

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 mlíčím rýnského lososa – **NUKLEIN**

3. Altman (1899)

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

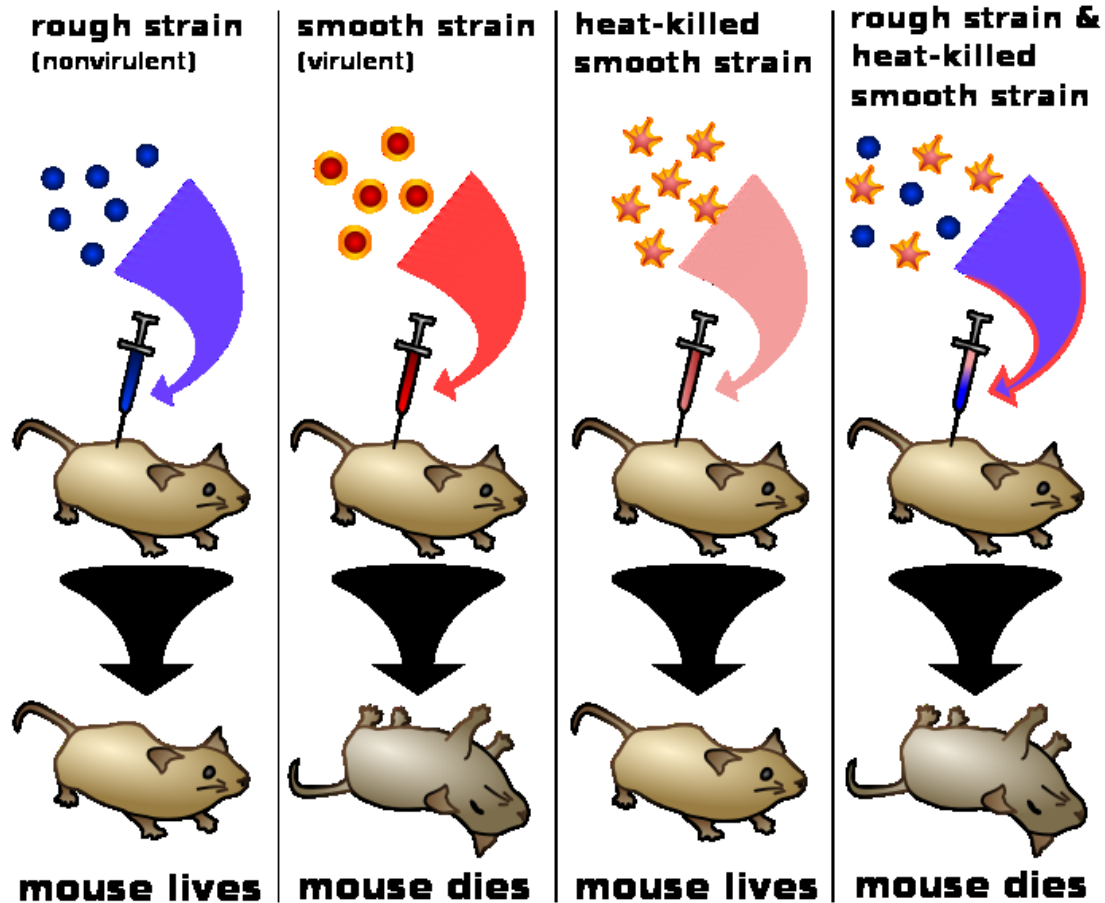
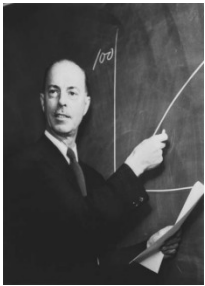
4. Leven (1909)

– **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 :

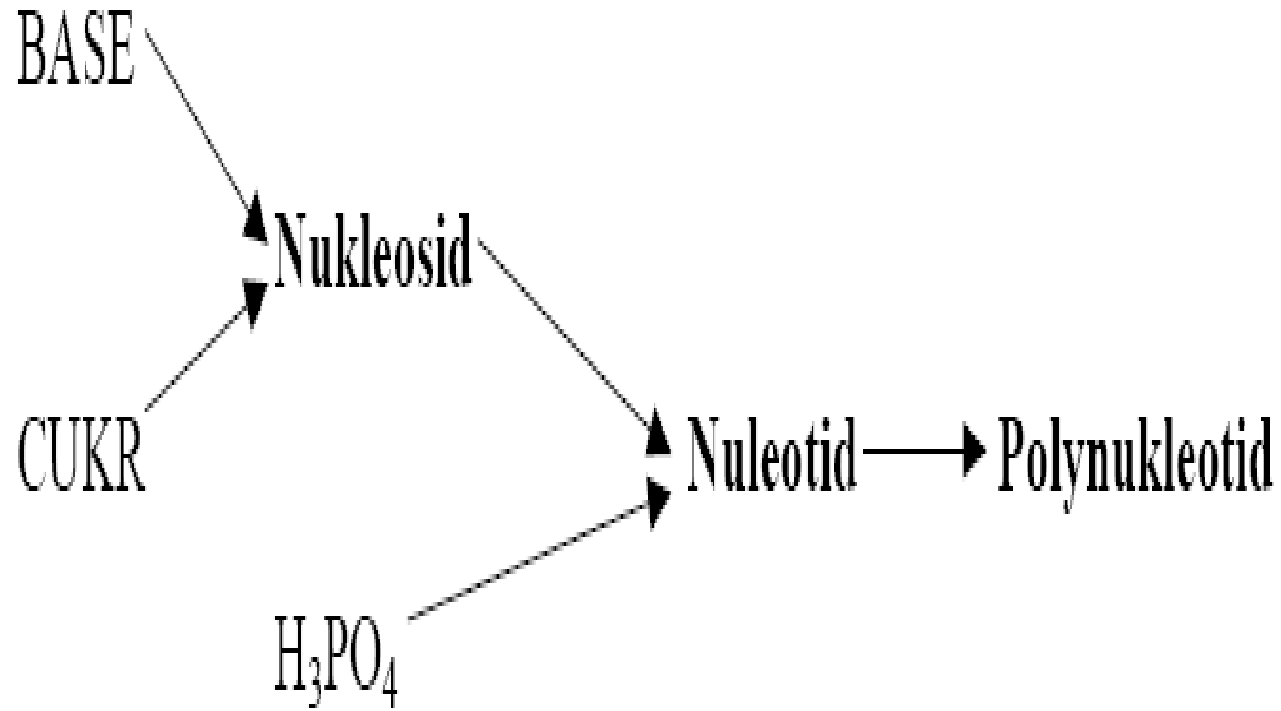
- Dusíkaté báze – purinové, pyrimidinové
 - Sacharid – ribosa, deoxyribosa
 - H_3PO_4
-
- DNA (AGCT)
- RNA (AGCU)

Funkce :

DNA – nositel genetické informace

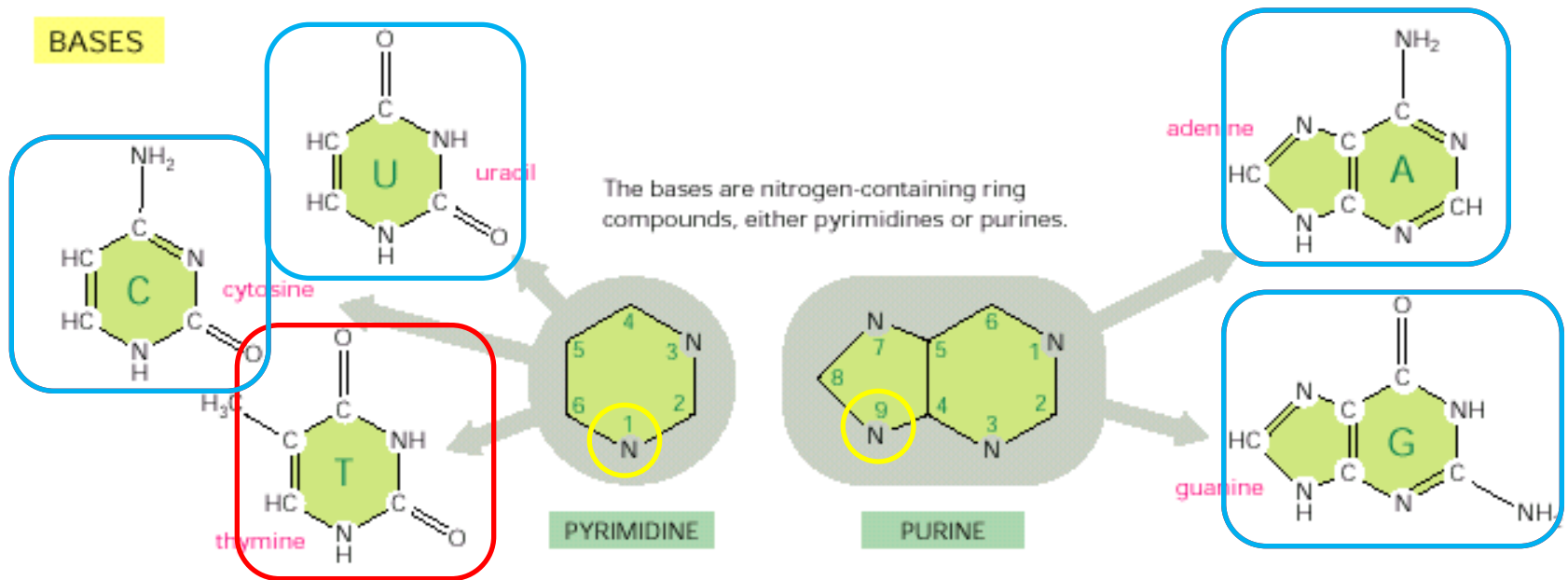
- Viry
- Prokaryonta –cytoplazma
- Eukaryonta – jádro, mitochondrie, chloroplasty

