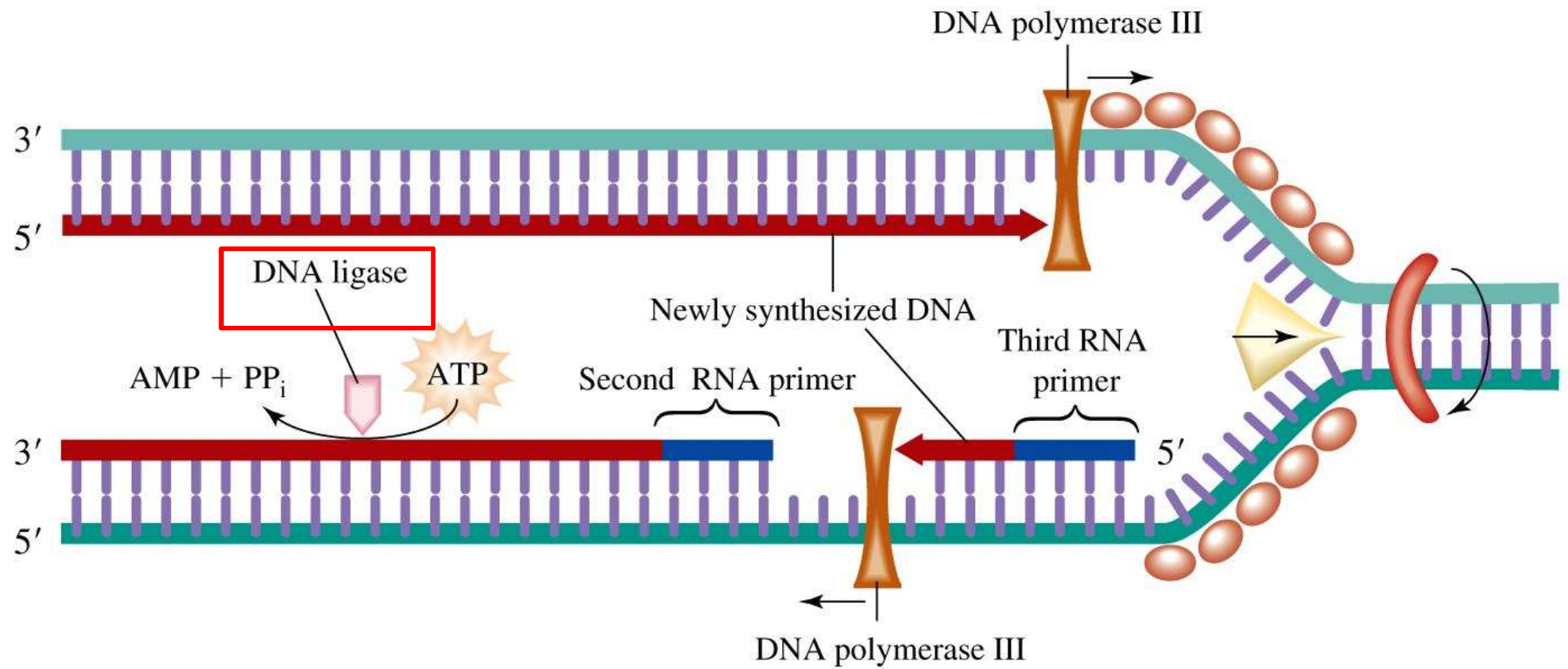
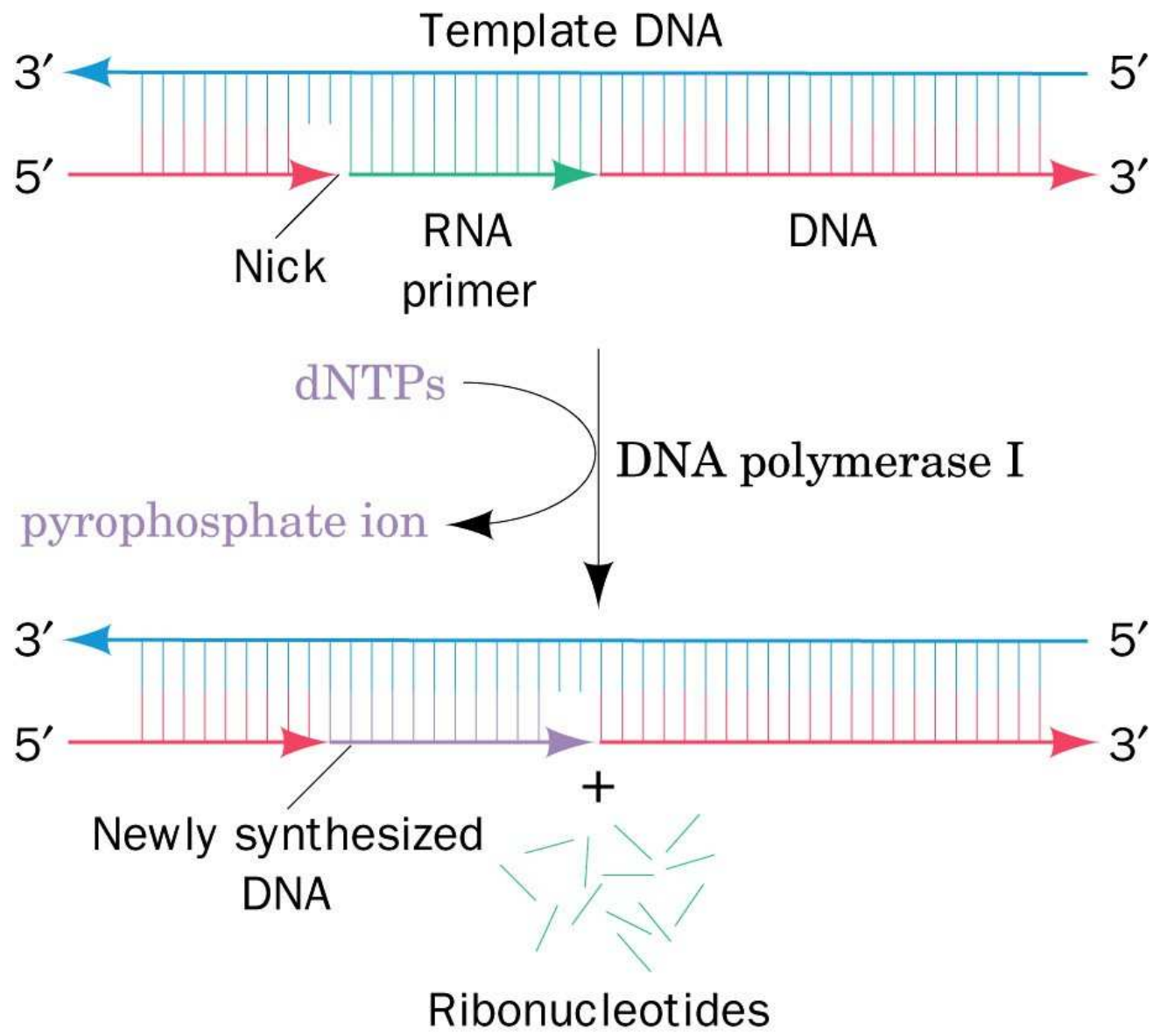
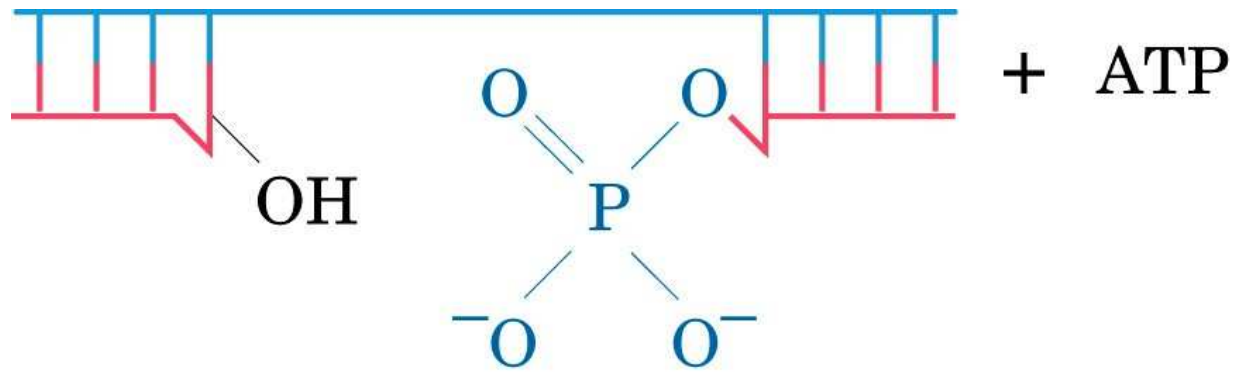
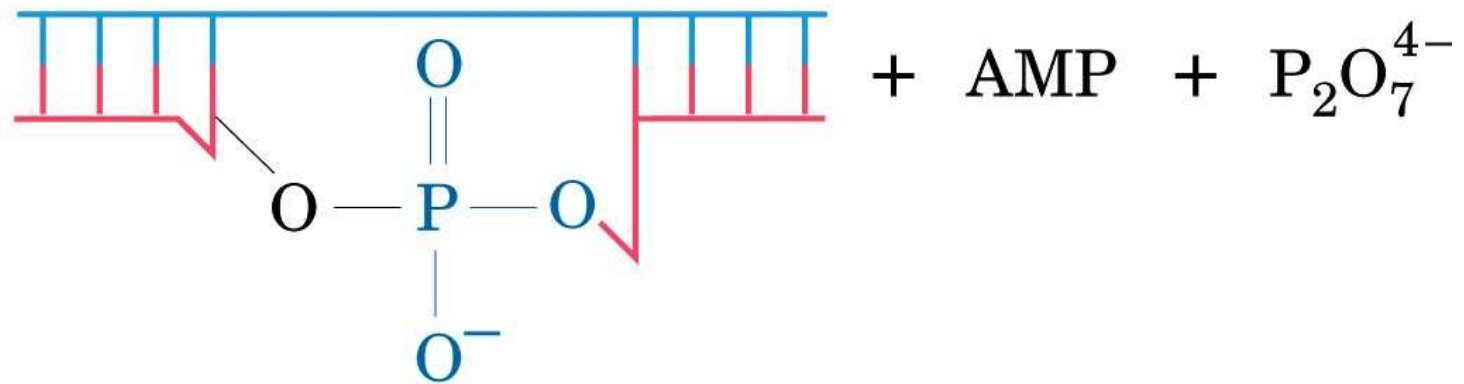


Figure 11-10f Concepts in Biochemistry, 3/e
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DNA ligase



Enzymová metoda sekvenace DNA

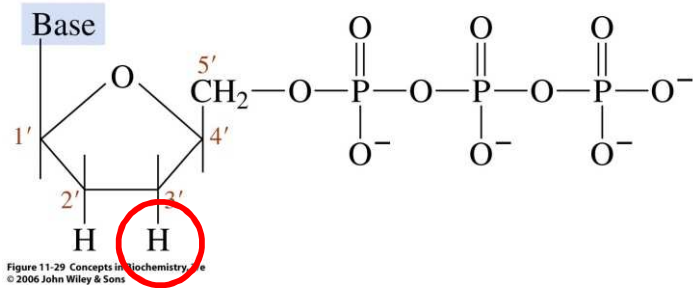
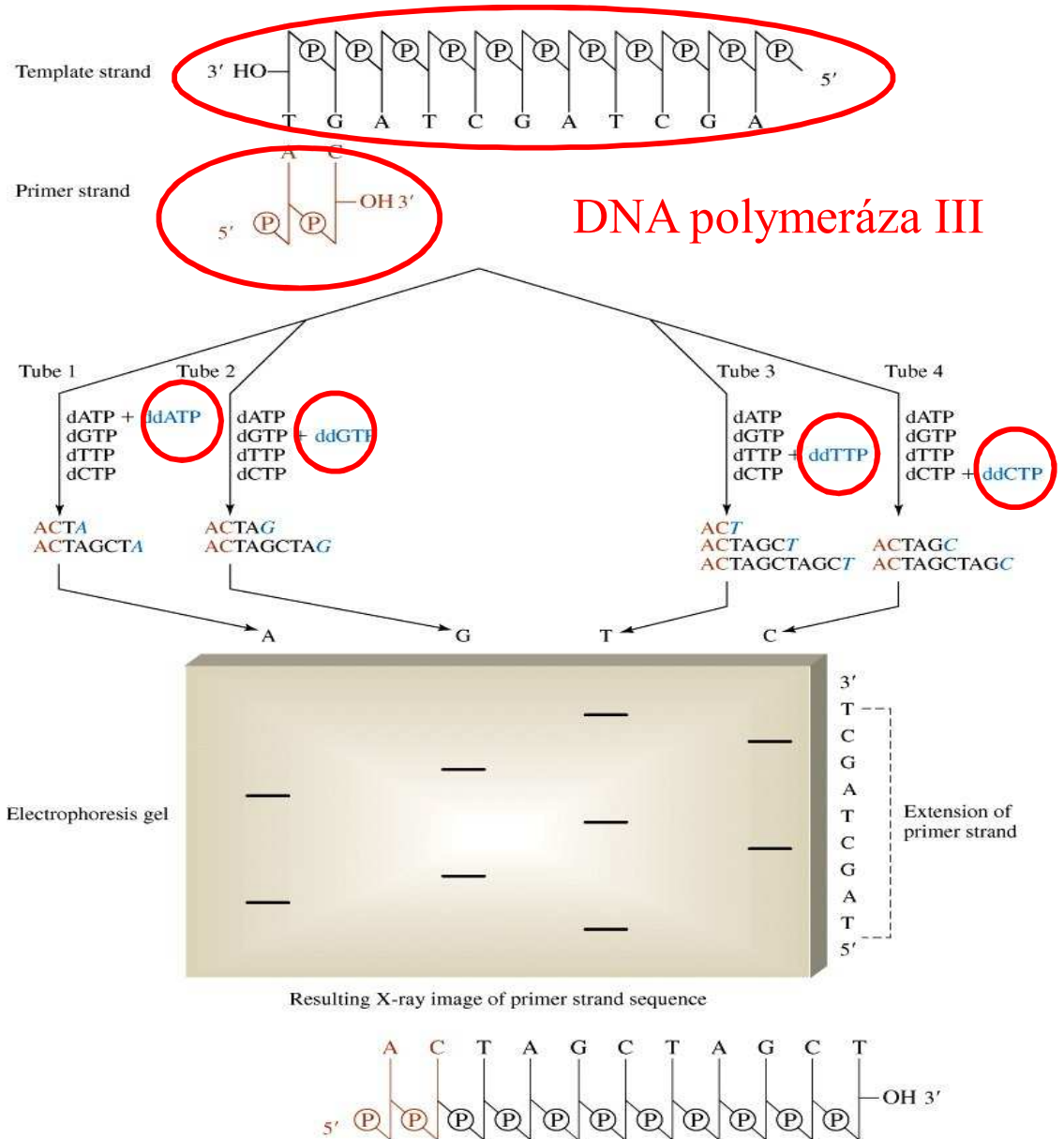


Figure 11-29 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons

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
ATCTGACTATA
90 TAATAATATG
TCCAAGTTTA



80

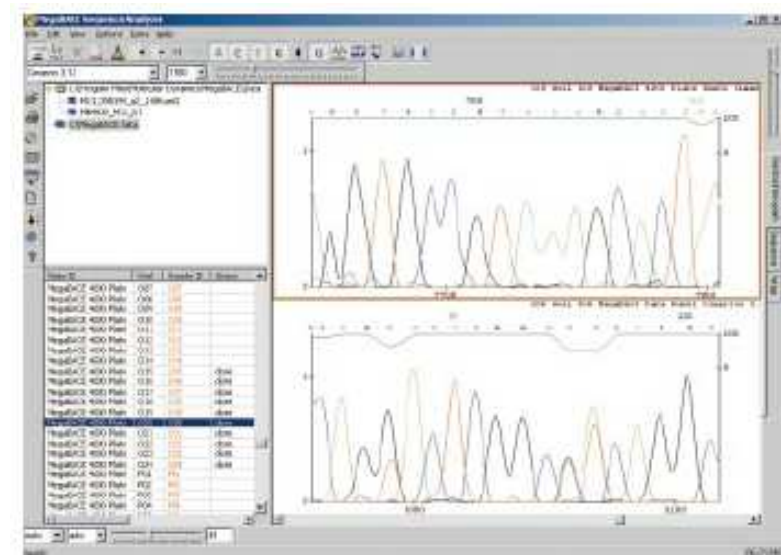
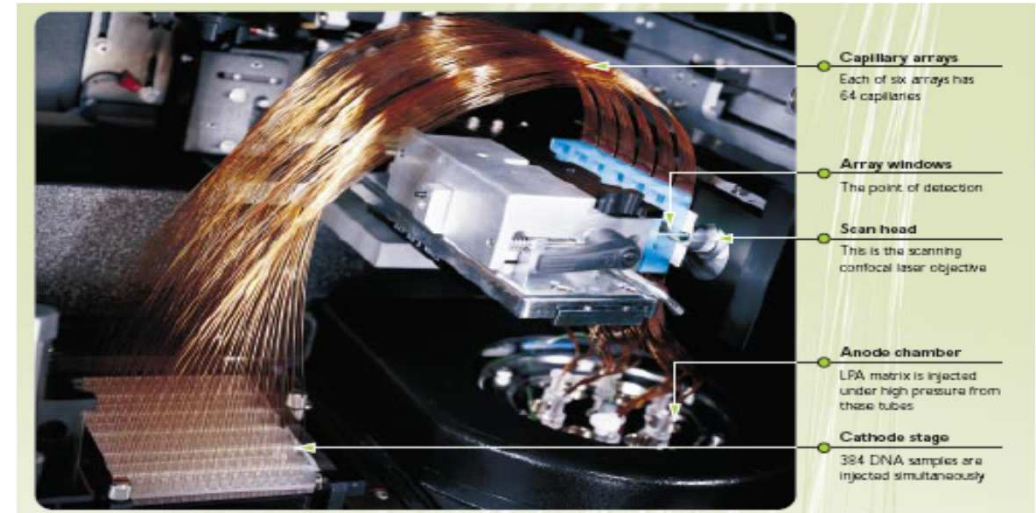
ATTGAACT
CAGATAGTAA
TTCCCTGAGTT
CCTGGGAGAA
AACATCCGAA
TGAACCTTTG
TACCCATCAT
TCGTACTTGT

1989- 2003 Projekt lidského genomu

Paralelní sekvencování



Automatizace

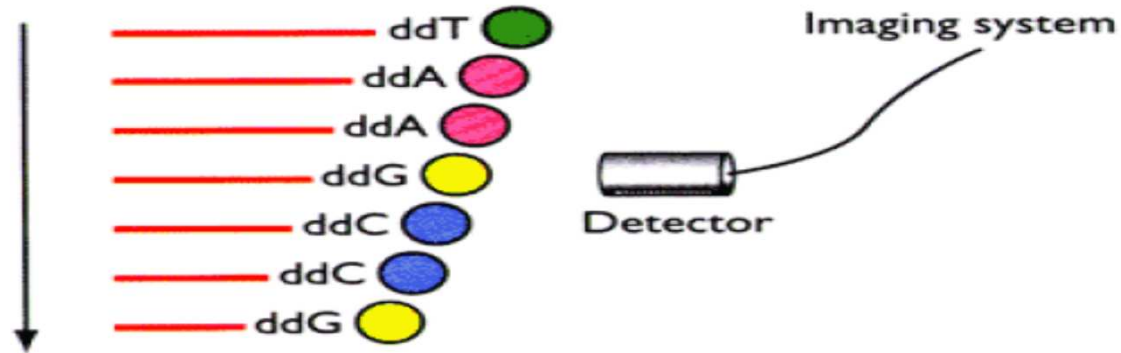


1 DNA – 1 vzorek

(A)

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

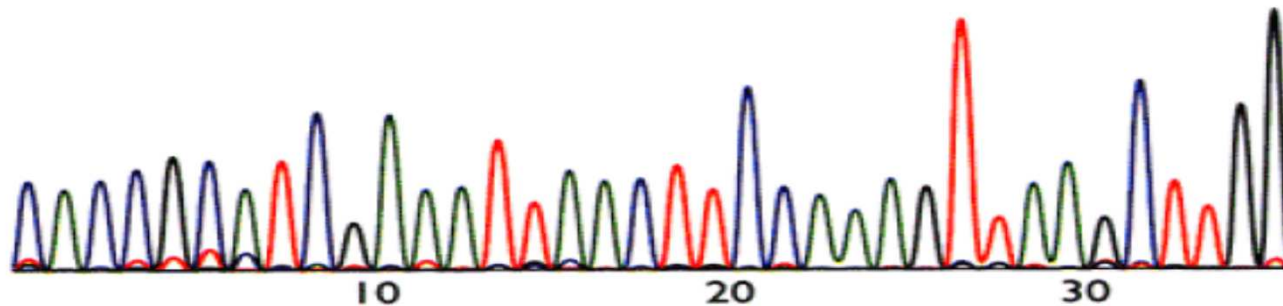
Sequencing reactions,
fractionation of products



Fluorescent bands
move past the detector

(B)

CACCGCATCGAAATTAAC TTCCAAAGTTAAGCTTGG



300 sekvenátorů

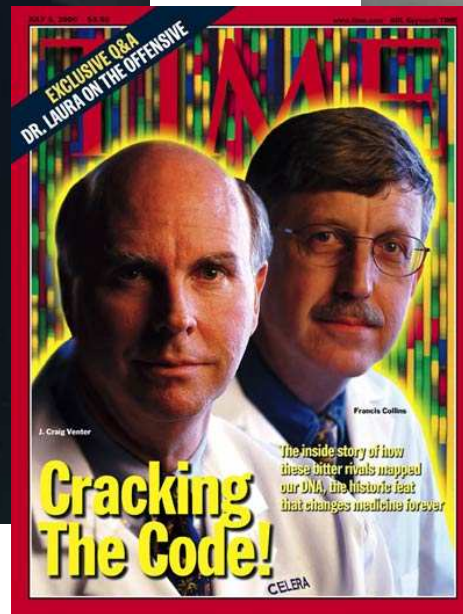
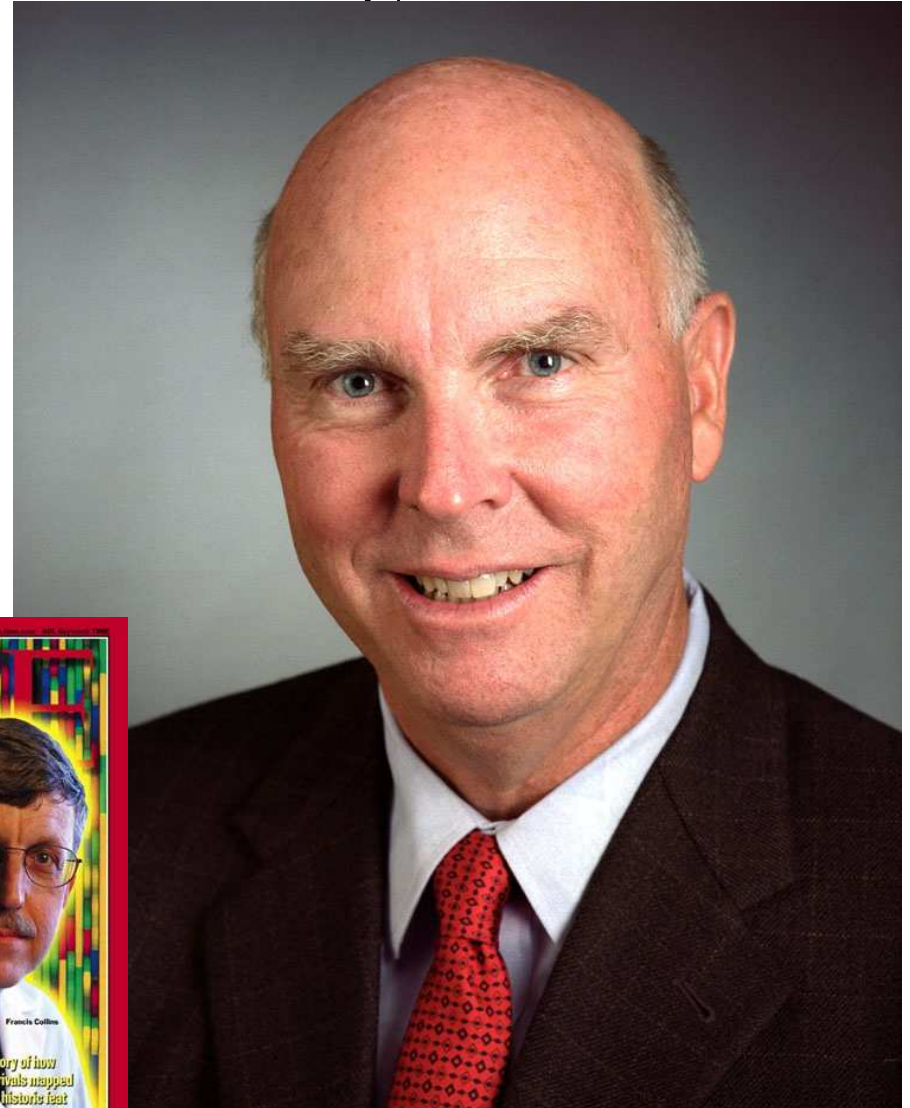


Veřejné consortium x Celera Genomics

Francis Collins



Craig Venter



Clone-by-clone

Hierarchical Shotgun Sequencing Method

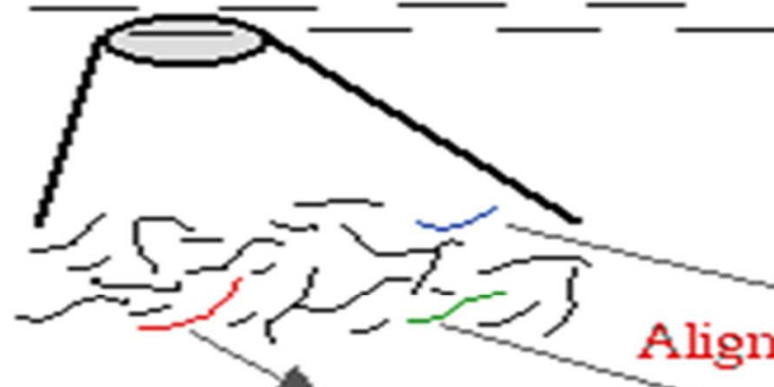


Genomic DNA



BAC Library

Create Contig Map



Sequence Each Contig
with Shotgun Approach

Align Contiguous Sequences

GCATTTTCGAGTTACCTGGACAACCAAGTG

CCAGTGGTACTGAGGACGCAGAGGGCTTGA

GCTTGATTGGCCAATAATAGTATAT

GCATTTTCGAGTTACCTGGACAACCAAGTGGTACTGAGGACGCAGAGGGCTTGATTGGCCAATAATAGTATAT

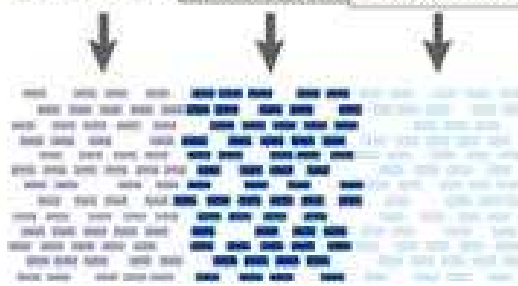
Generate Finished Sequence



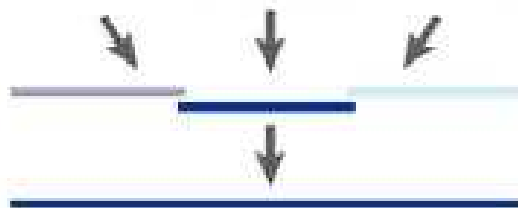
Construct
clone map
and select
mapped
clones

AGTTGGTAACCTA	TGGCAATTTAGA	CGATCGATGACTA
ATTTGACTTGGAA	TAACTTGCATGCT	CACTAGCCTGAT
CGATCGATGACTG	TGATCGATGTACT	ATCTGACTGTAG
CTTGAATCGATTA	GGATCTTSCAGGT	ATRACCTGCTTTG
ACTGGGATCCTAC	GGATTAAAACCA	CGAGCCTTGGCG
TGGCCTATAGCCC	AACTTAAATCGA	ATCGATGTACTGG
AACTCGATGCGAT	TAGCACATGGCGT	ATTTTACAGATTA
ATACAGCTTCTAT	ATAGCCCTAGAT	CGTTAGATCGATA
TGATCGATGAAT	CTGTATCGATAT	GCACATGCGGTAT

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

GCTTGATTGGCCATAATAGTATAT

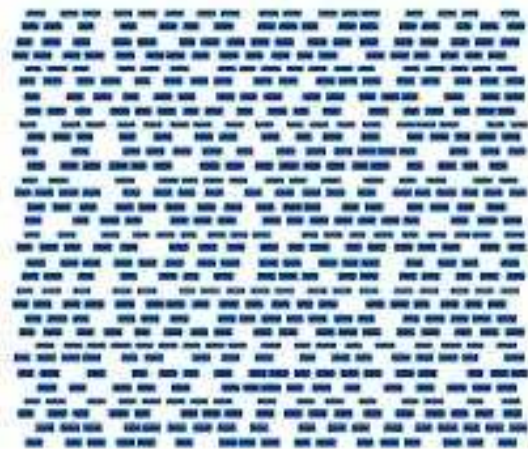
Align Contiguous Sequences

GCATTTTCGAGTTACCTGGACACCAGTGGTACTGAGGACGCARAGAGGCTTGGATTGGCCATAATAGTATAT

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.**

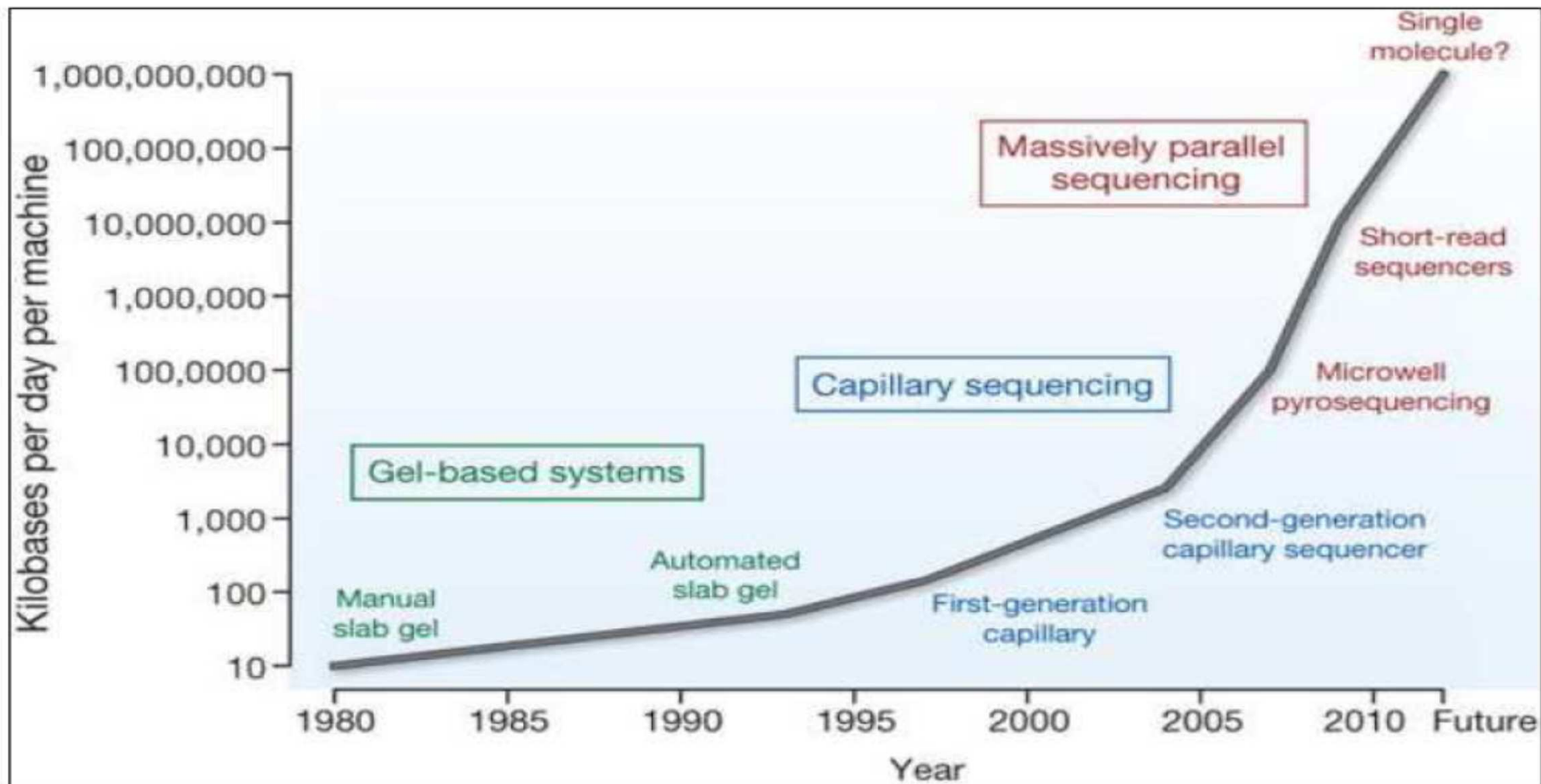
Lidský genom - 2003

- 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



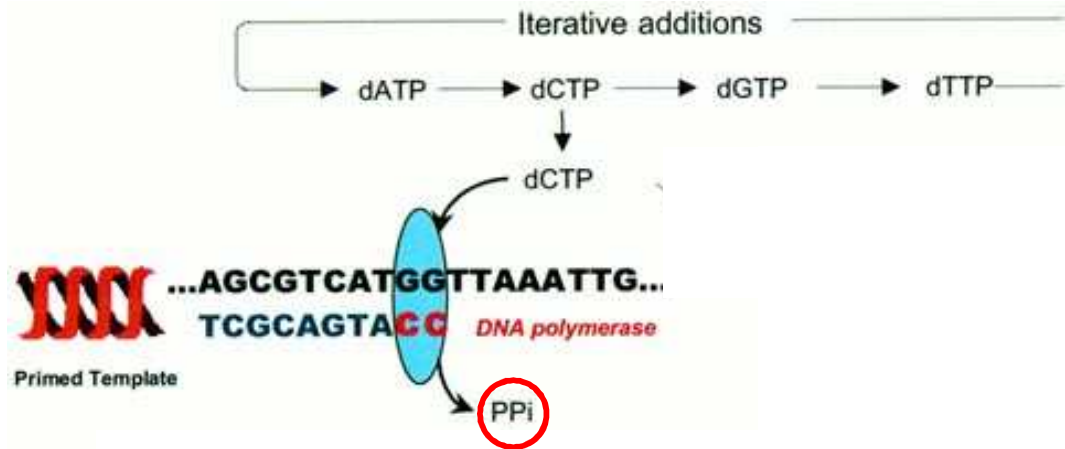
Pyrosekvenování

sekvenování druhé generace

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



Pyrosekvenování



Pyrosekvenování

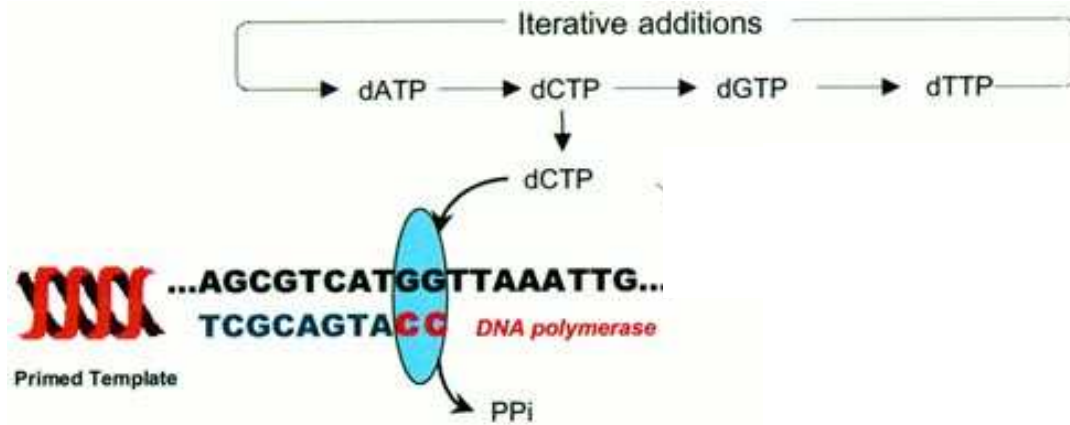
- První reakcí je DNA polymerace pomocí DNA polymerasy, kdy dochází k zařazení příslušného deoxynukleotid trifosfátu (dNTPs) za uvolnění pyrofosfátu.



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

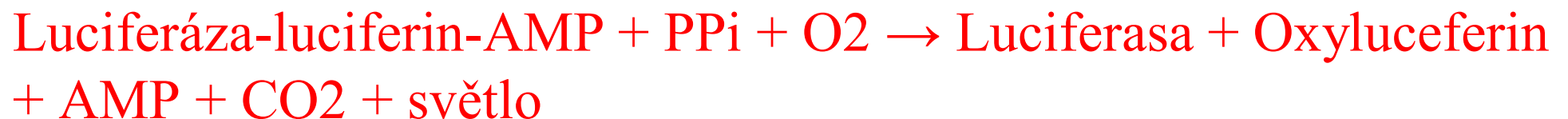


Pyrosekvenování

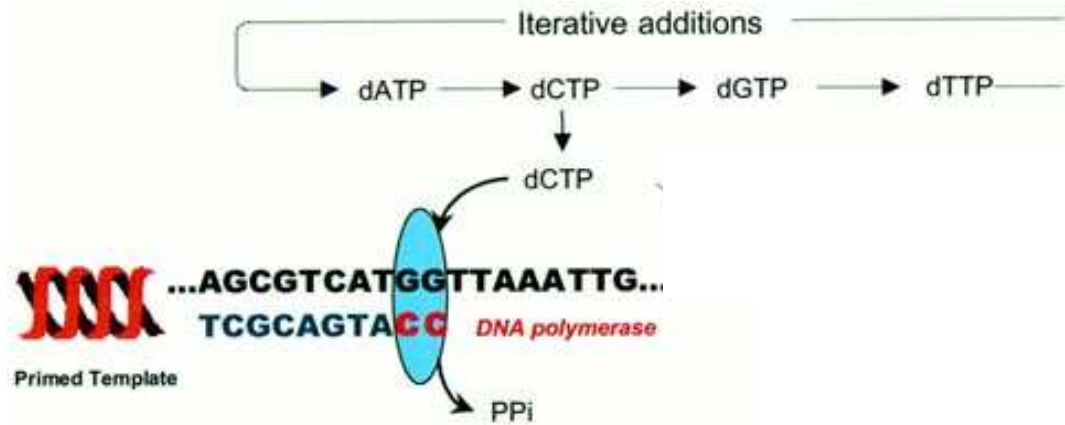


Pyrosekvenování

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



Pyrosekvenování

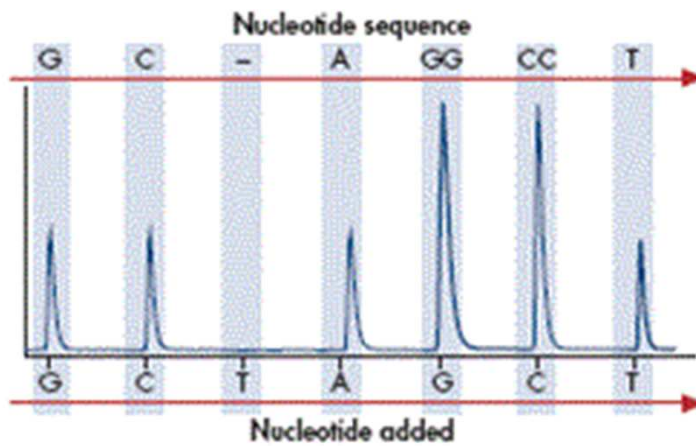
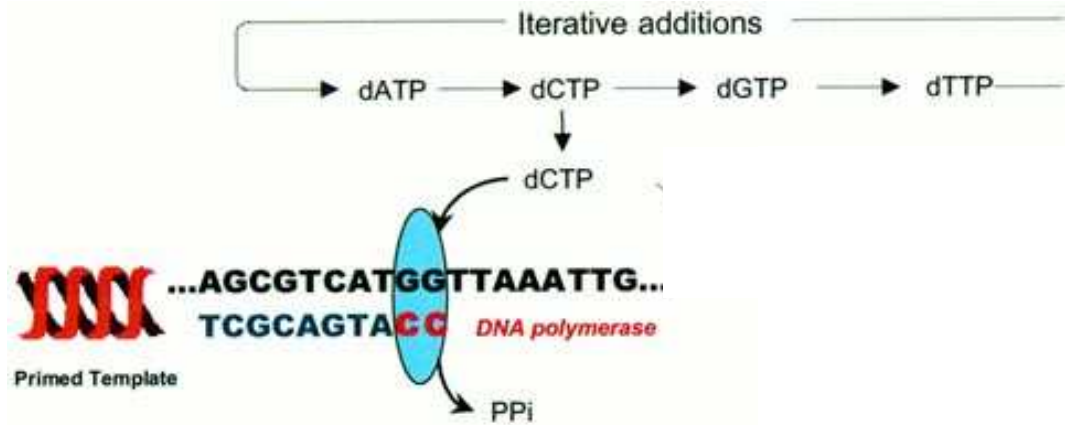


Pyrosekvenování

- 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.)

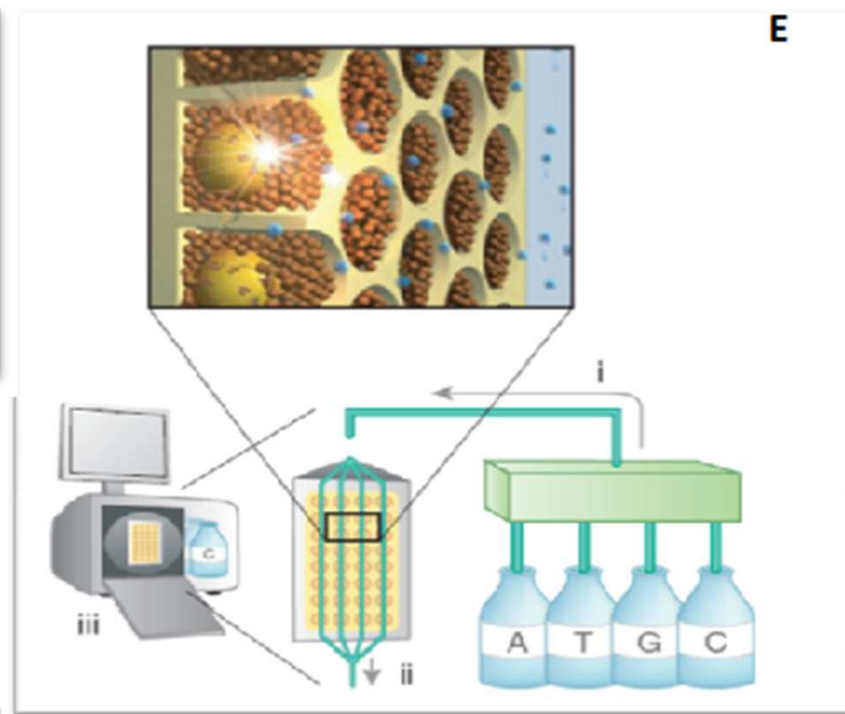
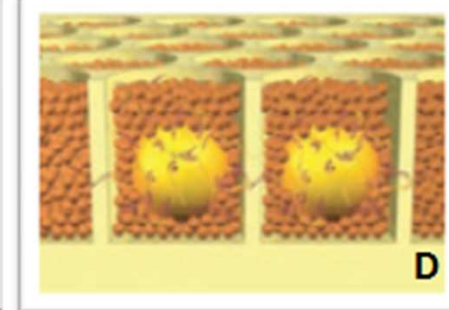
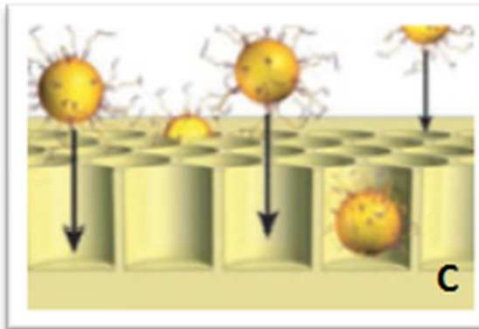
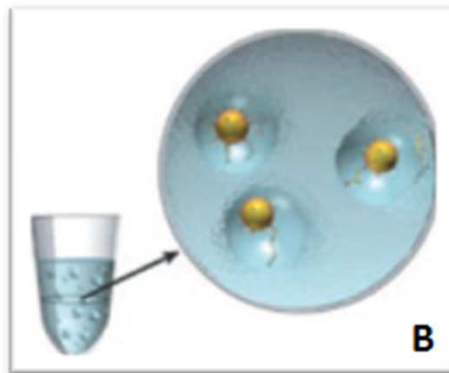
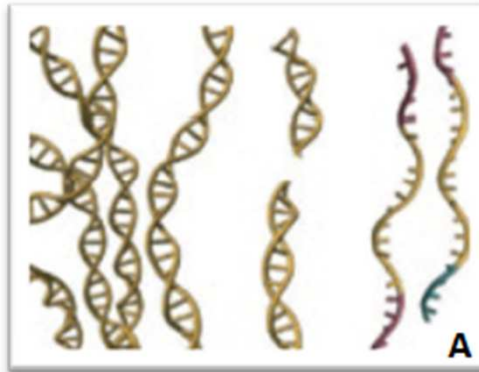
- tzv. ampikonové sekvenování. Vlastní sekvenaci předchází PCR zacílená na 16S nebo 18S geny prokaryot a eukaryot

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

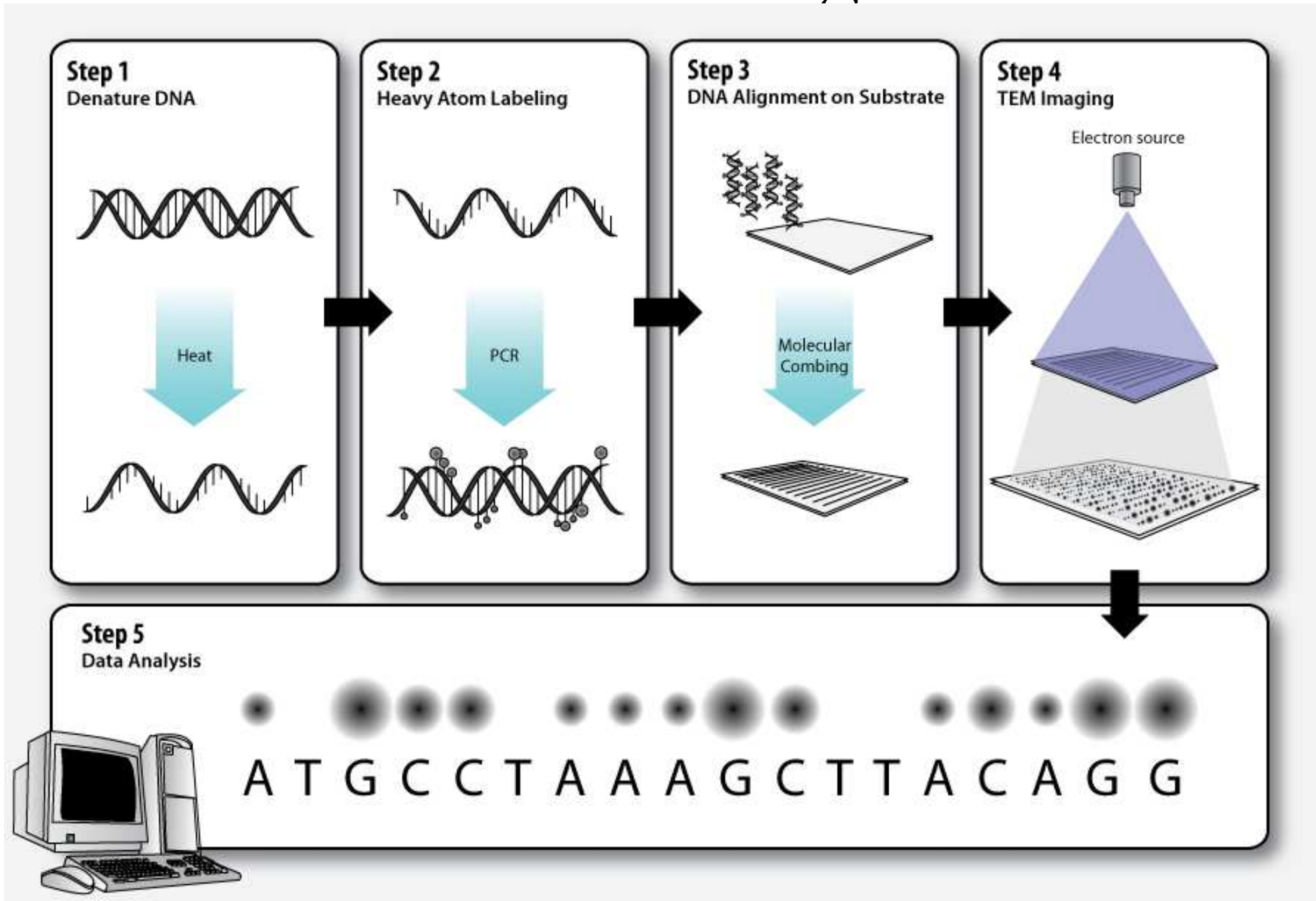


Zařízení je velmi nákladné (cca 17 mil. Kč), analýzy jsou ale dostupné komerčně, takže většina laboratoří v současnosti využívá služeb externích sekvenačních středisek.