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

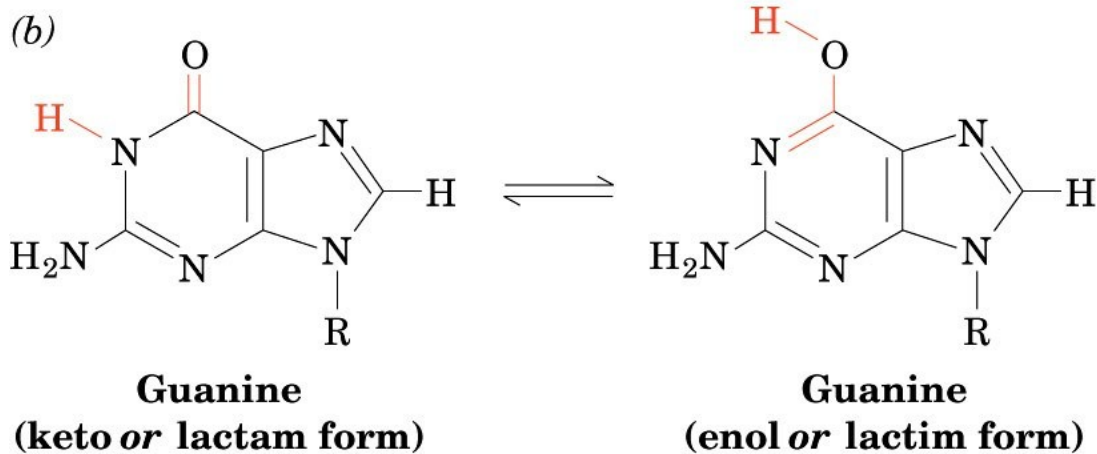
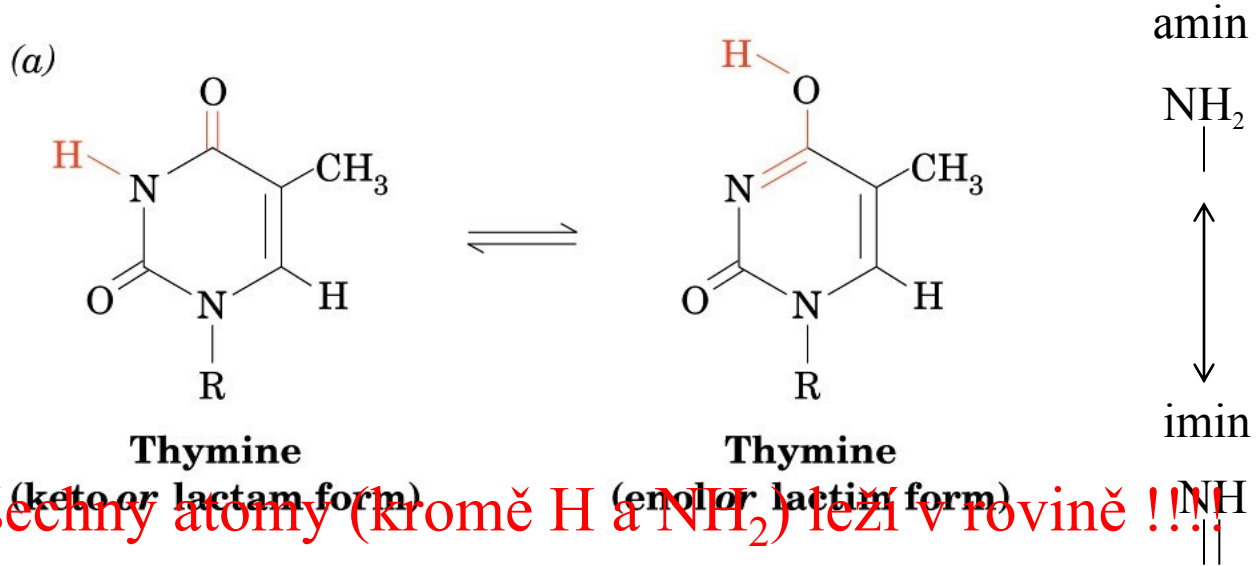


Báze

BNA



Báze -tautomerie

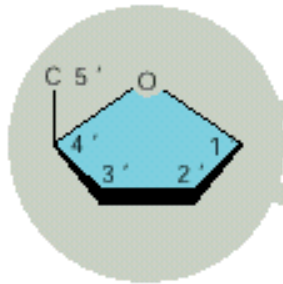


Monosacharidy

SUGARS

PENTOSE

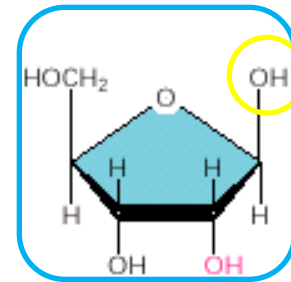
a five-carbon sugar



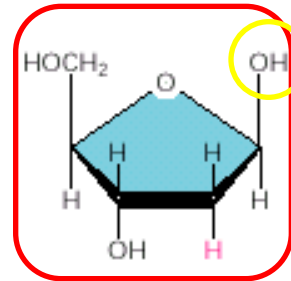
two kinds are used

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

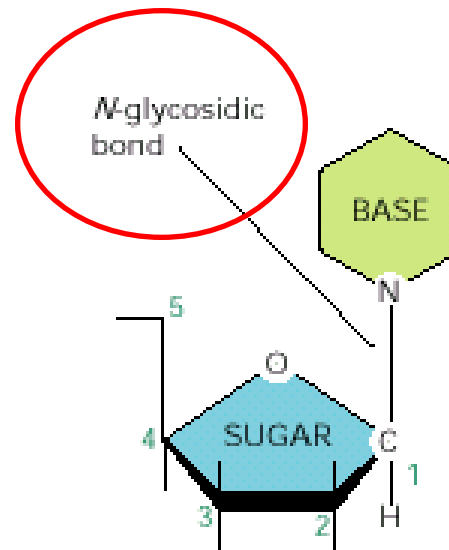


β -D-2-deoxyribose
used in deoxyribonucleic acid

DNA

Nukleosid

BASIC SUGAR LINKAGE

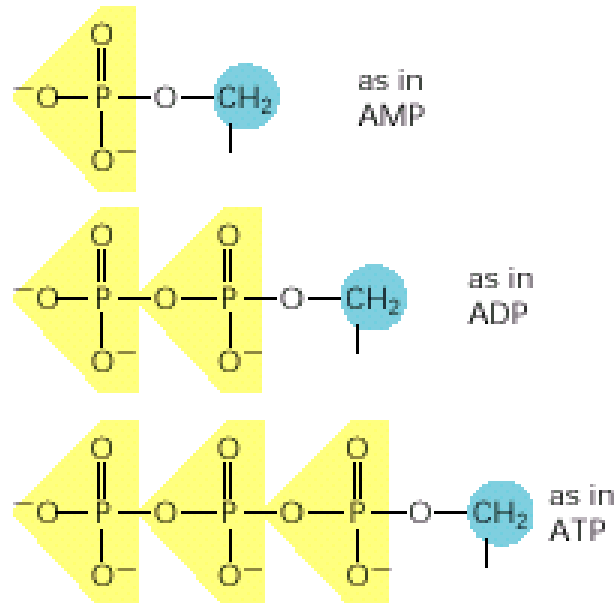


The base is linked to the same carbon (C1) used in sugar-sugar bonds.

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

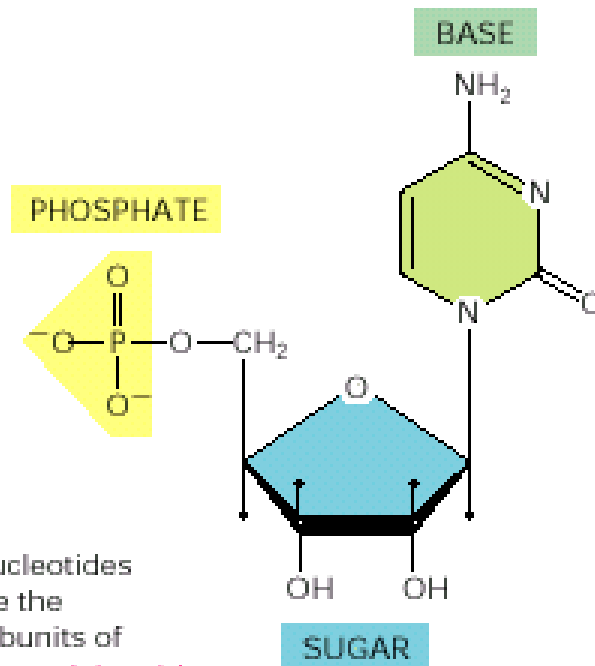
Anhydrid

The phosphate makes a nucleotide negatively charged.

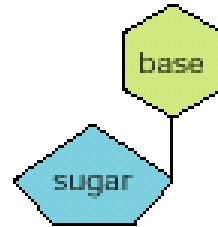
Nukleotid

NUCLEOTIDES

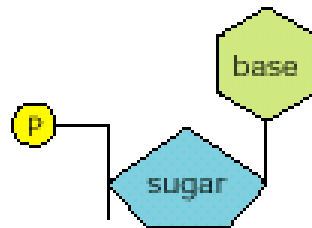
A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.



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

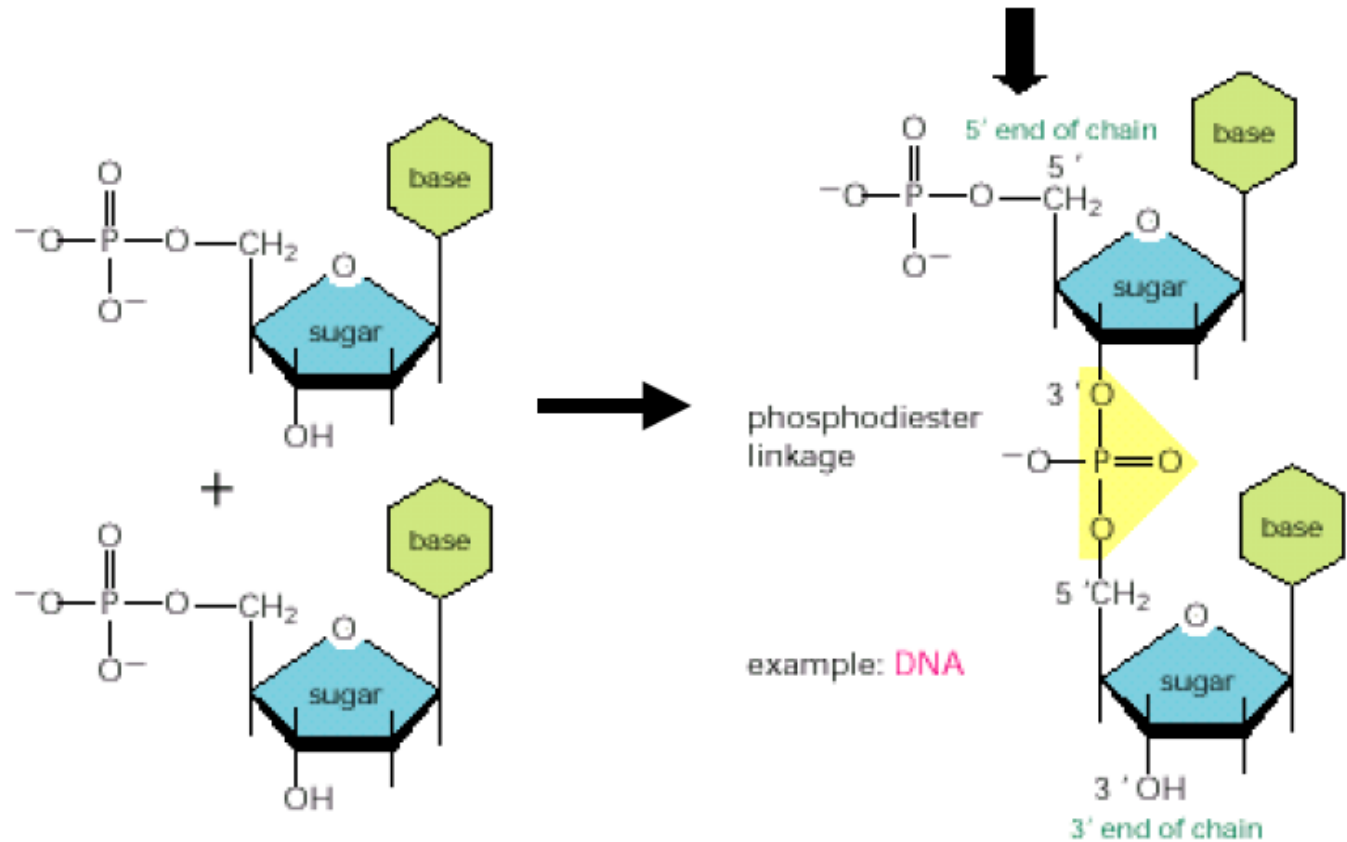
UDP = uridine diphosphate

ATP = adenosine triphosphate

Funkce nukleotidů

- přenašeče energie (ATP, GTP)
- fosforylační činidla (ATP – kinasy)
- aktivátory meziproductů biosyntéz – UDP-glukosa, CTP-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

A,T,G,C + deoxyribosa

Primární struktura – sekvence basí

Sekundární struktura – Watson, Crick (1953) – dvojšroubovice

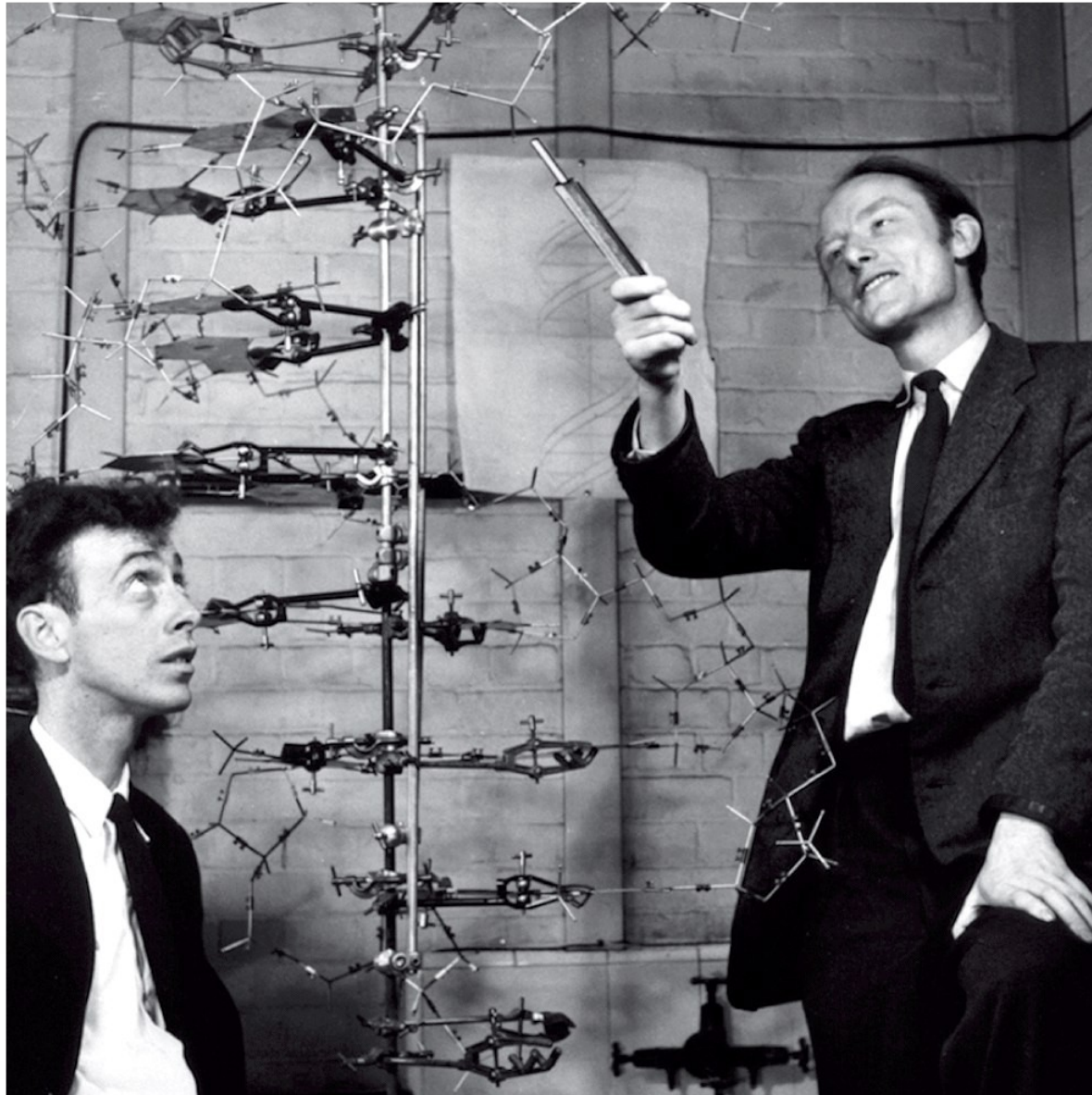
- Chragaffovy pravidla – poměr basí v DNA