454 Pyrosekvenování



Sekvenování třetí generace



Sekvenování

Sequencing Costs in the Genome Era

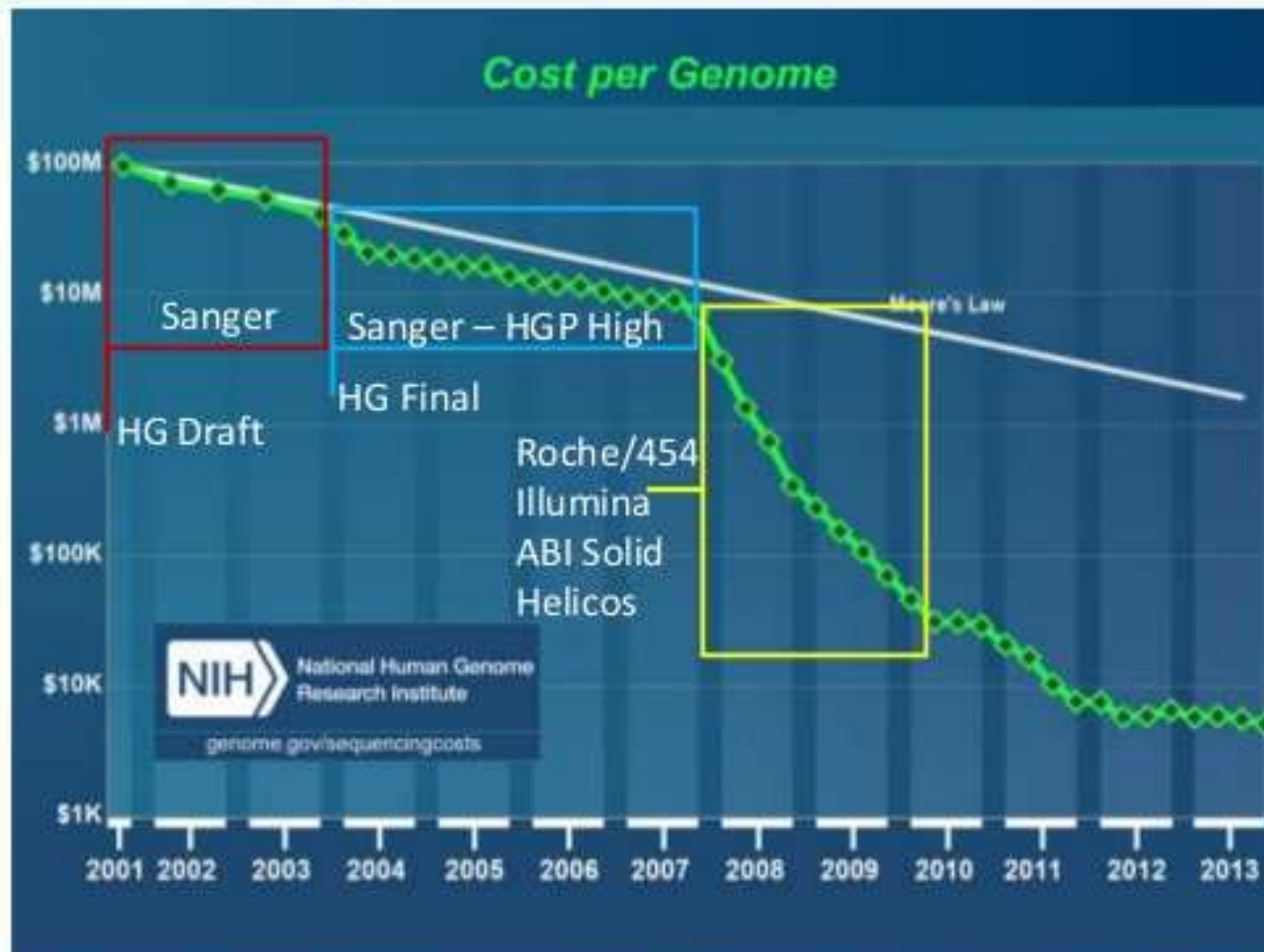


Image credit: NIH

PCR Mullis NC1993

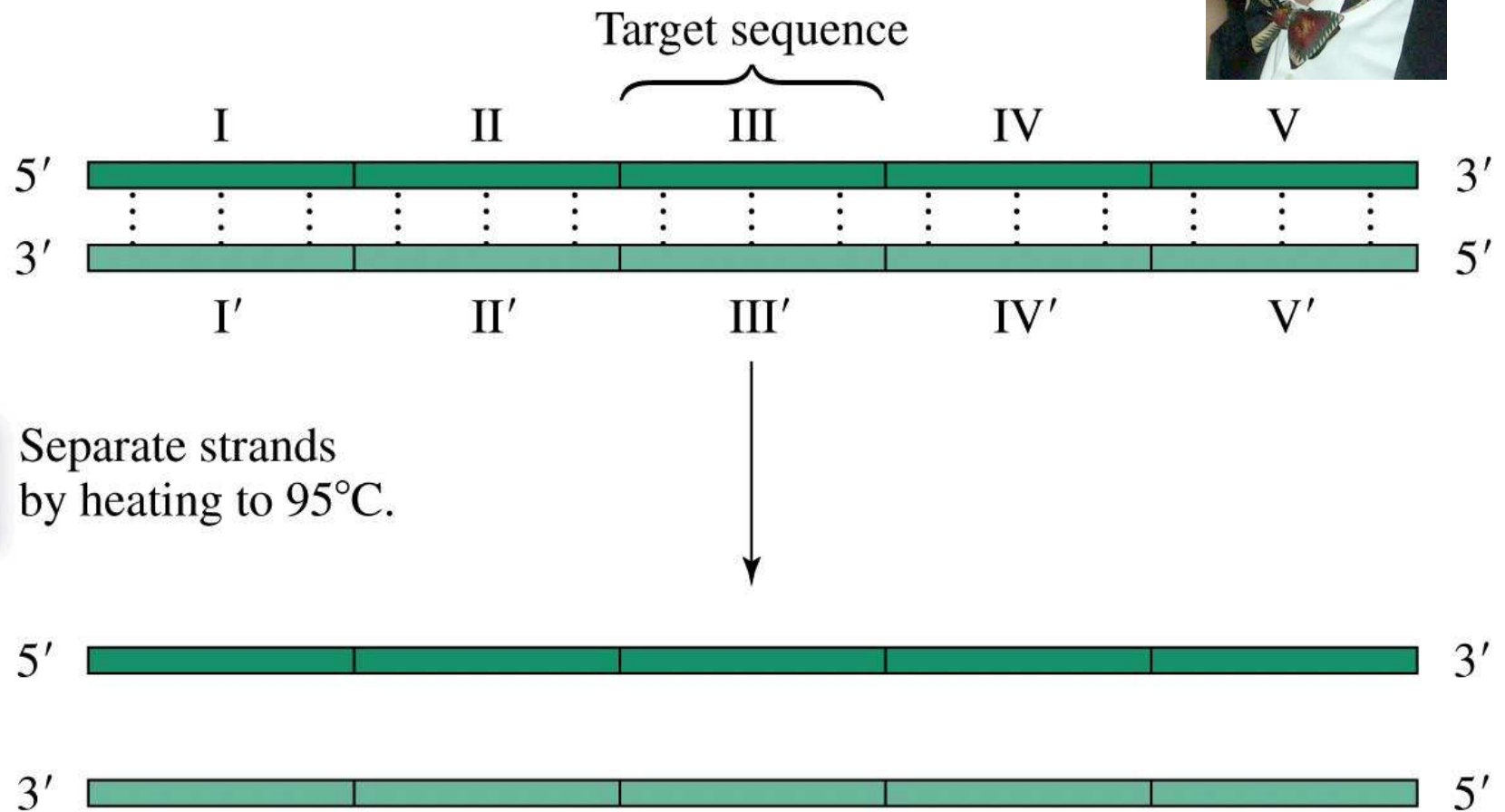
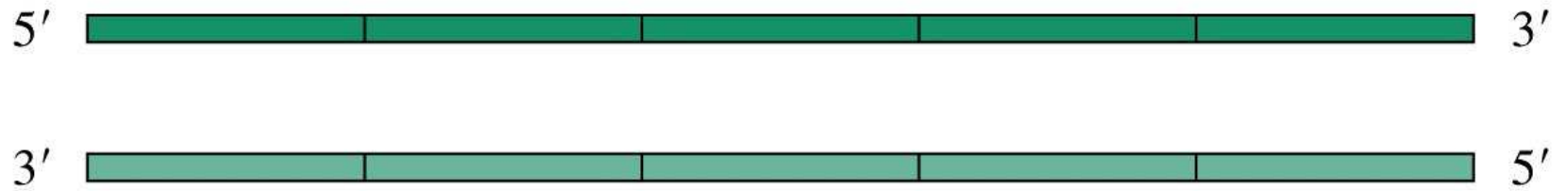


Figure 13-11 part 1 Concepts in Biochemistry, 3/e
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Step 2

Hybridize primers by cooling to 50°C.

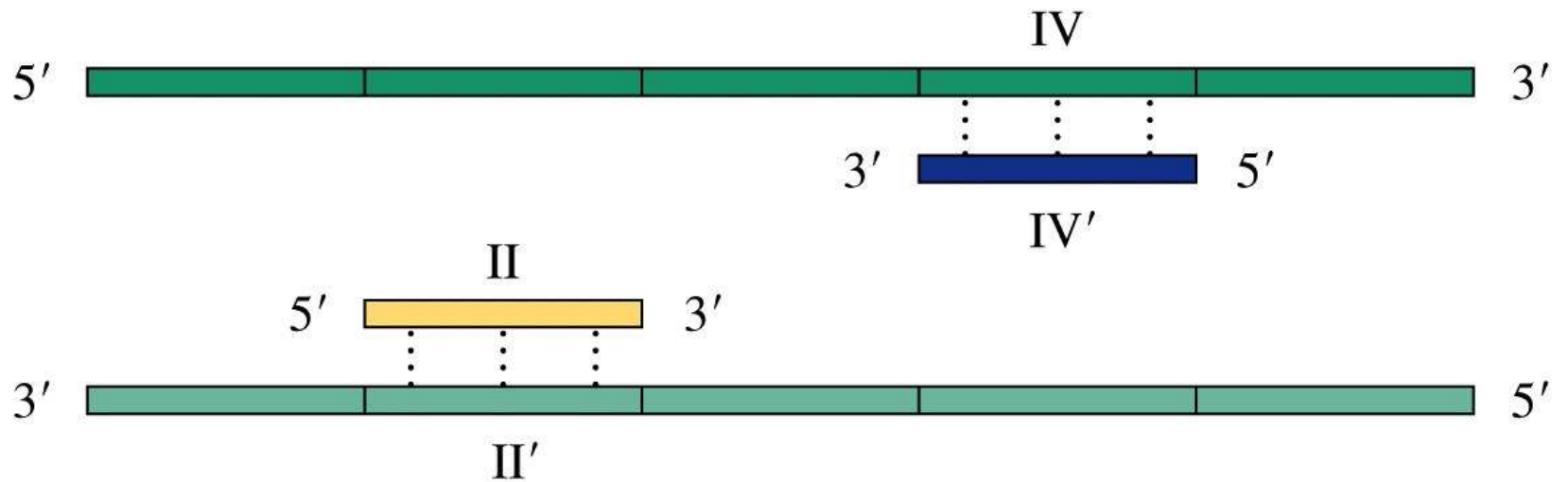
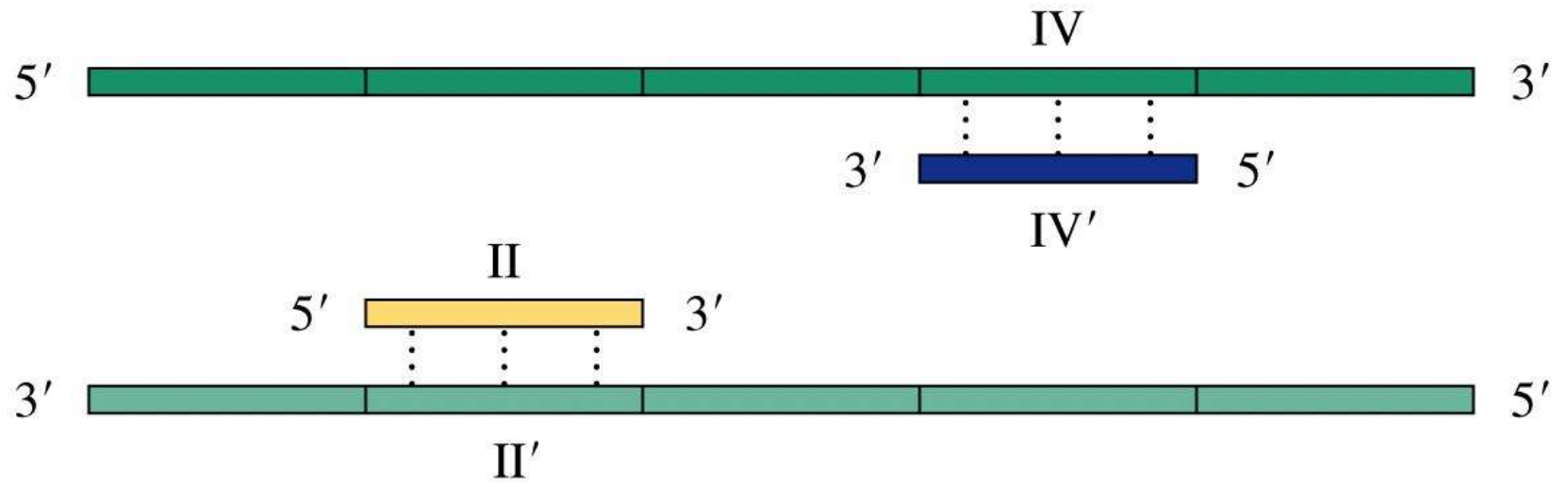


Figure 13-11 part 2 Concepts in Biochemistry, 3/e
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Step 3

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

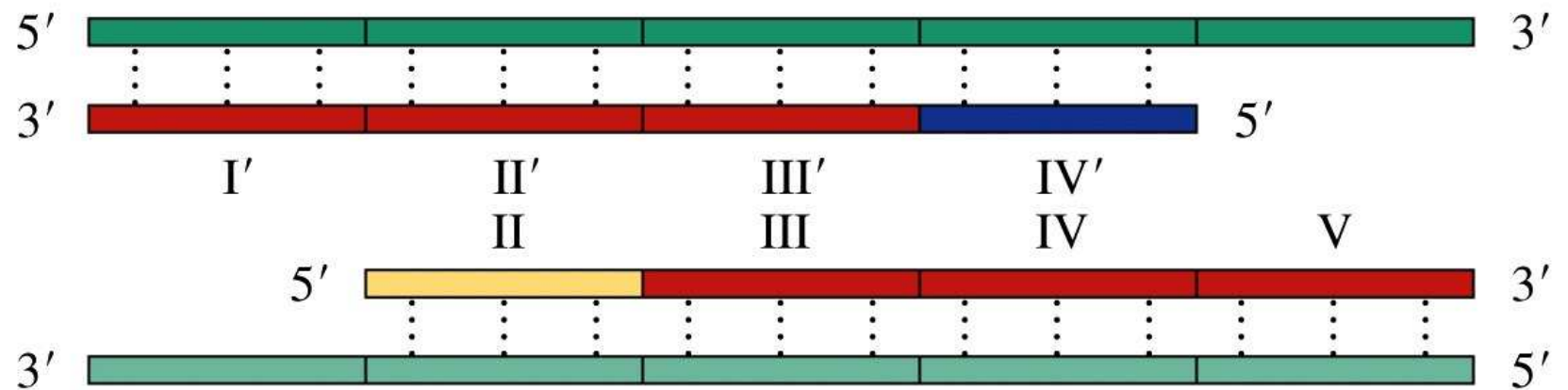
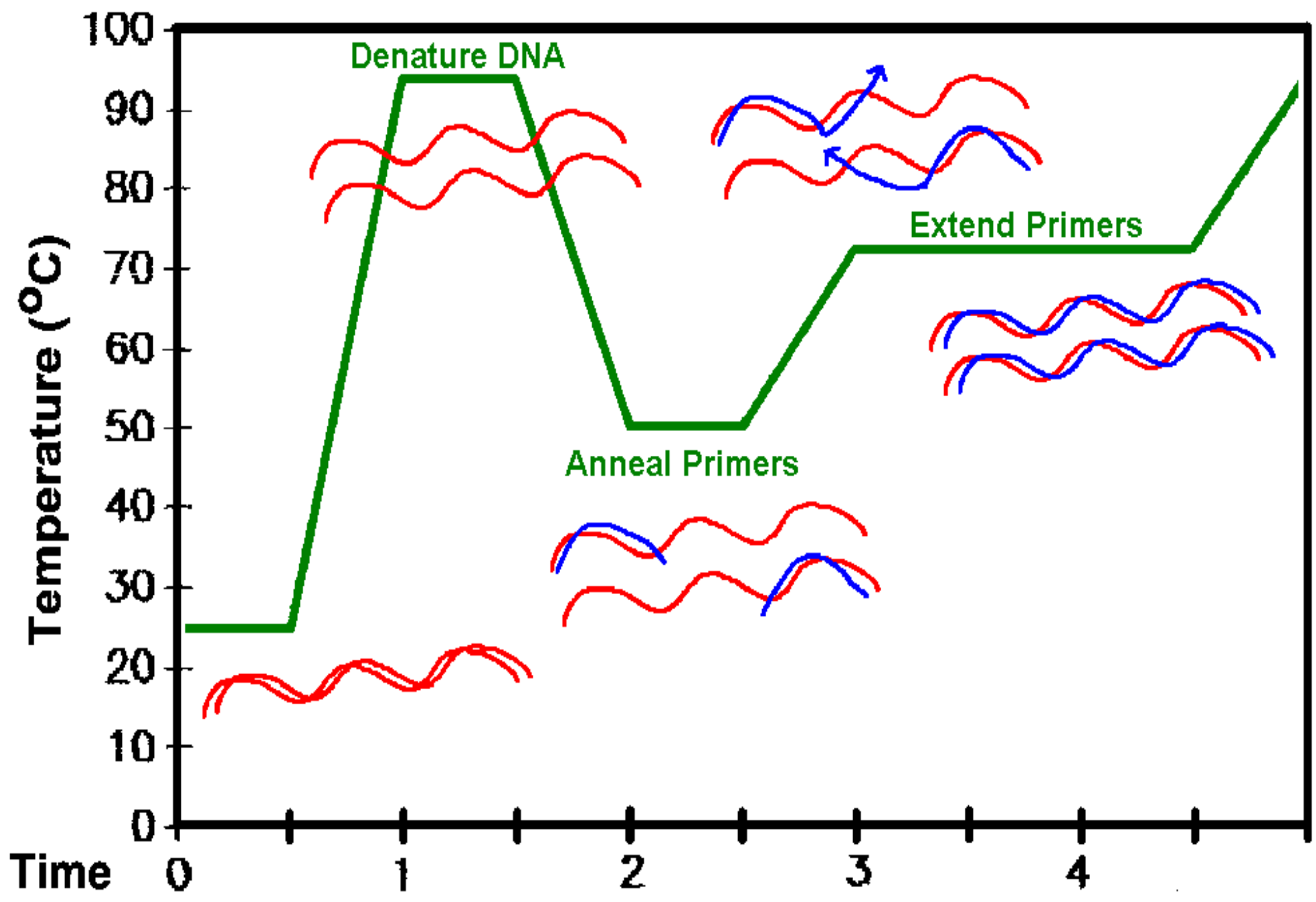
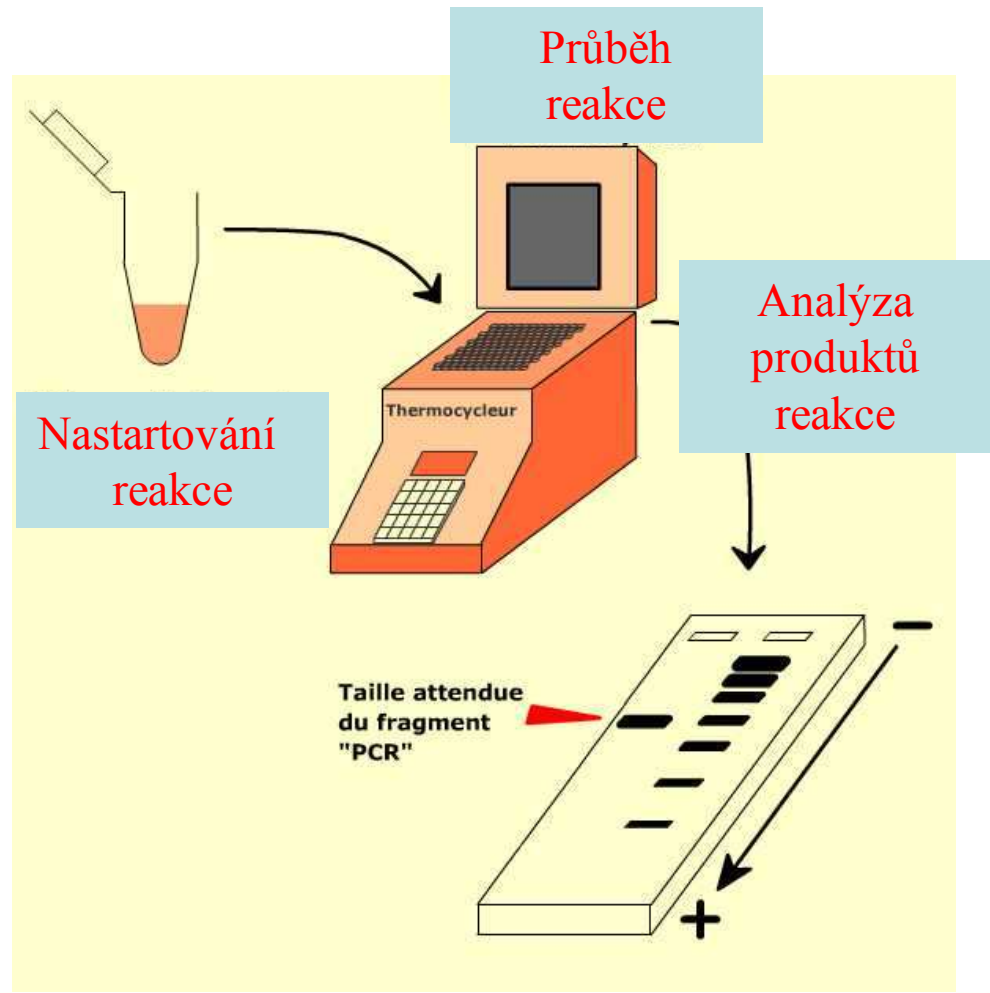


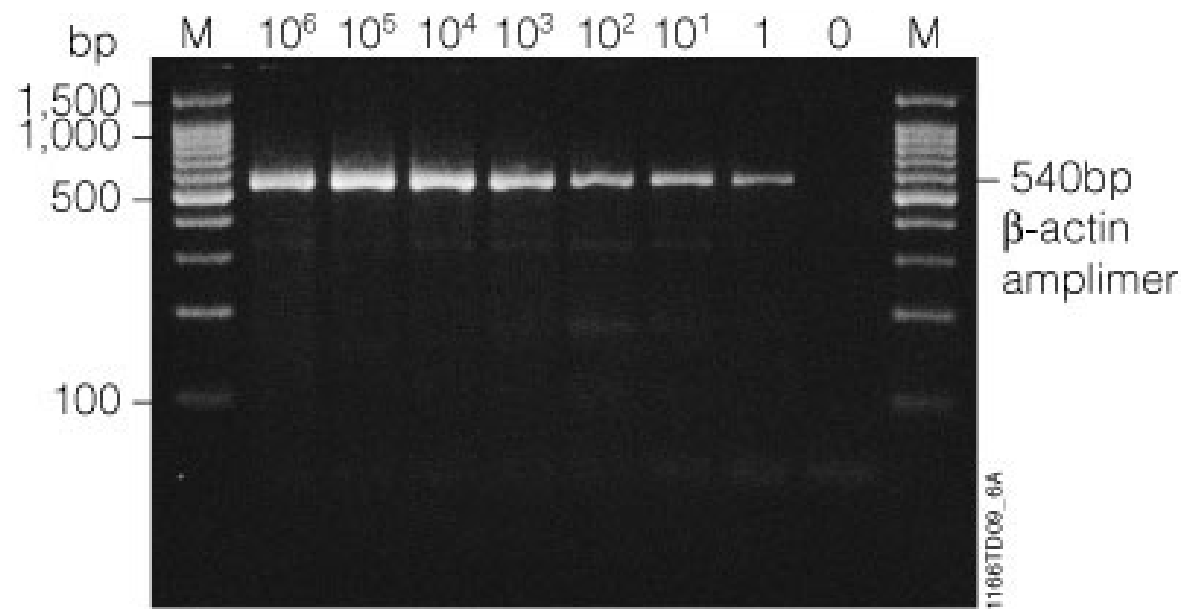
Figure 13-11 part 3 Concepts in Biochemistry, 3/e
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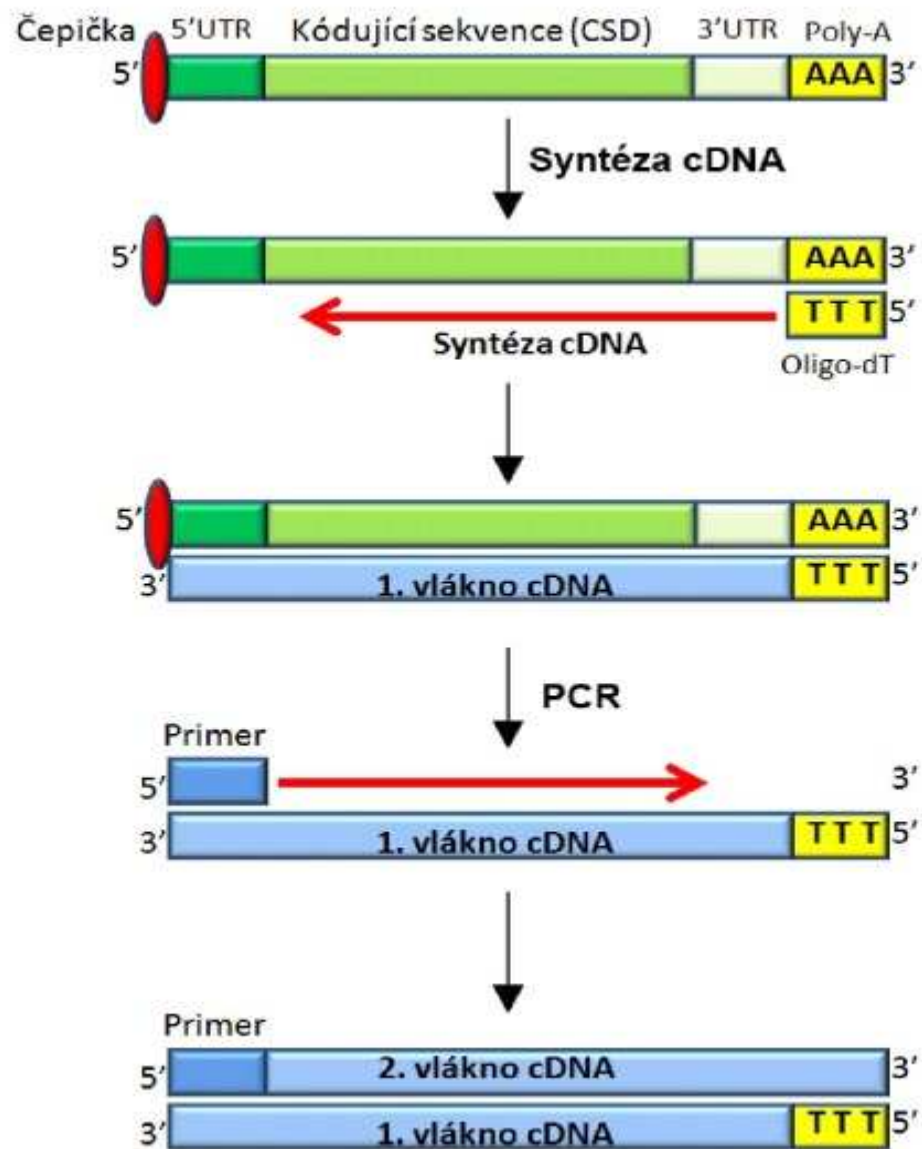
Thermocycler





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

PCR mRNA – reverzní transkripce



Real time PCR

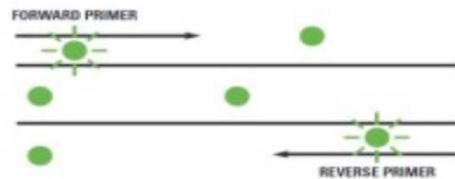
1. **Navázání:** SYBR® Green I se váže během každého cyklu na dvouvláknovou DNA.



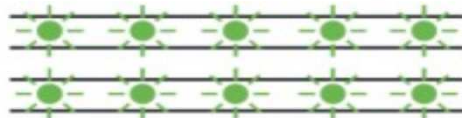
2. **Denaturace:** Ve fázi denaturace DNA je SYBR® Green I uvolněn z vazby na DNA a celková fluorescence dramaticky klesá.



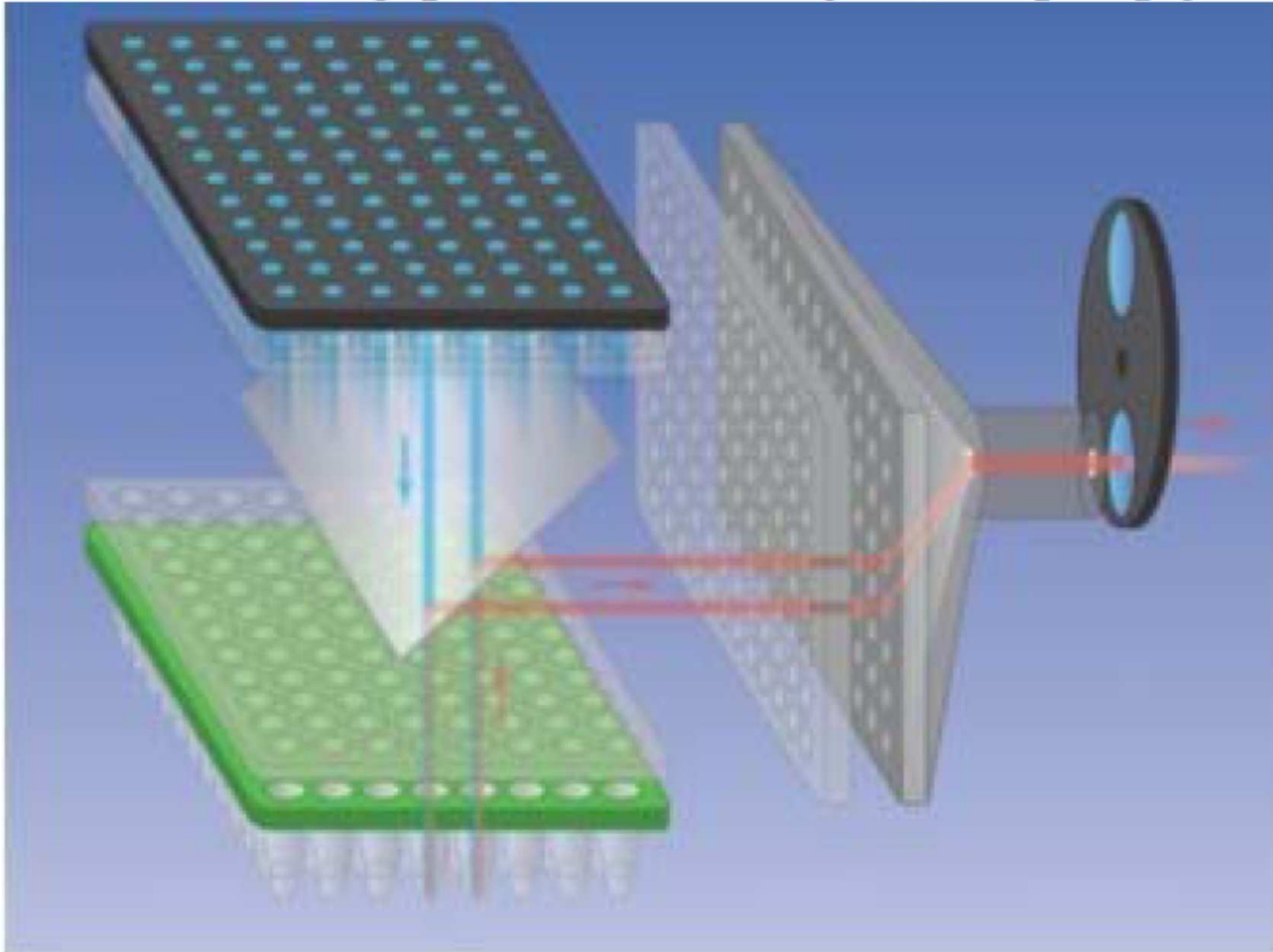
3. **Polymerizace:** Během annealingu primerů a elongace řetězce se Sybr Green opět začíná navazovat na vznikající dvouvláknovou DNA - fluorescence stoupá.



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



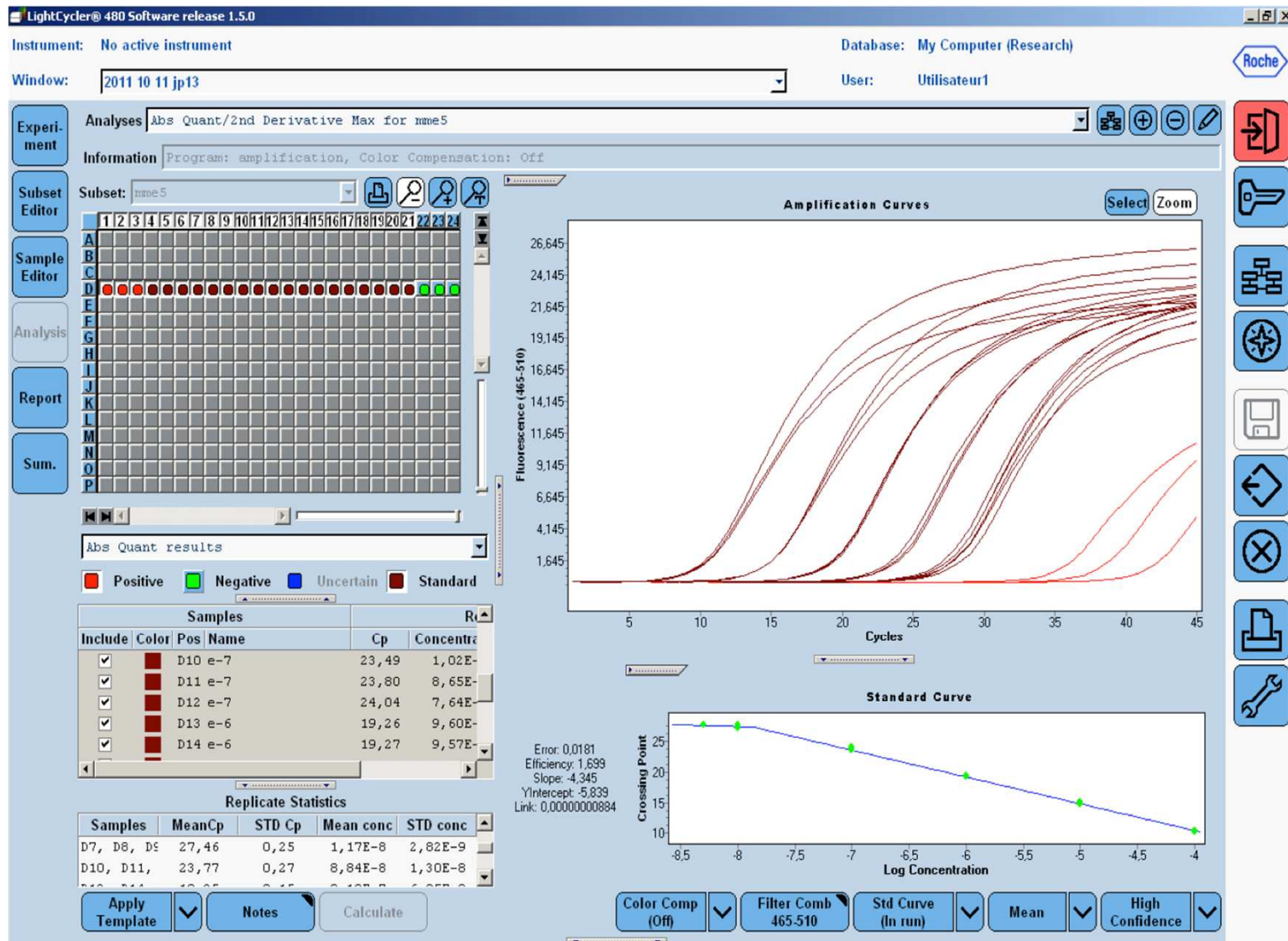
Real time PCR

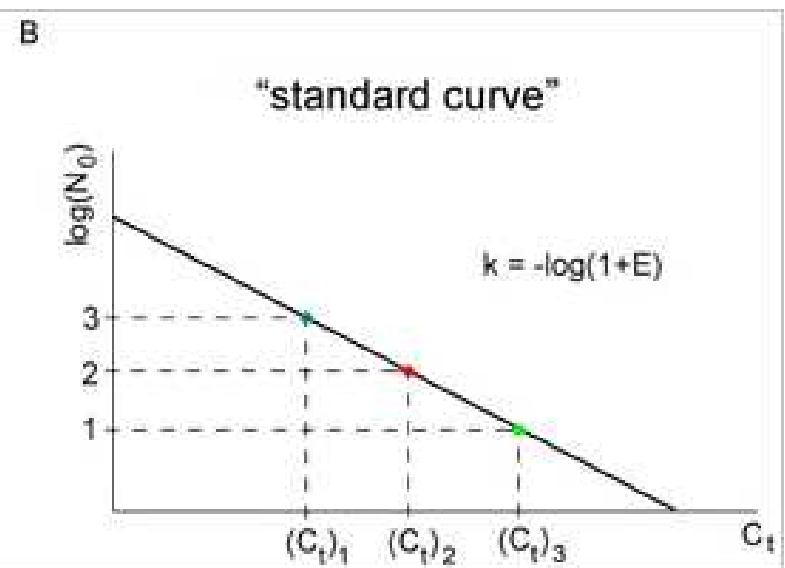
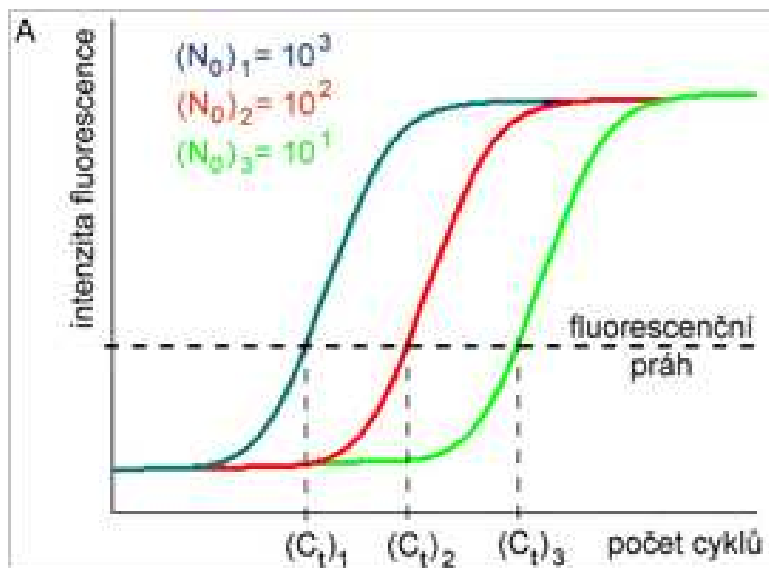


Real time PCR



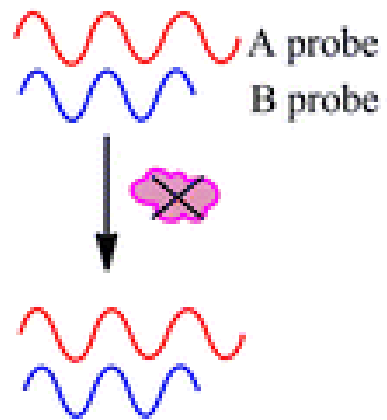
Real time PCR



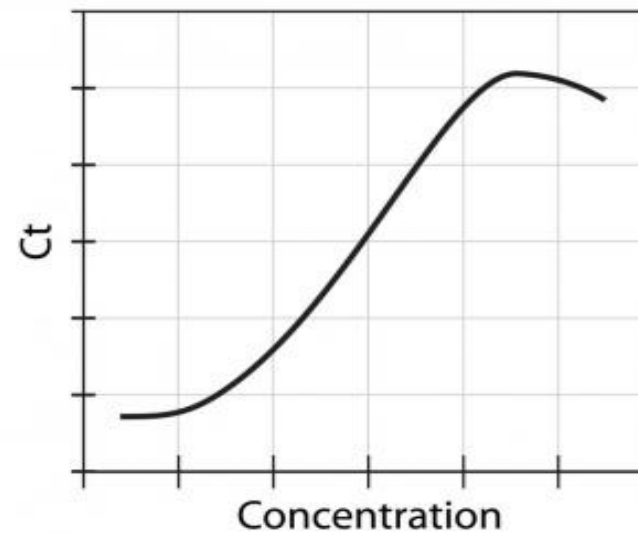
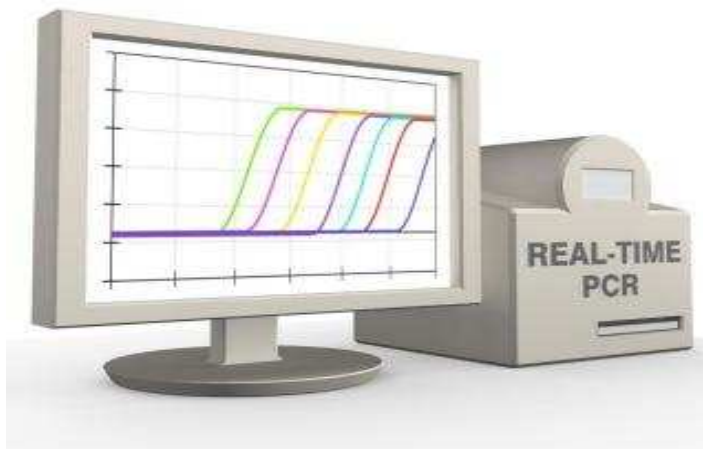
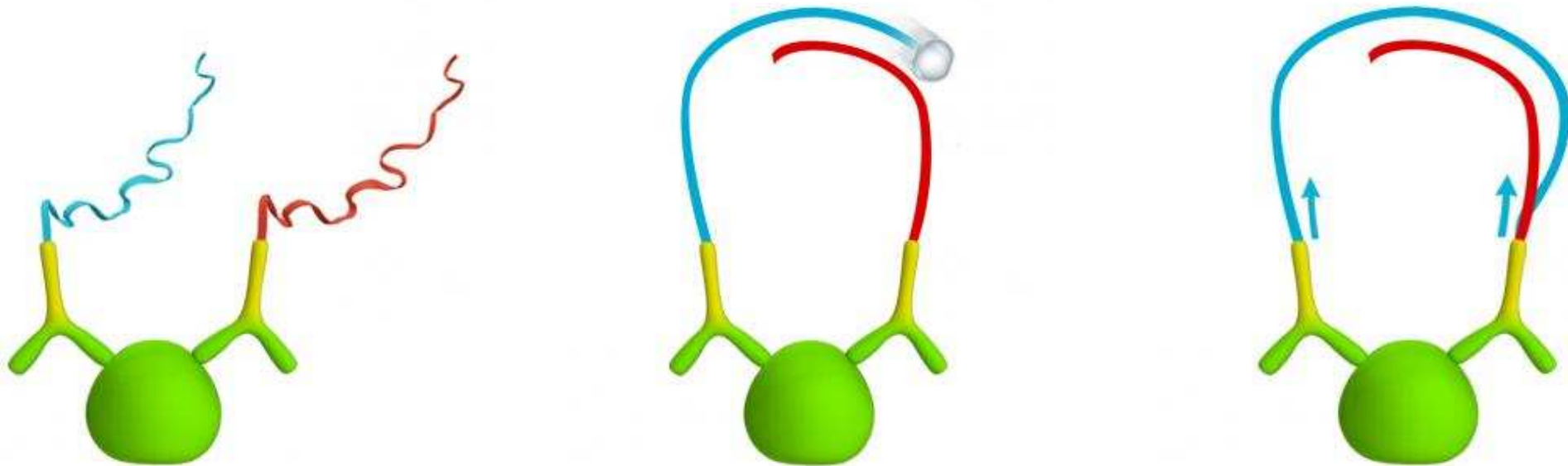


Real time PCR proteinů

Protein → DNA

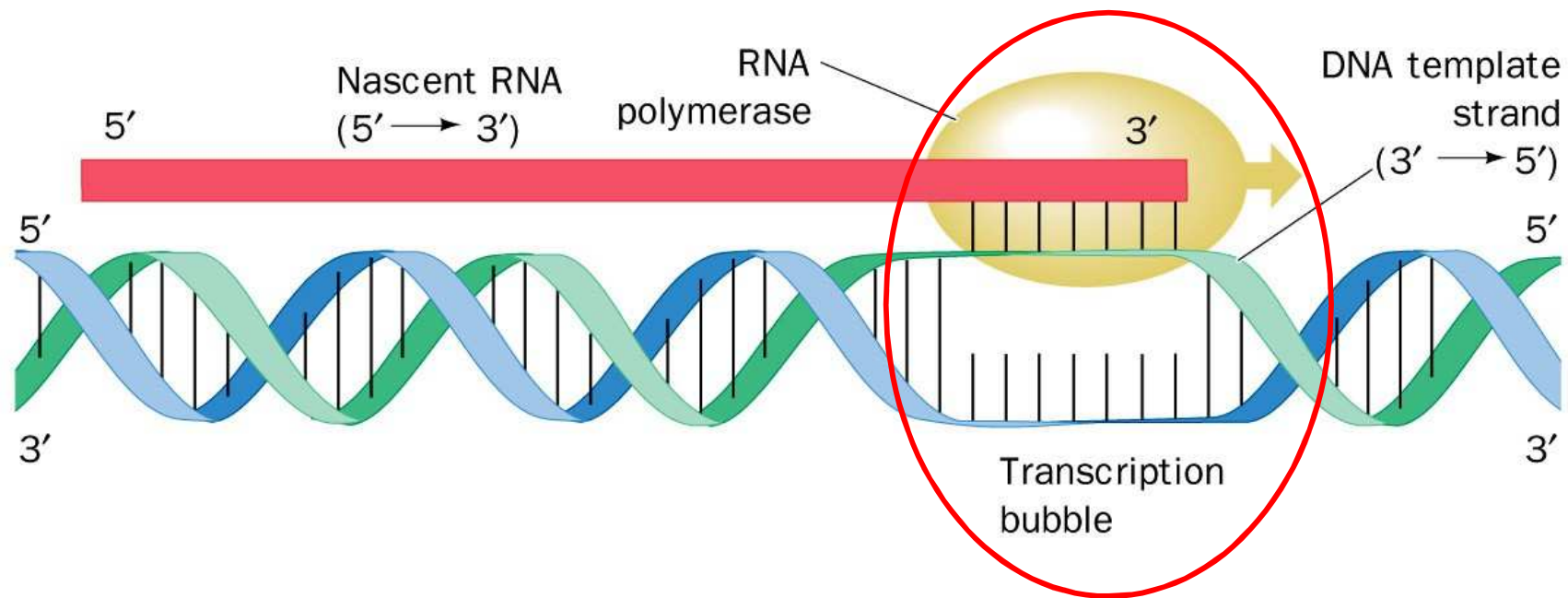


Real time PCR proteinů



Transkripce – přepis - DNA → RNA

DNA + NTP + 1 enzym



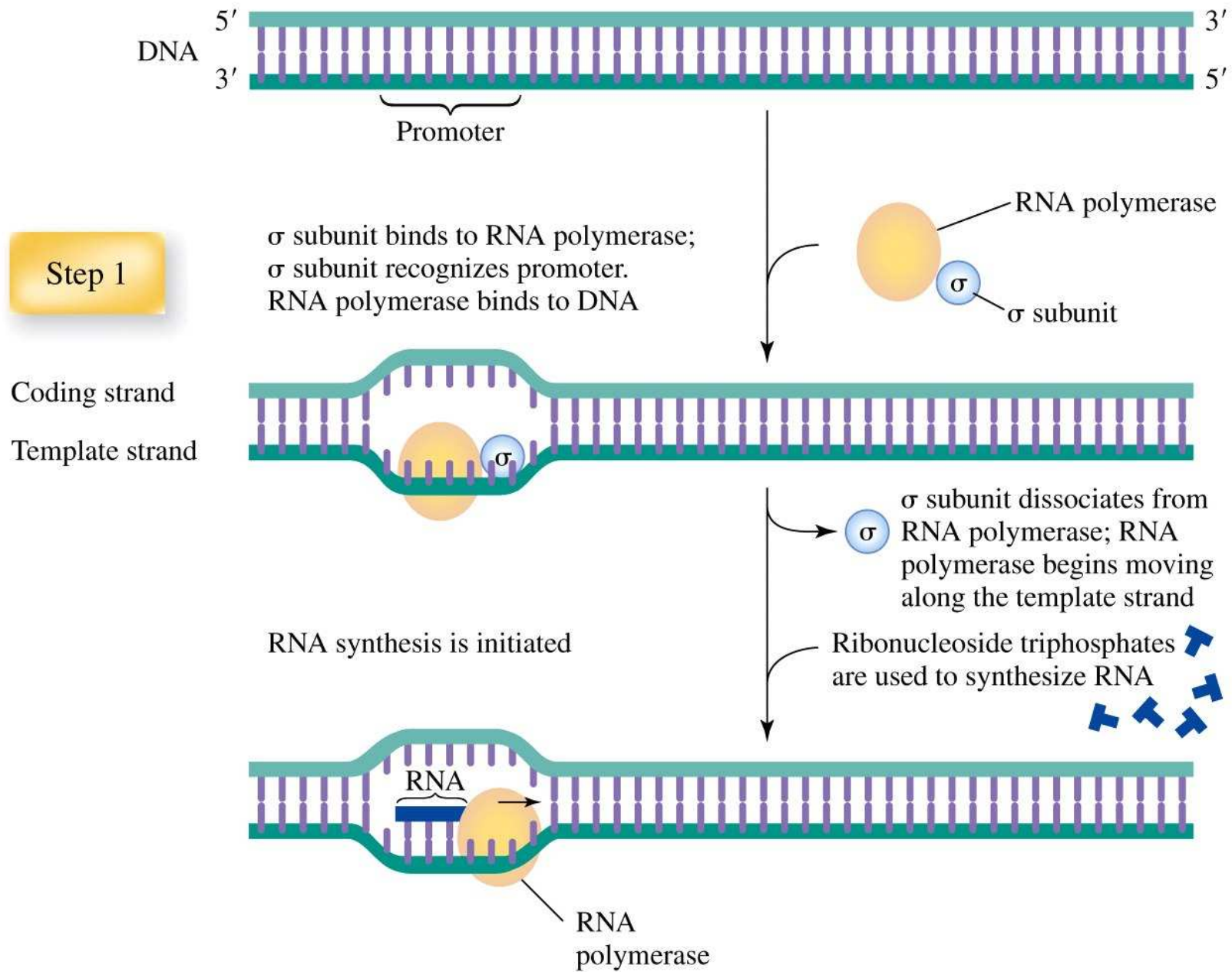


Figure 11-22 part 1 Concepts in Biochemistry, 3/e
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Step 2