$A+G=T+C$ $A=T$ $G=C$ $A+C=G+T$

- Donohue – báze v tautomerních ketoformách

- Franklinová – RTG difrakční analýza

Watson



Crick

Unnumbered figure pg 9 Concepts in Biochemistry, 3/e

Chargaffovy pravidla

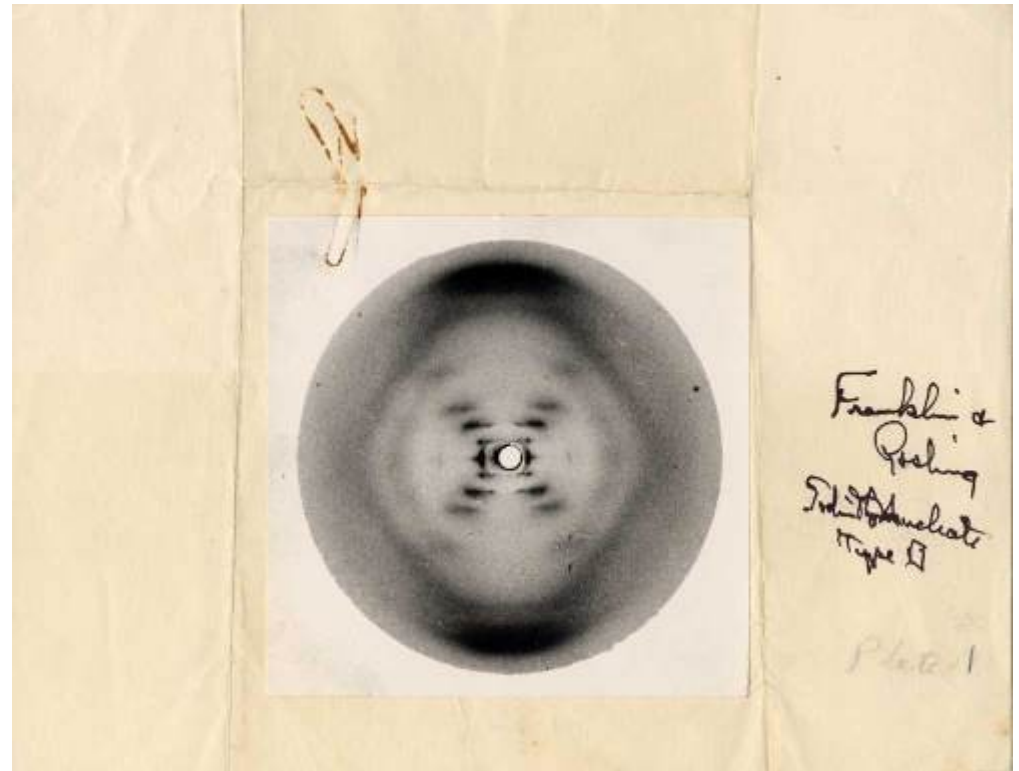
$$A+G=T+C \quad A=T \quad G=C$$

$$A+C=G+T$$

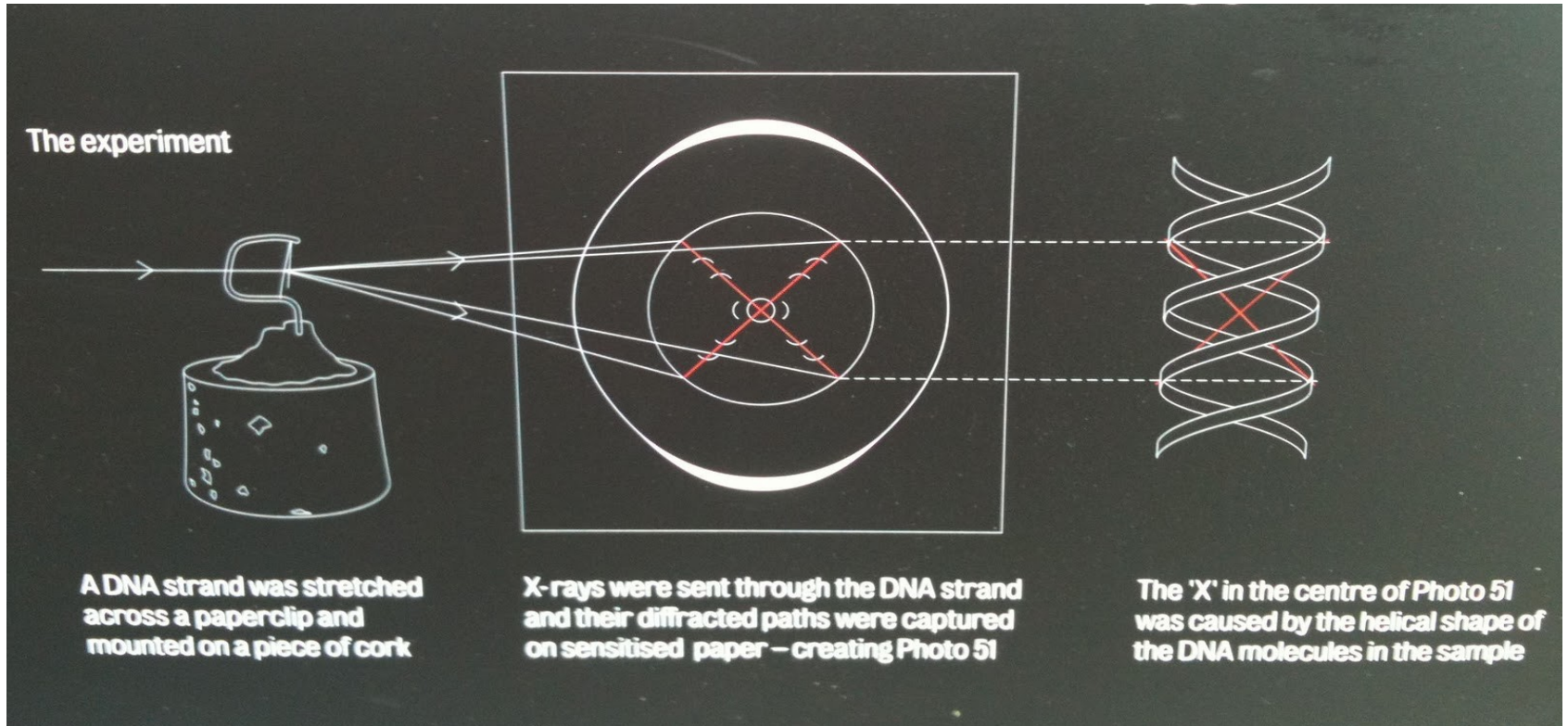
Zastoupení basí v DNA (molární %)