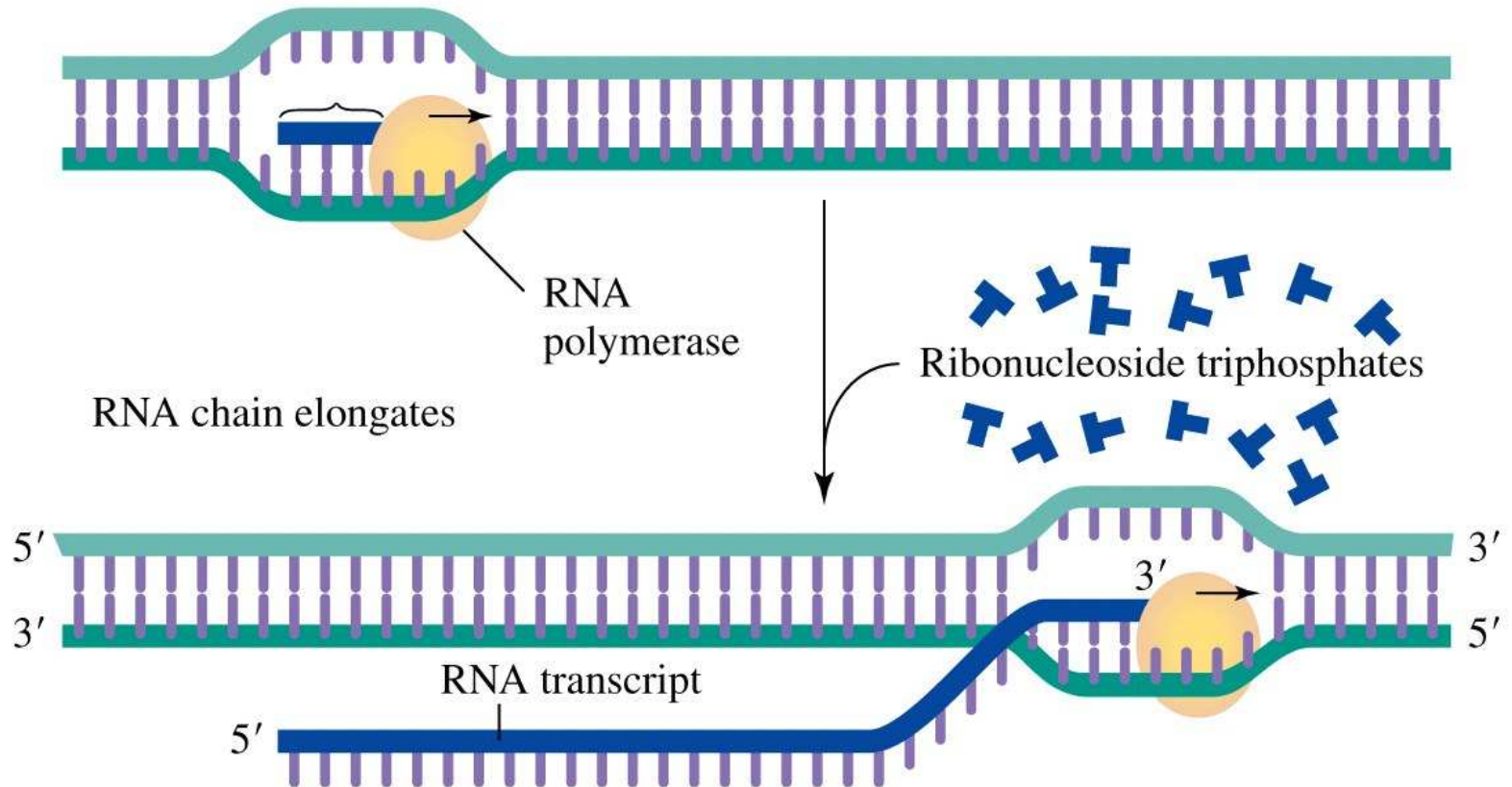


Figure 11-22 part 2 Concepts in Biochemistry, 3/e
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Step 3

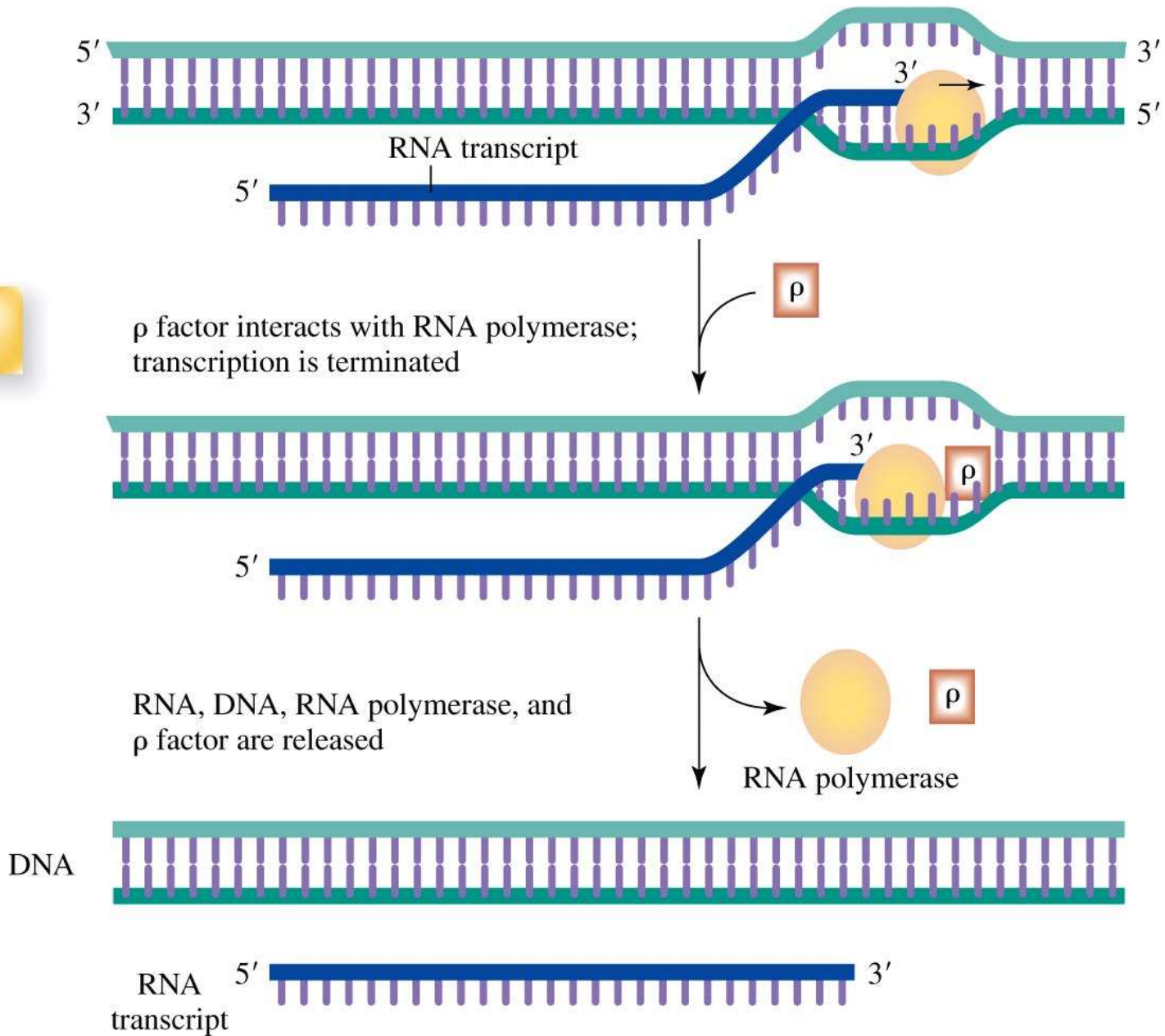
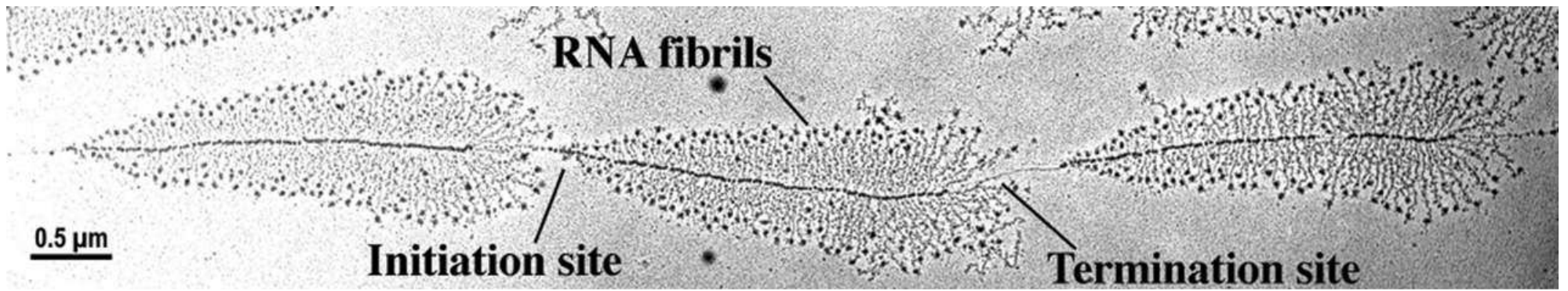
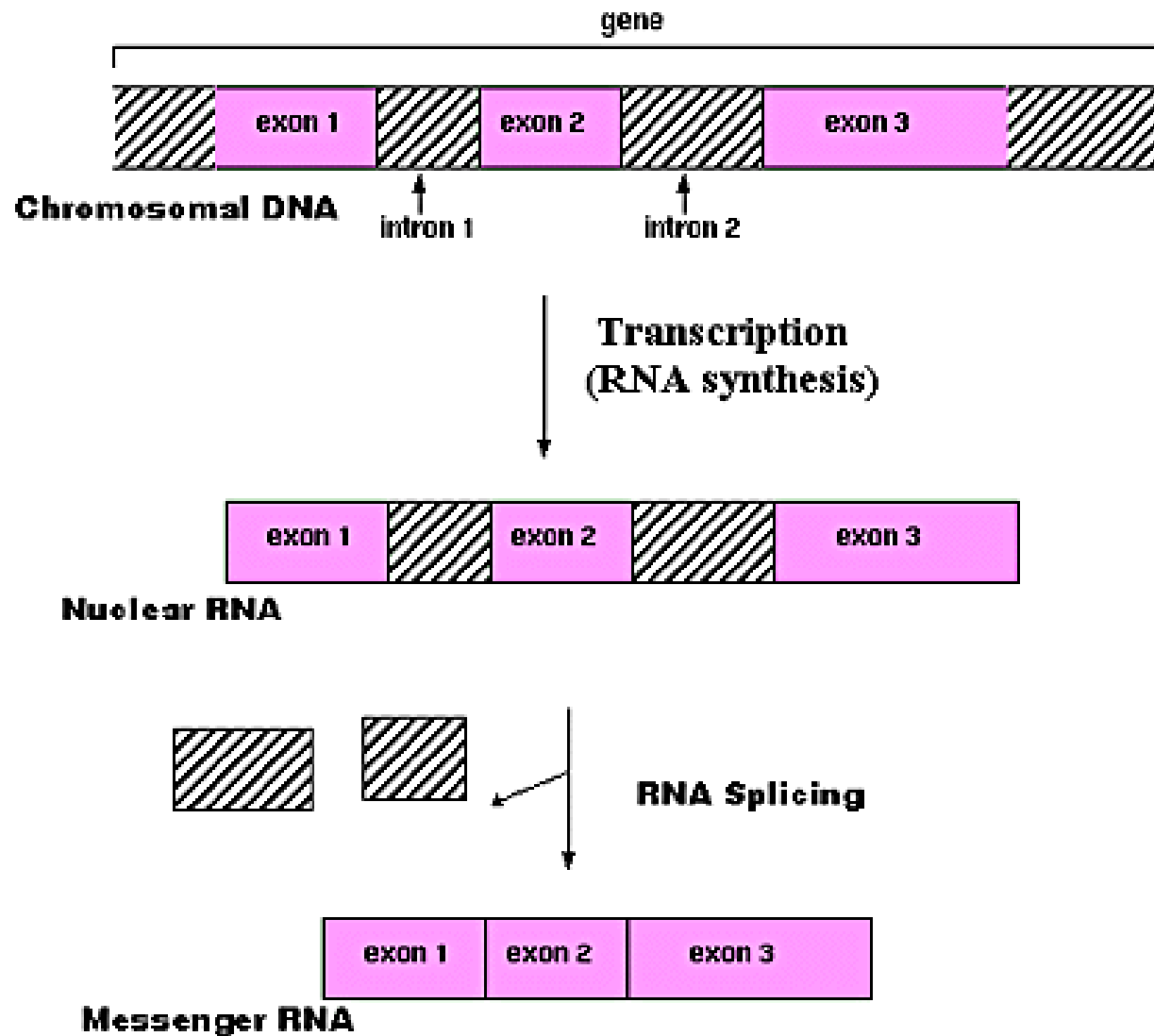


Figure 11-22 part 3 Concepts in Biochemistry, 3/e
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Courtesy of Ulrich Scheer, University of Würzburg, Germany

Syntéza eukaryontní RNA



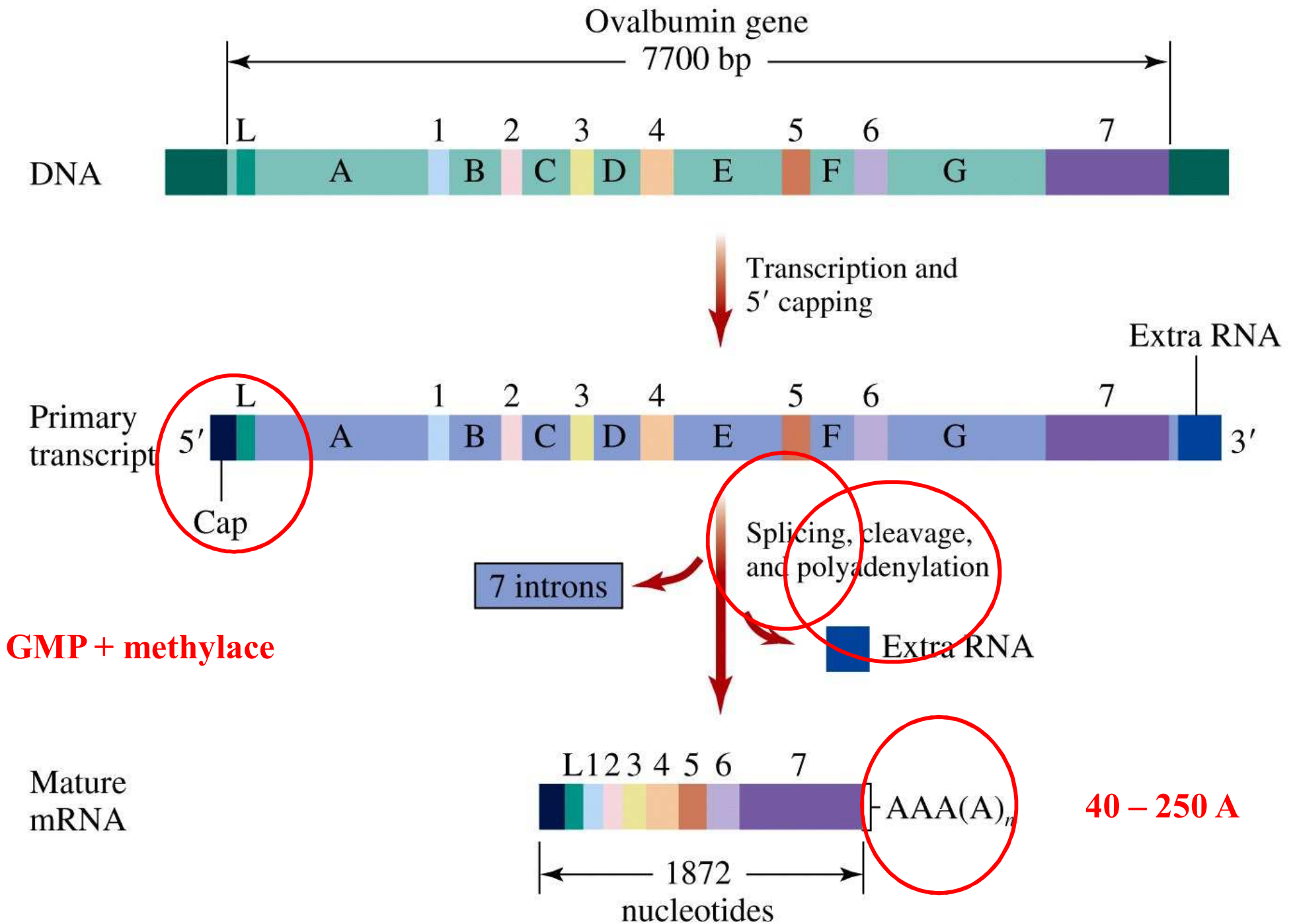
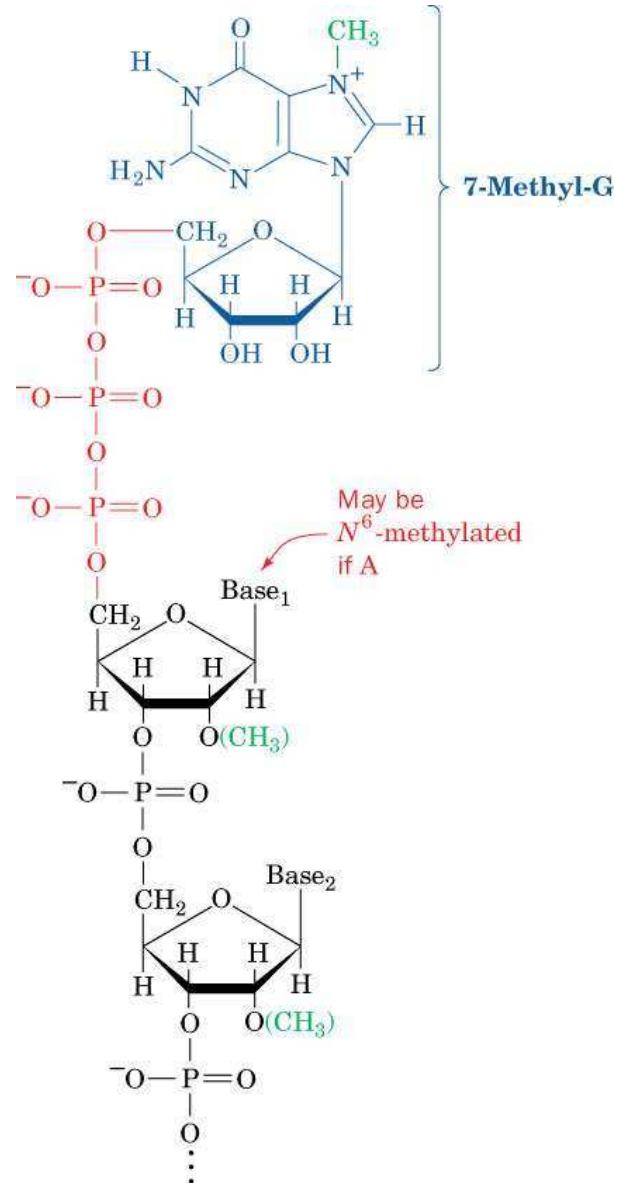


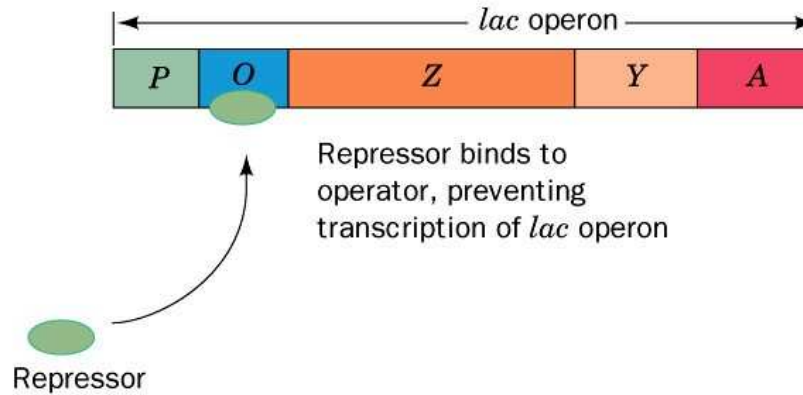
Figure 11-28 Concepts in Biochemistry, 3/e
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Cap

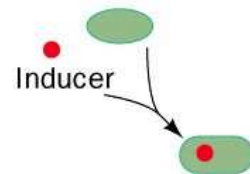
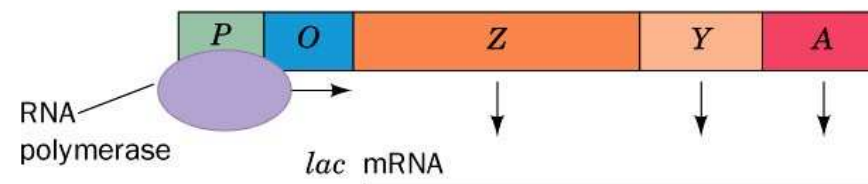


Regulace translace

(a) Absence of inducer



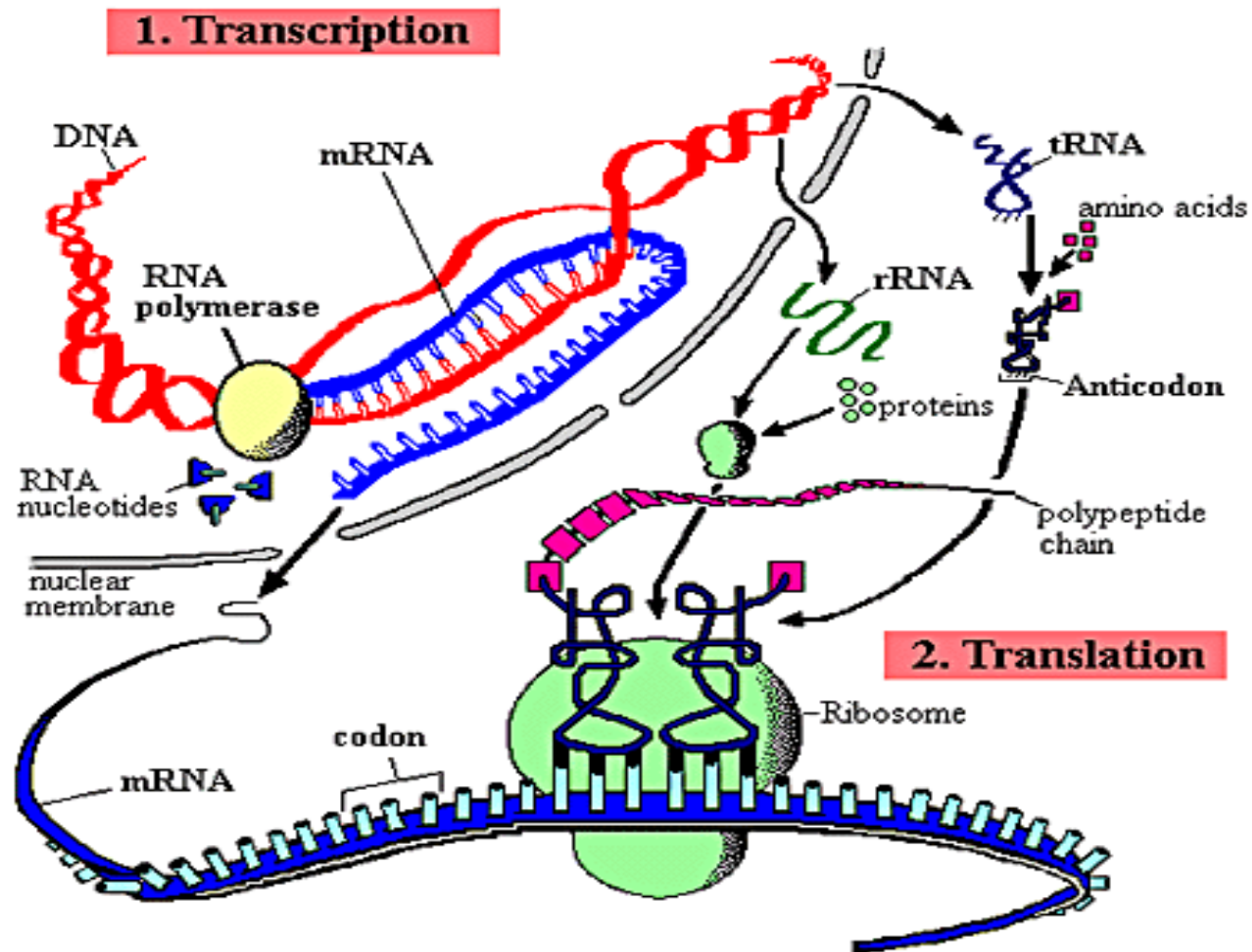
(b) Presence of inducer



Transcription of *lac* structural genes

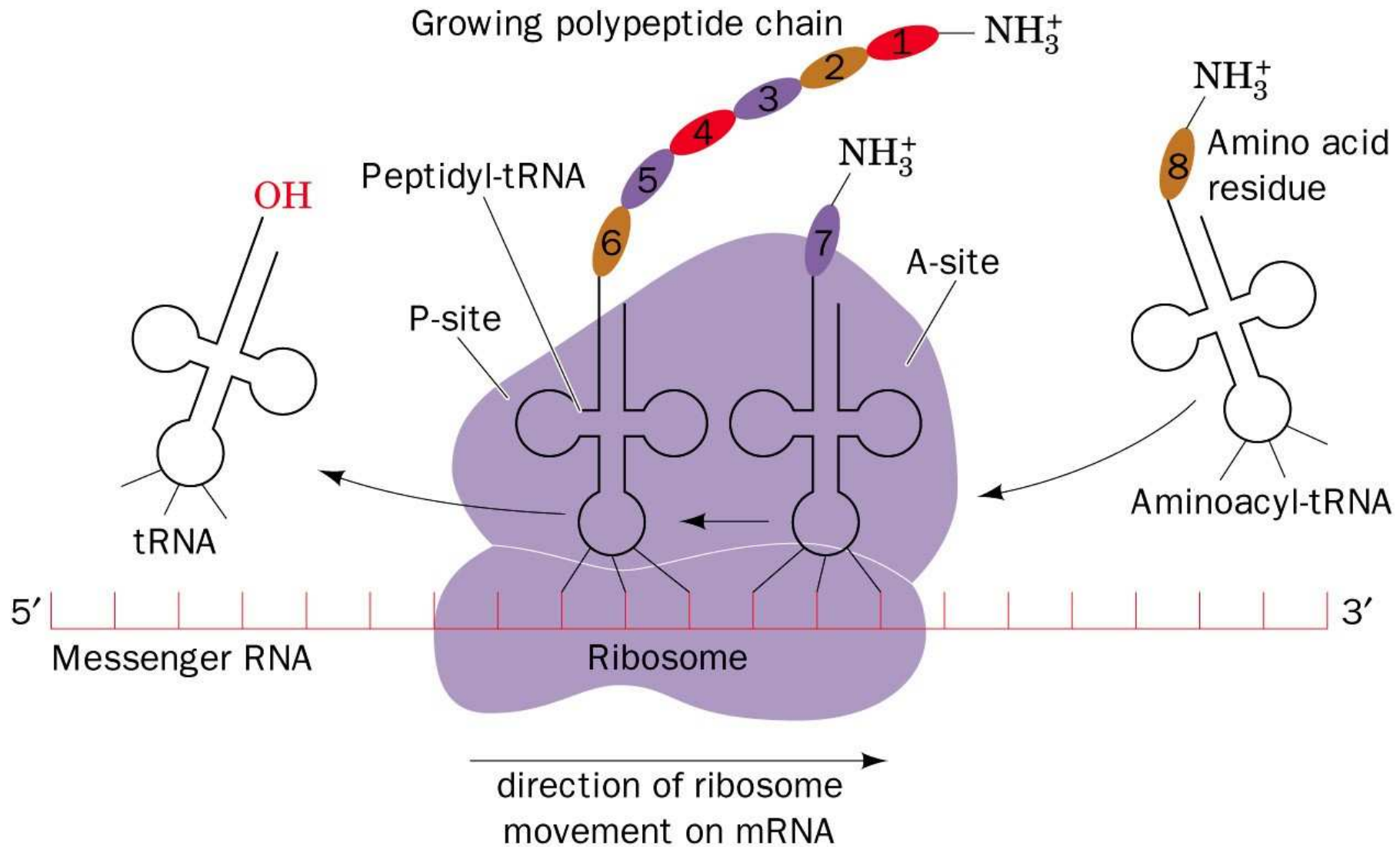
Inducer-repressor complex does not bind to operator

Syntéza bílkovin



Translaci – překladi - RNA → protein

mRNA + ribozomy + (tRNA-AMK)_n + IF_n + EF + RF_n



Ribozomy (rRNA + proteiny)

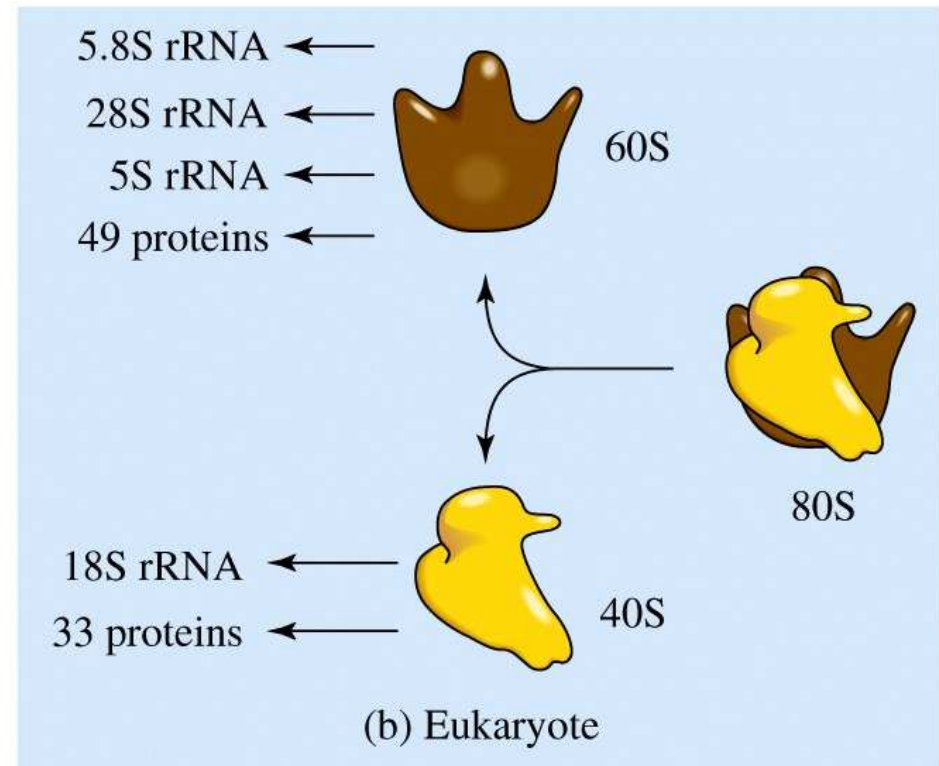
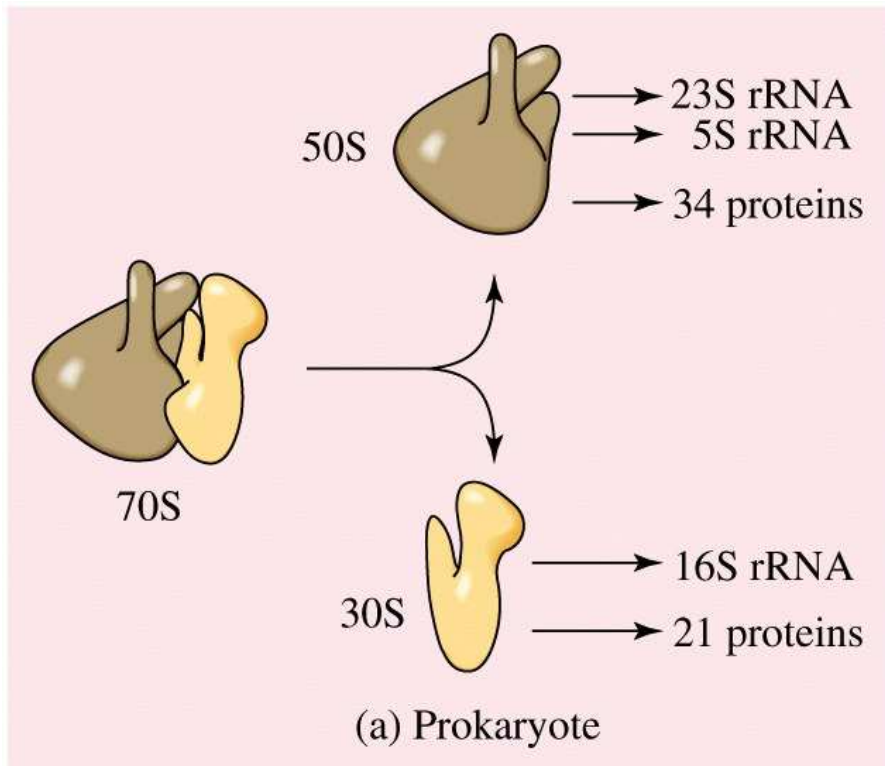
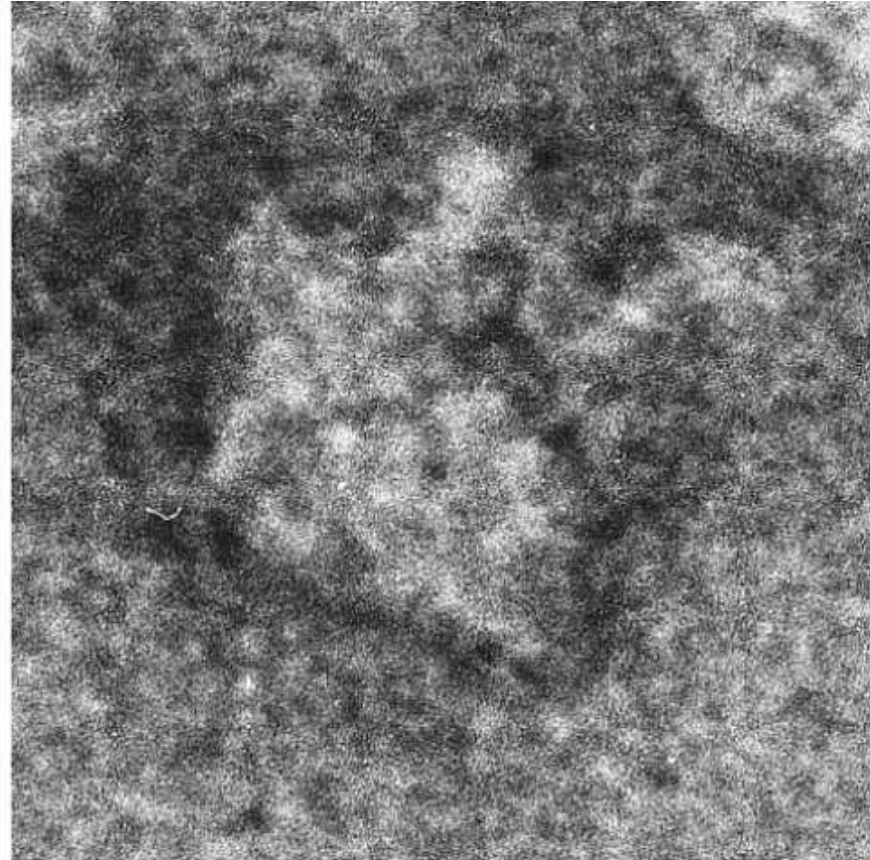
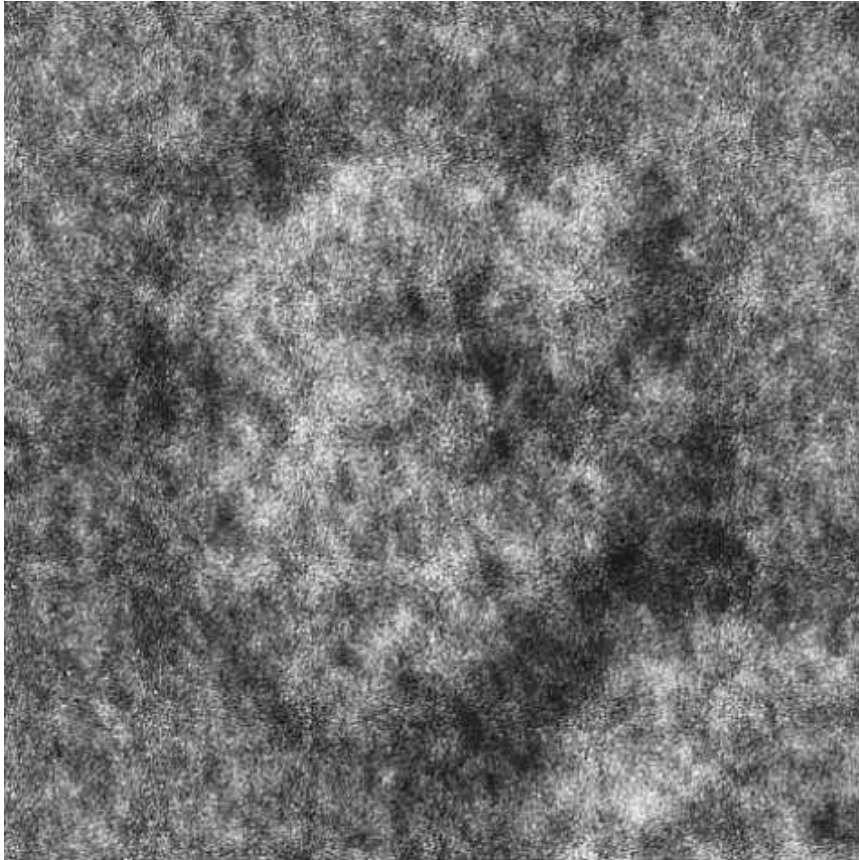


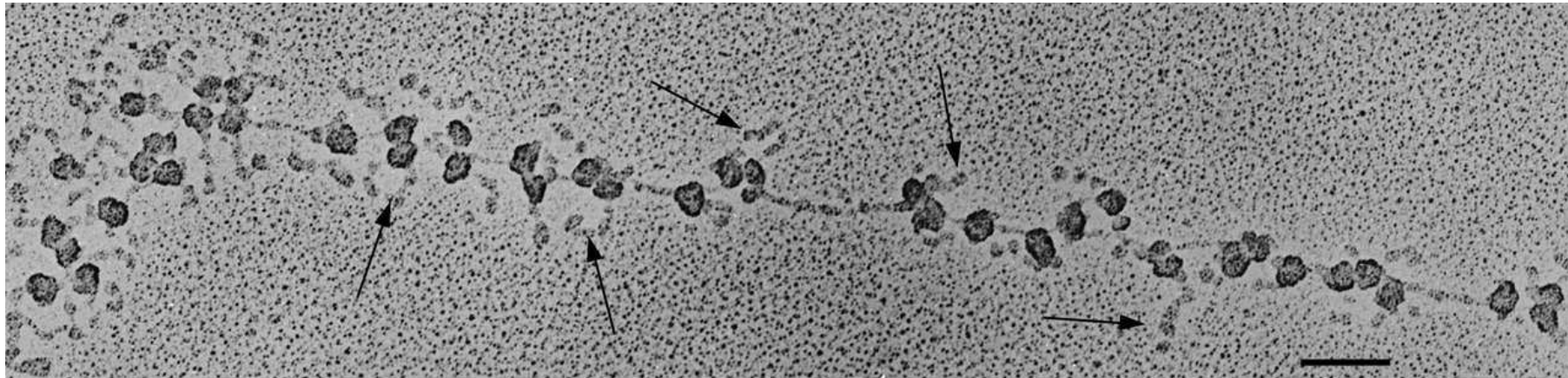
Figure 12-1 Concepts in Biochemistry, 3/e
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Ribozomy E.coli