<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

Rosalind Franklin



Obrázek 51



Nobelova cena 1962



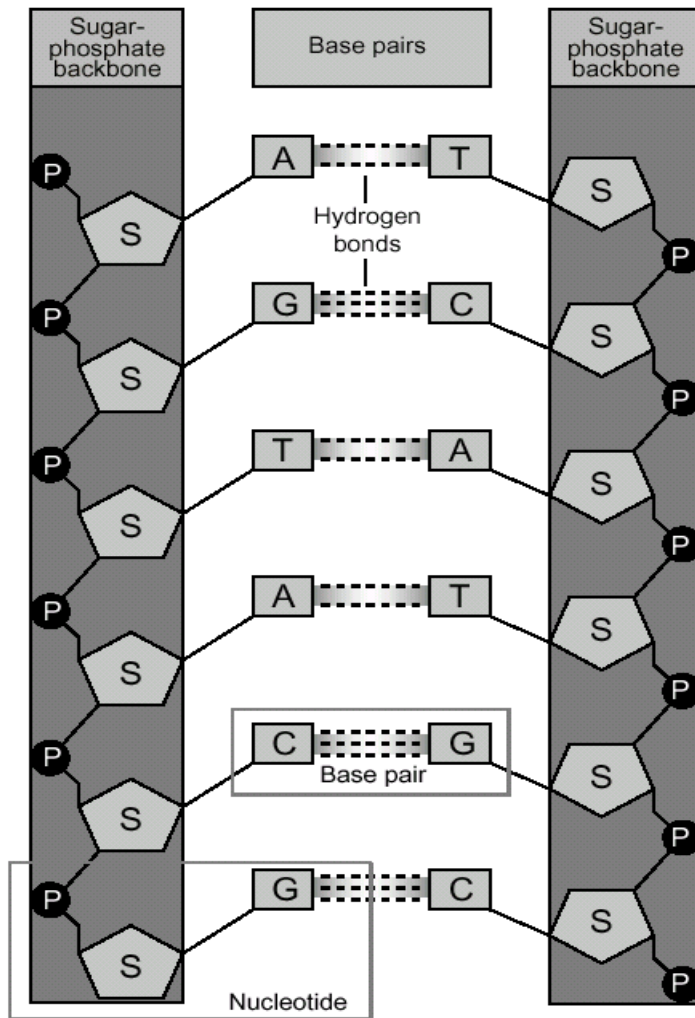
**Francis Harry
Compton Crick
(1916-2004)**



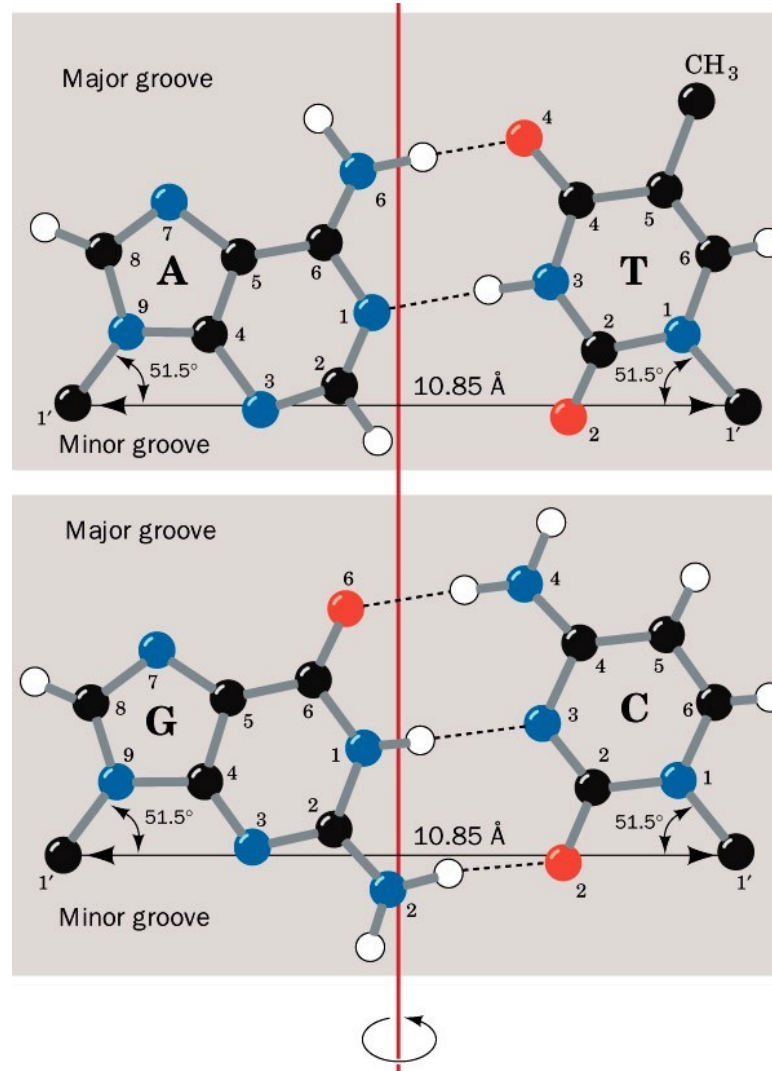
**James Dewey
Watson
(1928 -)**



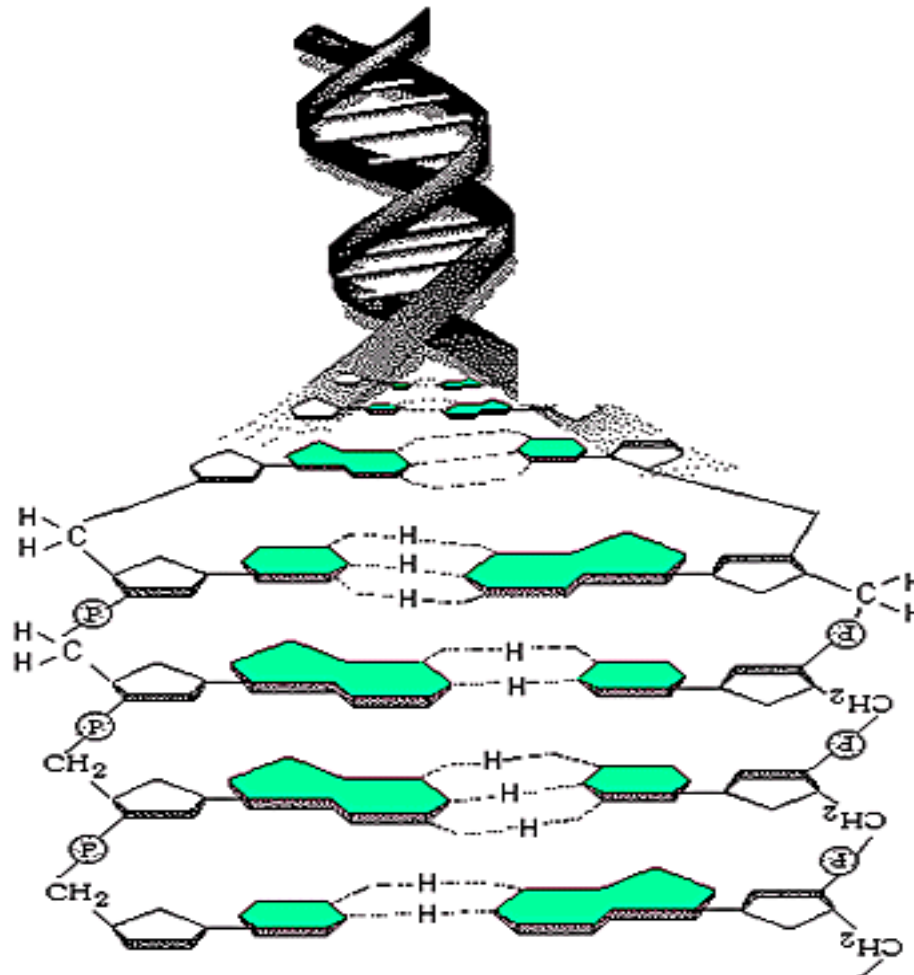
**Maurice Hugh
Frederick Wilkins
(1916-2004)**