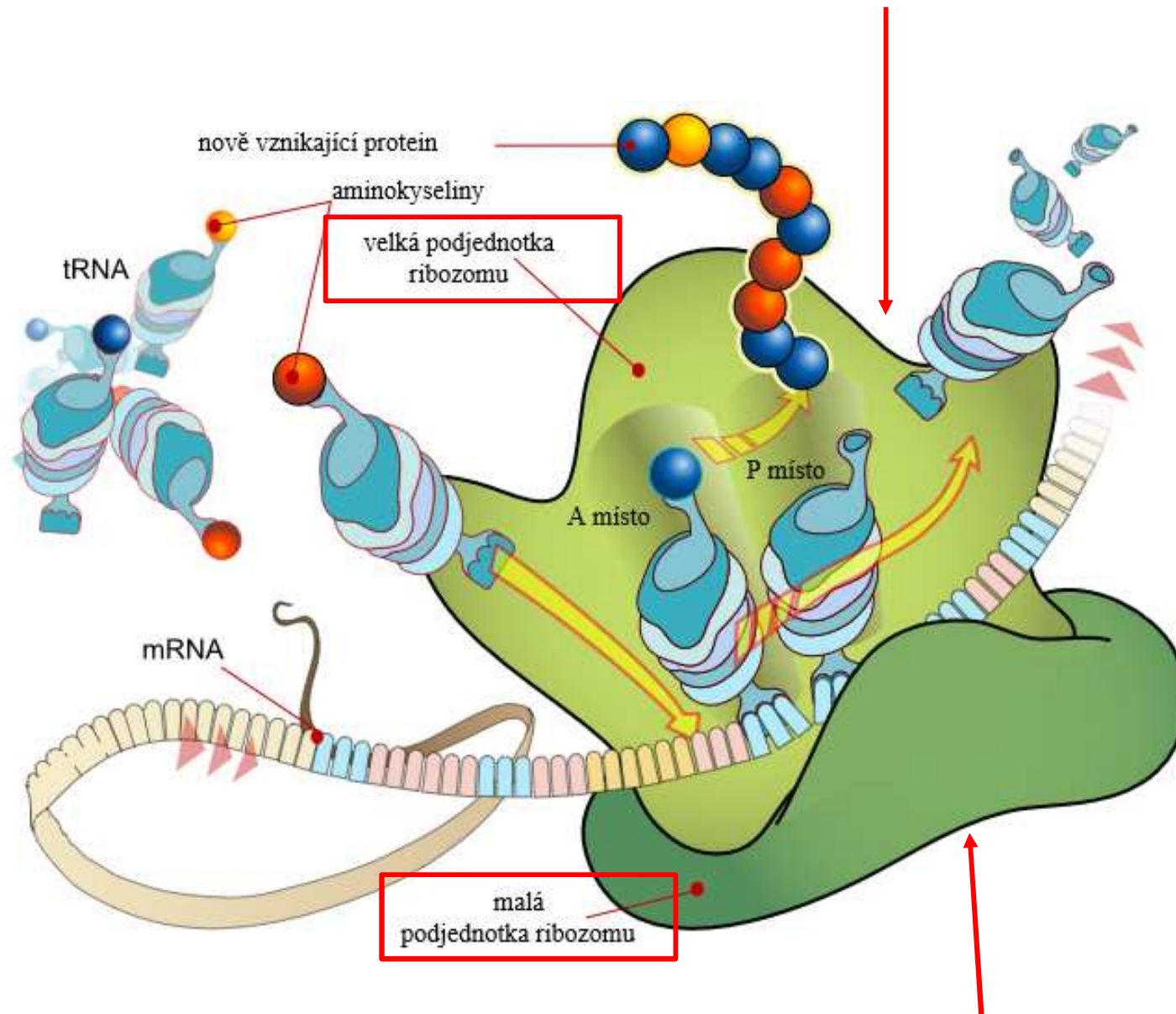
Courtesy of James Lake, UCLA

Polyribozomy *Bombyx mori*

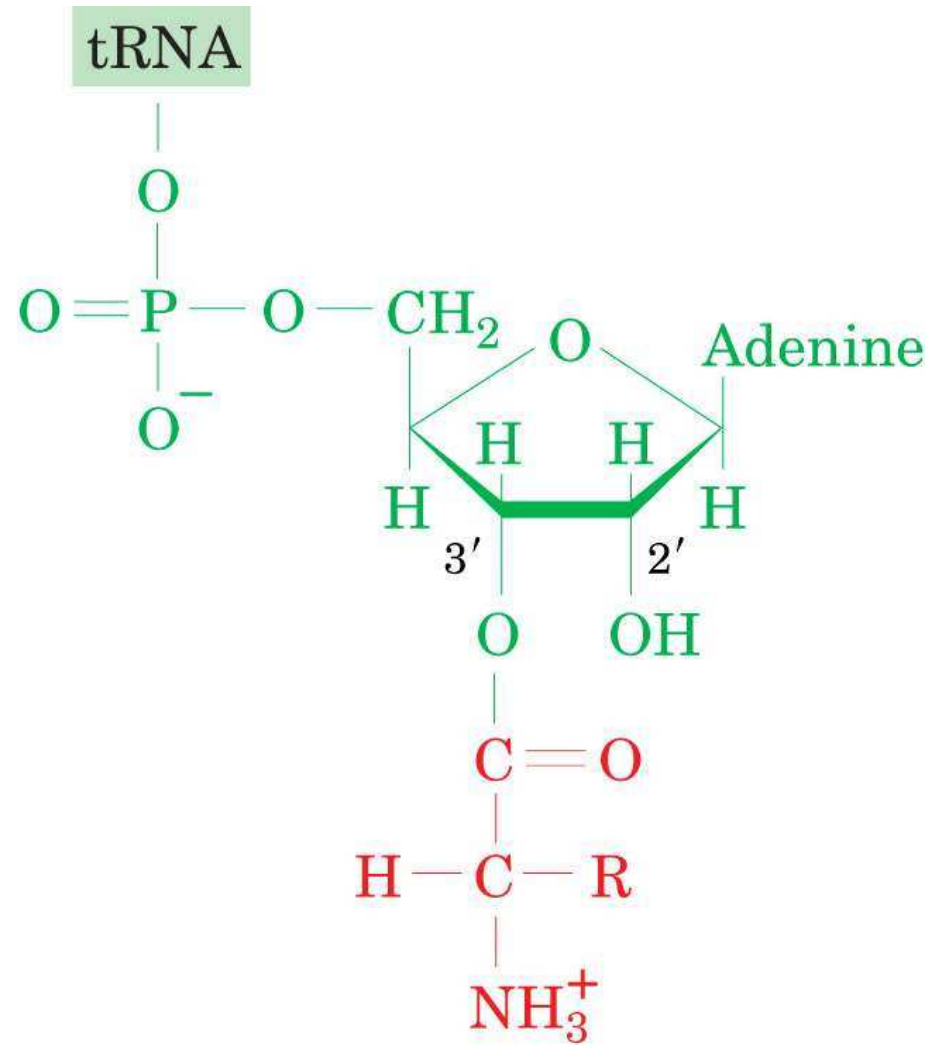


Courtesy of Oscar L. Miller, Jr. and Steven L. McKnight, University of Virginia

Katalytická funkce – peptidyltransferáza



Rozpoznávací funkce



Aminoacyl-tRNA

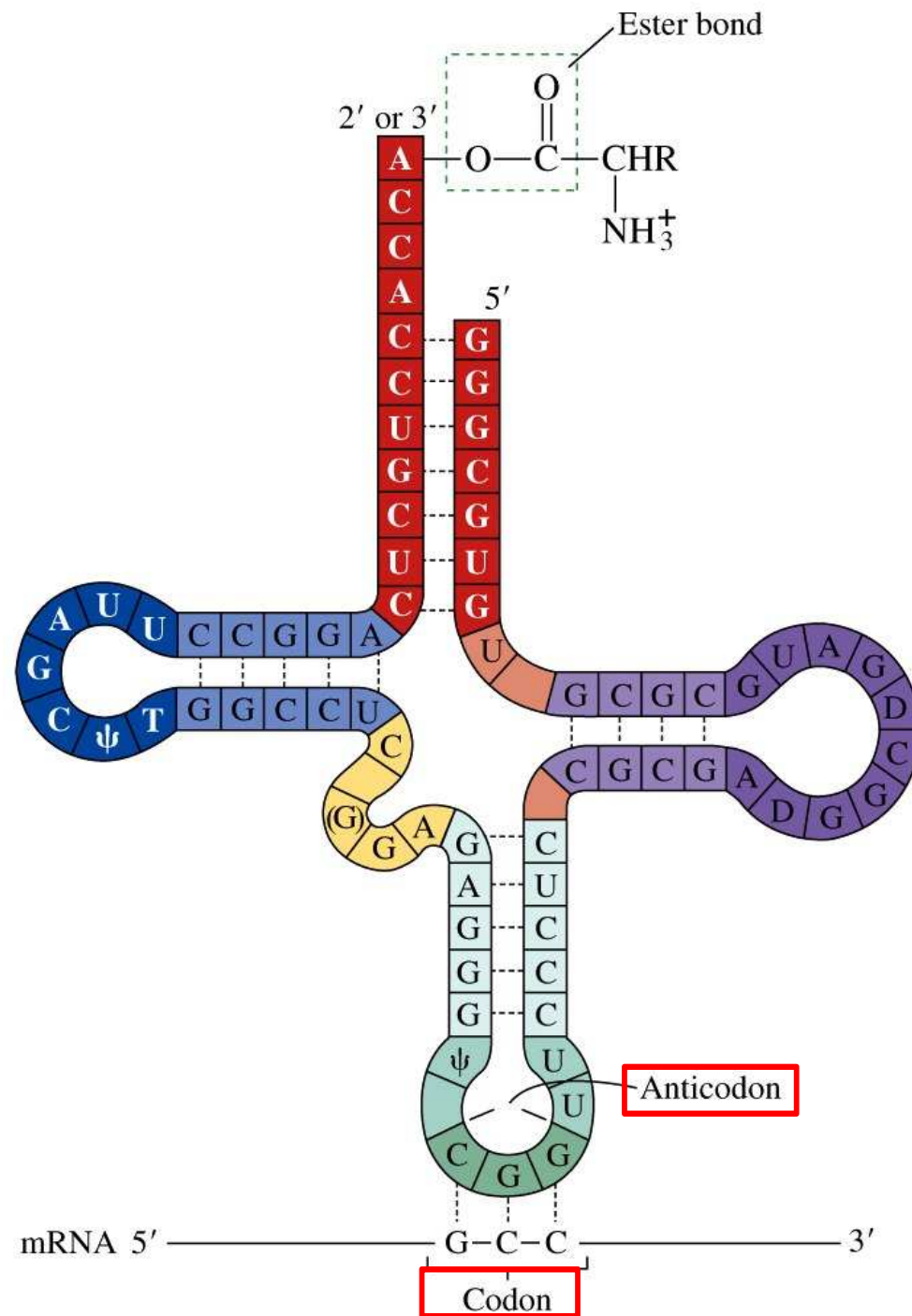
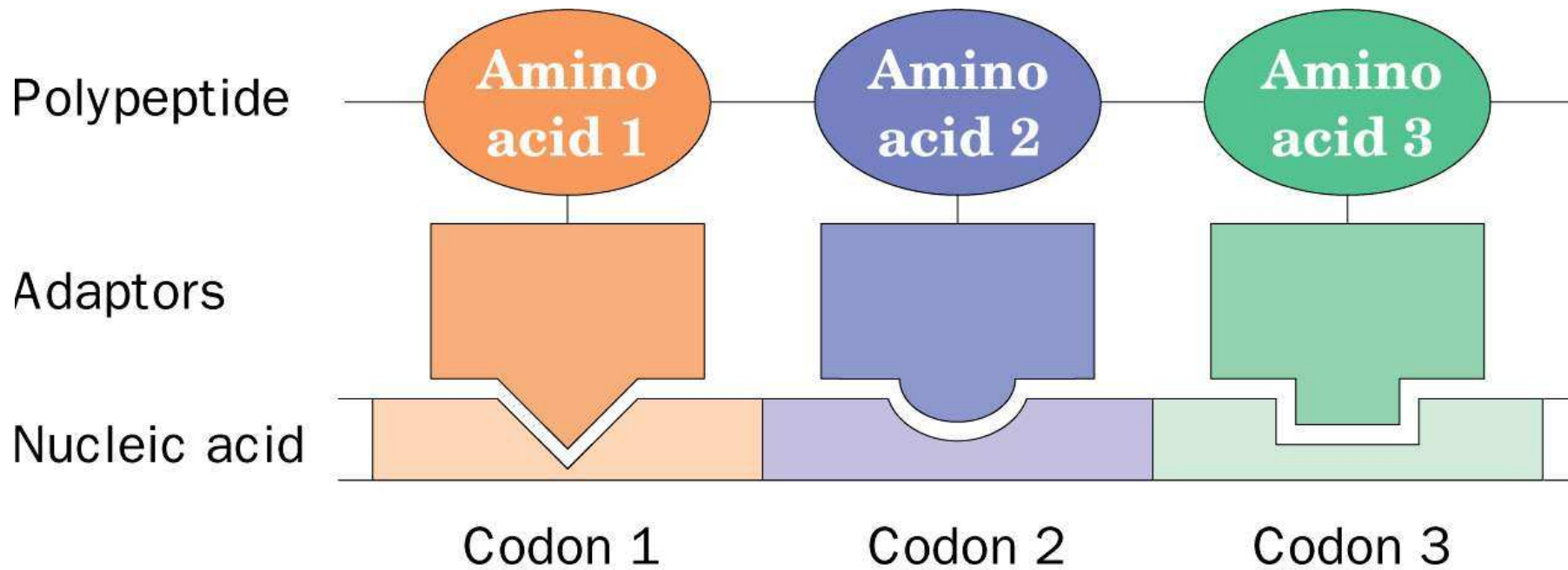
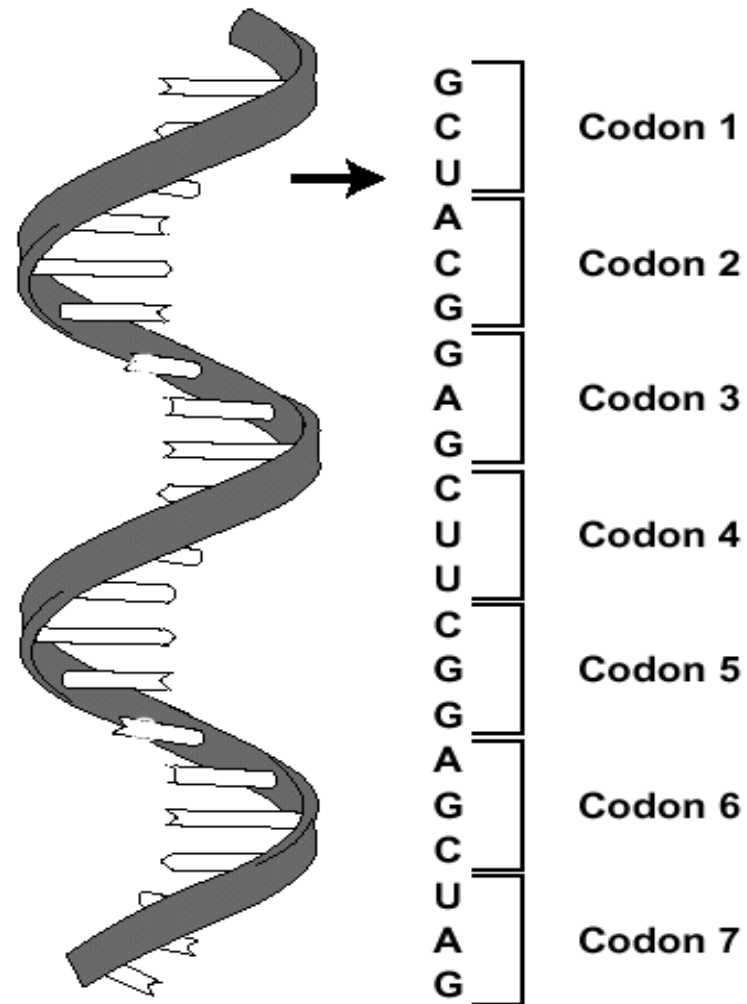


Figure 12-3 Concepts in Biochemistry, 3/e
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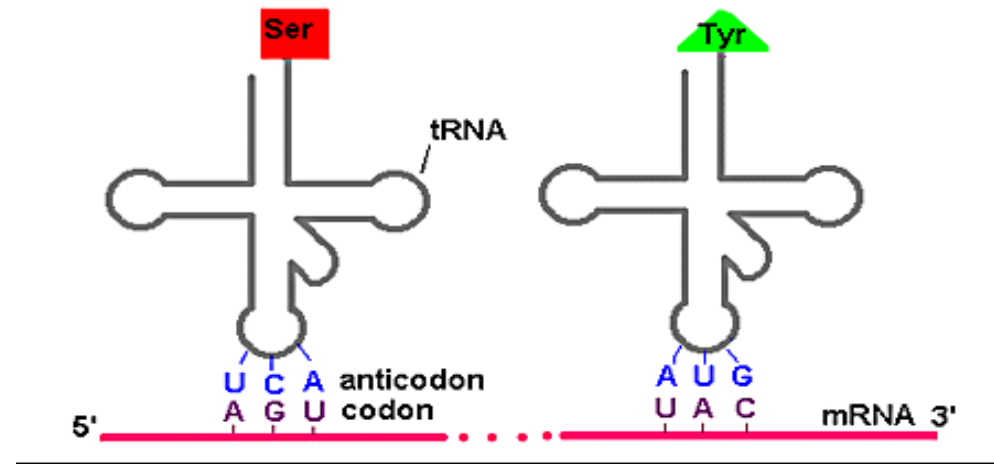


Kodón



Genetický kod

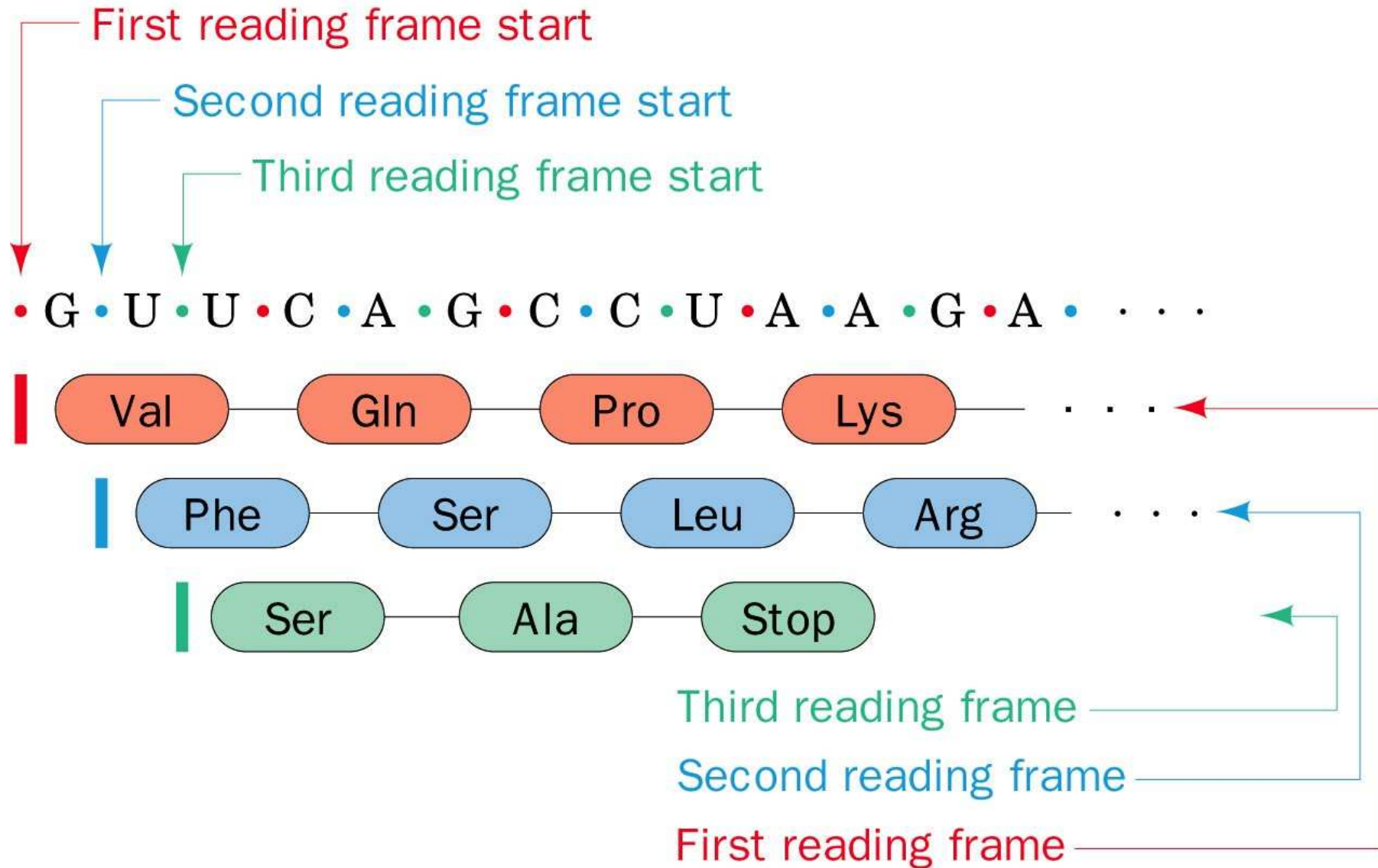
- Tripletový $4^3 = 64$
- Degenerovaný
- Universální
- Nepřekrývající se



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

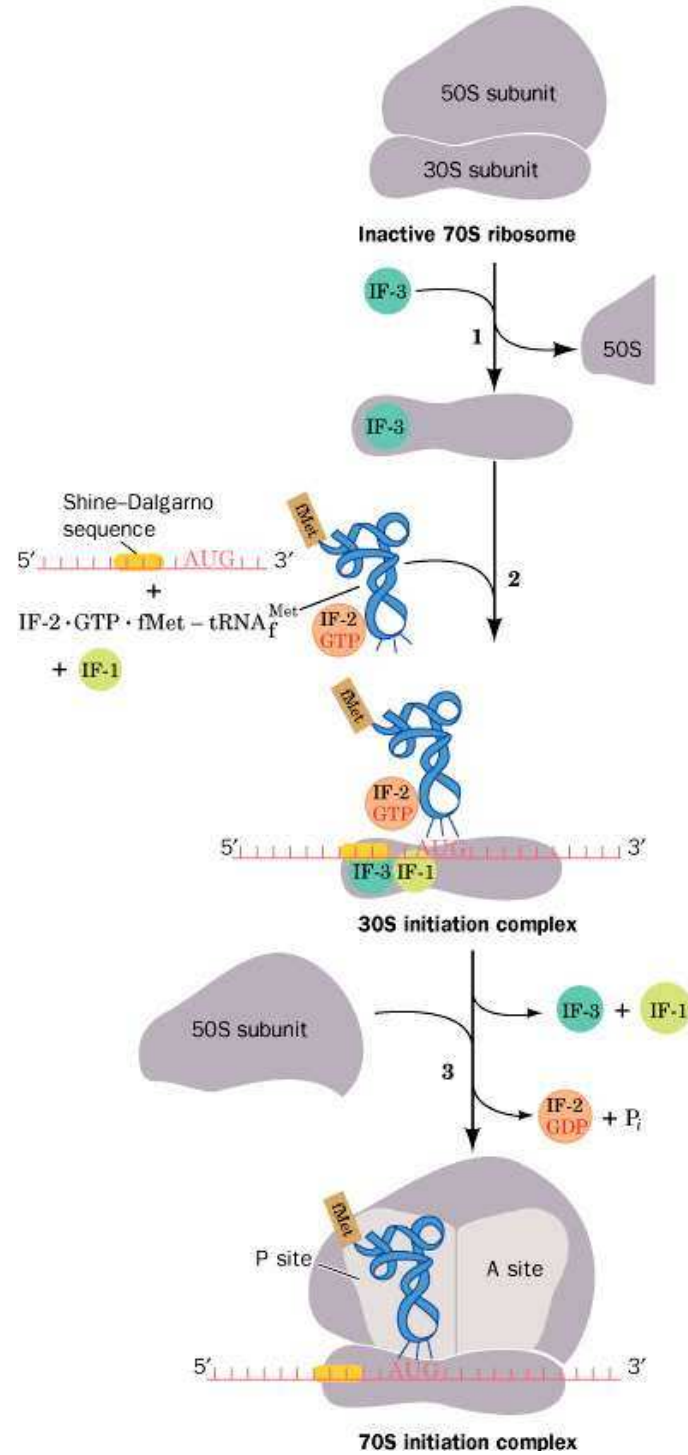
Stop

Third reading frame

Second reading frame

First reading frame

Inicializace



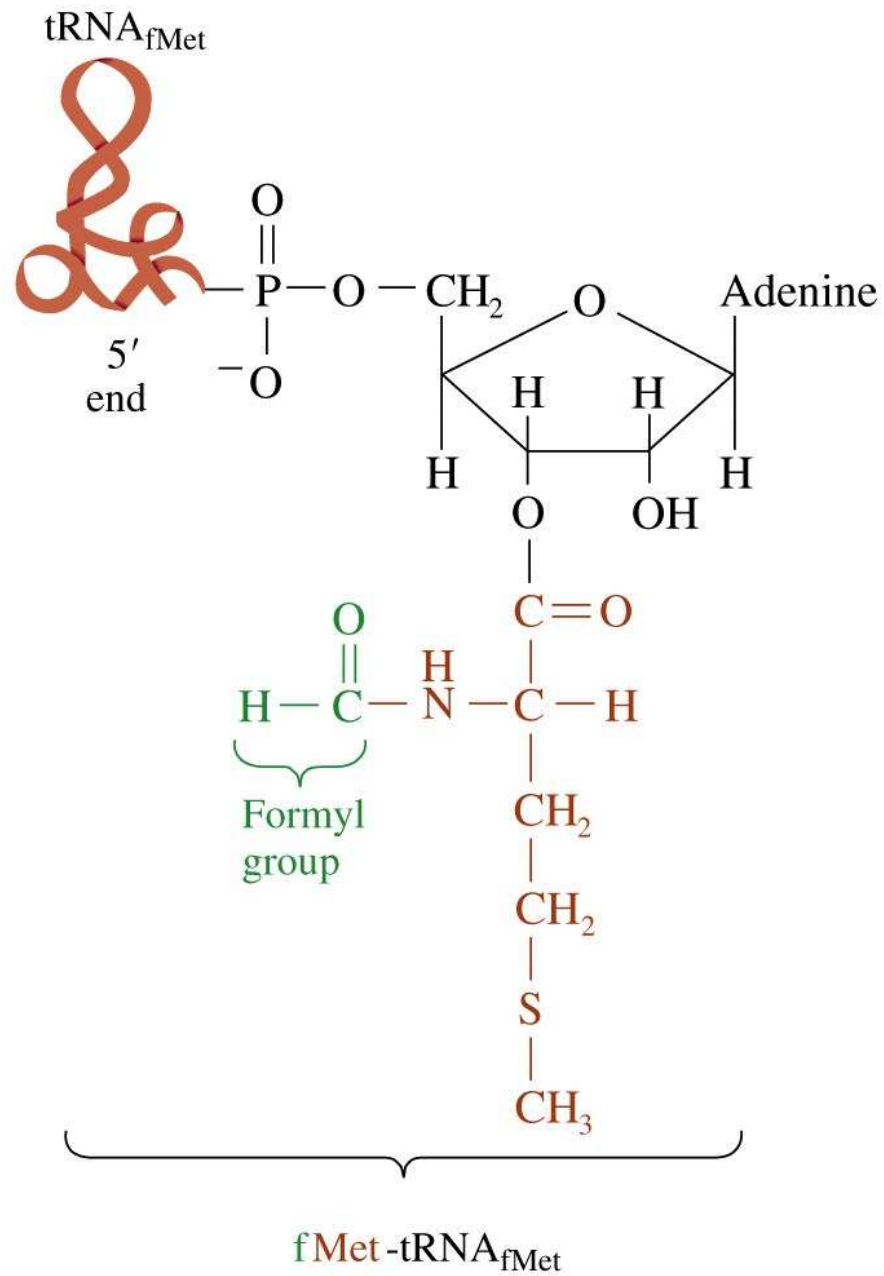
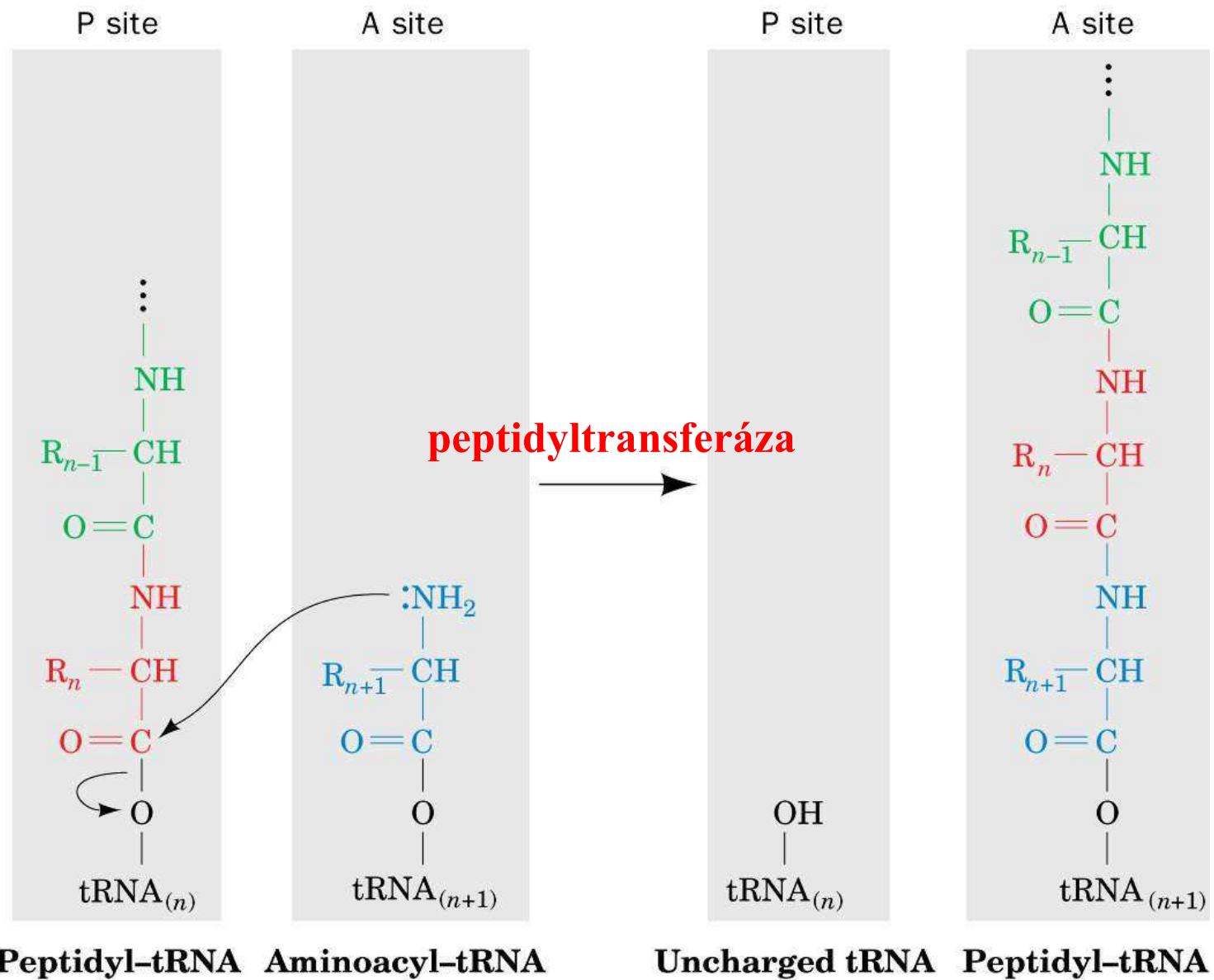


Figure 12-6 Concepts in Biochemistry, 3/e
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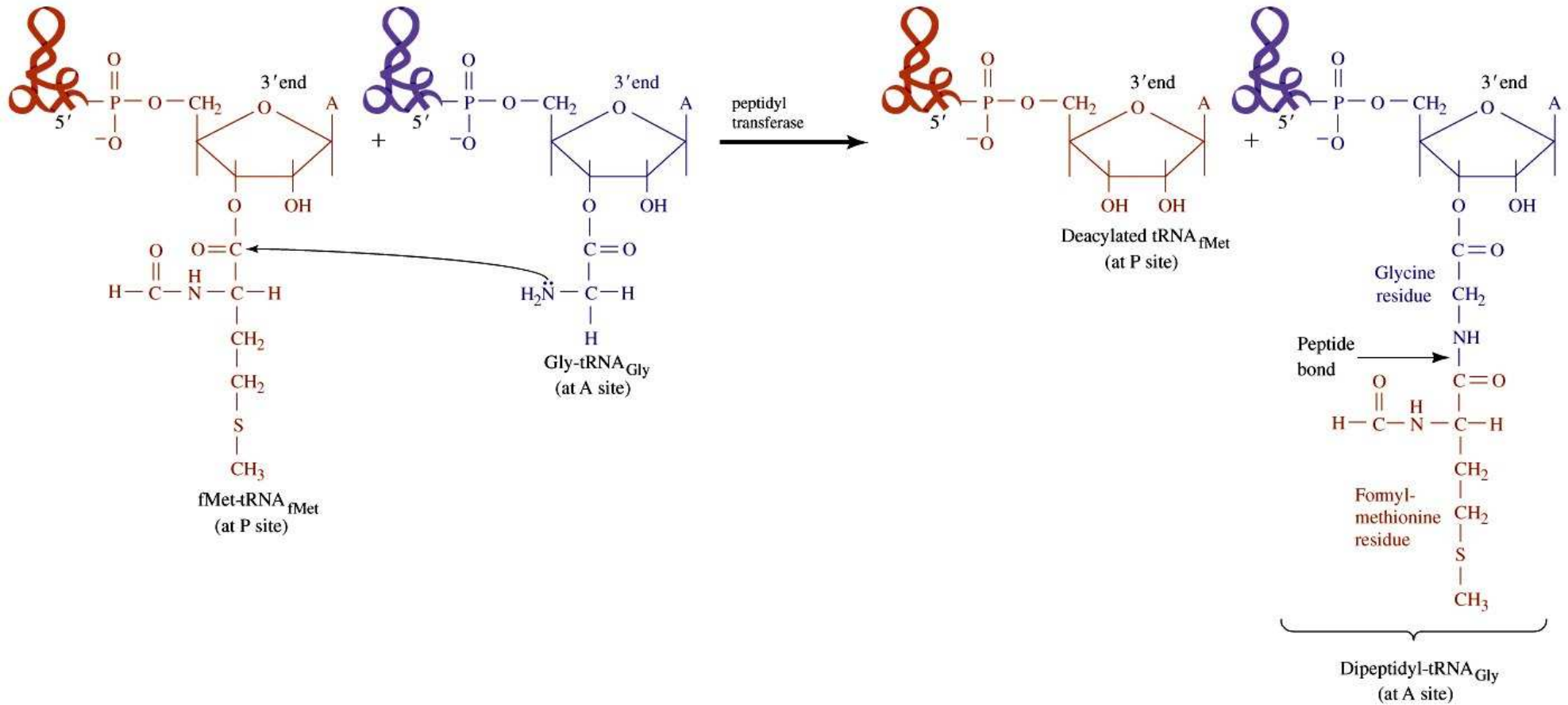
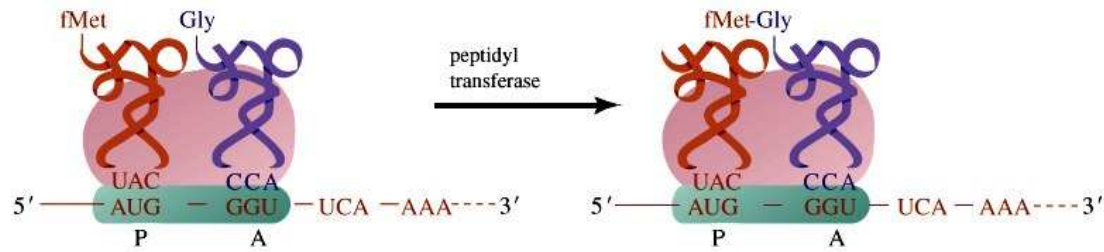
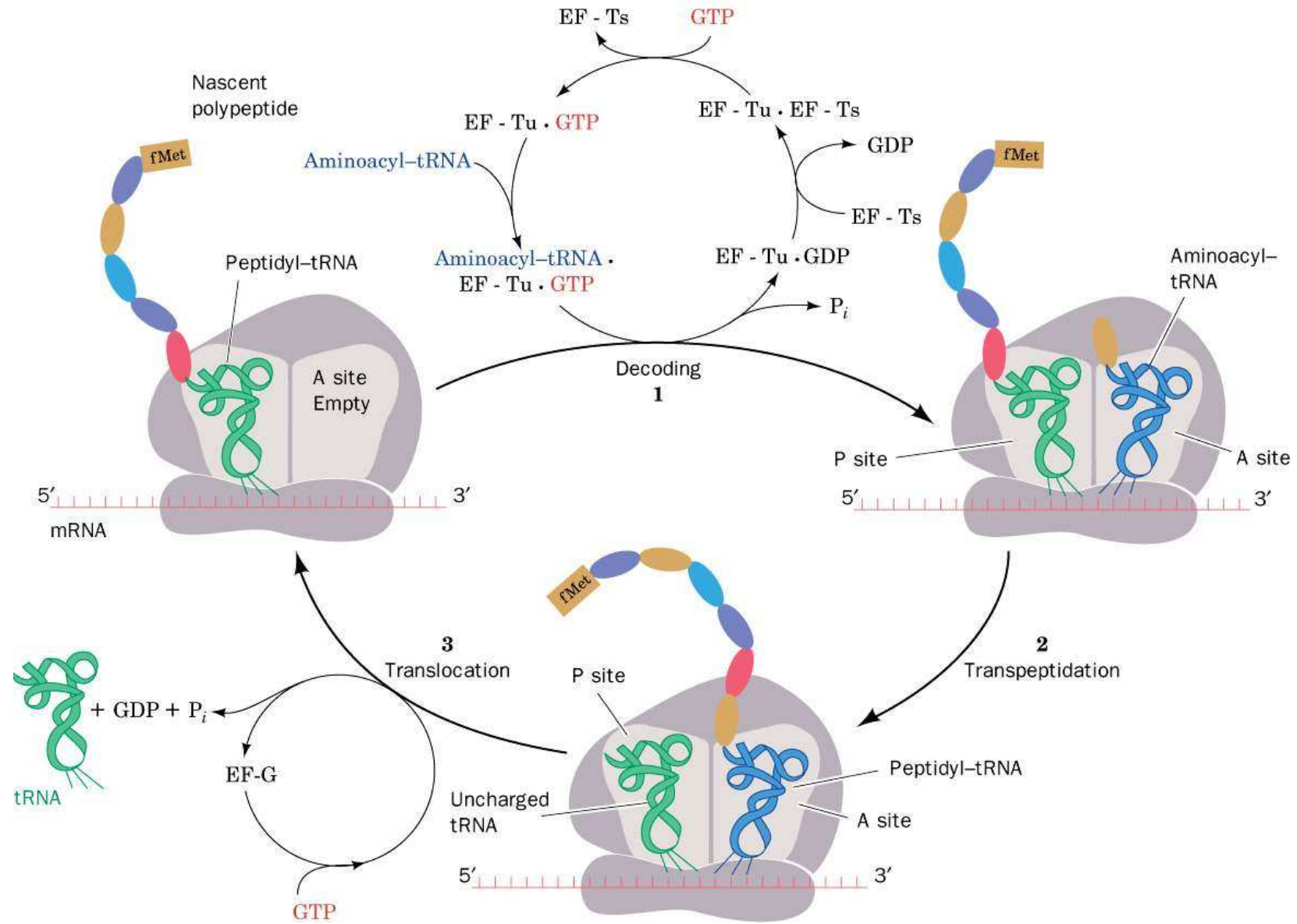
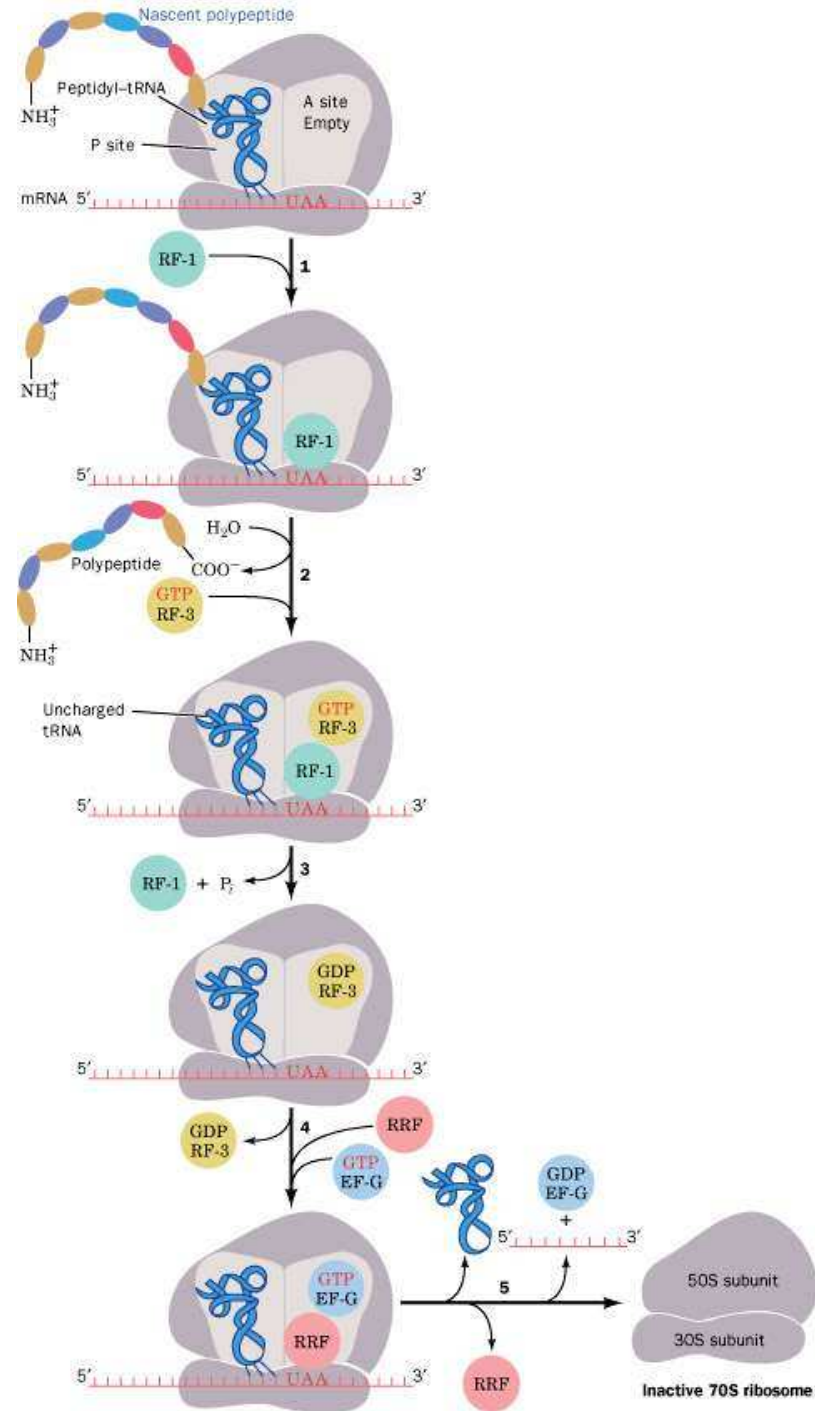


Figure 12-7 Concepts in Biochemistry, 3/e
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Elongate



Terminace



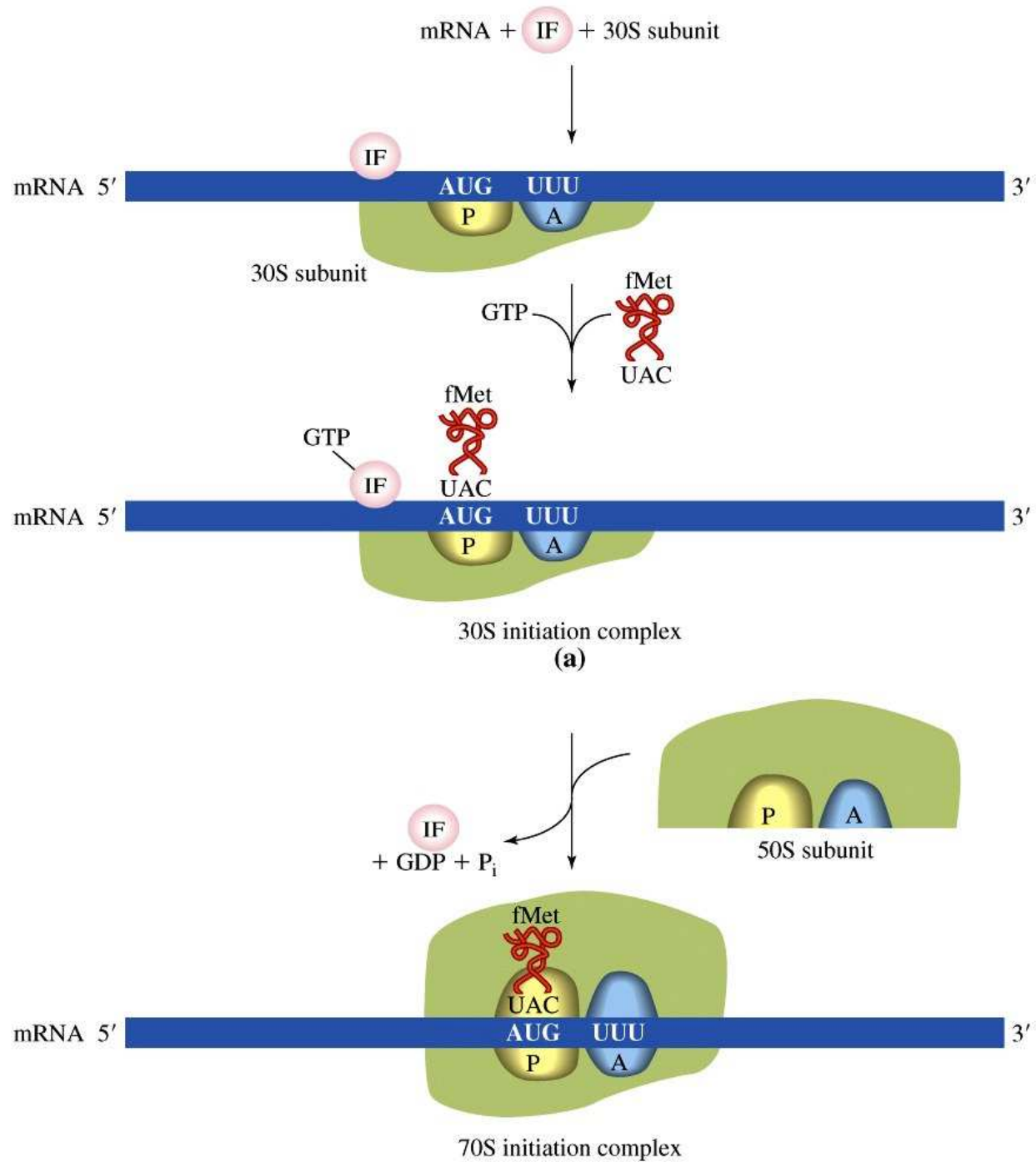


Figure 12-5a Concepts in Biochemistry, 3/e
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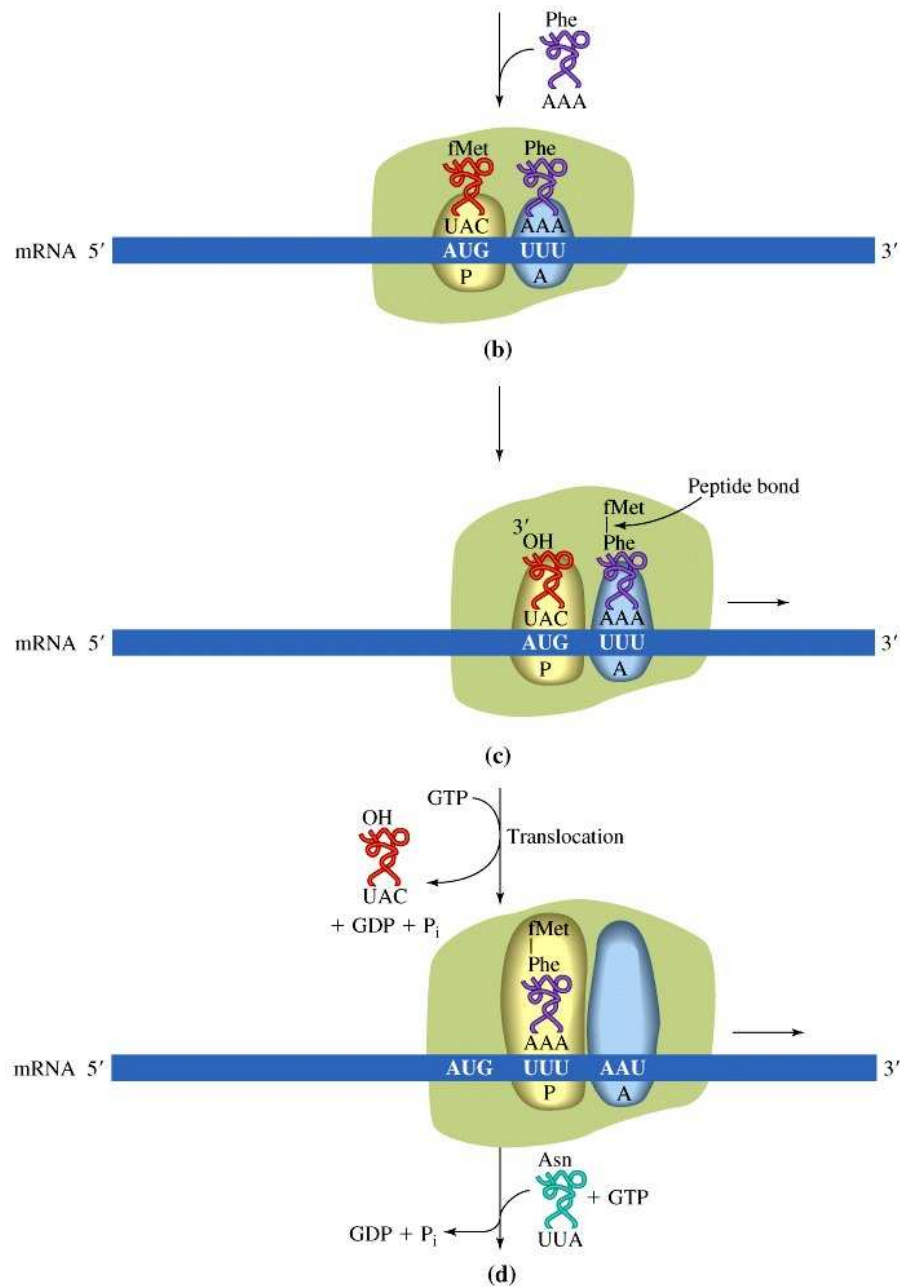


Figure 12-5b Concepts in Biochemistry, 3/e
 © 2006 John Wiley & Sons

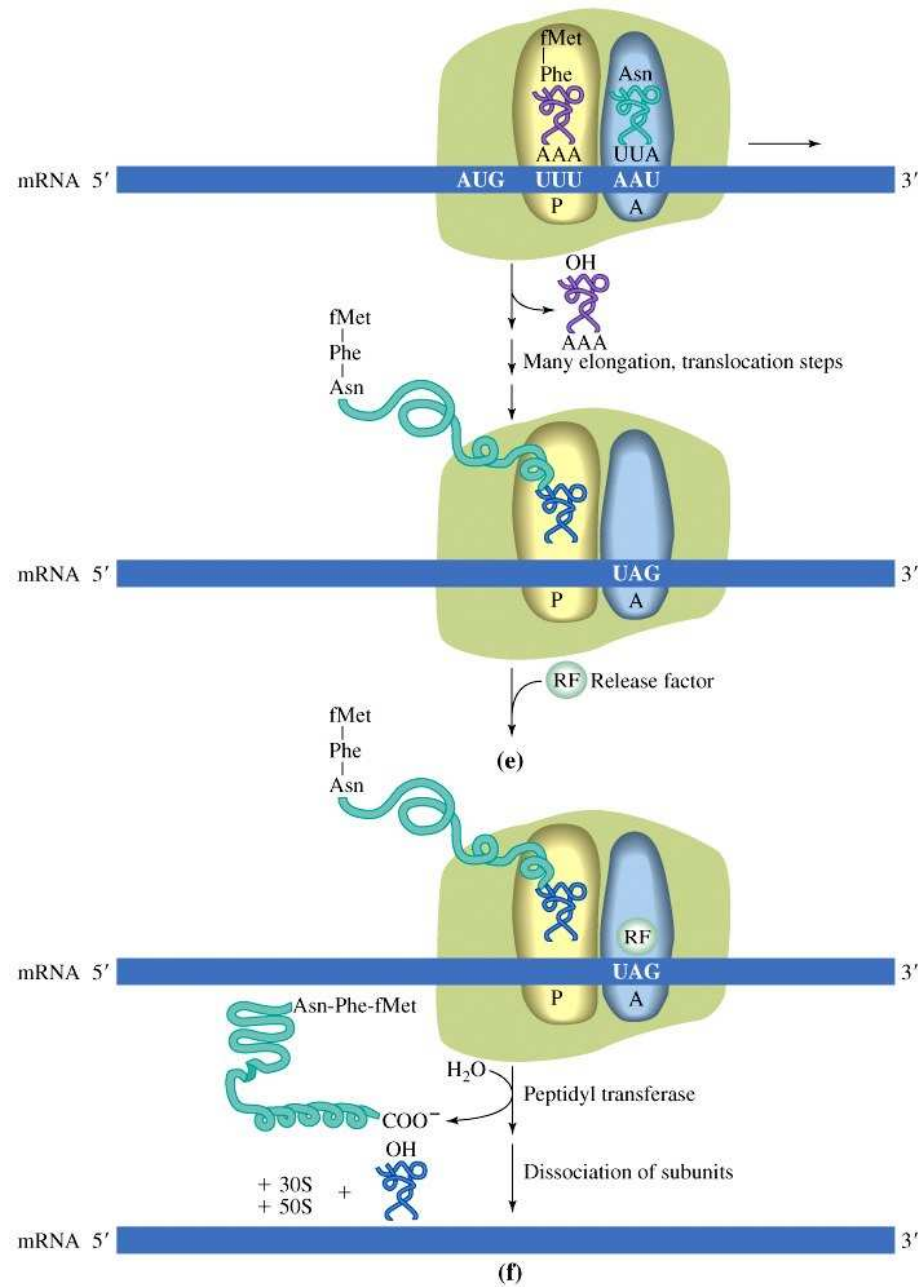


Figure 12-5c Concepts in Biochemistry, 3/e
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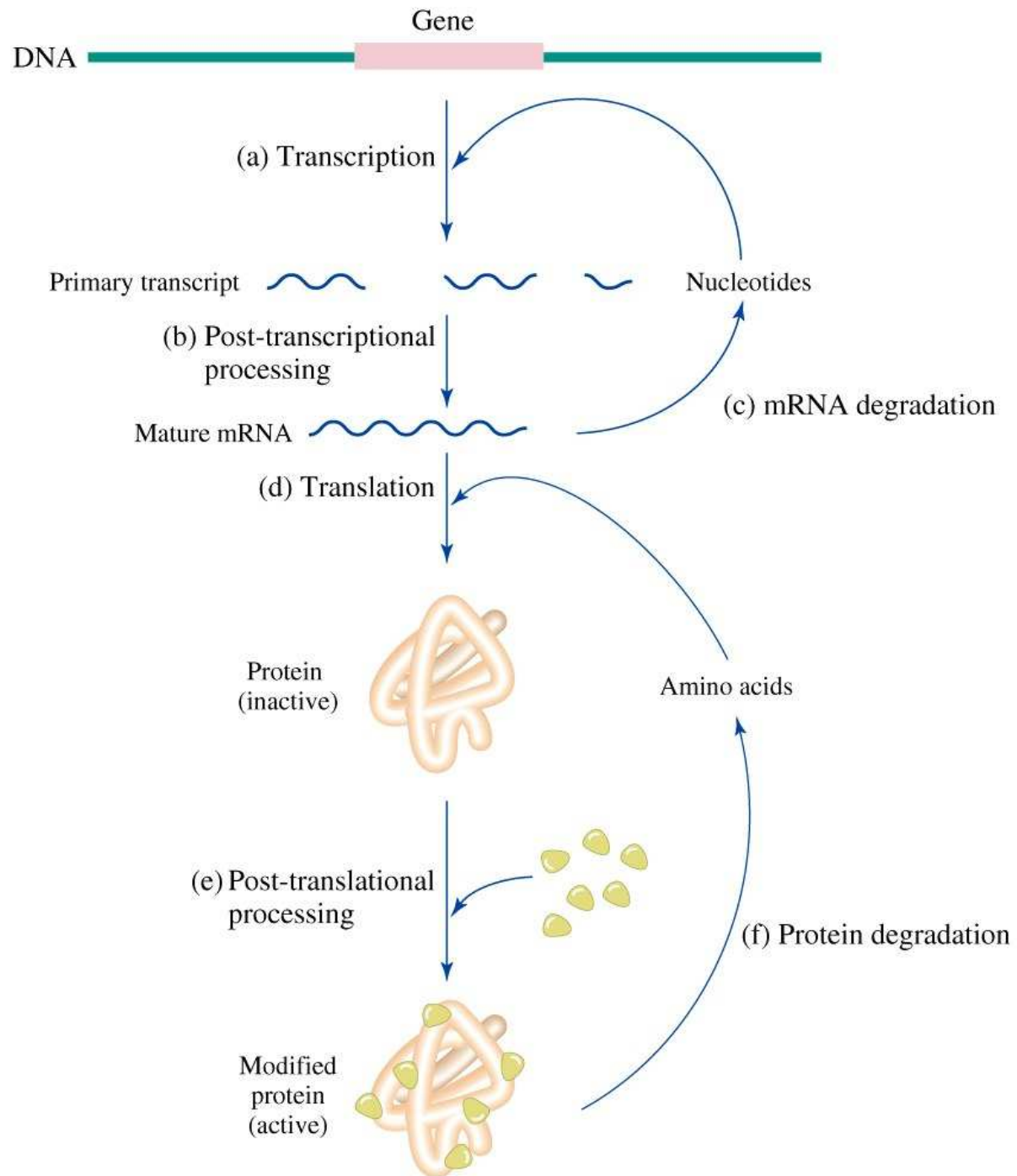


Figure 12-16 Concepts in Biochemistry, 3/e
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Posttranslační modifikace

- fosforylace/defosforylace – enzymy kinázy a fosfatázy připojují či odpojují fosfátovou (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 -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 -SH skupiny cysteinu na -S-S-
- vazba prostetických skupin – např. FAD, FMN, hem, nutné pro funkci některých enzymů

Kontrola exprese genu

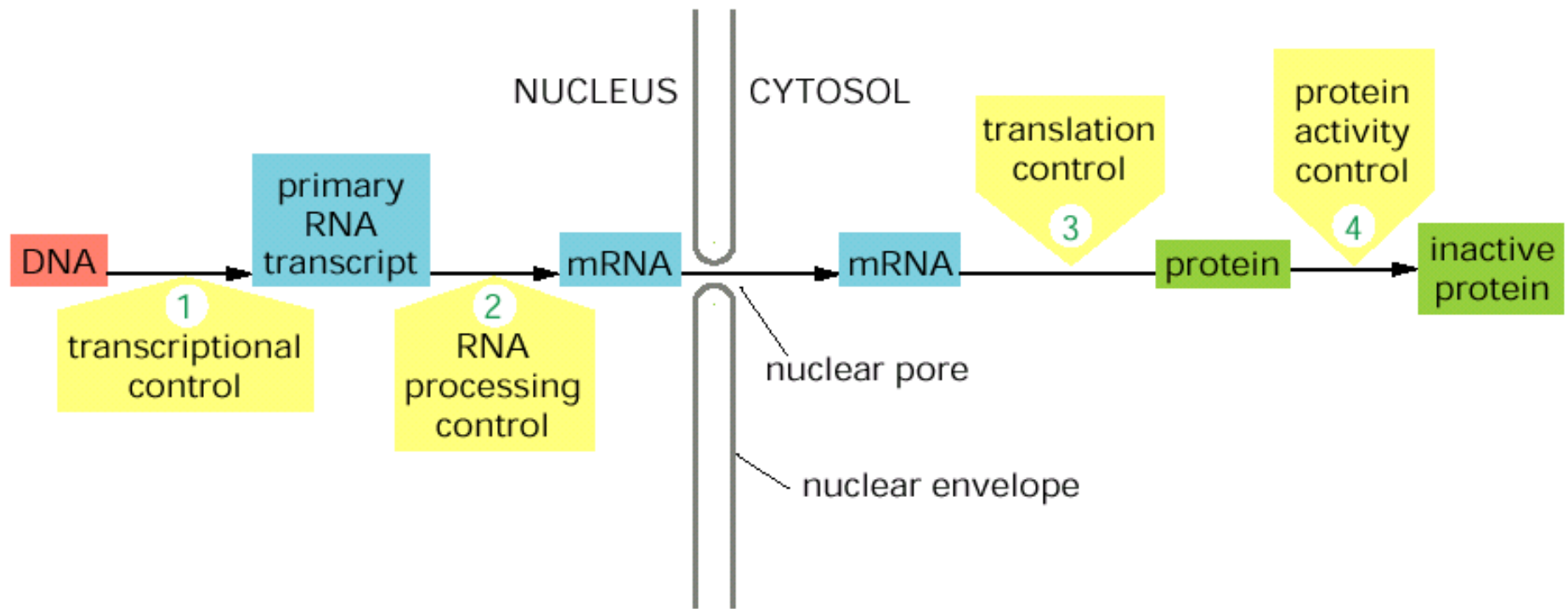


Table 12.4**Antibiotic inhibitors of protein synthesis**

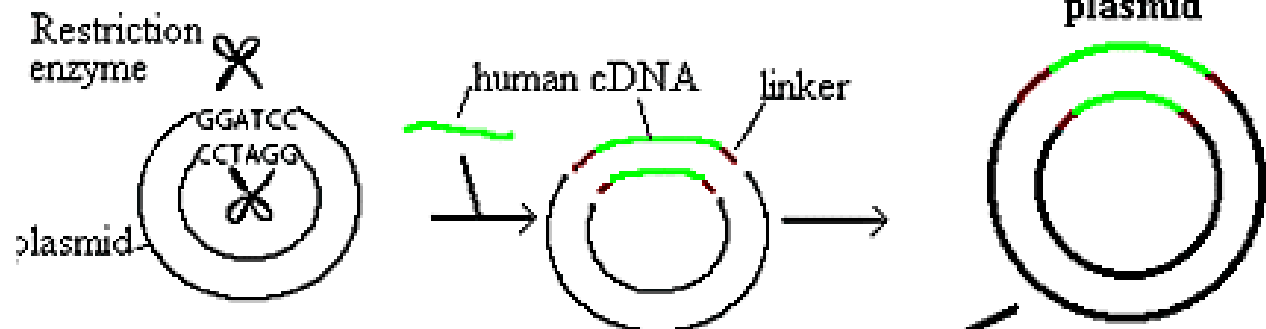
Antibiotic	Mode of Action
Puromycin	Causes early termination by mimicking the action of an aminoacyl-tRNA; acts on prokaryotes and eukaryotes
Streptomycin	Causes misreading of mRNA and inhibits initiation; acts on prokaryotes
Tetracycline	Binds to the A site of ribosomes and blocks entry of aminoacyl-tRNAs; 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

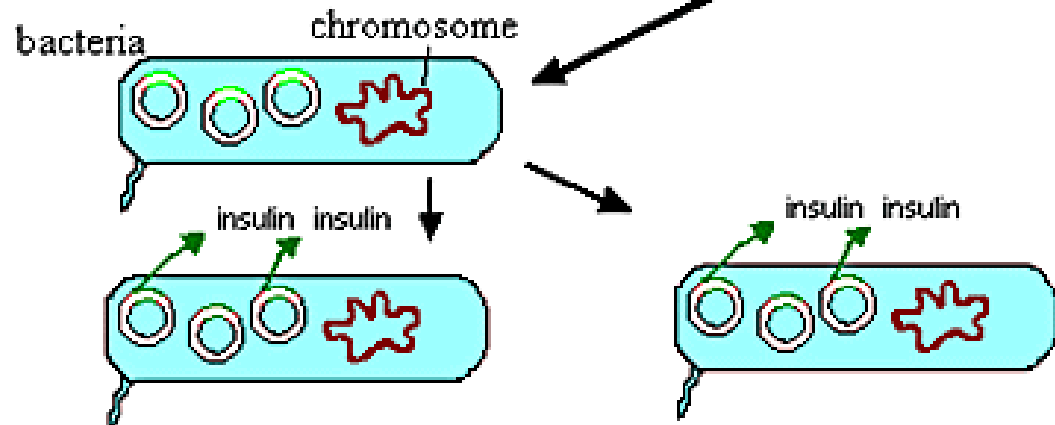
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Genetické inženýrství

Transfer of the Insulin gene



Cloning the Insulin Gene



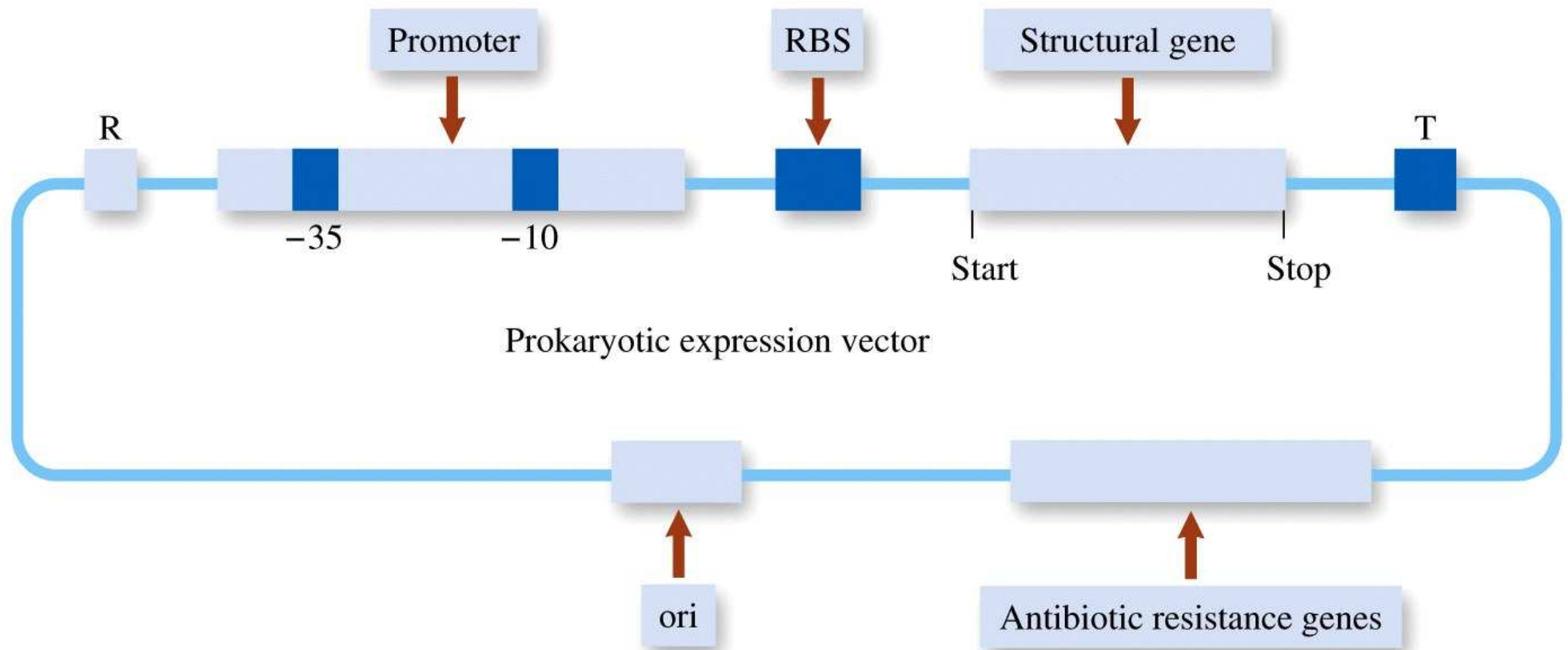


Figure 13-3 Concepts in Biochemistry, 3/e
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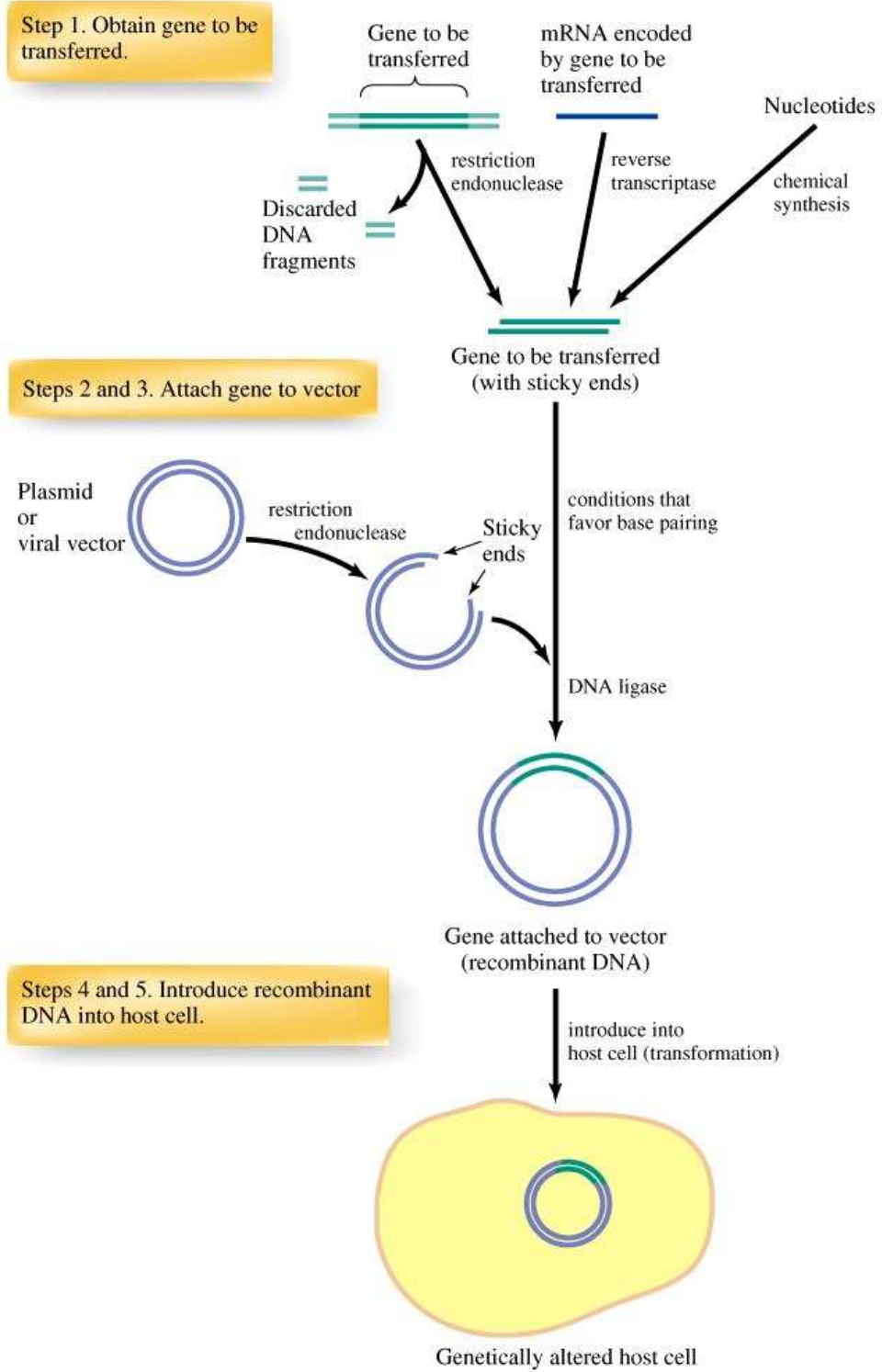


Figure 13-1 Concepts in Biochemistry, 3/e
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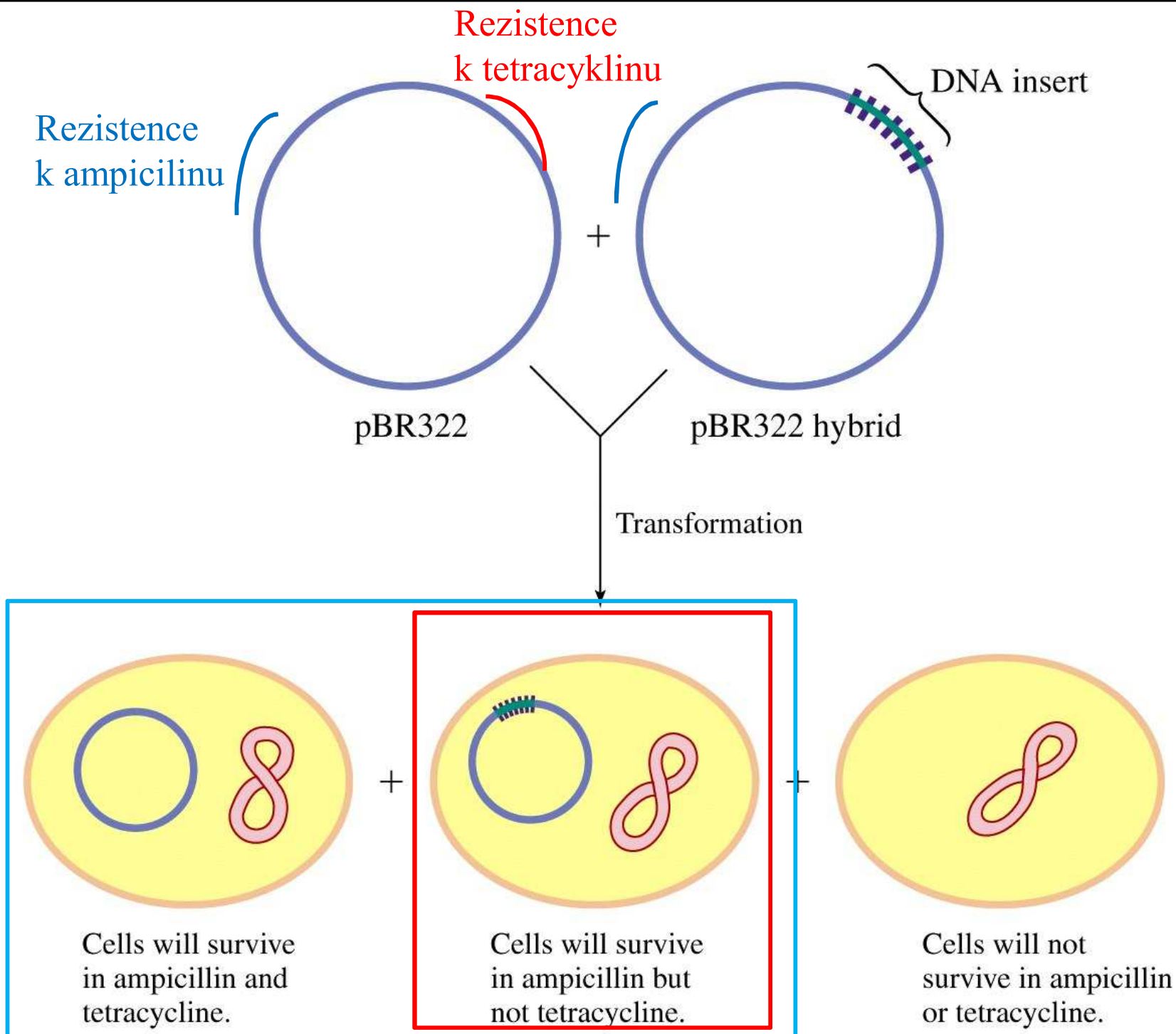


Figure 13-6 part 3 Concepts in Biochemistry, 3/e
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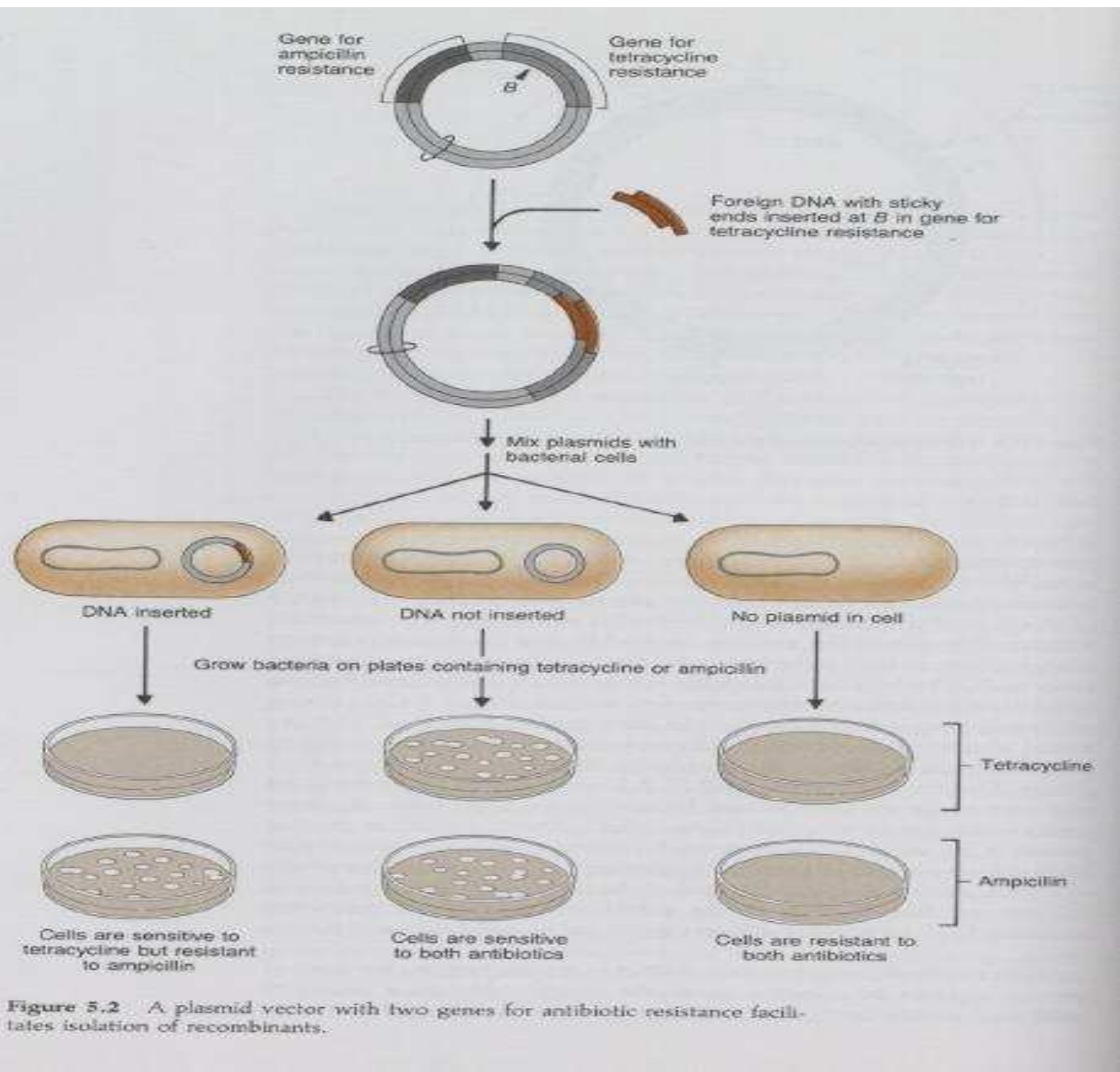
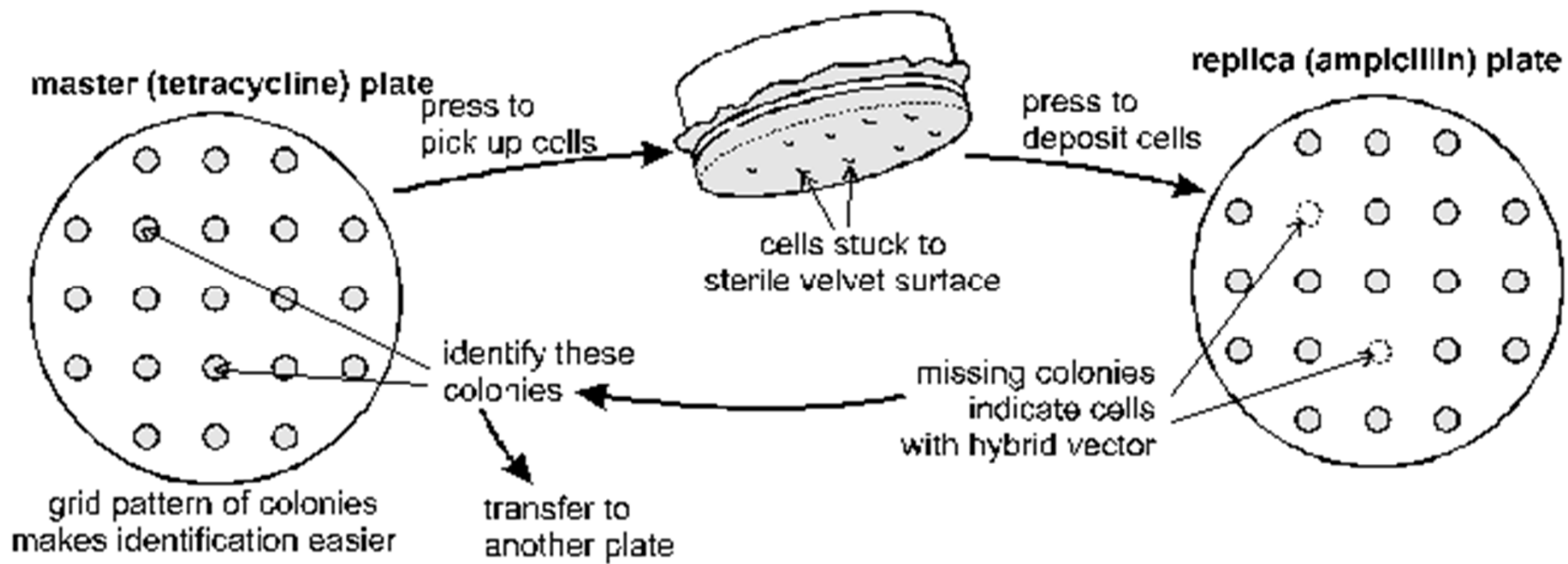


Figure 5.2 A plasmid vector with two genes for antibiotic resistance facilitates isolation of recombinants.

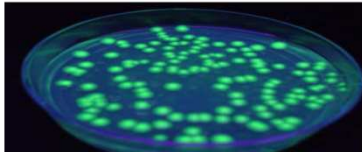


Inklusní tělíska



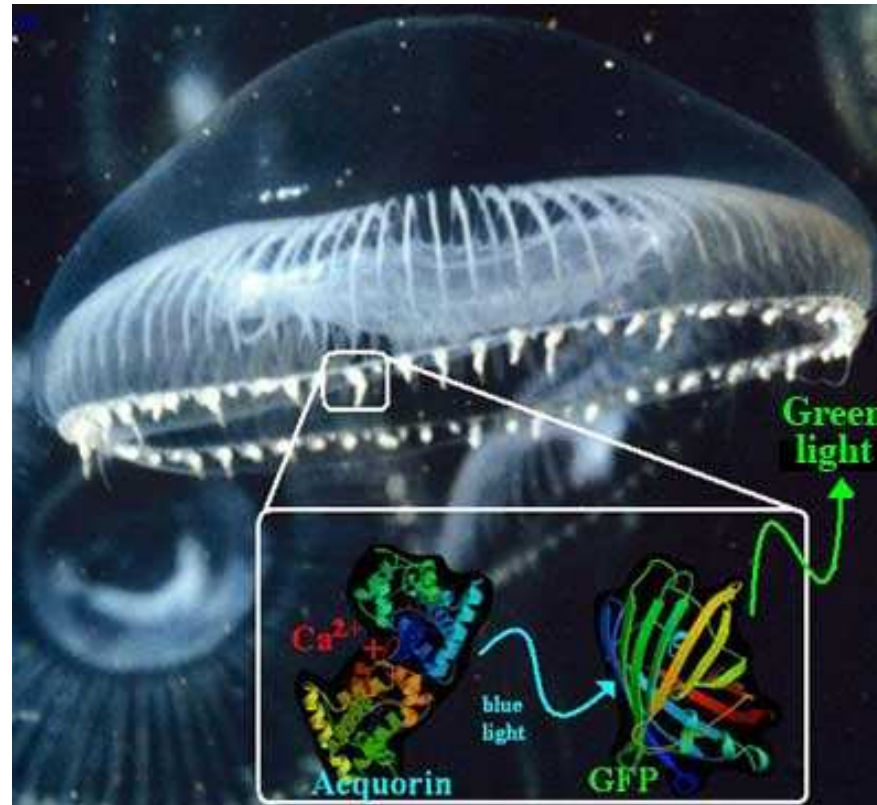
Courtesy of Teruhiko Beppu, Nihon University, Japan

Využití AC pro purifikaci rekombinantních proteinů

řízní kotva	imobilizovaný ligand	podmínky vazby	podmínky eluce
Glutathion S-transferasa GST	redukovaný glutathion	Neutrální pH, nedenaturující prostředí, glutathion musí být redukovaný a GST musí být aktivní	volný redukovaný glutathion
Histidinová kotva His-tag	Chelatovaný nikl nebo kobalt	Neutrální pH bez redukčních a oxidačních látek	>200 mM Imidazol, nízké pH, silné chelatační činidlo
Maltose Binding Protein MBP	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 GFP	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

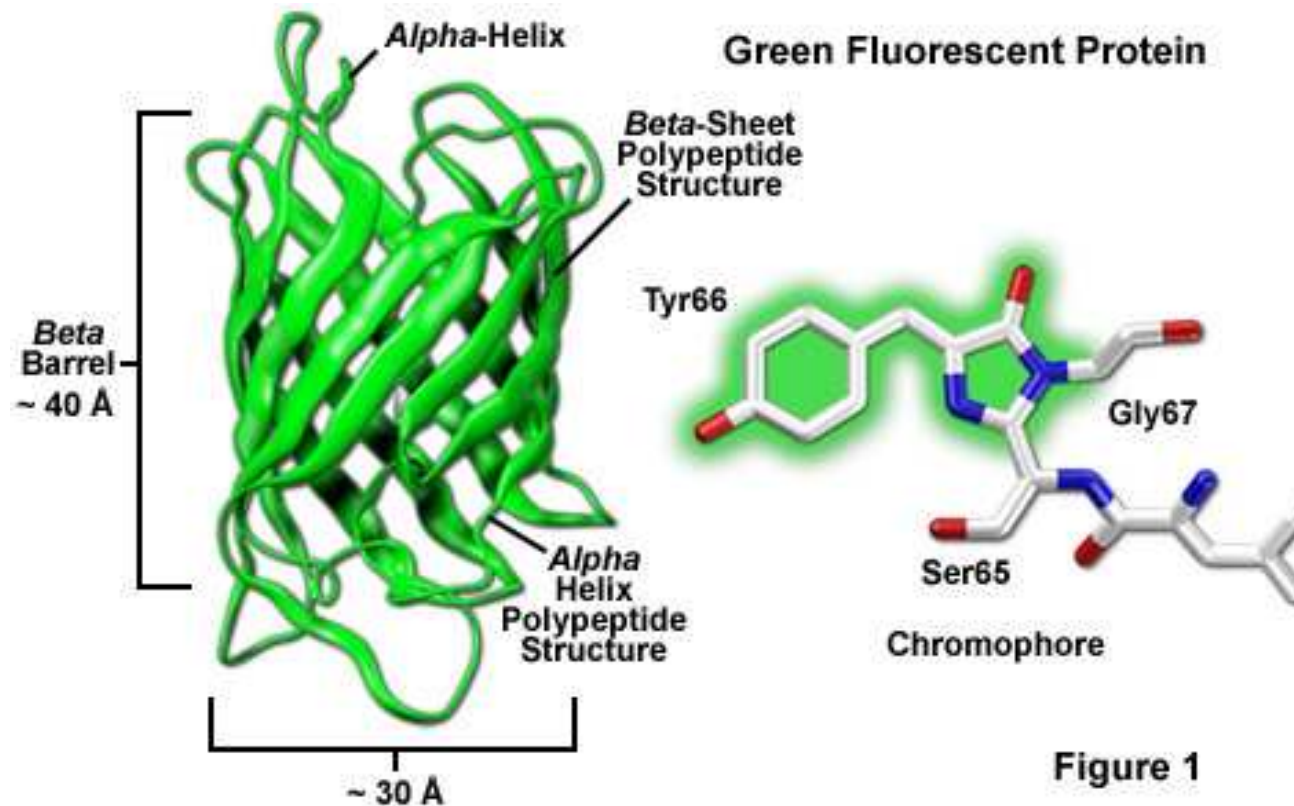


vysvětlil fluorescenci

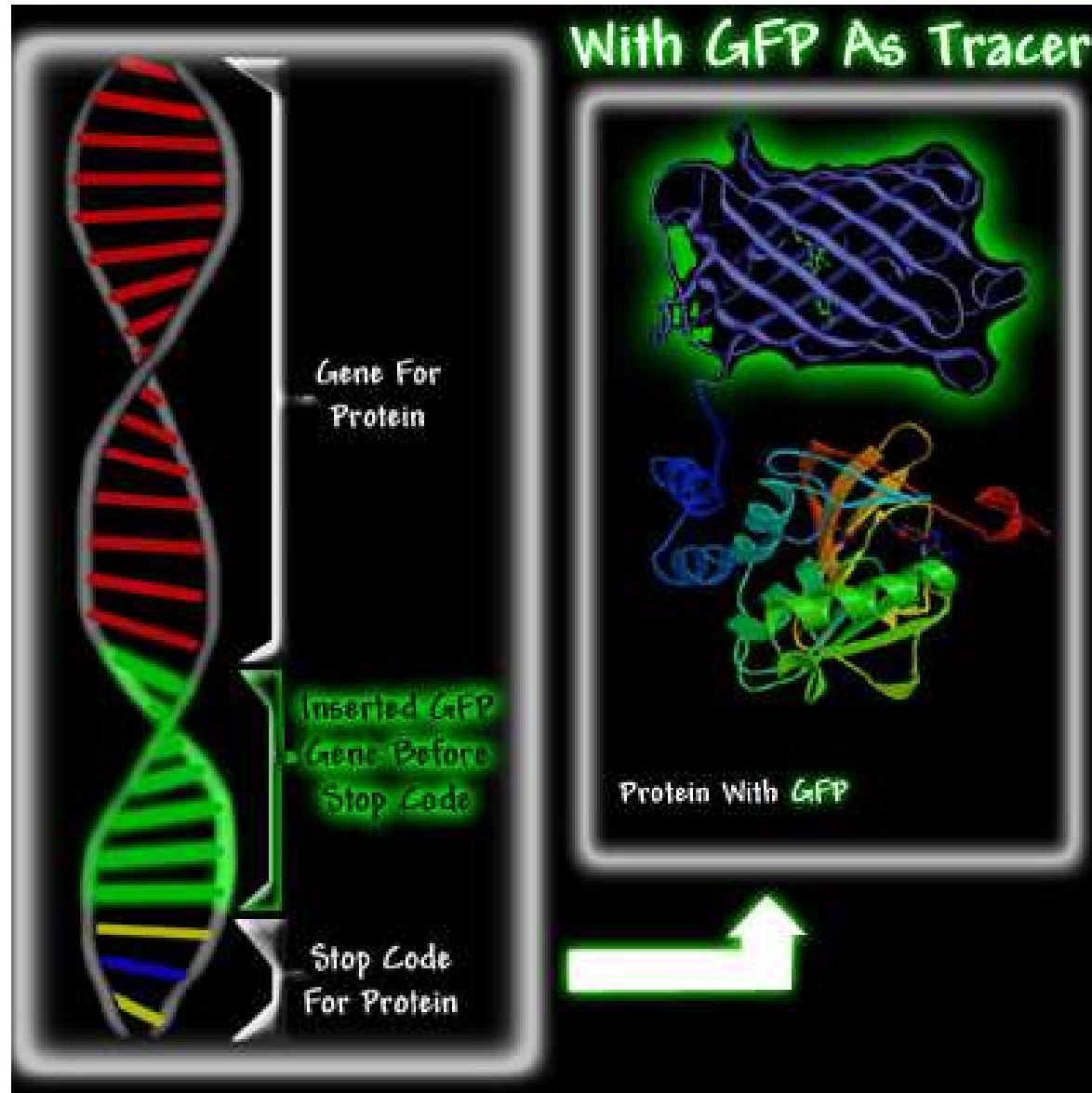
Green Fluorescent Protein

GFP AMK sekvence – 238 AMK

MSKGEELFTGVVPVLVELDGDVNGQKFSVSGEGEGDATYGKLTNFICT
TGKLPVPWPTLVTTFSYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTI
FYKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKMEYNYNS
HNVYIMGDKPKNGIKVNFKIRHNIKDGSVQLADHYQQNTPIGDGPVLLP
DNHYLSTQSALS KDPNEKRDHMILLEFVTAARITHGMDELYK



Green Fluorescent Protein

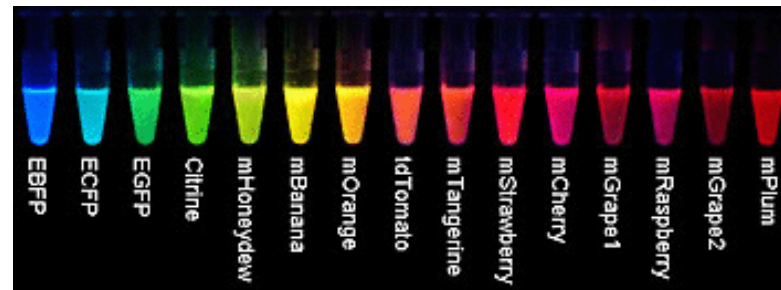


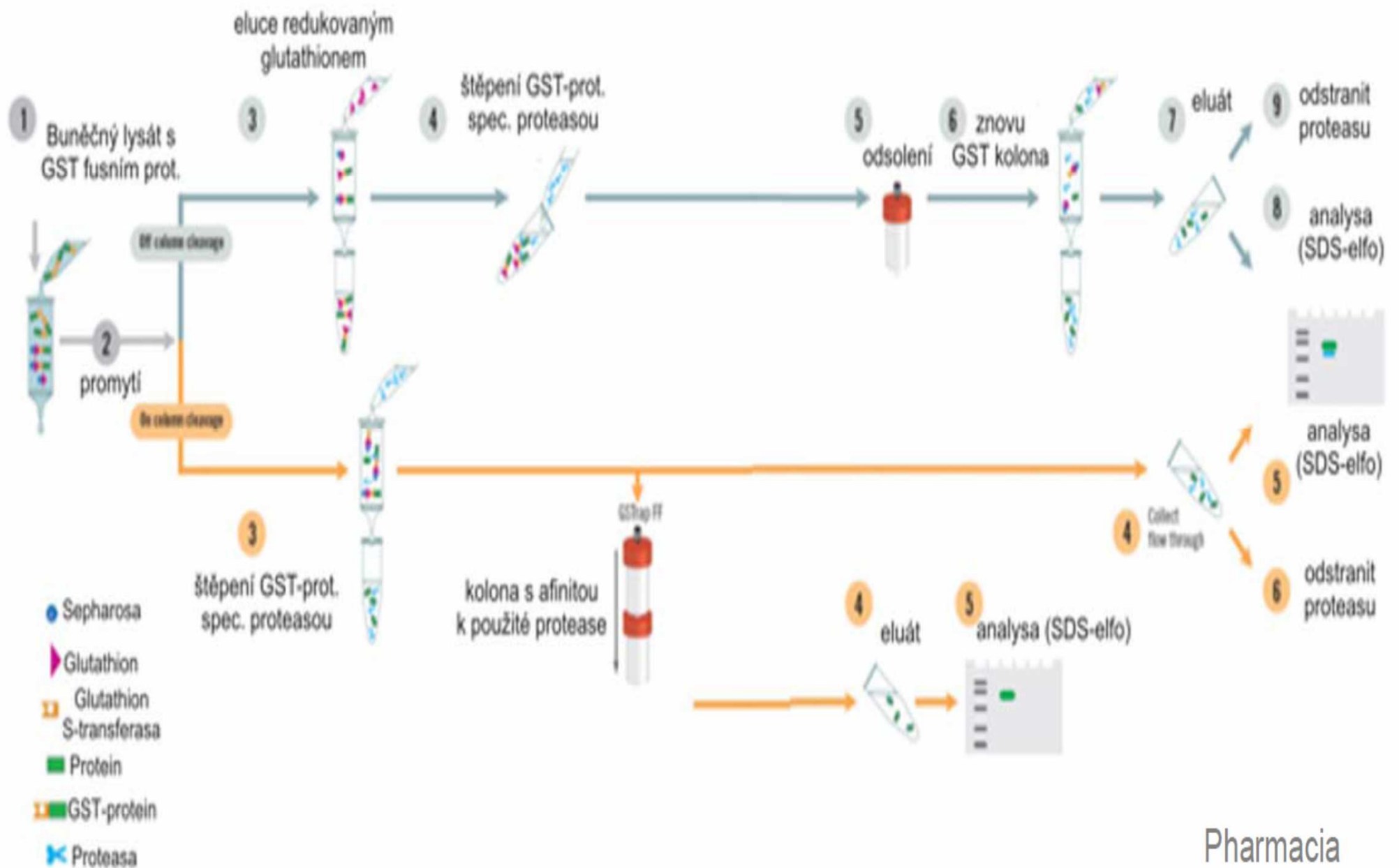
Reportérový gen

Green Fluorescent Protein



Green Fluorescent Protein



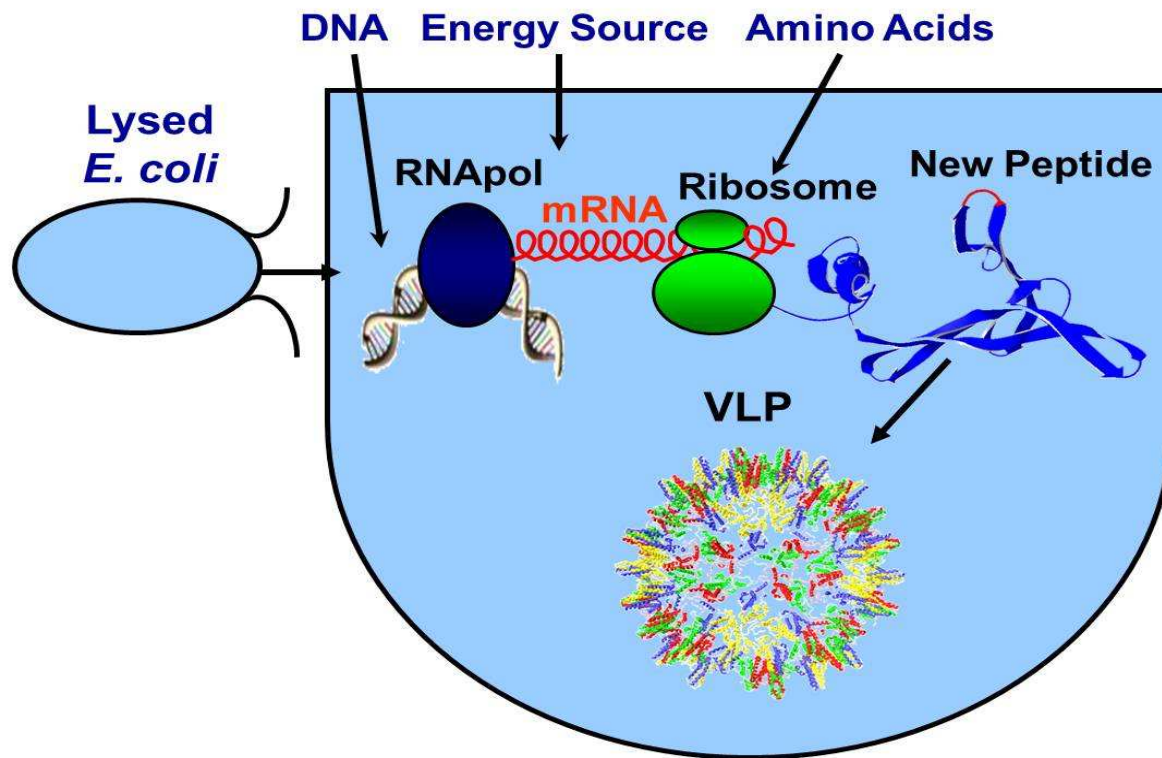


Pharmacia

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

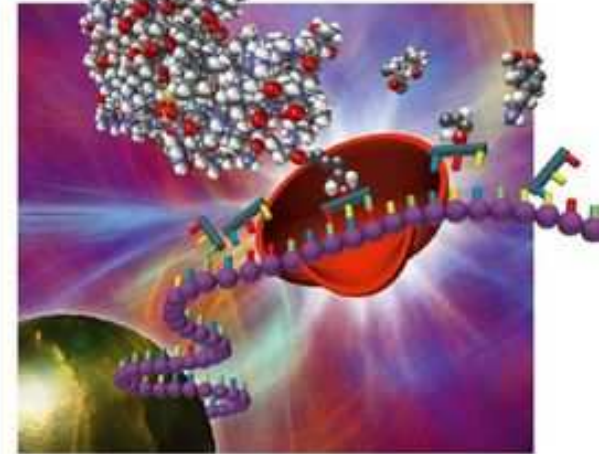


Edited by Alexander S. Spirin
and James R. Swartz

WILEY-VCH

Cell-free Protein Synthesis

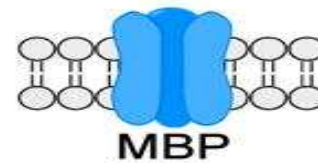
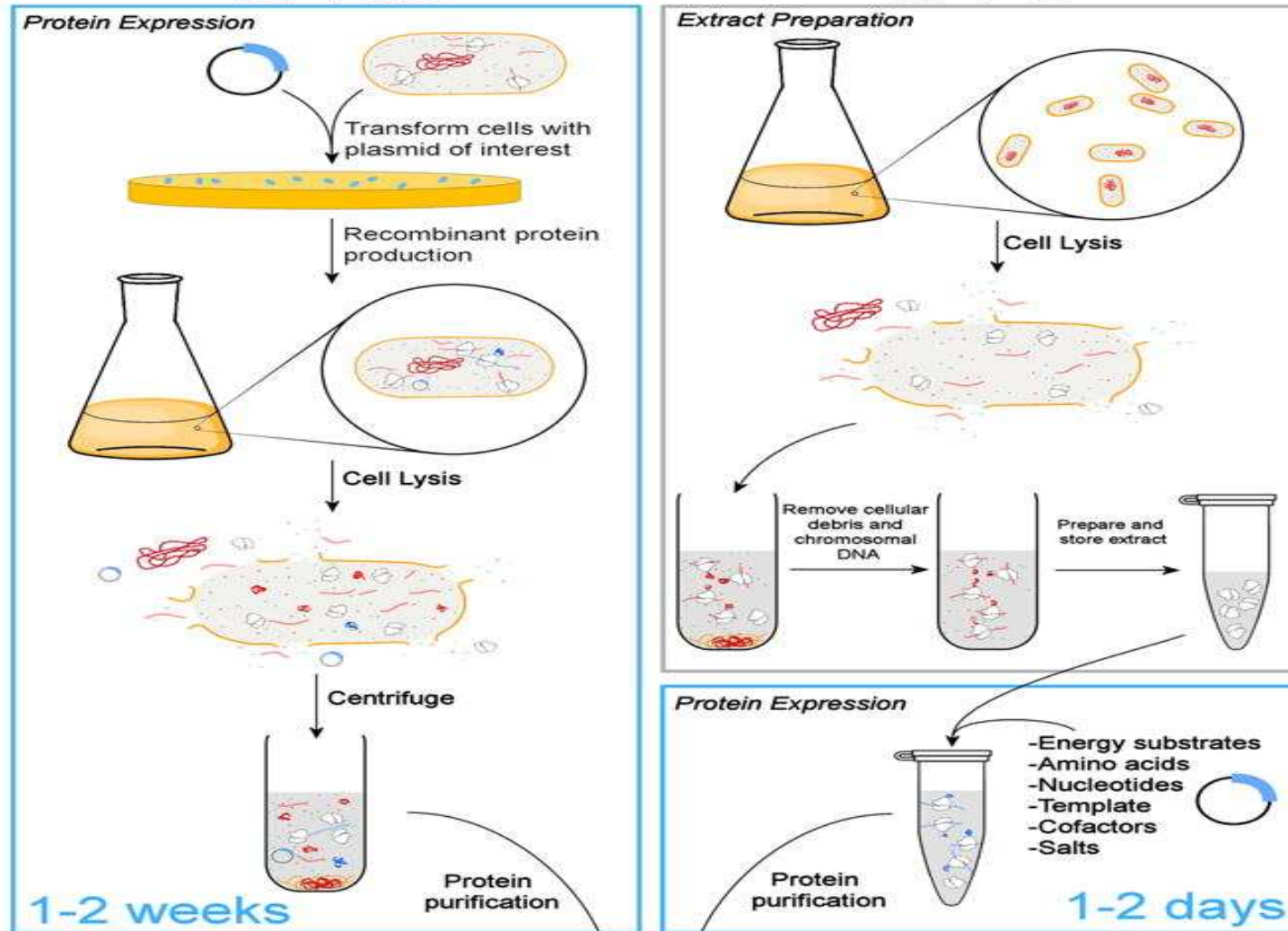
Methods and Protocols