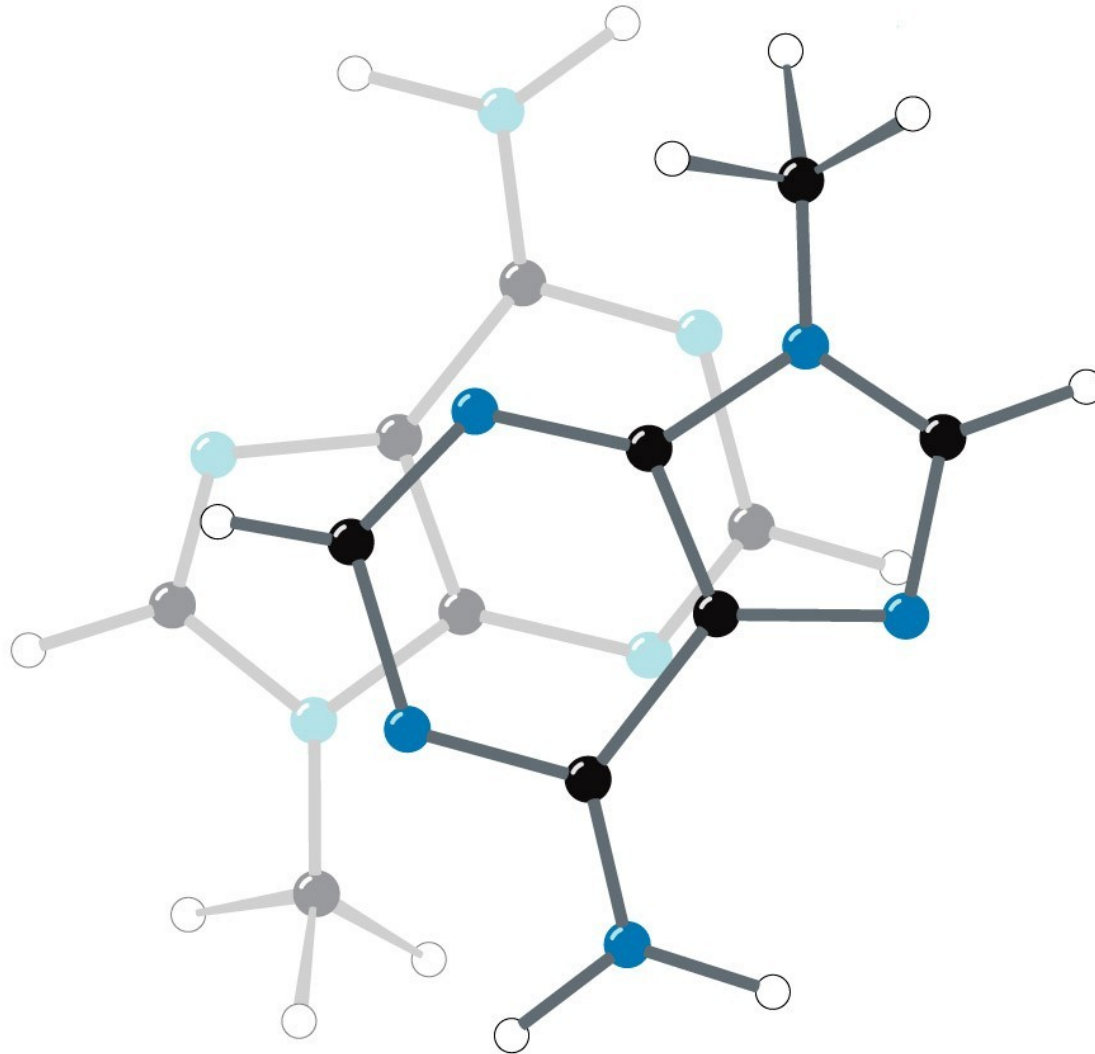
Párování basi – H můstky



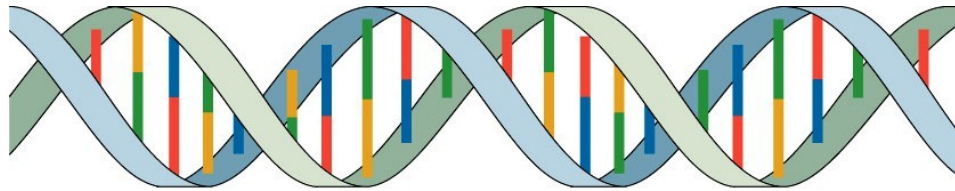
Stabilizující vazby v DNA



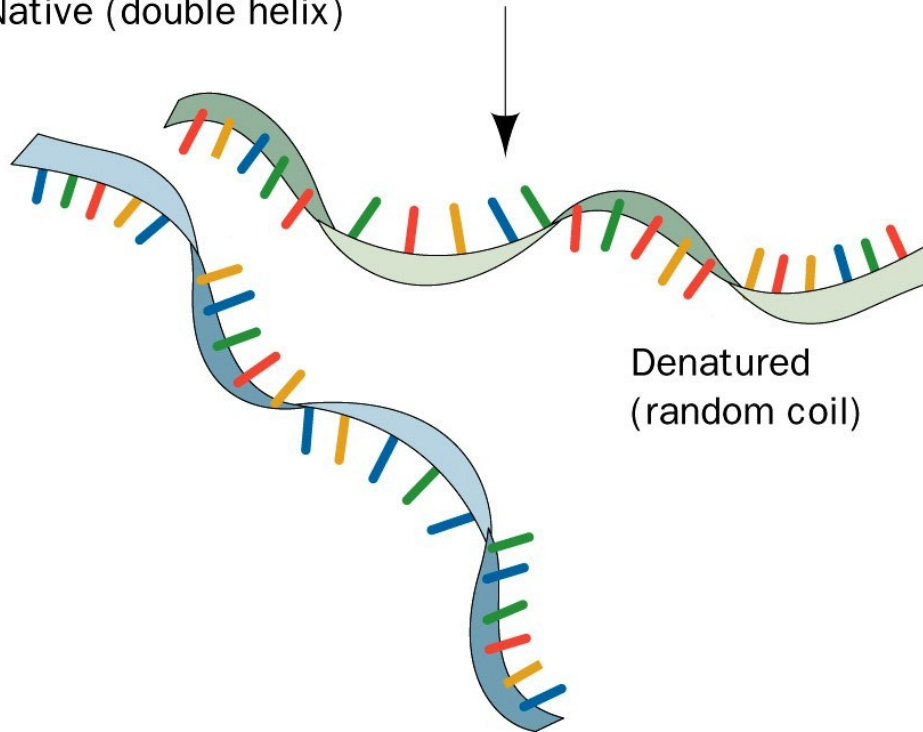
Stohování bází



Denaturace - renaturace

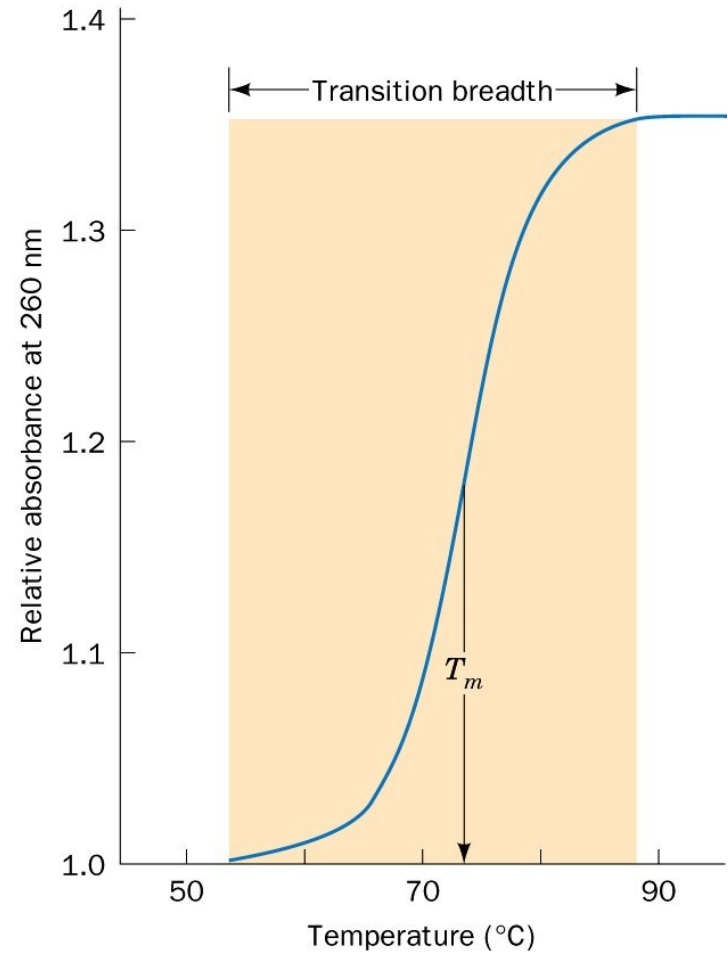
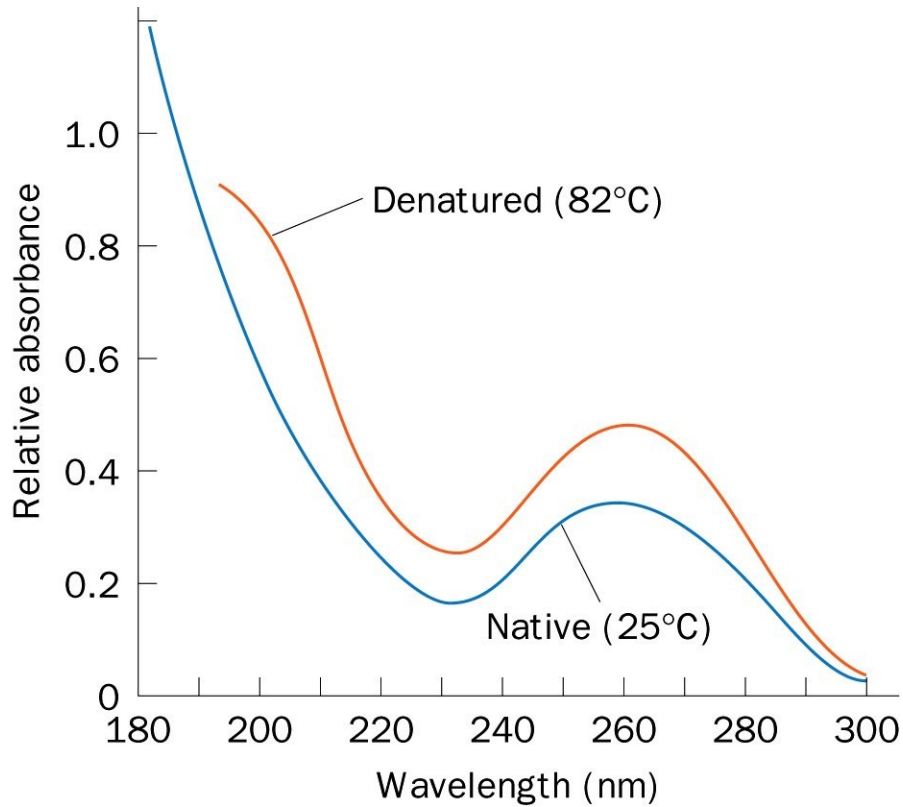


Native (double helix)



Denatured
(random coil)

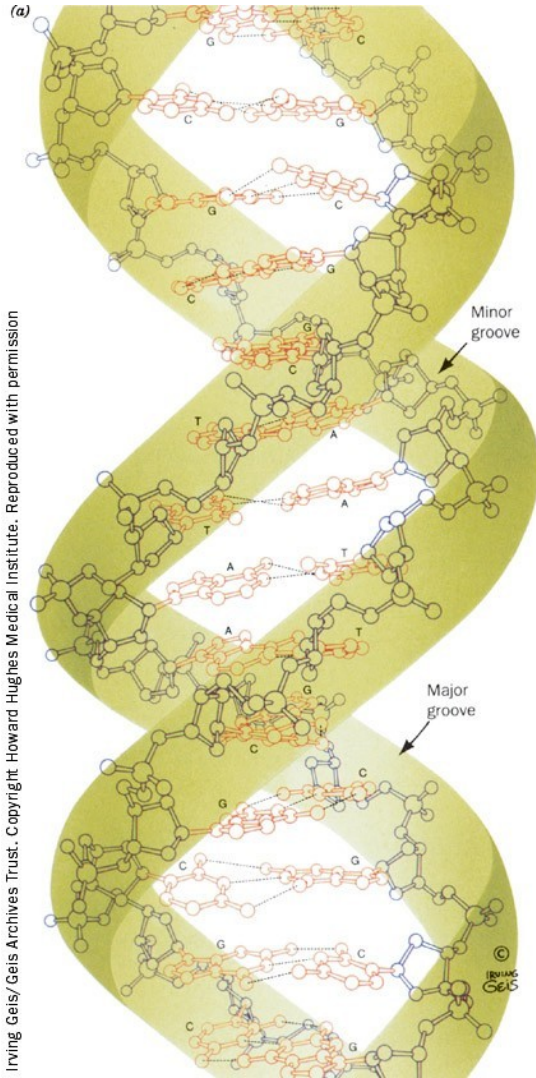
Denaturation - renaturation



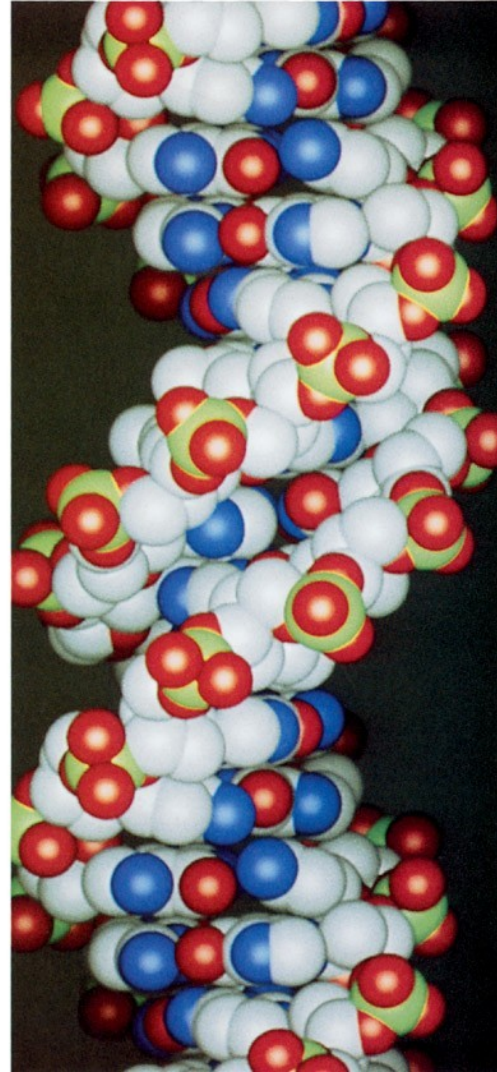
Formy DNA

- 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

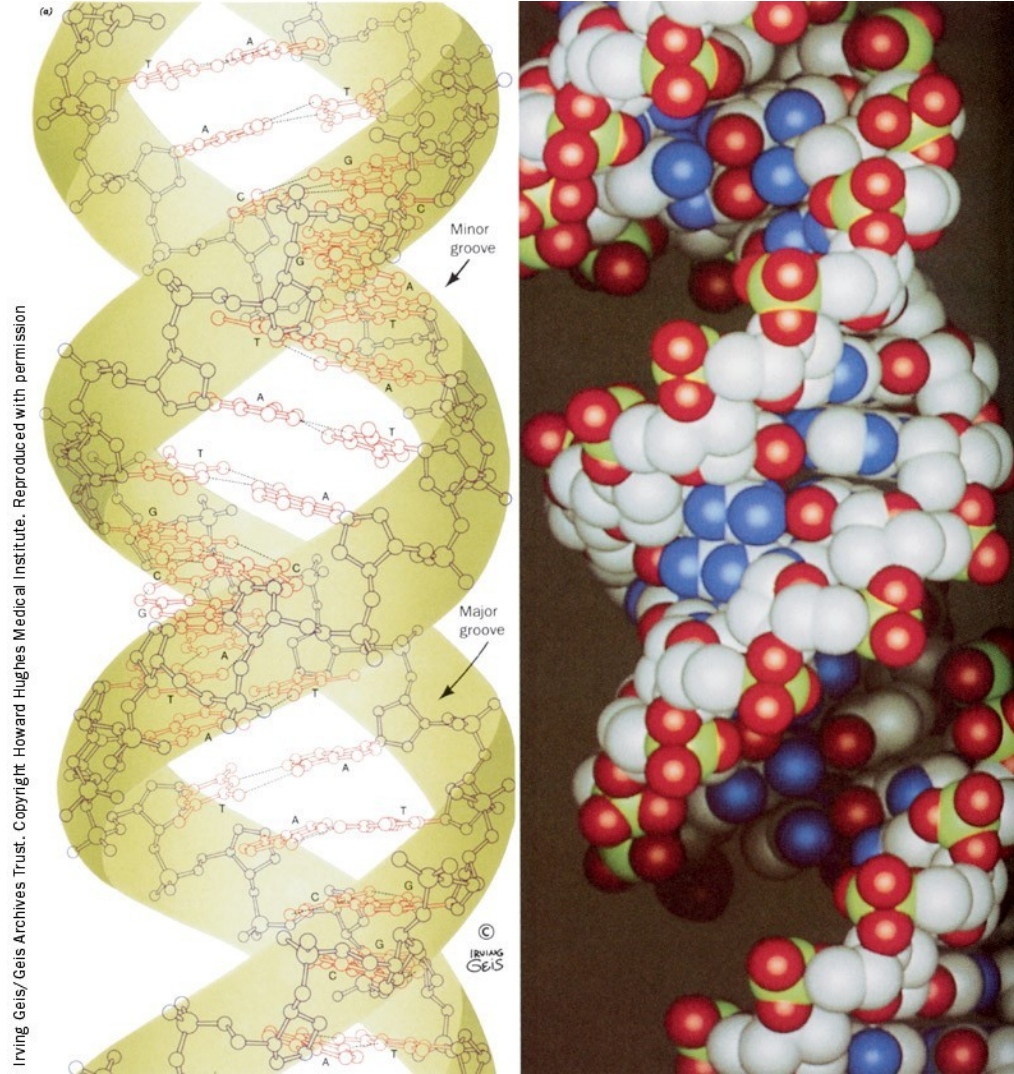
B DNA



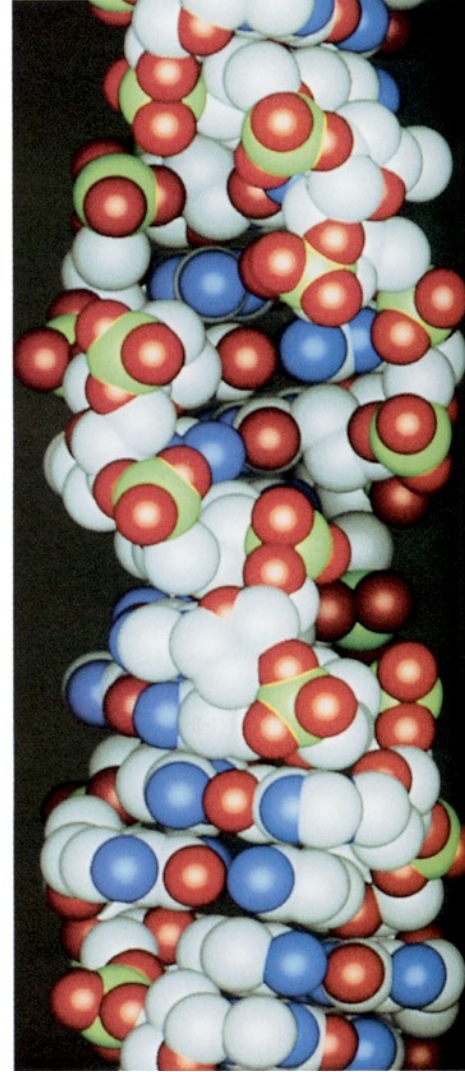