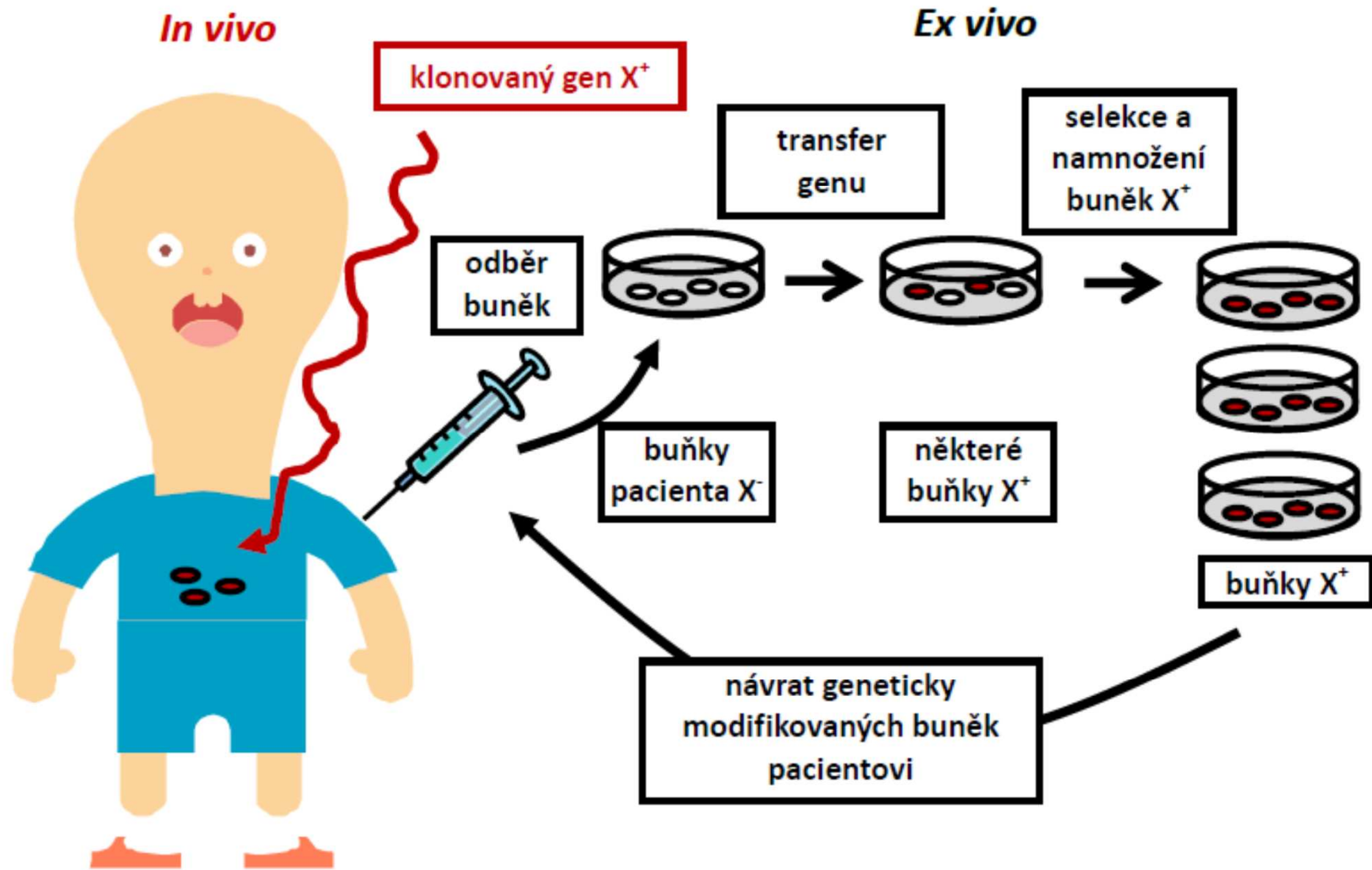
Cell-free protein synthesis

In vivo

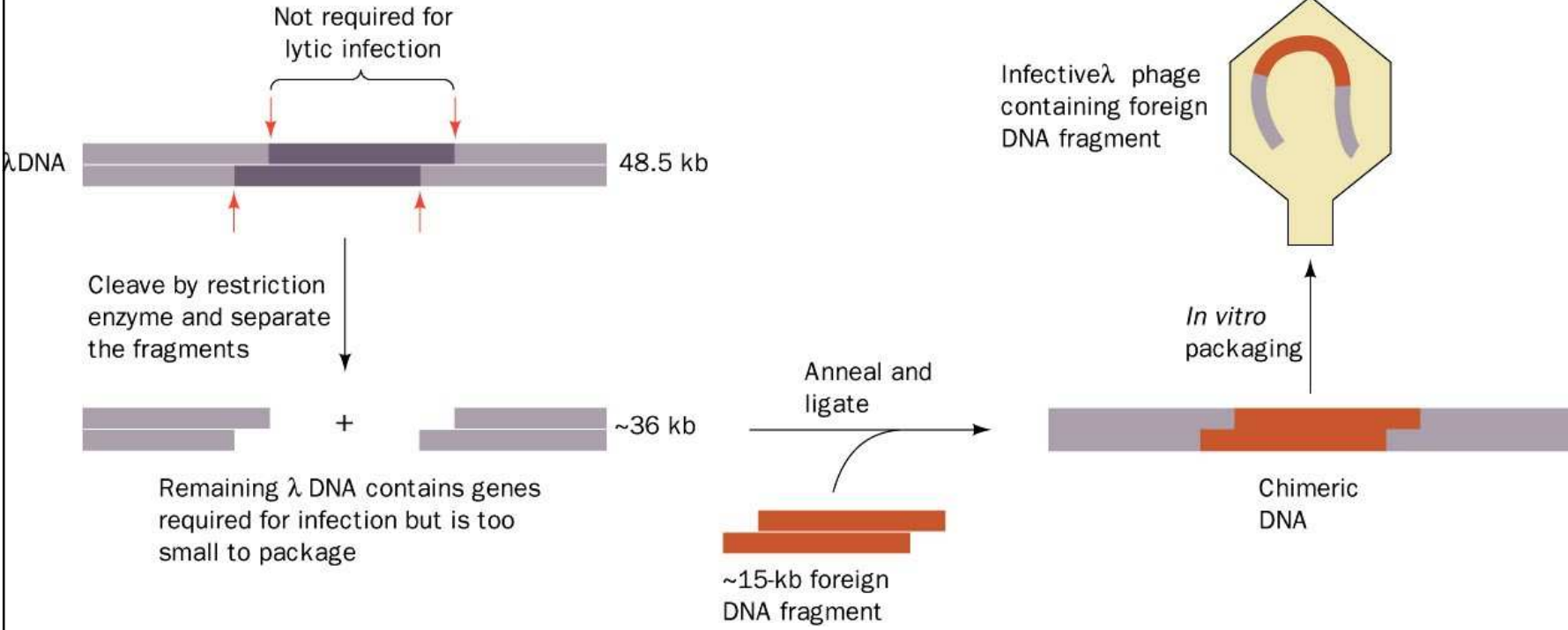
CFPS



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

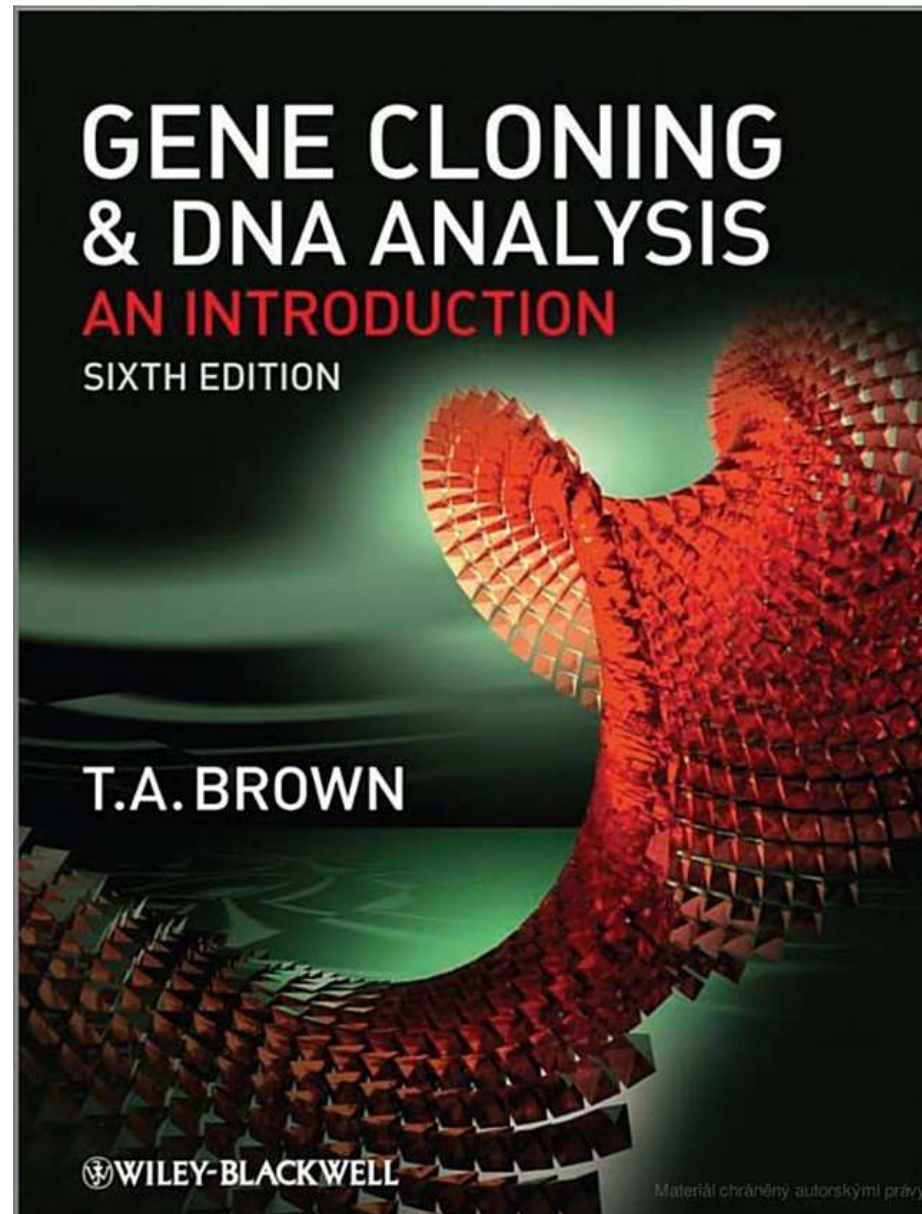
Člověk: možní kandidáti na genovou terapii

- 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
β -Thalassemia	β -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	α_1 -Antitrypsin
Cystic fibrosis	A product that unclogs lung mucus is inhaled in a nasal spray
Cancer	E1A and p53 tumor suppressor genes

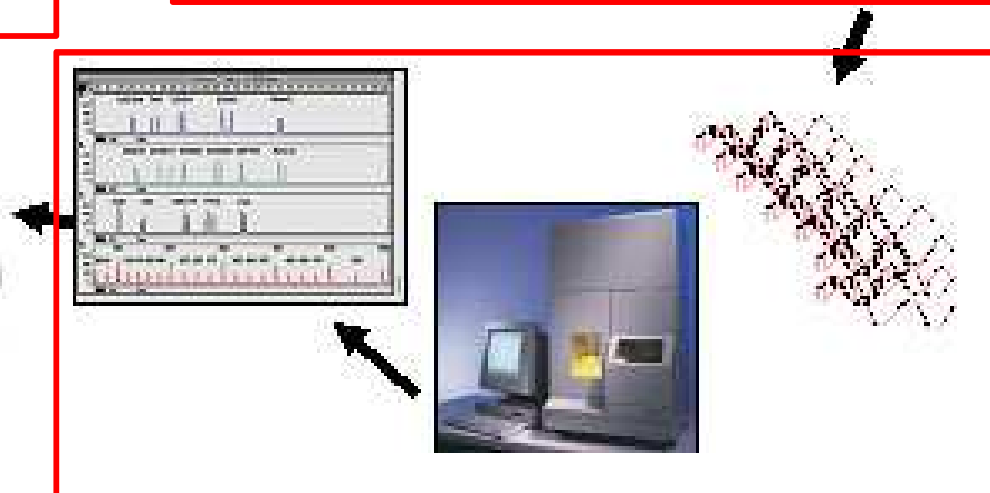
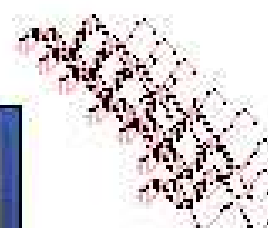
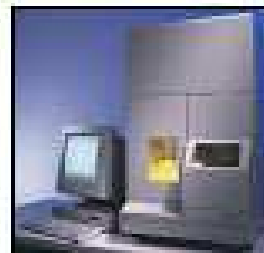
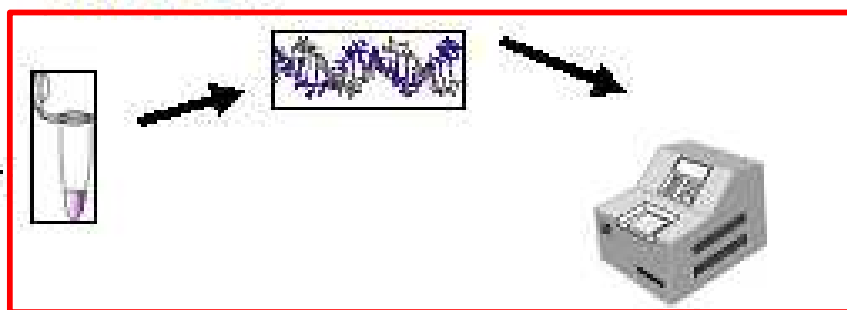
Literatura



Genetická daktyloskopie

The Process of DNA Typing
via PCR

1 ng or less of starting material

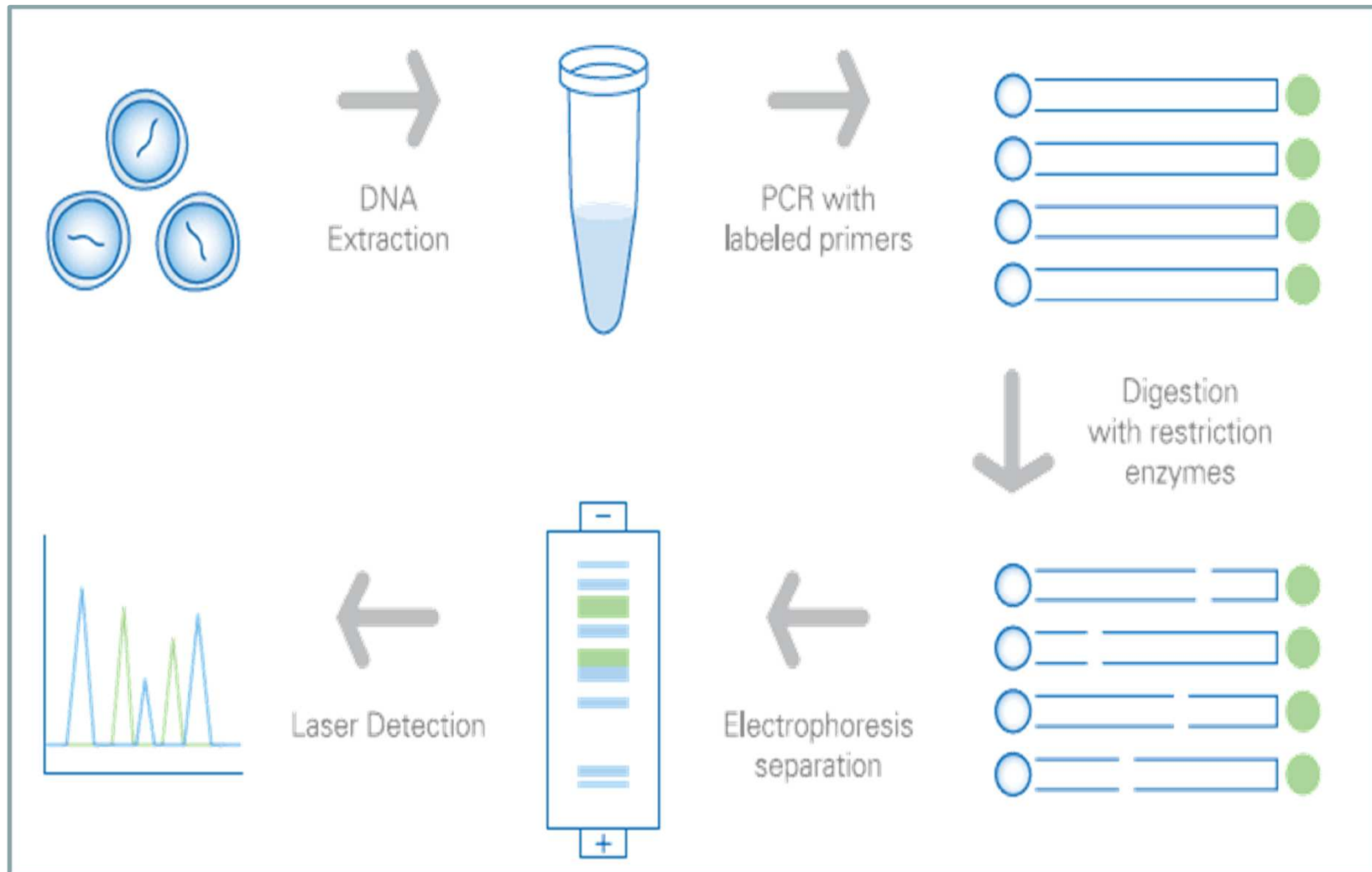


Brief History of Forensic DNA Typing

- 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

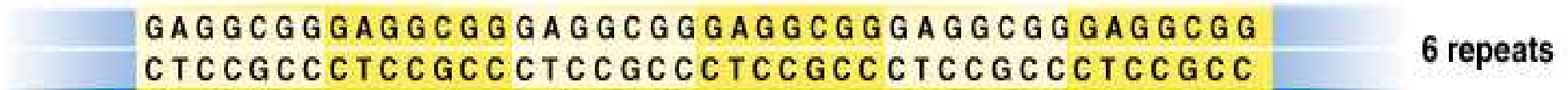
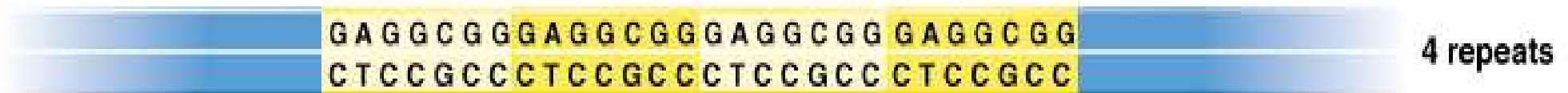
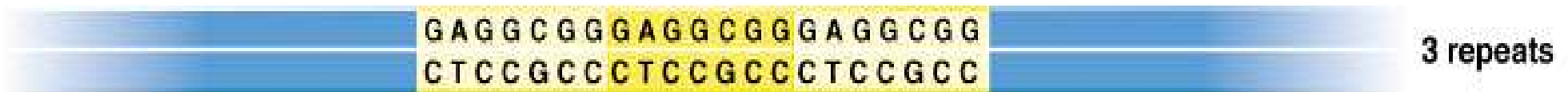
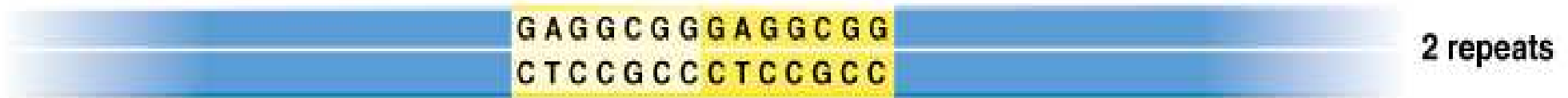
Použití restrikčních enzymů - RFLP

Restriction Fragment Length Polymorphism



Variable Number Tandem Repeats

9 – 100 pb

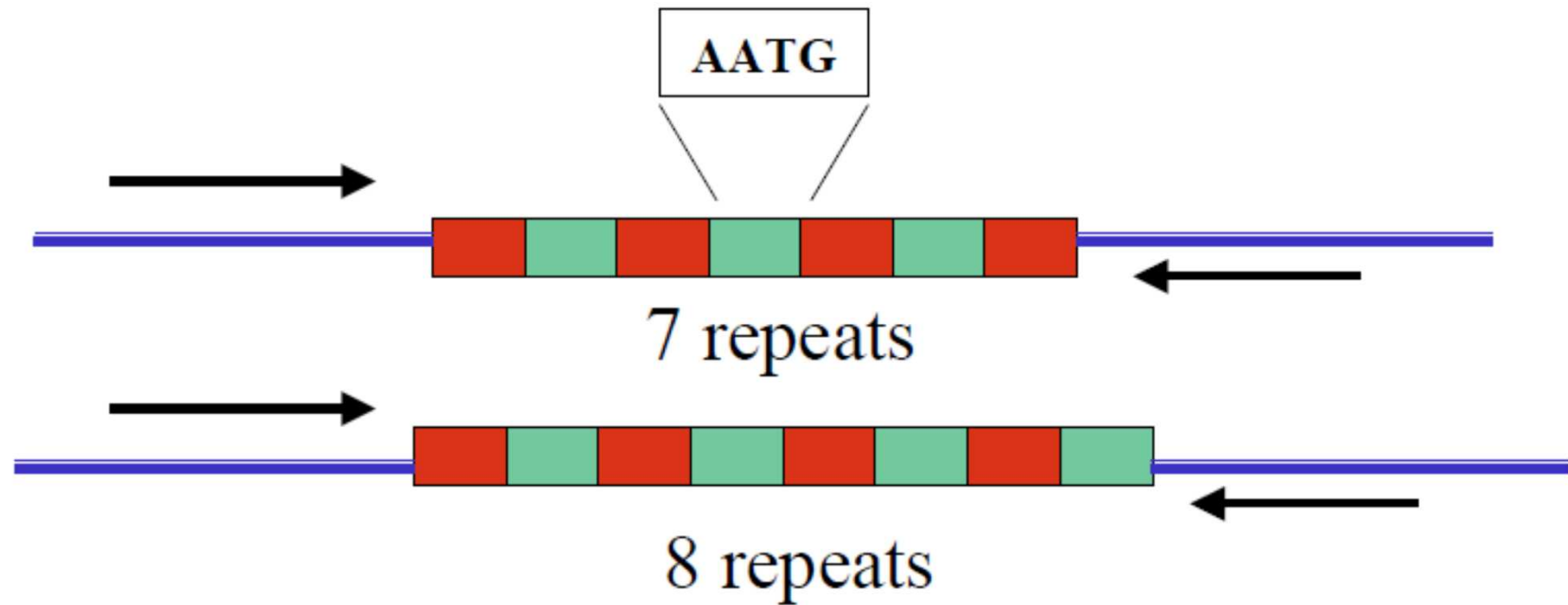


Short tandem repeats

2 – 6 pb

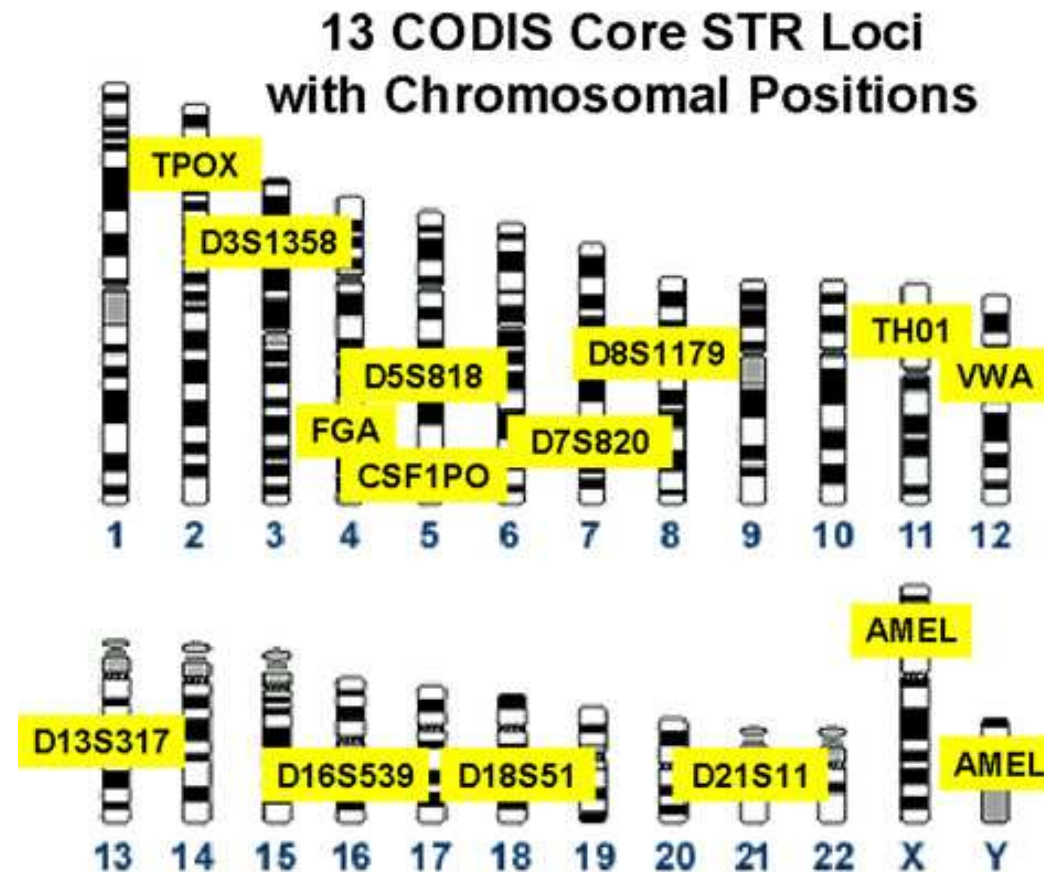
Repeat monomer	P [%]	REI [%]	α_B	α_R
A/T	59	1.75	1.0	0.2
C/G	41	0.12	1.1	0.4
AG/CT	27	0.27	2.6	0.24
AC/GT	20	0.66	2.6	
AT/TA	25	0.78	2.6	
CG/GC	10	0.02	2.6	
AAC/GTT	9	0.22	3.5	0.55
AAG/CTT	12	0.053	3.5	
AGG/CCT	11	0.077	3.5	

Short tandem repeats



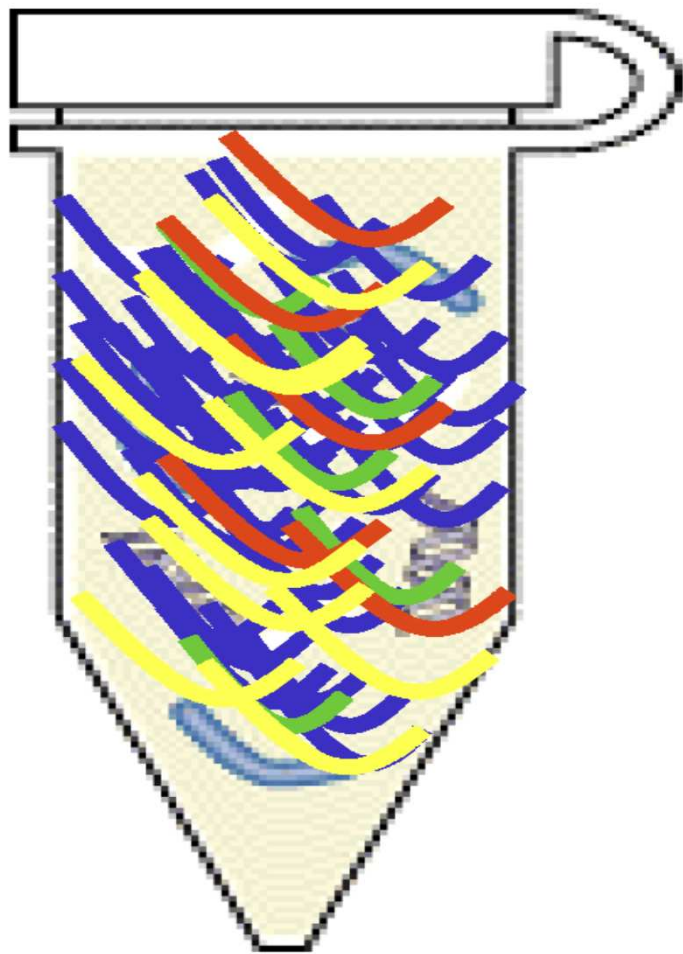
- Homozygot – obě alely stejné
- Heterozygot alely různě dlouhé

Short tandem repeats



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

Multiplex PCR

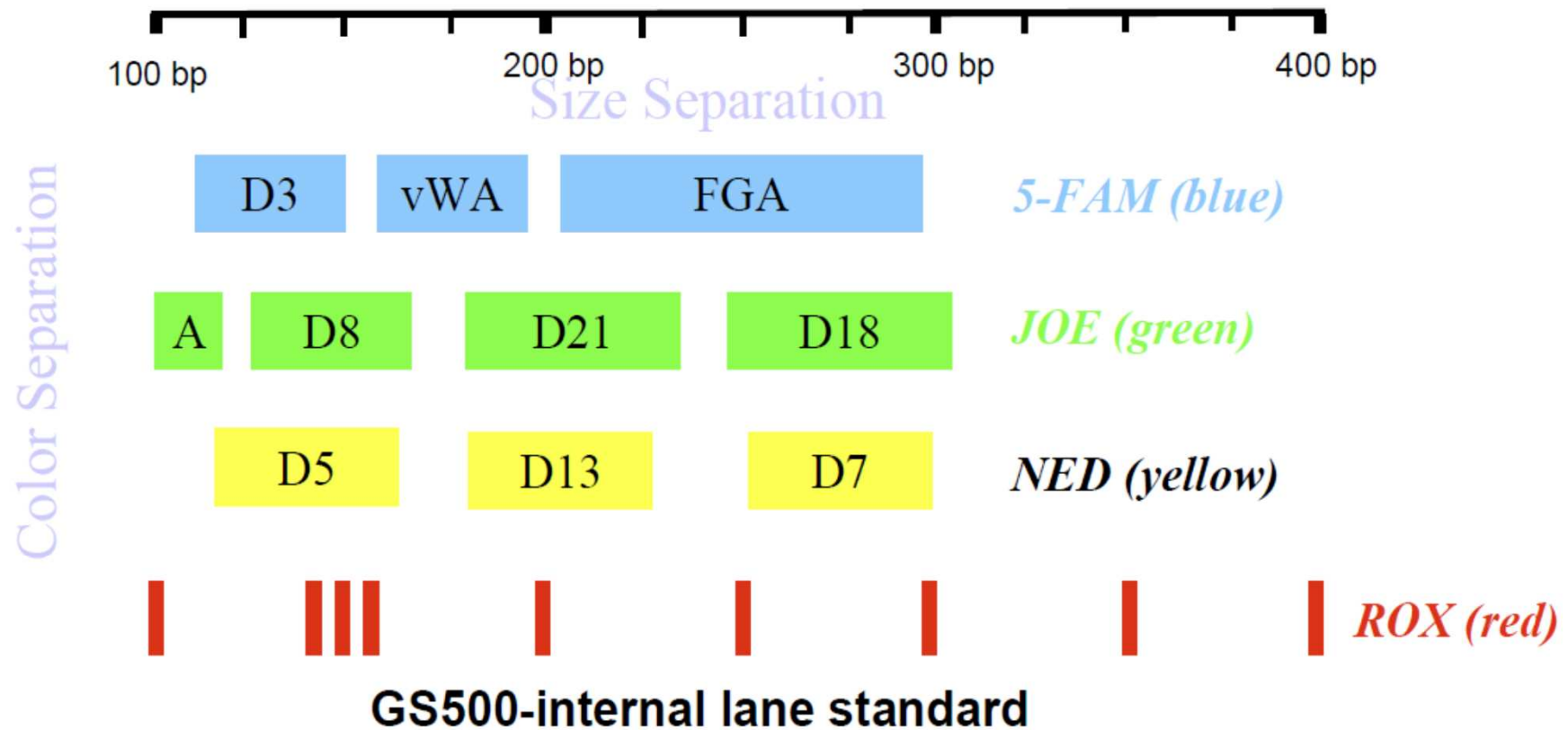


- 10 STR najednou
- Citlivost 1 ng DNA
- Možnost zpracovat směsi a degradované vzorky
- Různé fluorescenční barvičky pro překrývající se alely

An Example Forensic STR Multiplex Kit

AmpFISTR® Profiler Plus™

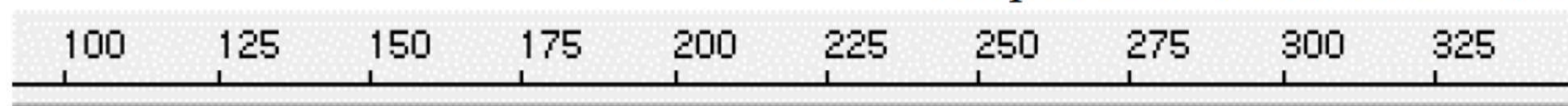
Kit available from PE Biosystems (Foster City, CA)



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

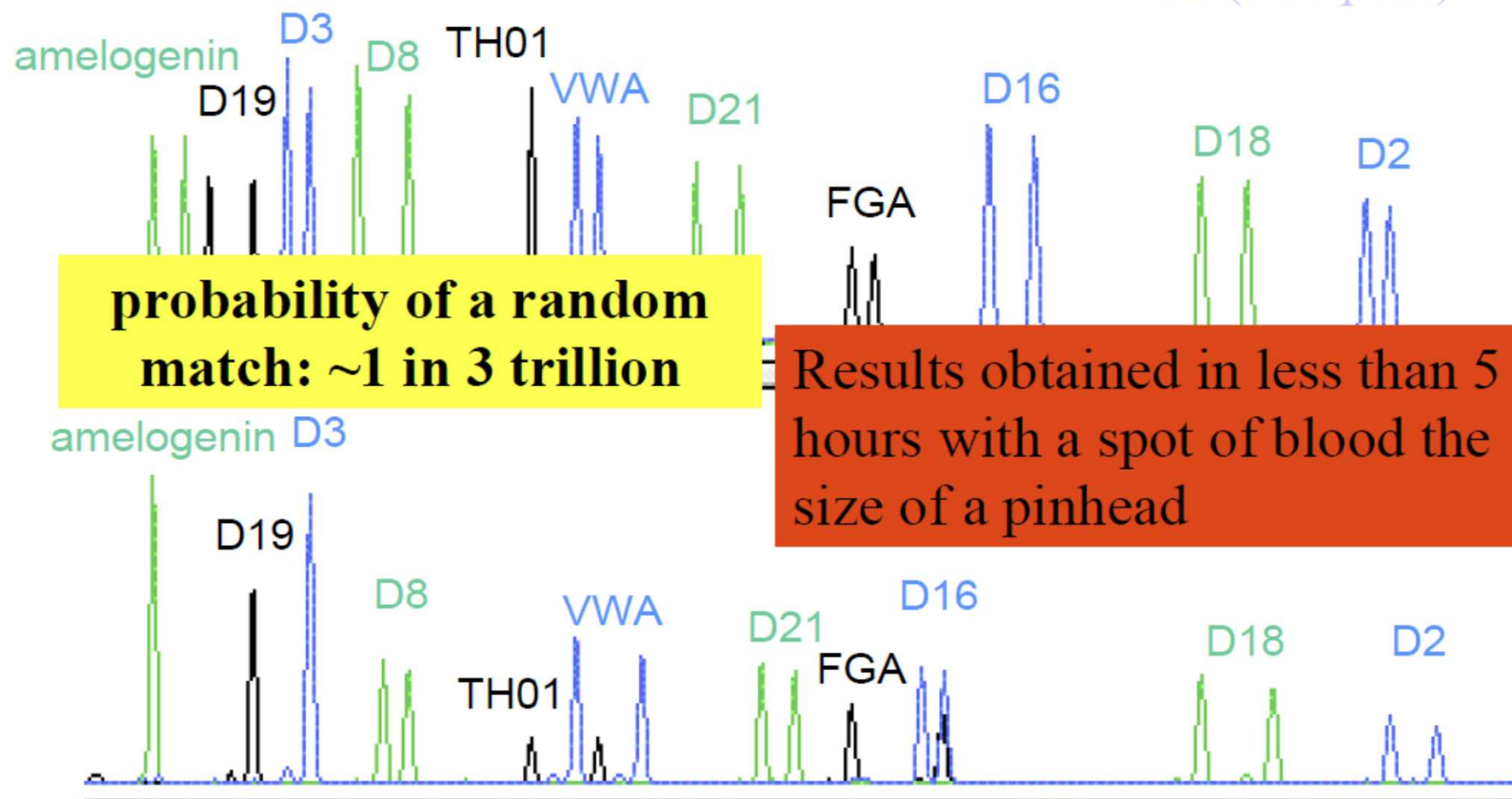
Human Identity Testing with Multiplex STRs

AmpFISTR® SGM Plus™ kit



DNA Size (base pairs)

Two different individuals



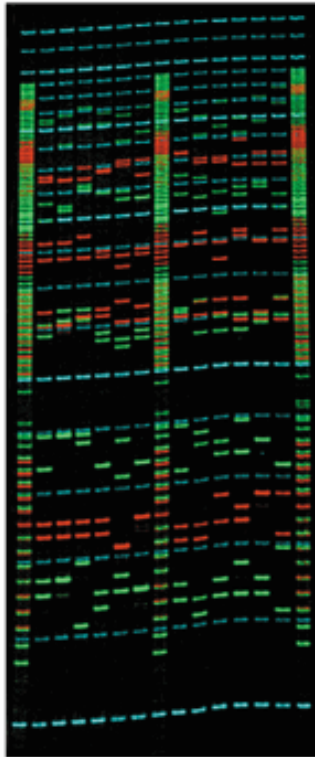
probability of a random match: ~1 in 3 trillion

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

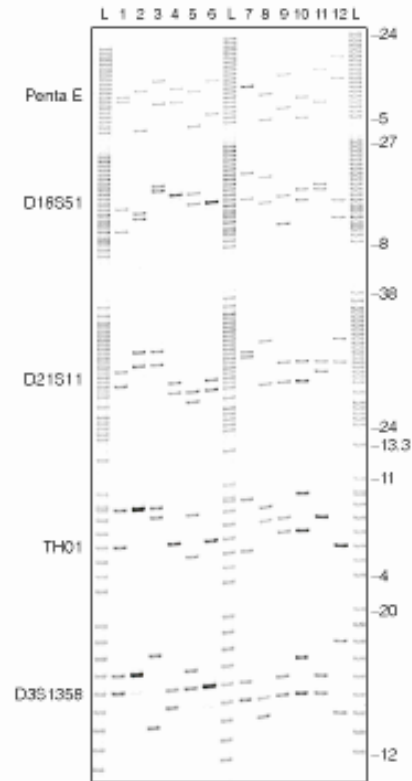
Simultaneous Analysis of 10 STRs and Gender ID

Short tandem repeats

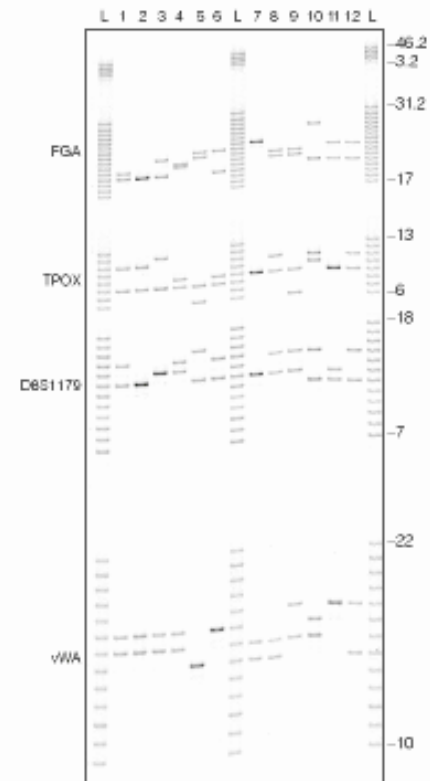
A.



B.

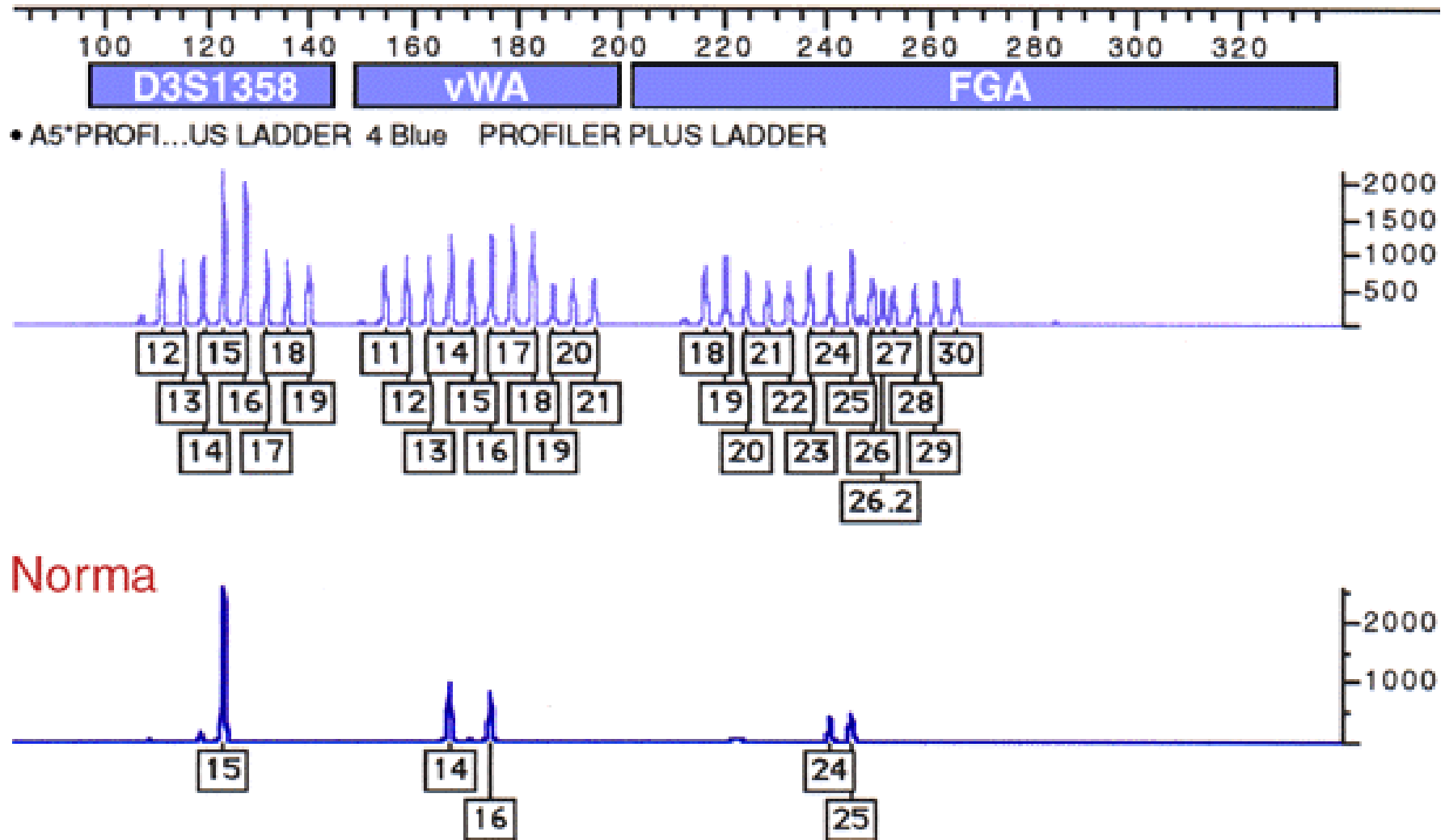


C.





Short tandem repeats



Short tandem repeats



X - 106 Y - 112

Vzorky biologického materiálu

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



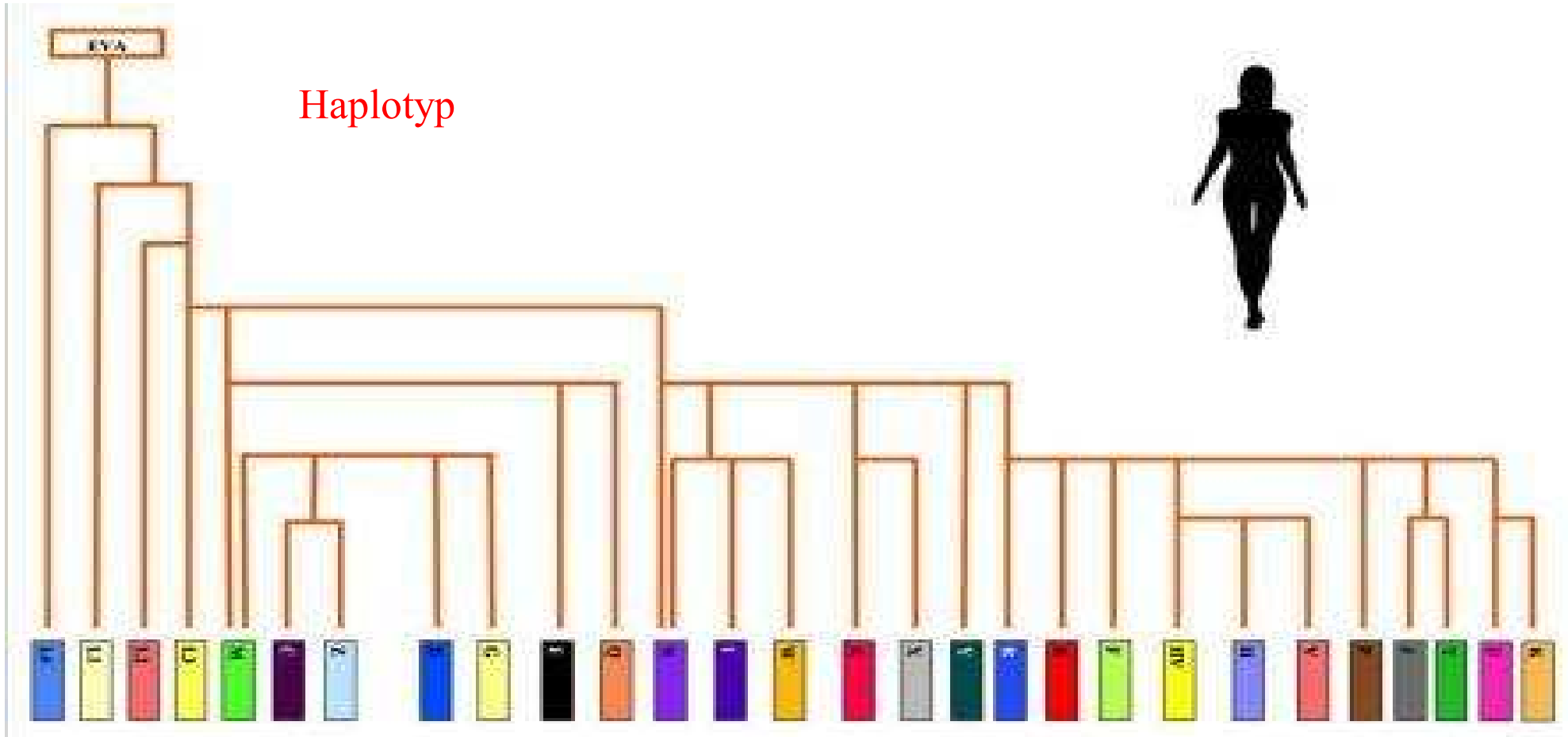
Testy paternity

Zjednodušené testy

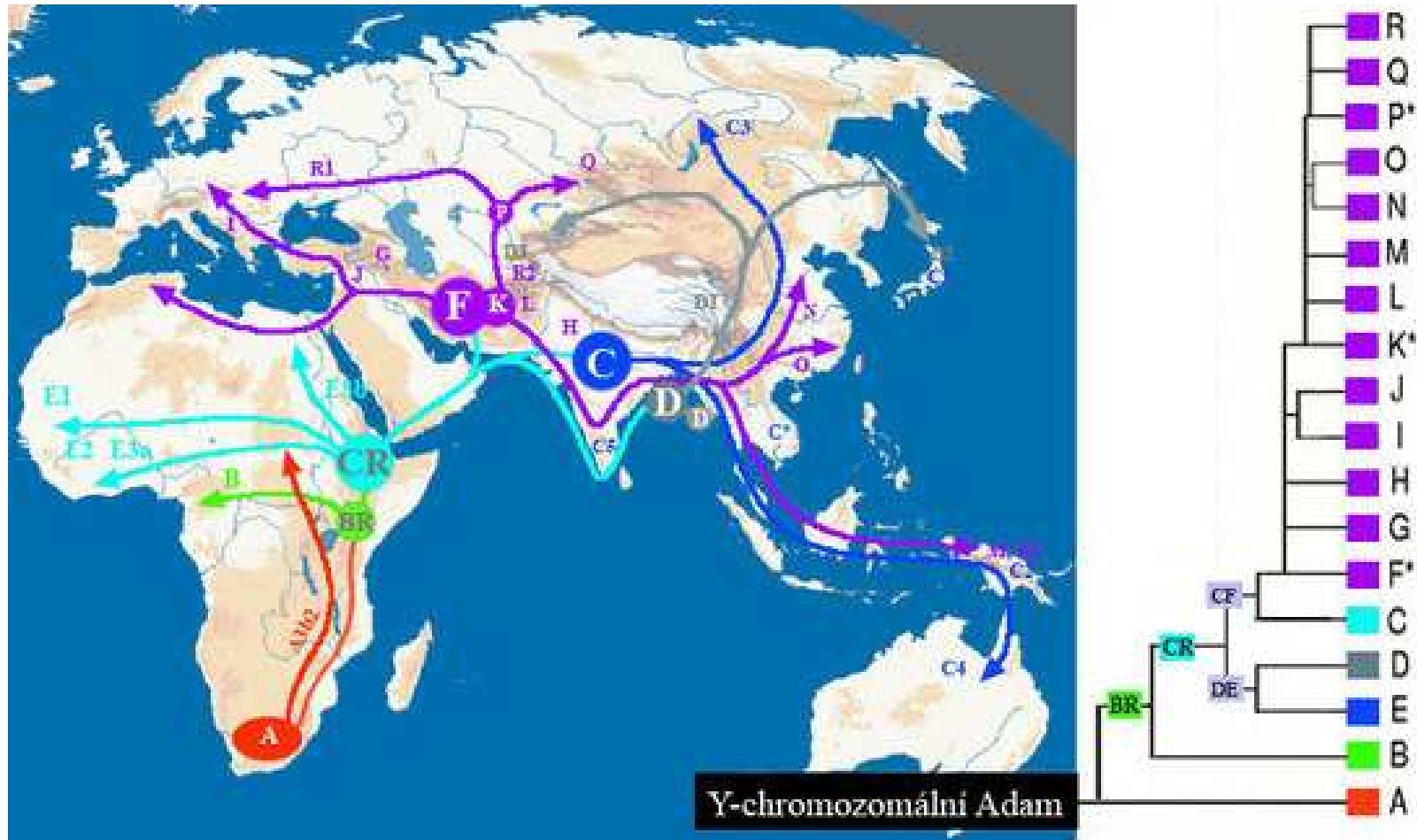
- STR na Y chromosomu – mužských potomků srovnání s otcem
- Mitochondriální DNA – dědí se po matce – matroklinní dědičnost

Mitochondriální Eva před 200 000 let v Africe

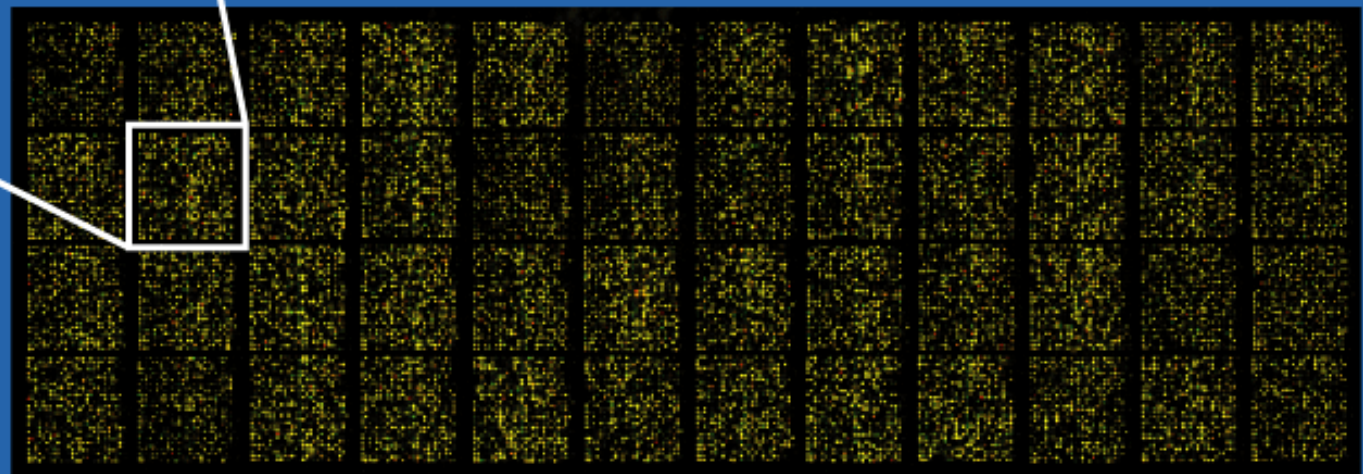
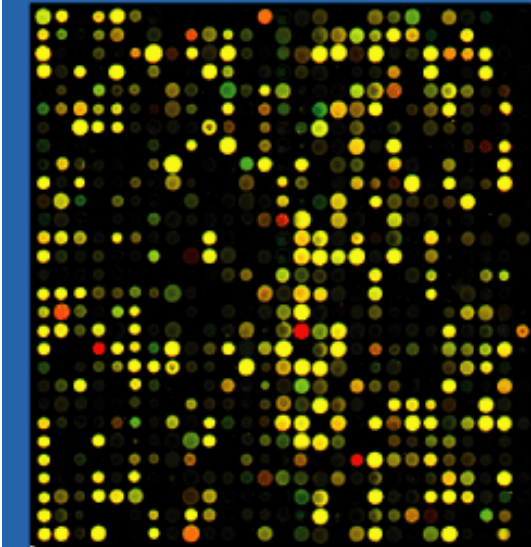
Haplotyp



Y-chromozomální Adam před 110 000 let v Africe



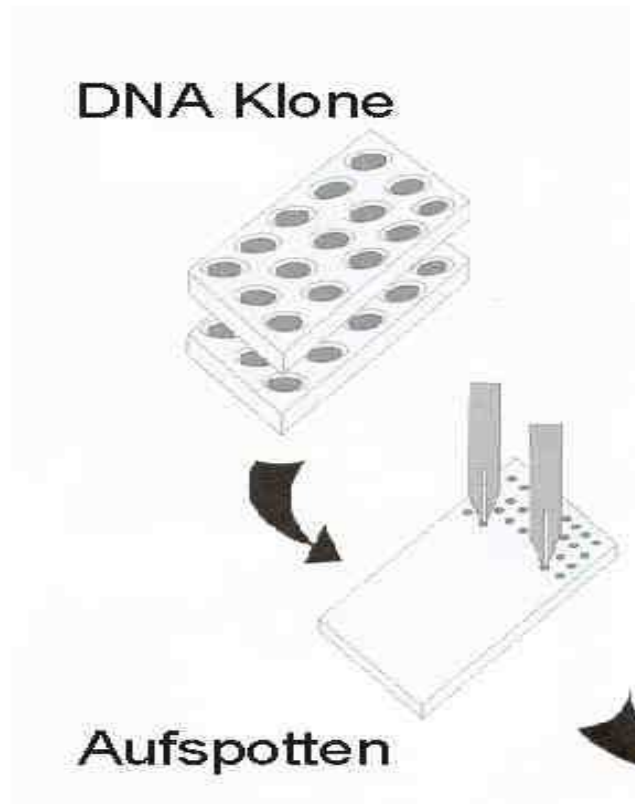
DNA chipy



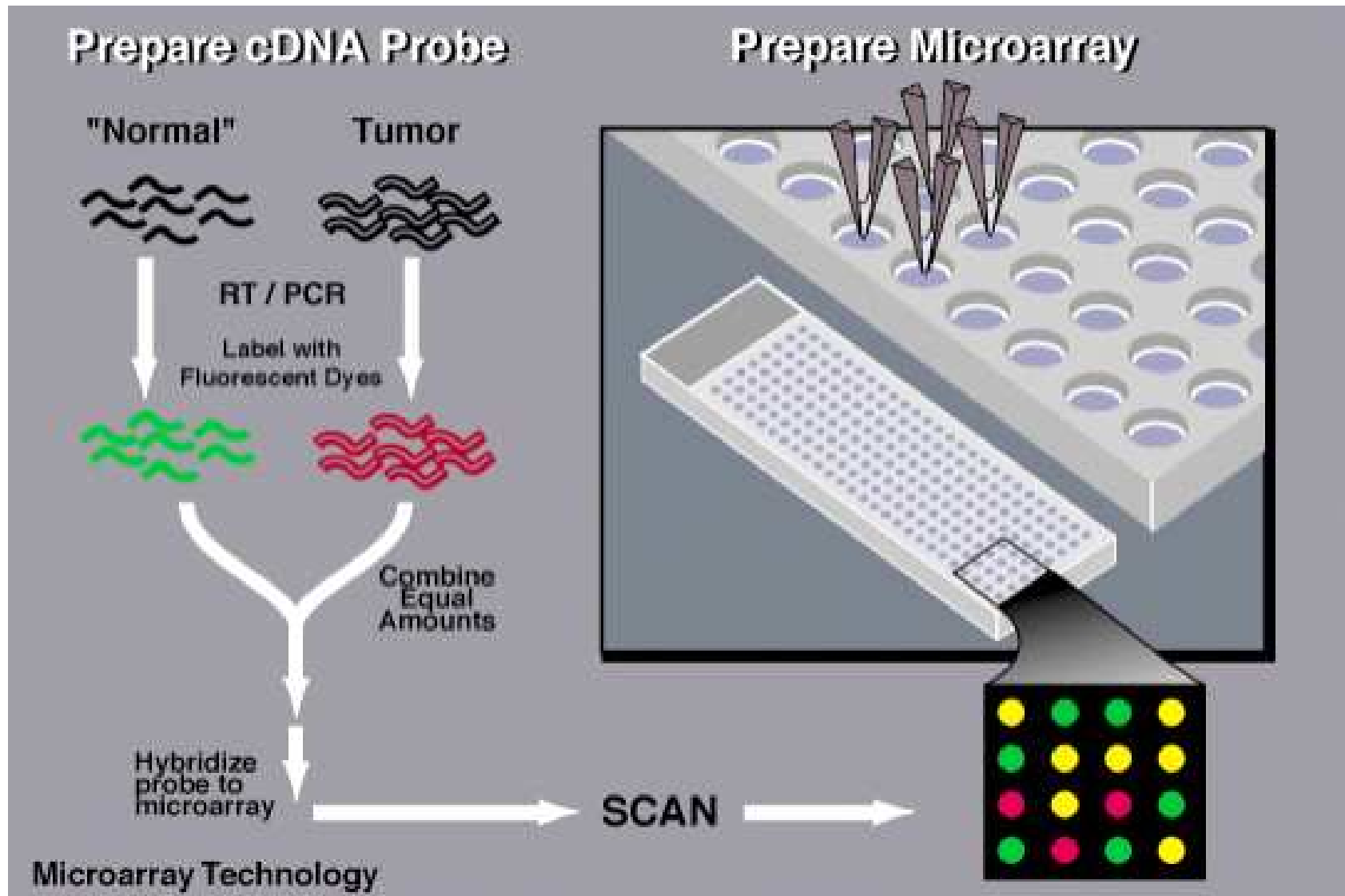
DNA chipy

Fotolitografie

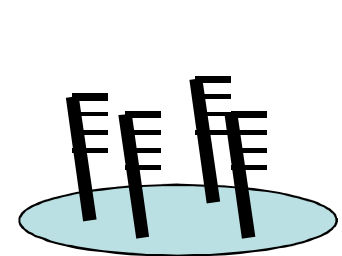
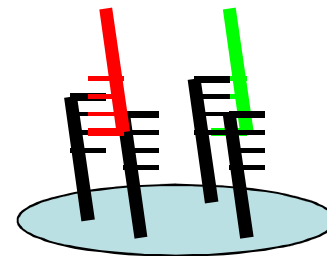
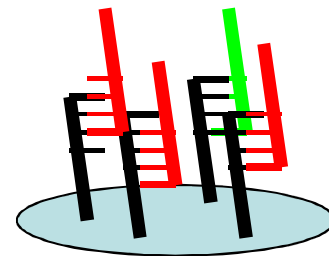
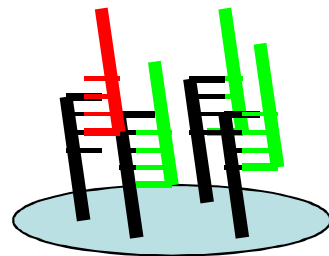
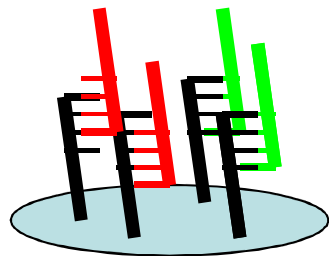
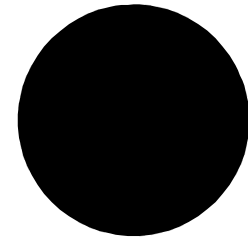
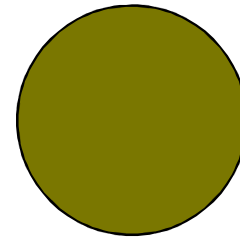
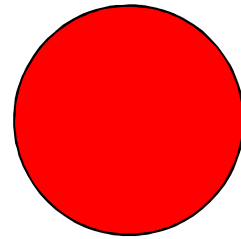
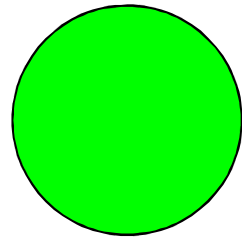
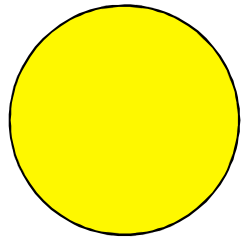
Kapkování



DNA chipy



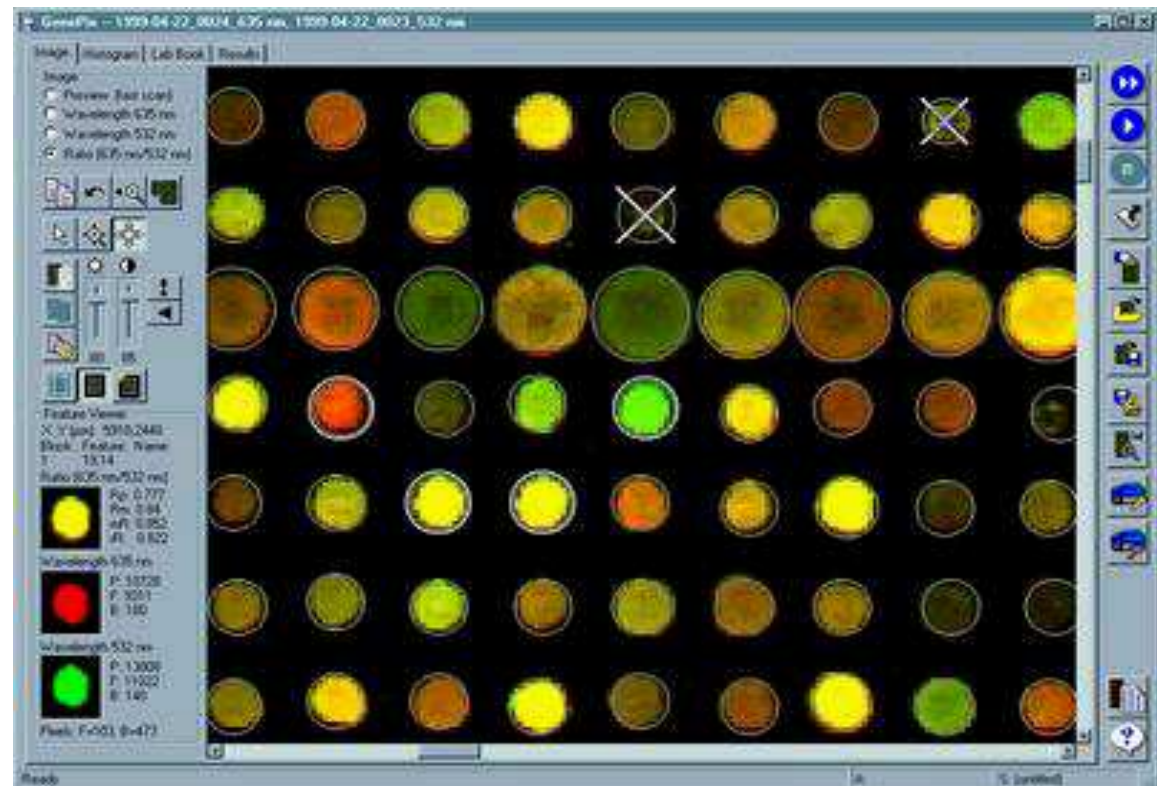
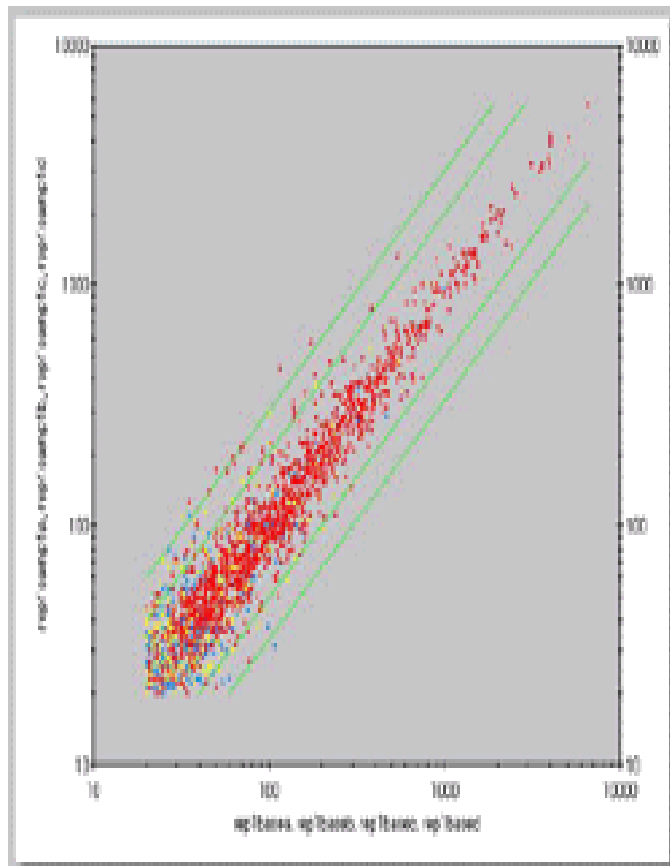
DNA chipy - barva skvrn



DNA chipy - vybavení



DNA chip software

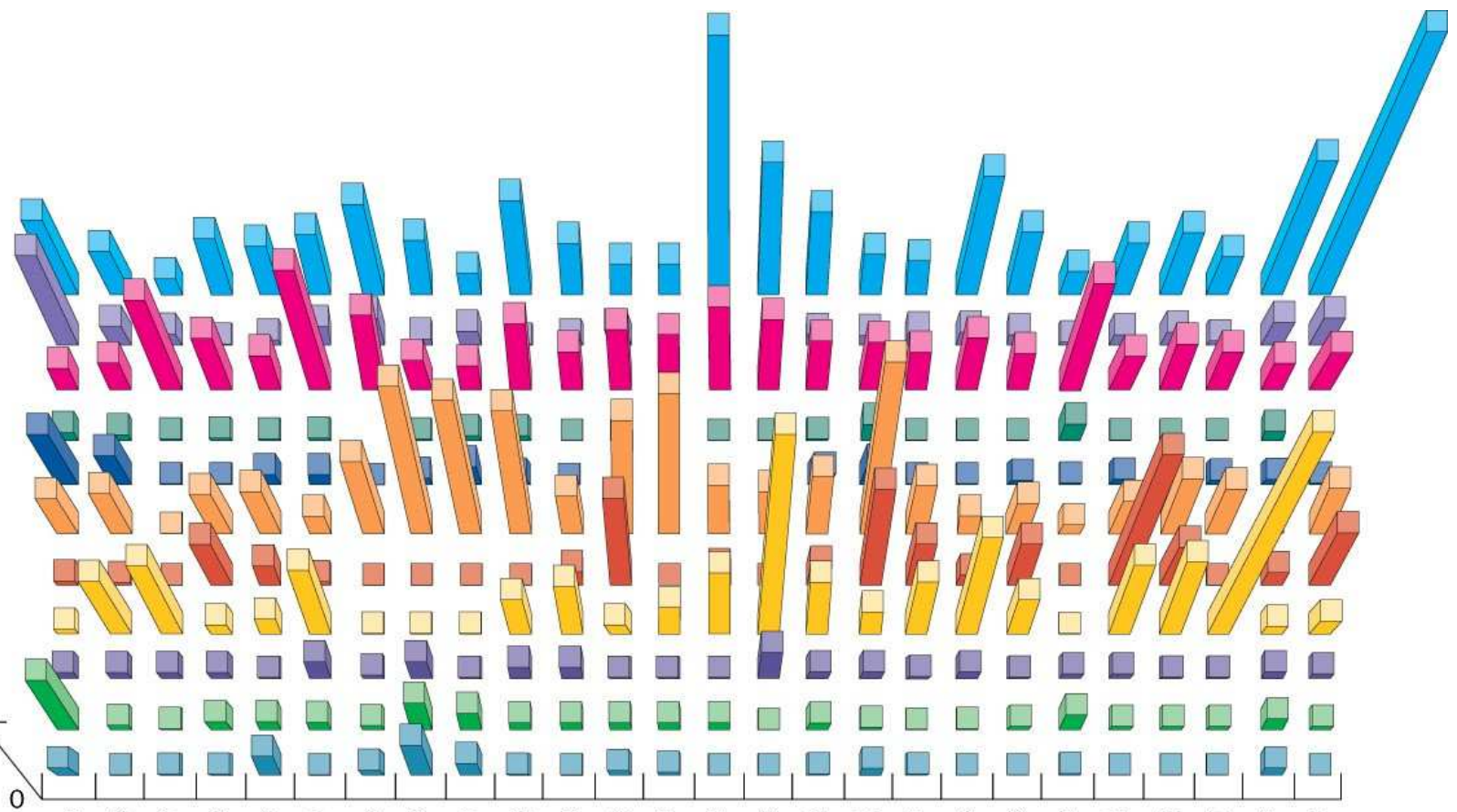


- Cyclin A
- Cyclin B
- Cyclin C
- Cyclin D1
- Cyclin D2
- Cyclin D3
- Cyclin E
- Cyclin F
- Cyclin G
- Cyclin H
- Cyclin I

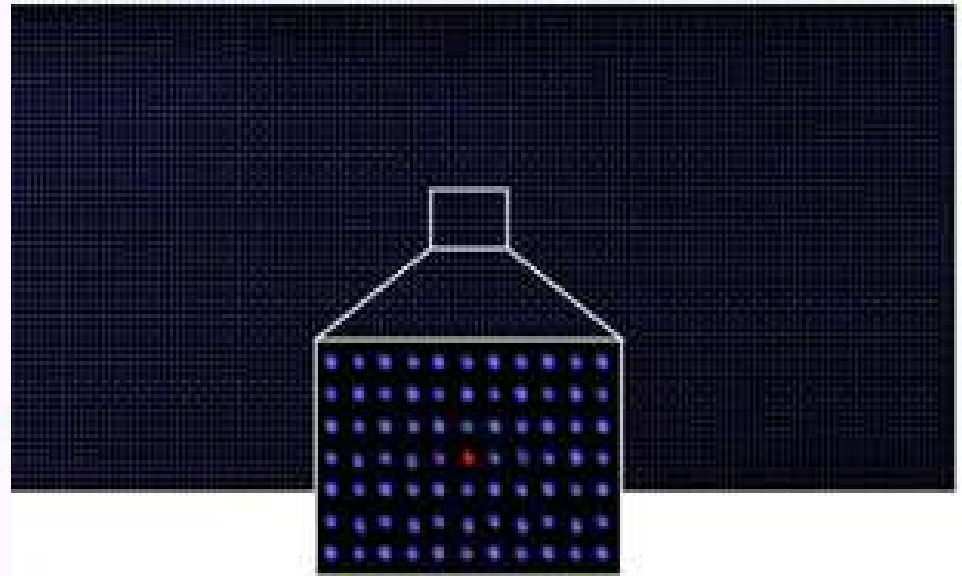
Expression
(arbitrary units)

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

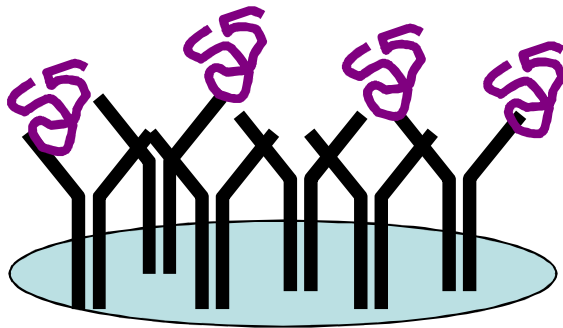


Proteinové chipy

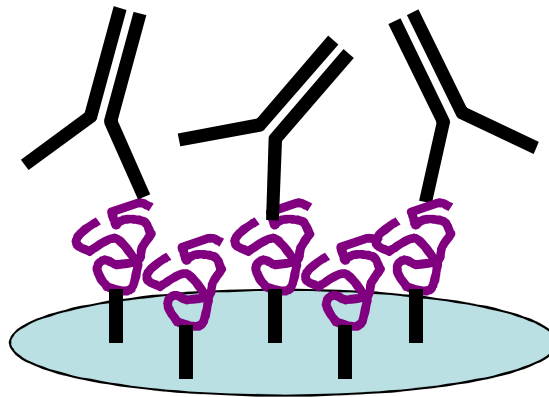


Proteinové chipy – typy interakcí

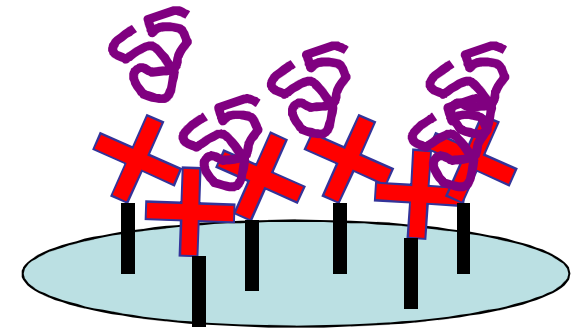
Protilátka



Antigen

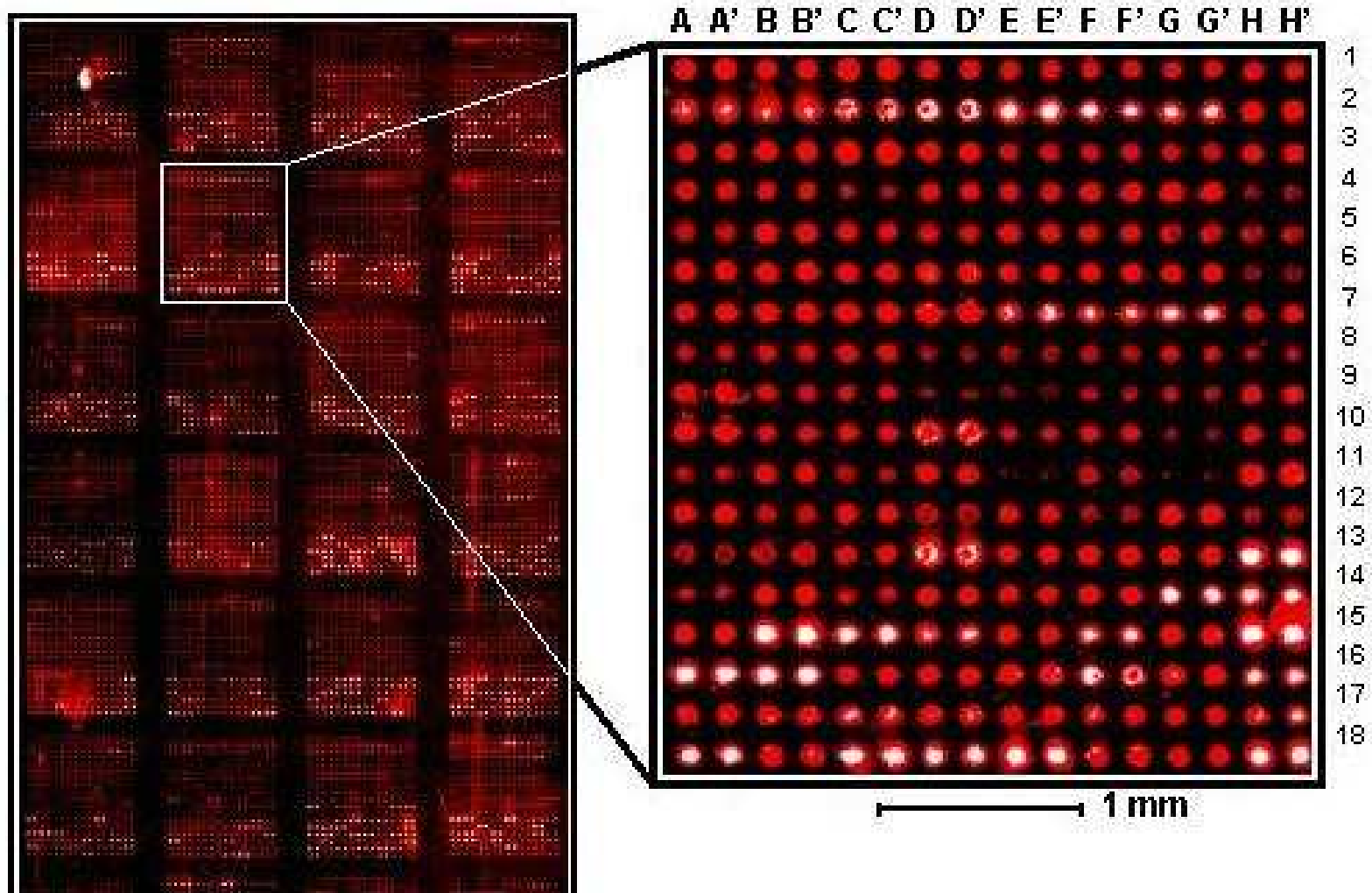


Ligand



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

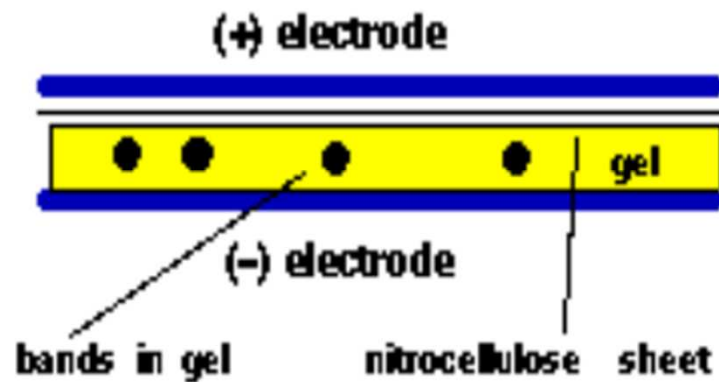
Anti-GST Probe



Blotting

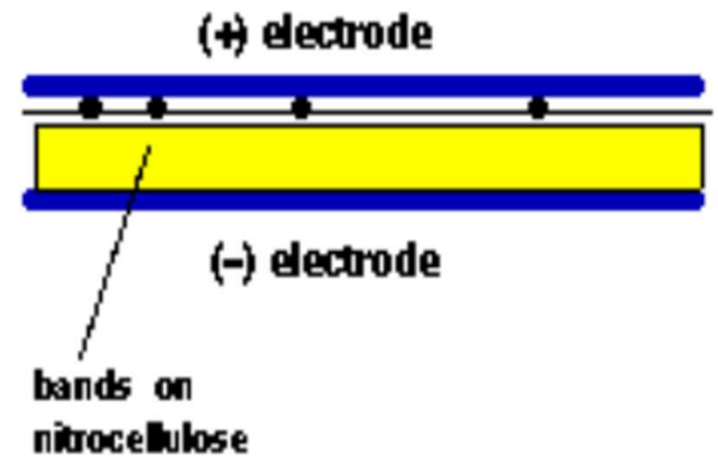
Side View:

Before Transfer:



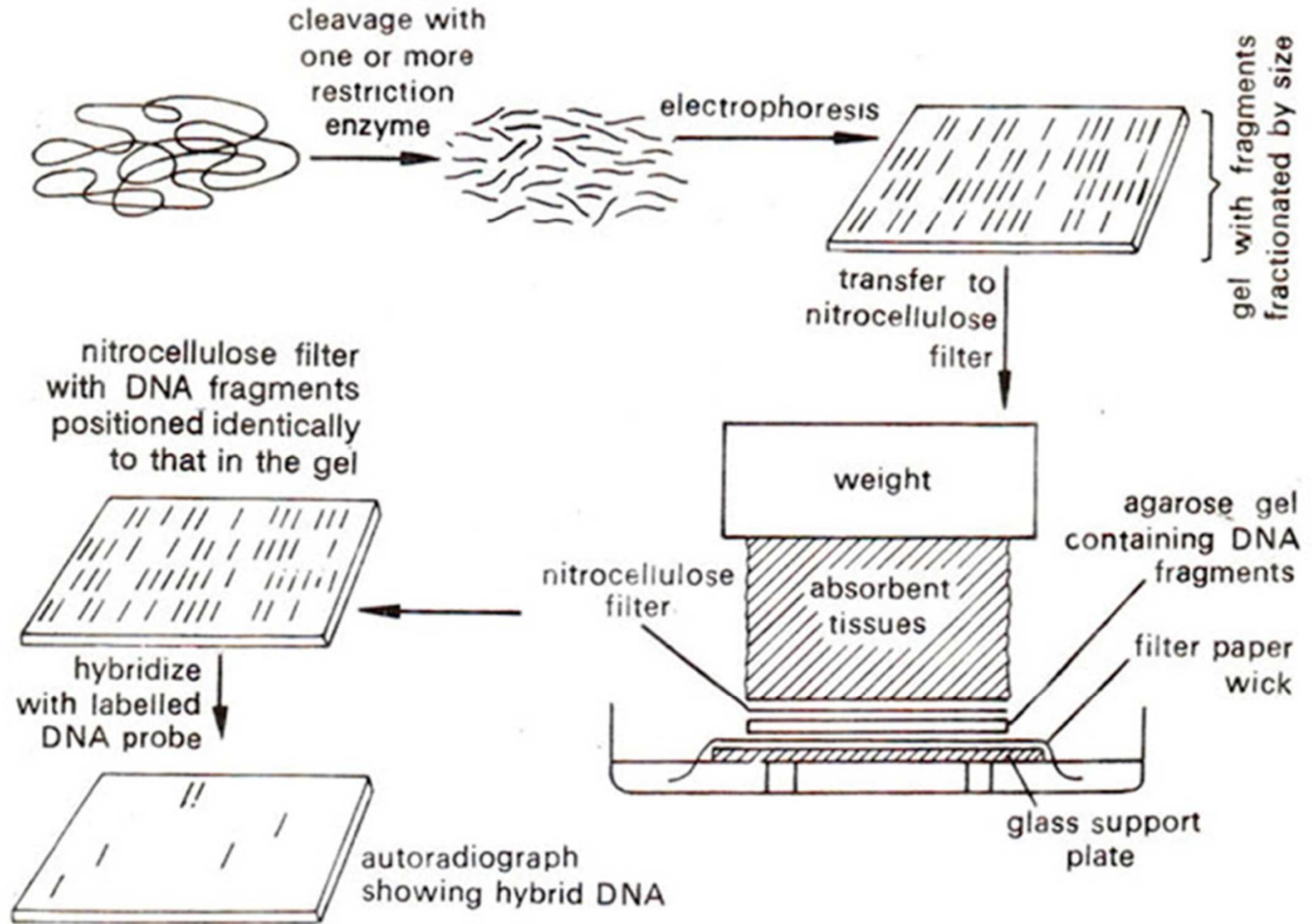
▲
Direction of
Transfer
(electric field)

After Transfer:



Note: All the layers are pressed tightly together.

Blotting NK



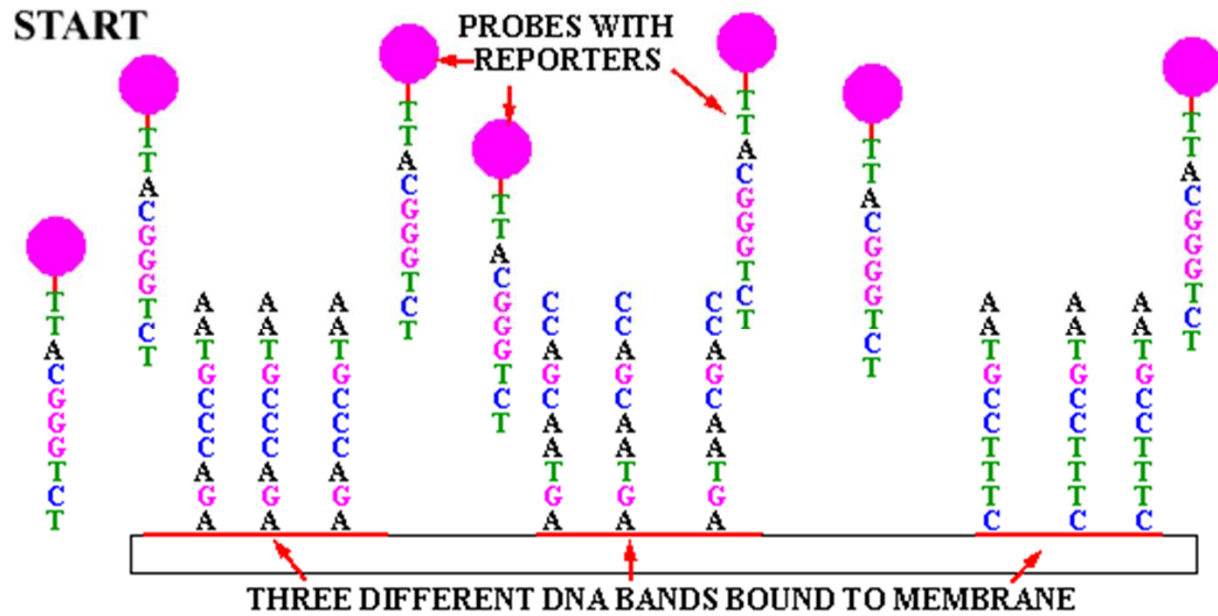
Detekce

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

Nejpoužívanější metody

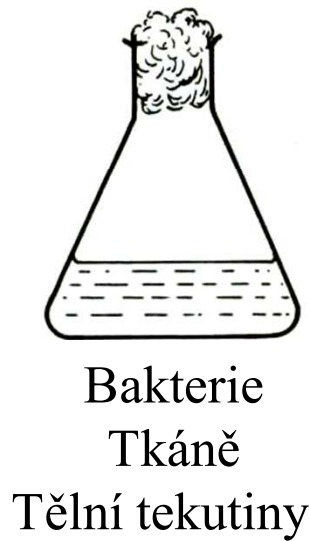
- na základě rozdílné rozpustnosti – extrakce, srážení
- na základě vlastností - chromatografie – polarita-adsorpční, náboj-ionexová
 - elektroforéza – velikost
- sedimentace - gradientová ultracentrifugace

Postup

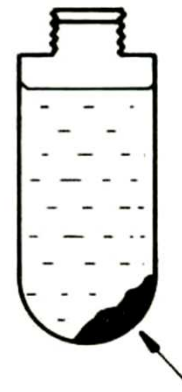
1. Rozbití buněk a membrán pro uvolnění NK
2. Inaktivace DNA- nebo RNA-degradujících enzymů (DNasy, RNasy).
3. Separace NK od dalších komponent uvolněných z buňky.
 - Extrakce/Precipitace
 - Chromatografie, elektroforéza
 - Ultracentrifugace

Extrakce/Precipitace

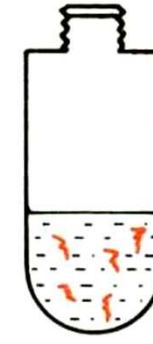
1 A culture of bacteria is grown and then harvested



Centrifugation



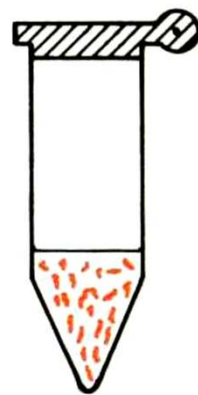
2 The cells are removed and broken to give a cell extract



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

- *Lysozym*
- 0.5% SDS + proteinase K
(55° několik hodin)

3. Fenolová extrakce

**Jemné třepání několik hodin
(pH 8)**

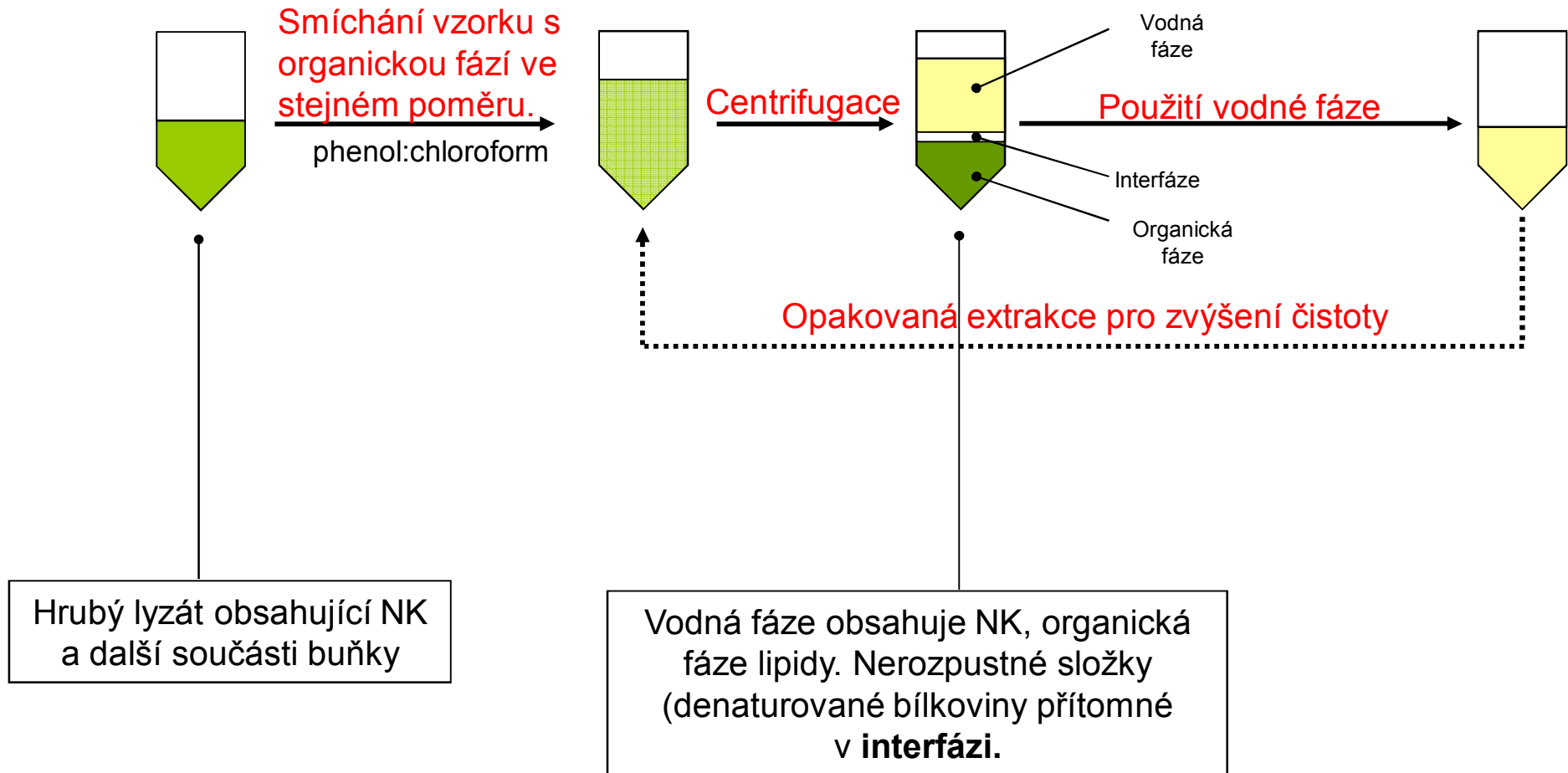
4. Ethanolová precipitace

5. Působení RNAsy a proteinasy K

6. Opakování kroku 3 a 4.

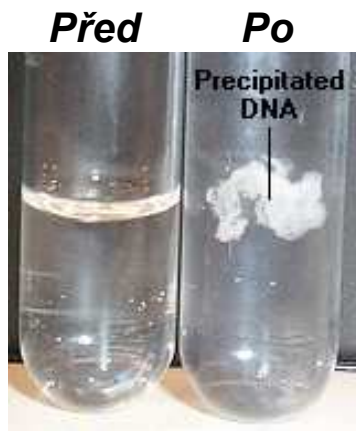
Extrakce/Precipitace

Krok 3: Organická extrakce



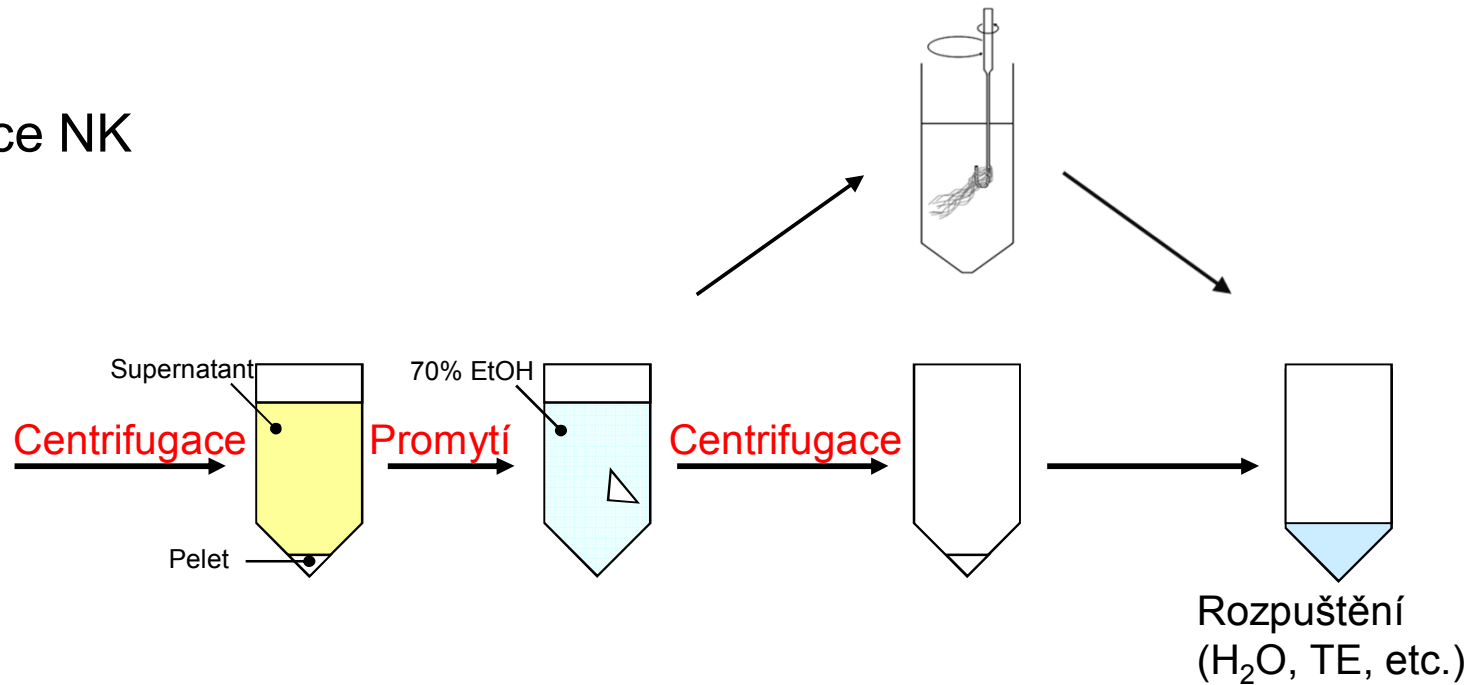
Extrakce/Precipitace

Krok 4: Precipitace NK



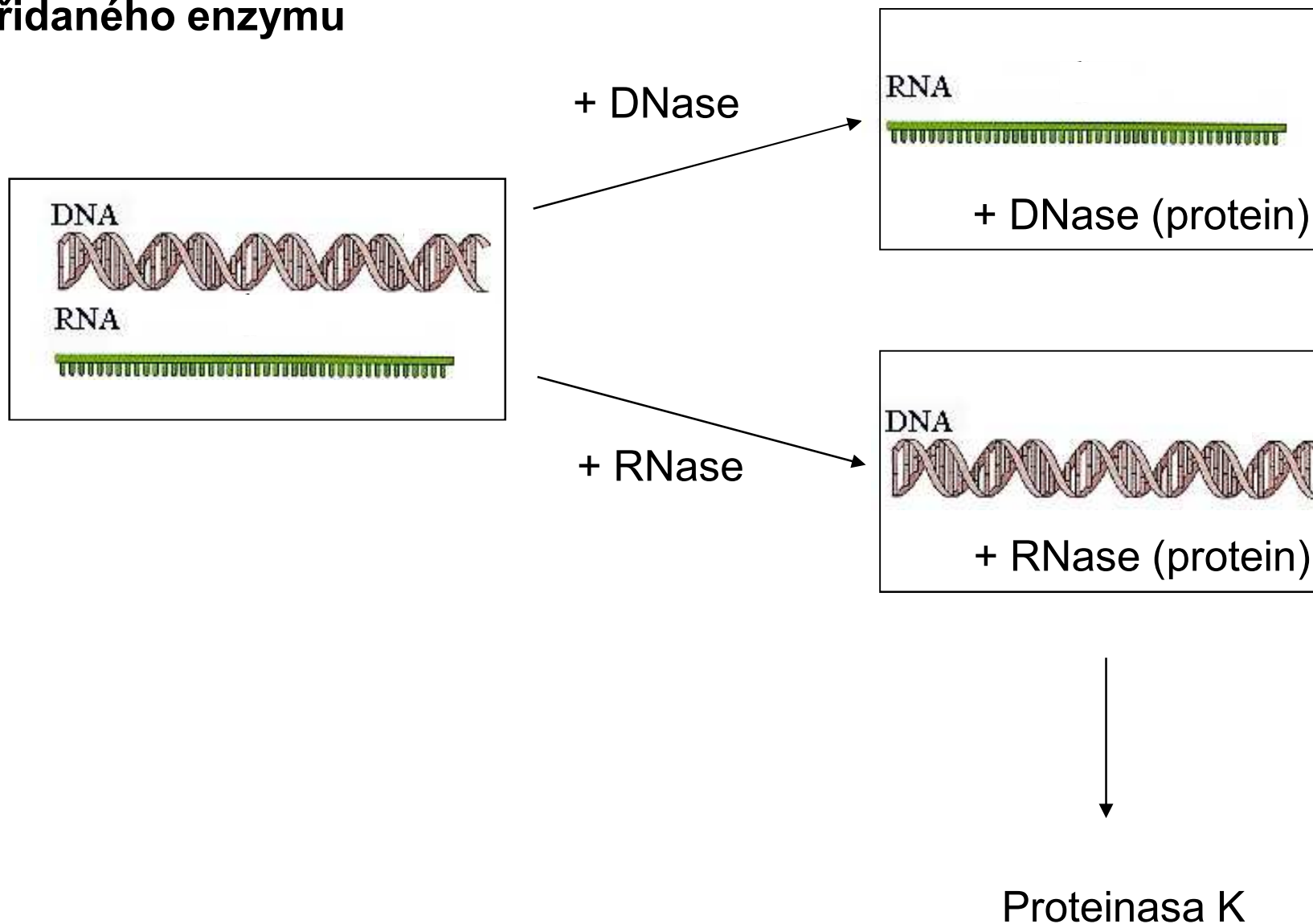
Přidání EtOH a soli

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



Detail kroku 5

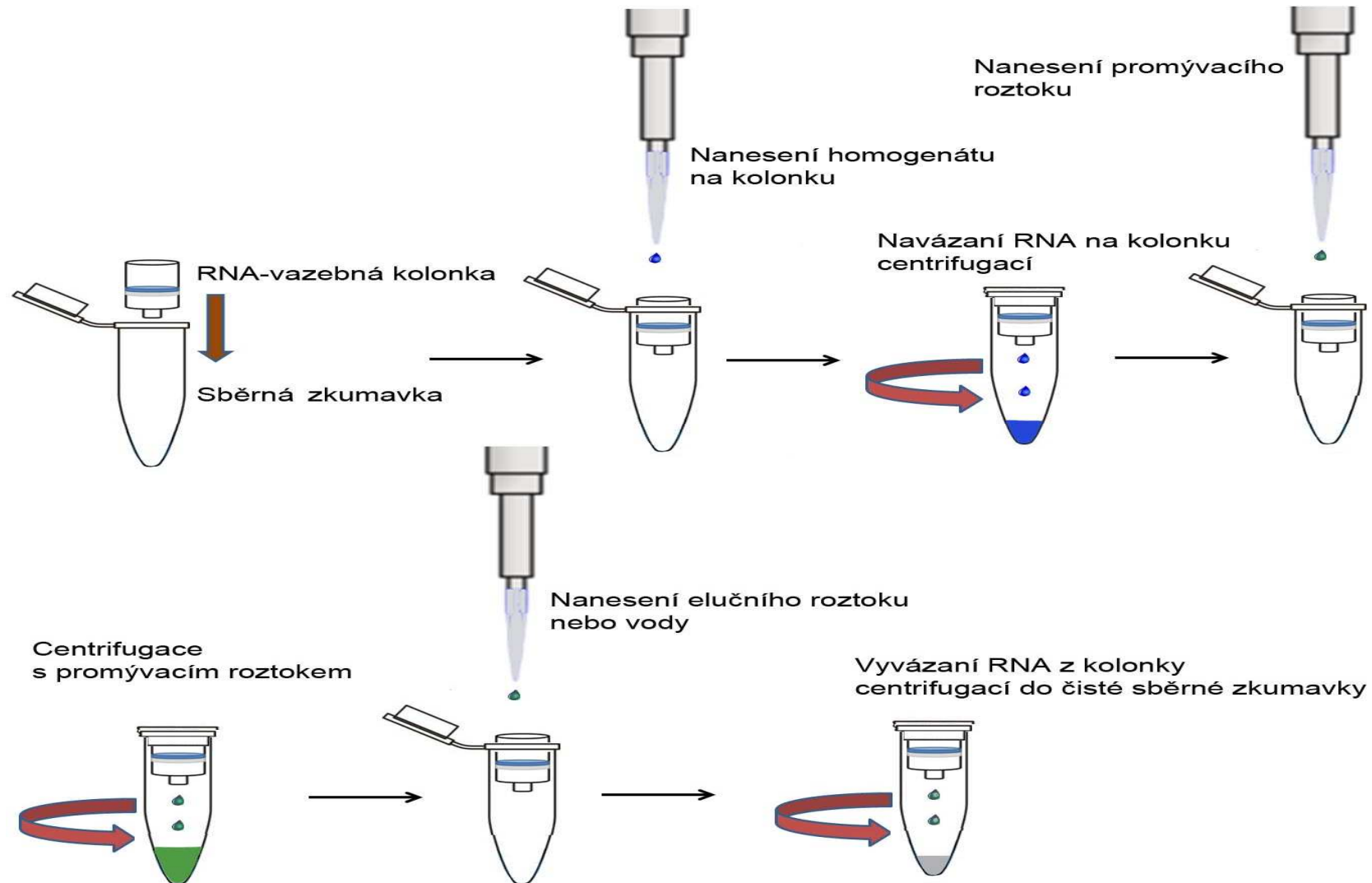
Použití nukleas pro odstranění nechtěné DNA nebo RNA
a proteas pro odstranění zbývajících proteinů včetně
přidaného enzymu



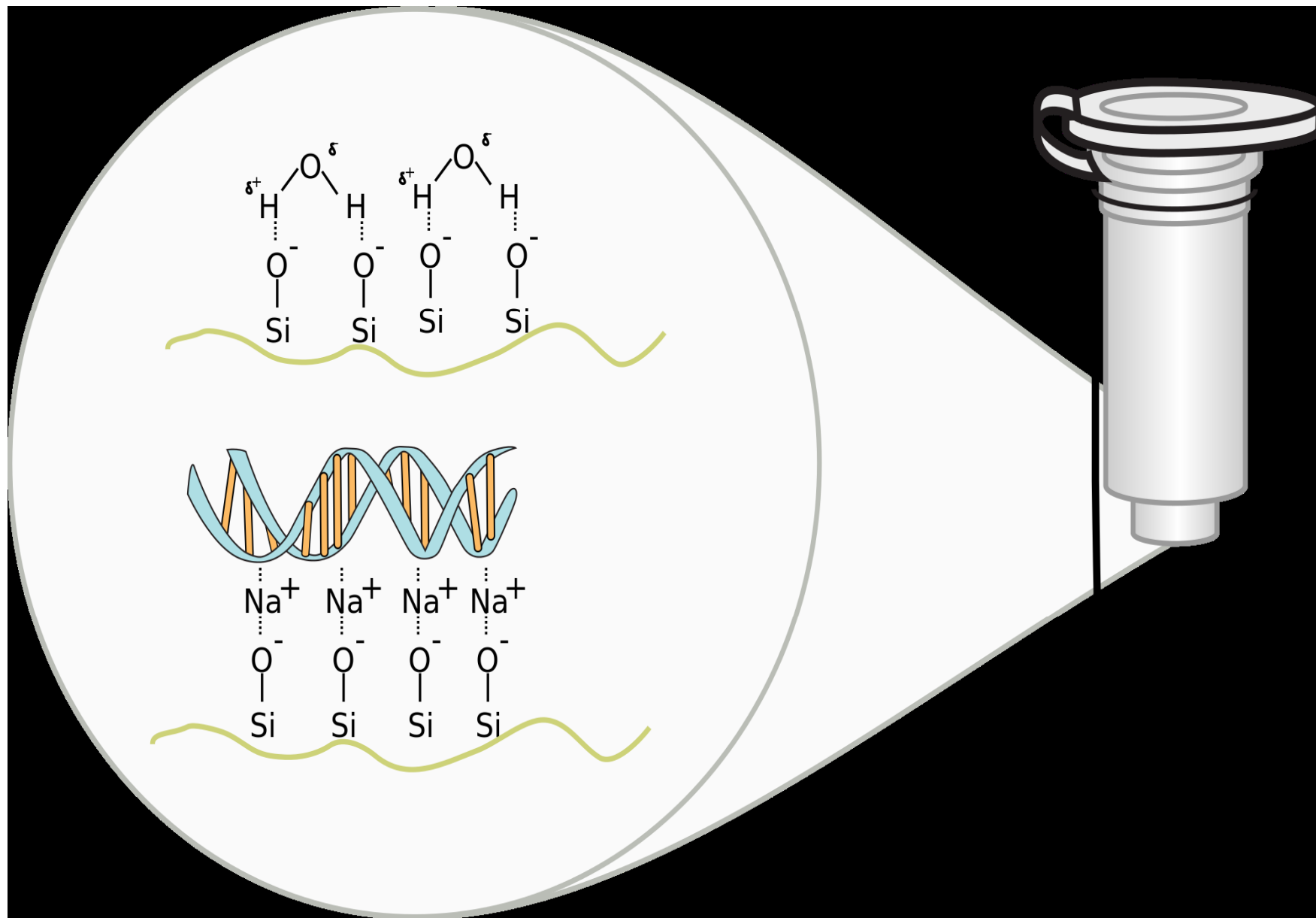
Chromatografie

Spin kolonky

Princip izolace RNA pomocí centrifugačních RNA vazebných kolonek

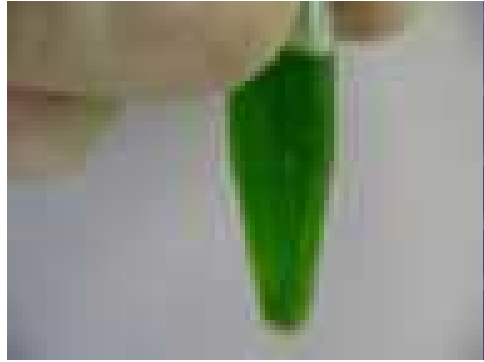


Adsorpční chromatografie



Adsorpční chromatografie

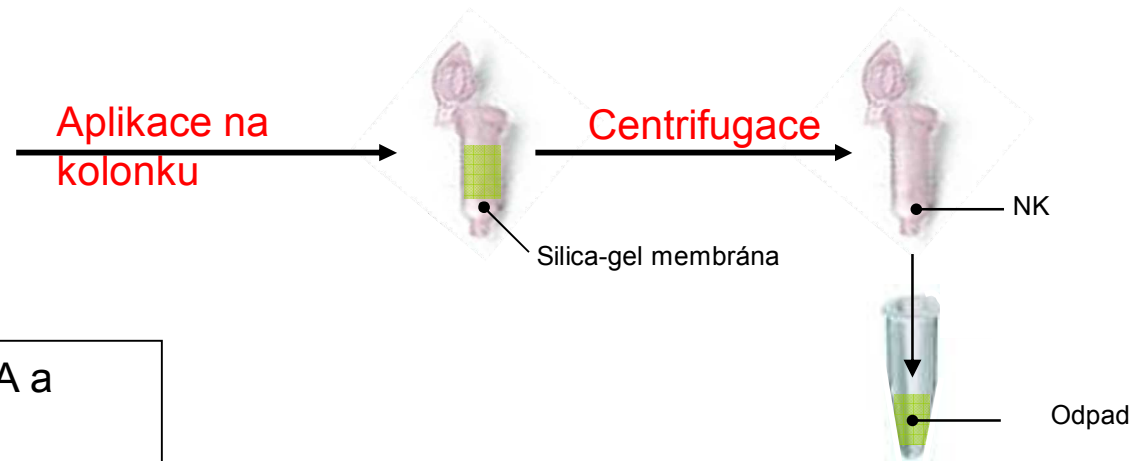
Krok 1: Příprava lyzátu



Extrakční pufr pro vazbu DNA a RNA na silikagel:

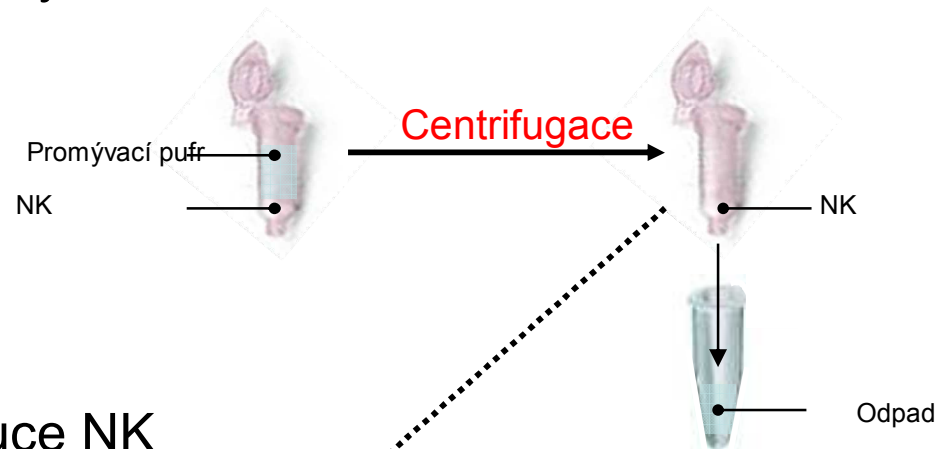
- nízké pH
- vysoká iontová síla
- chaotropní soli

Krok 2: Adsorpce na silikagel

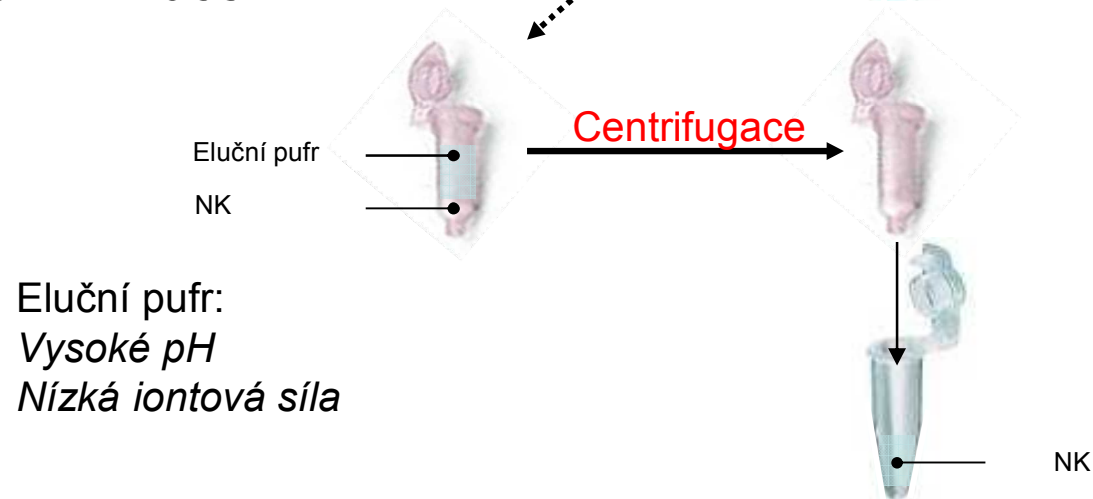


Adsorpční chromatografie

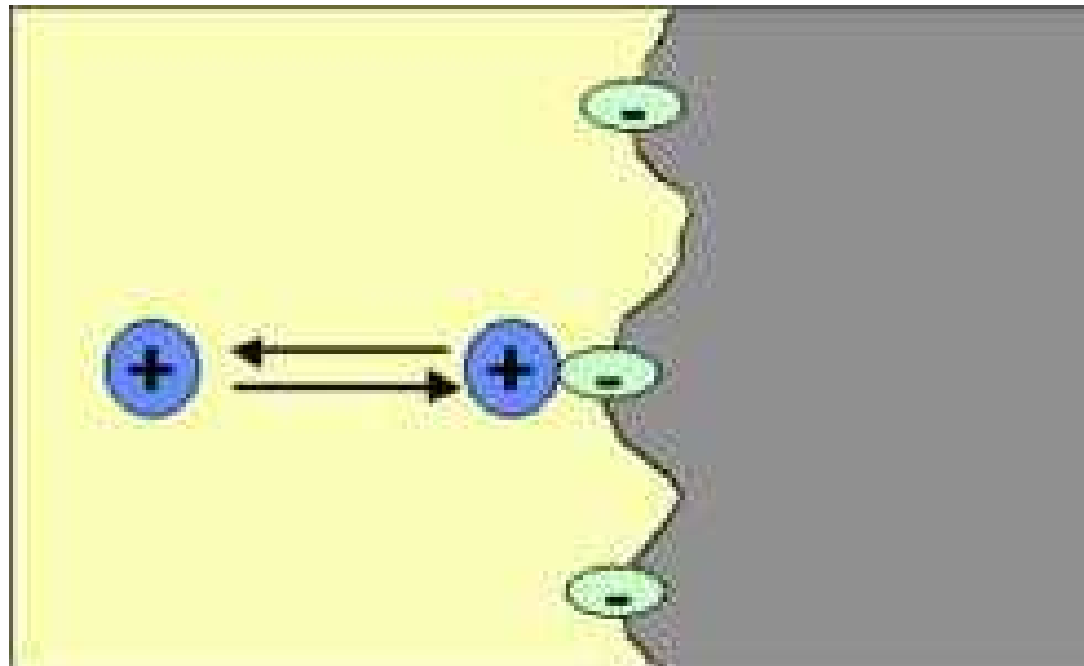
Krok 3: Vymytí kontaminant



Krok 4: Eluce NK

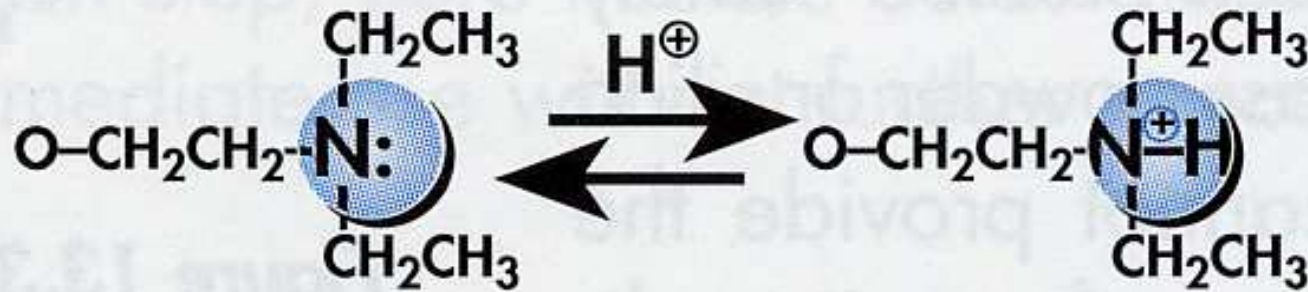


Ionexová chromatografie



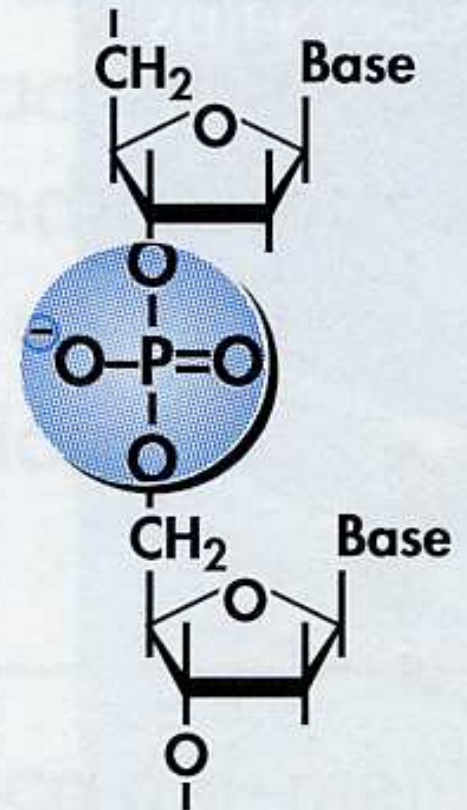
Ionexová chromatografie

Vazba při nízkém pH nízké I



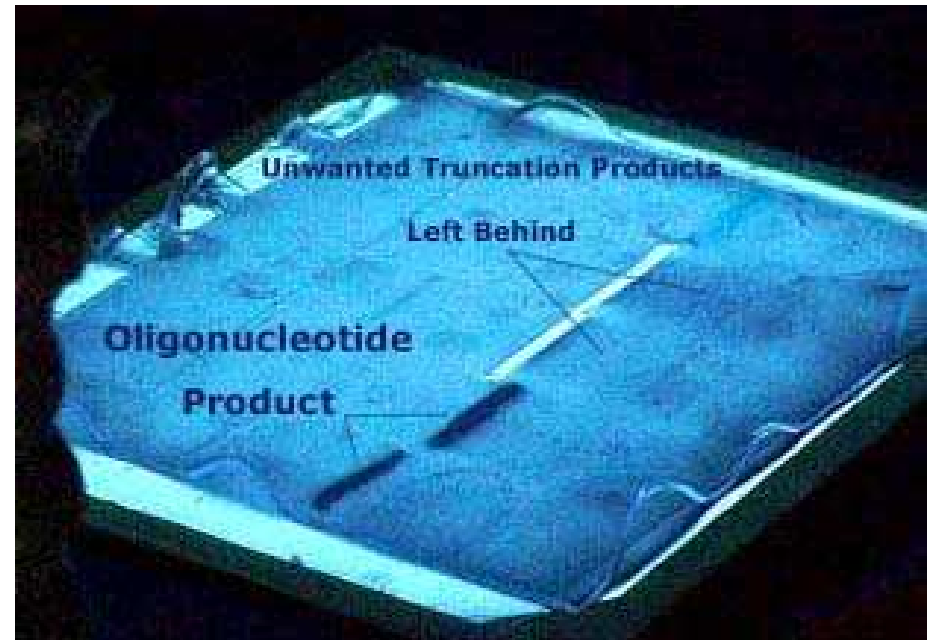
Eluce zvýšením pH nebo vysokou I

DEAE (diethylaminoethanol)

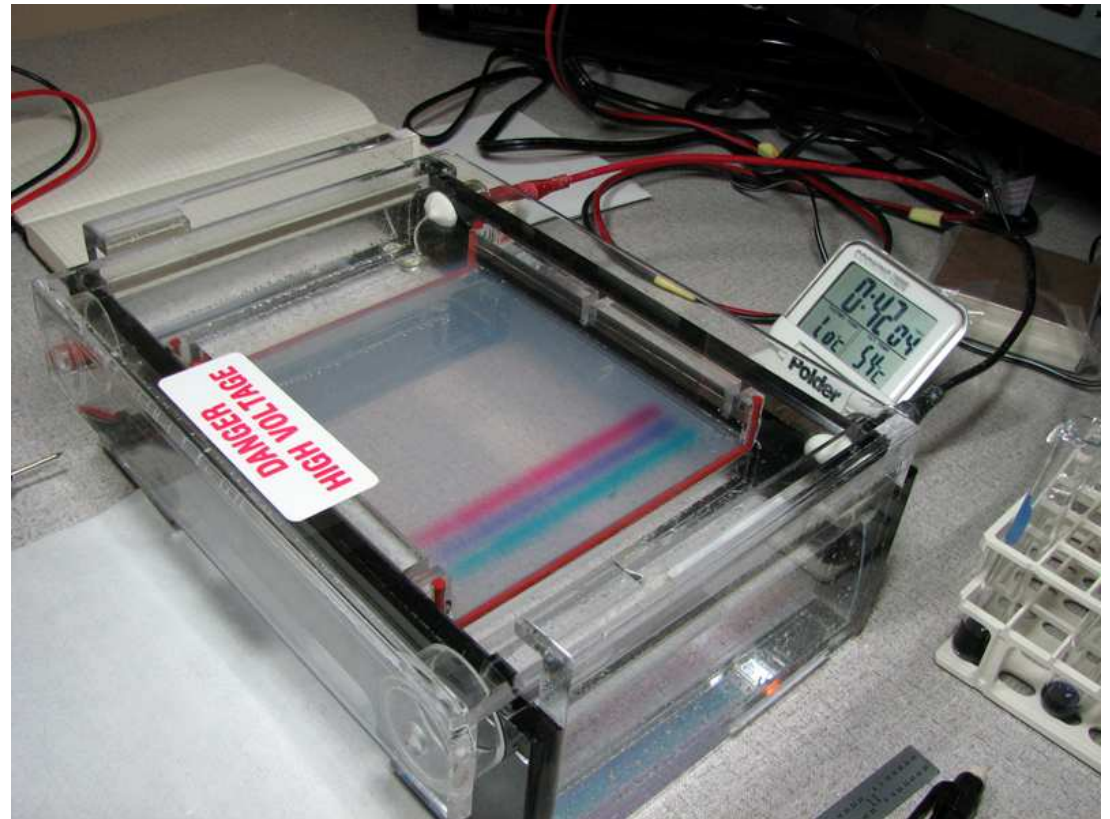
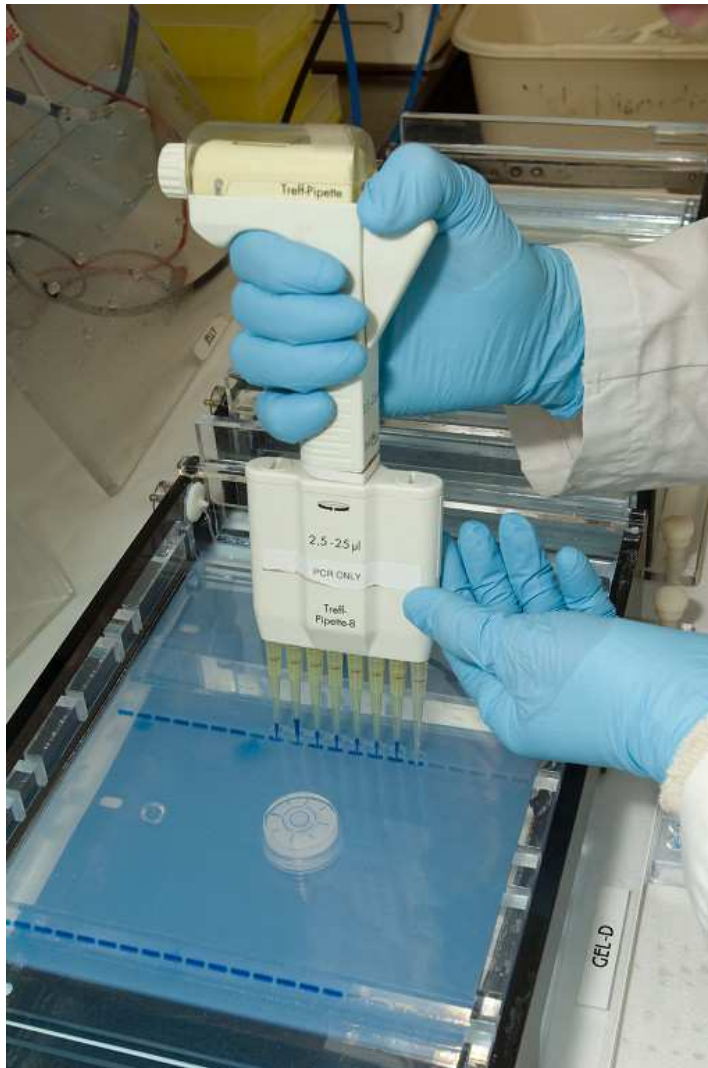


Chemical structure
of DNA

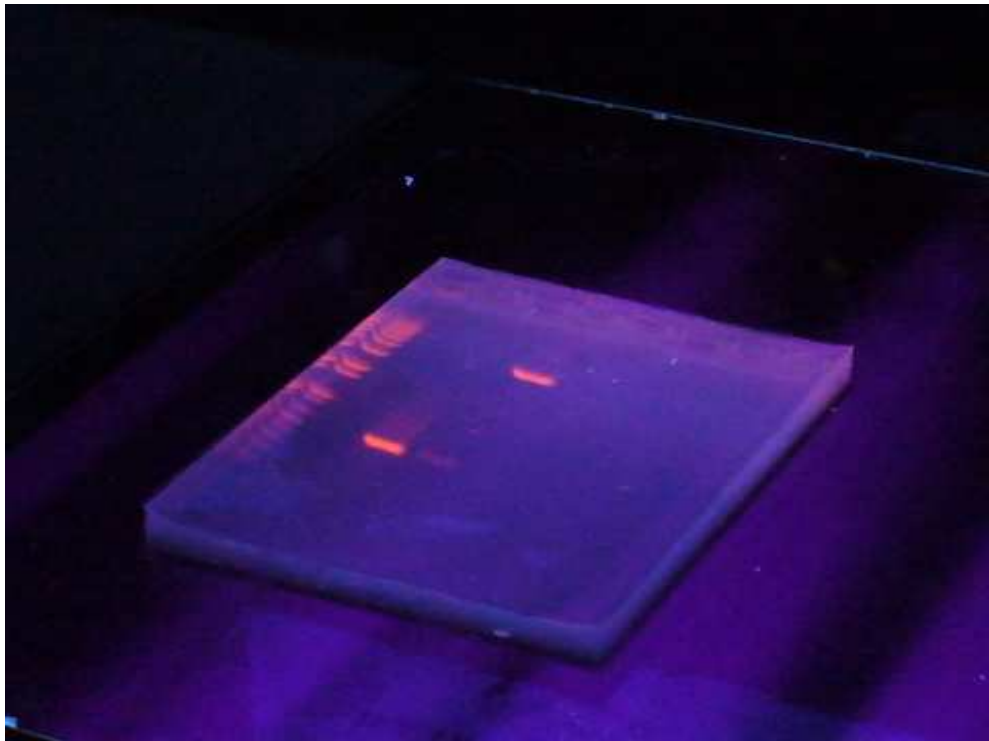
Preparativní elektroforéza



Preparativní elektroforéza



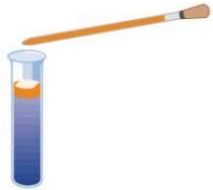
Preparativní elektroforéza



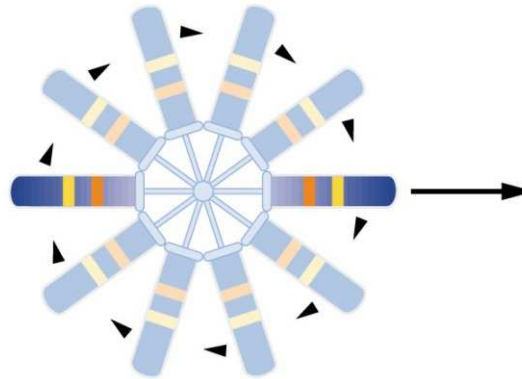
Separation of Nucleic Acids by CsCl Gradient Centrifugation



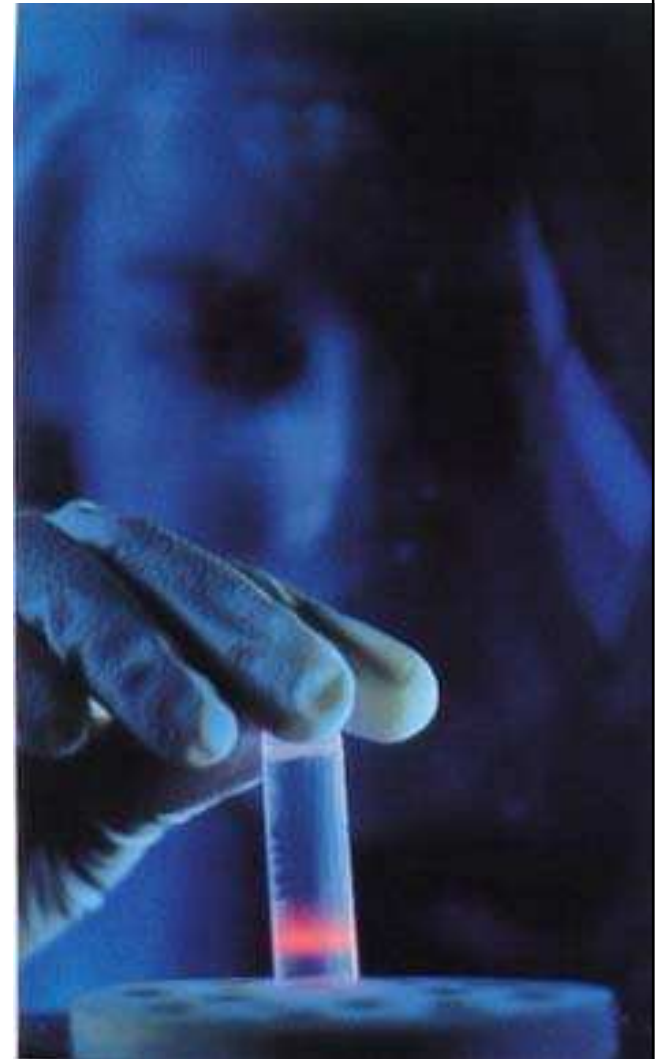
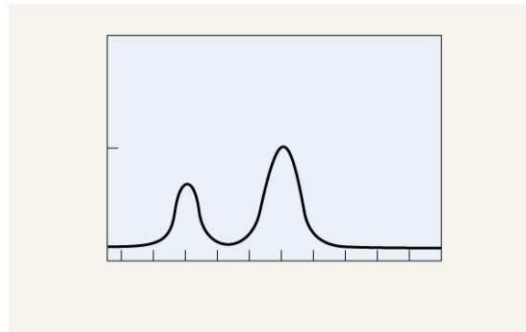
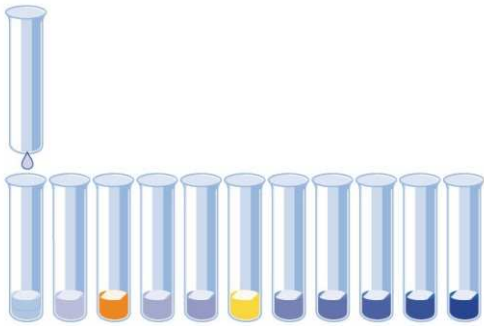
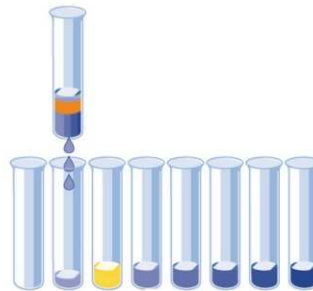
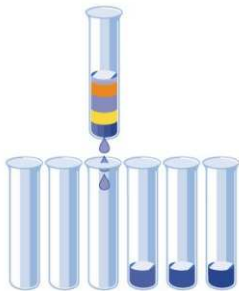
Sample of nucleic acid
Experiment begins

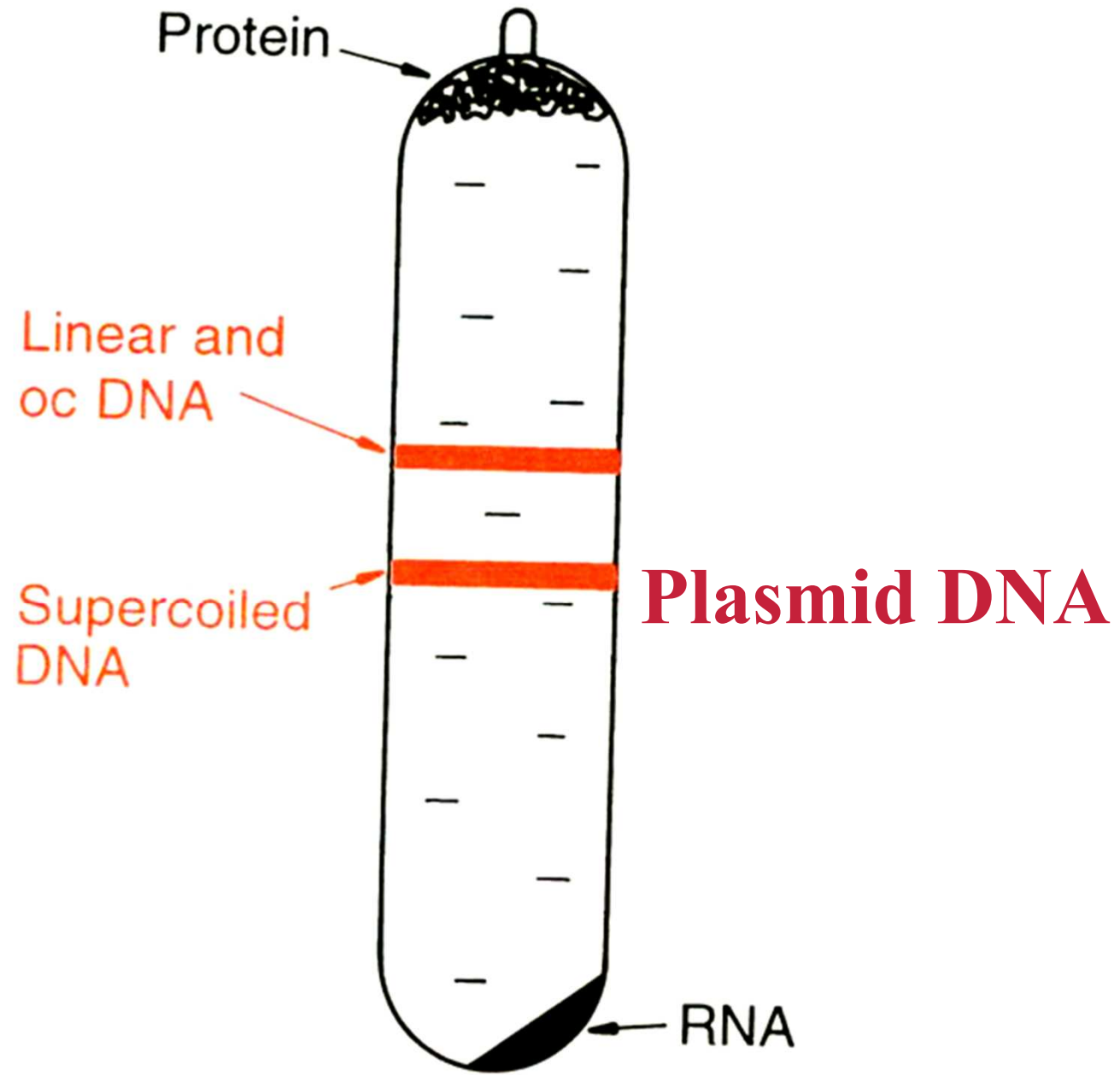


Sample loaded onto top of concentration gradient



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





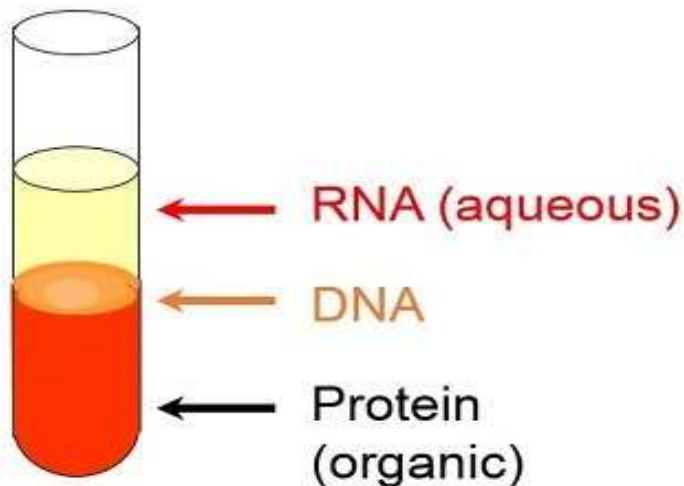
(a) An EtBr-CsCl density gradient

Izolace RNA - speciální přístupy

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

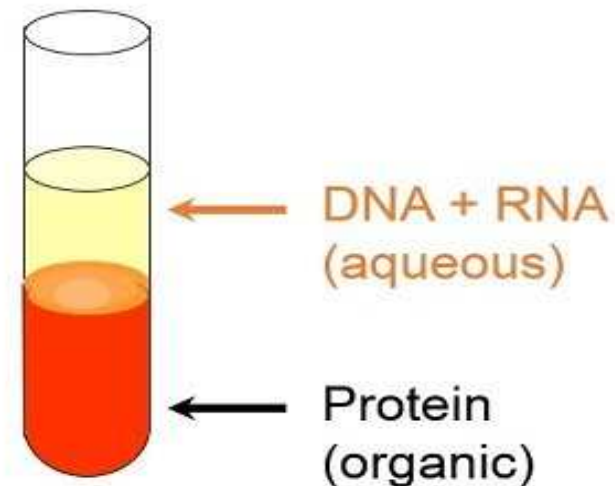
Traditional Phenol Extraction

Phenol, pH 4



RNA

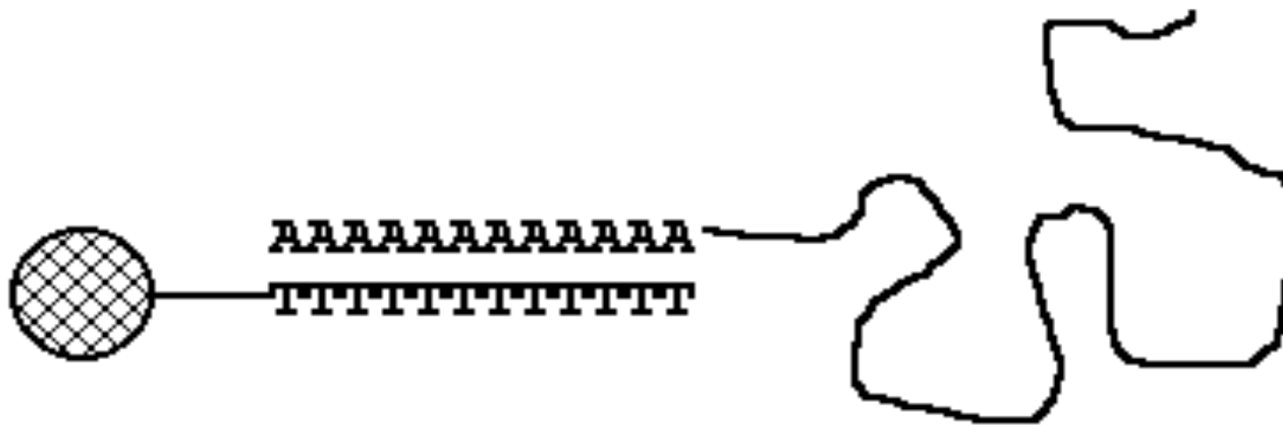
Phenol, pH 8



DNA

Izolace RNA - speciální přístupy

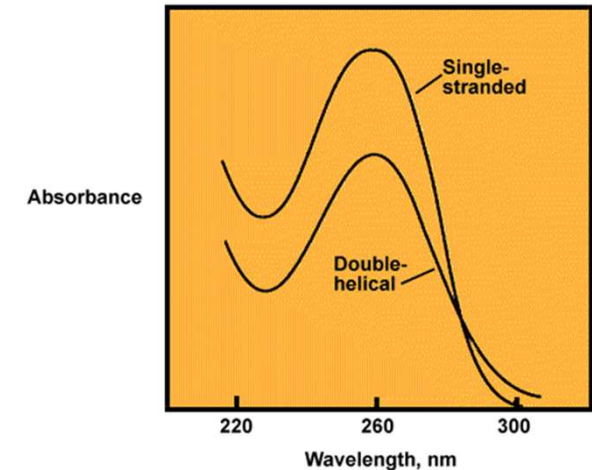
- působení RNase-free DNase
- selektivní precipitace rRNA, mRNA s LiCl
- oligo-dT afinitní chromatografie - mRNA



Kontrola čistoty a kvantifikace NK

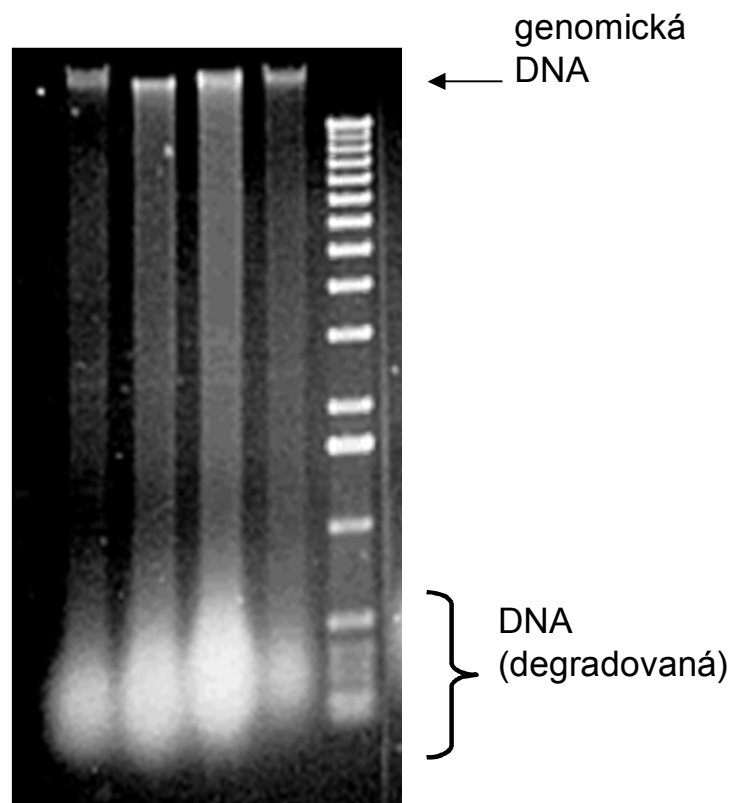
Kontrola NK

- 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}	~ 2.0

Kontrola degradace: DNA



Kontrola degradace: RNA