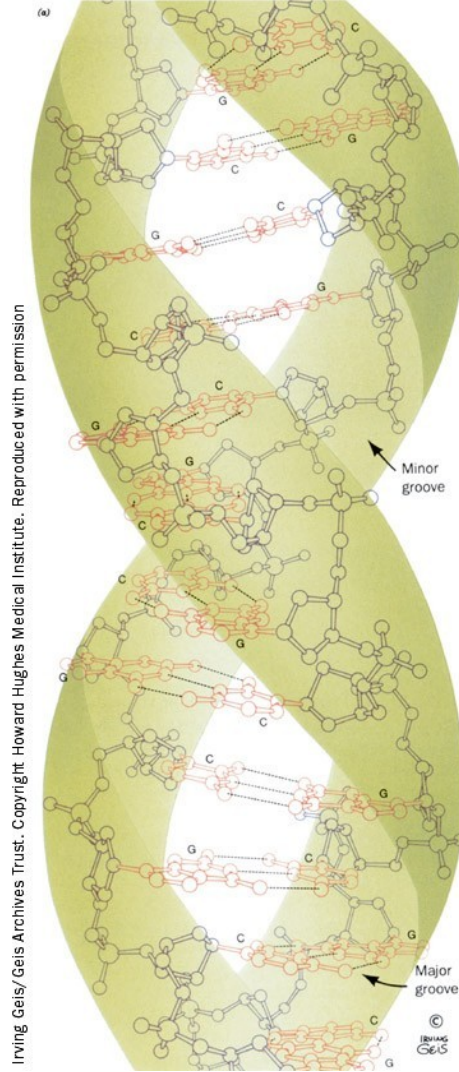
Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with permission



A DNA

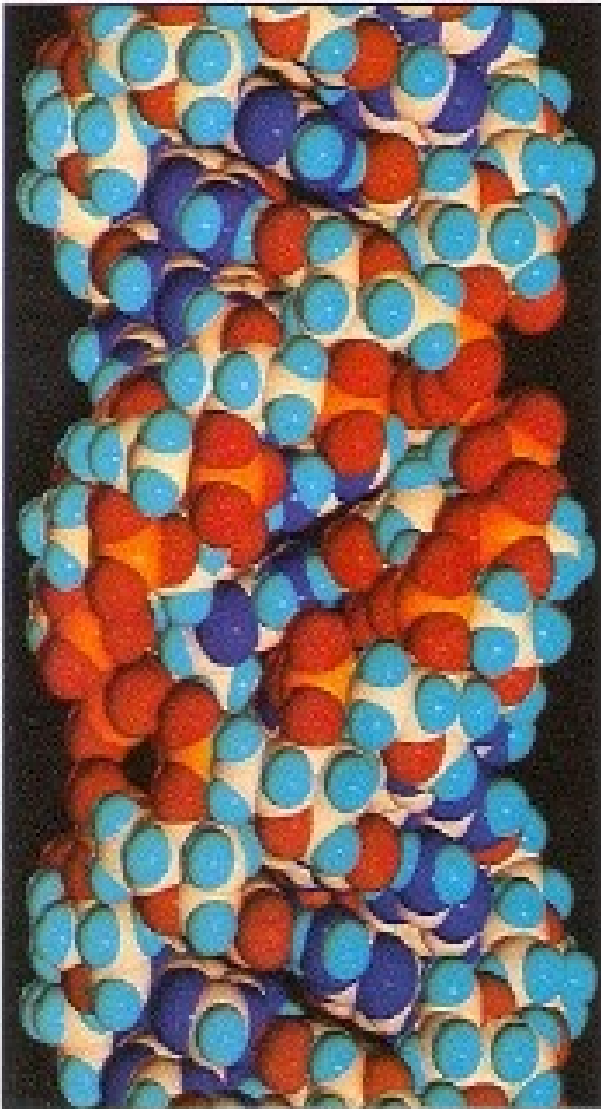


Z DNA

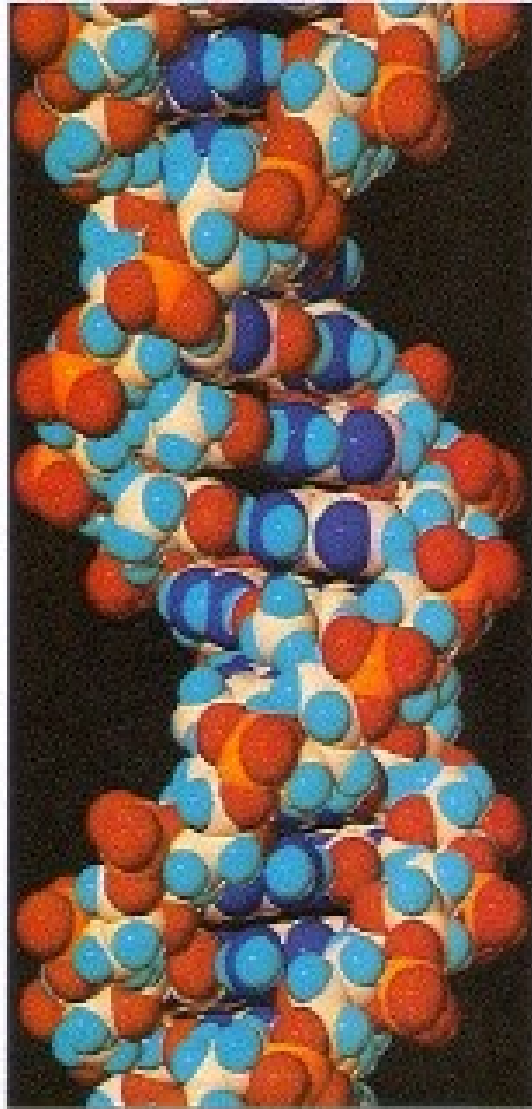


Základní číselné charakteristiky tří nejznámějších helikálních forem DNA

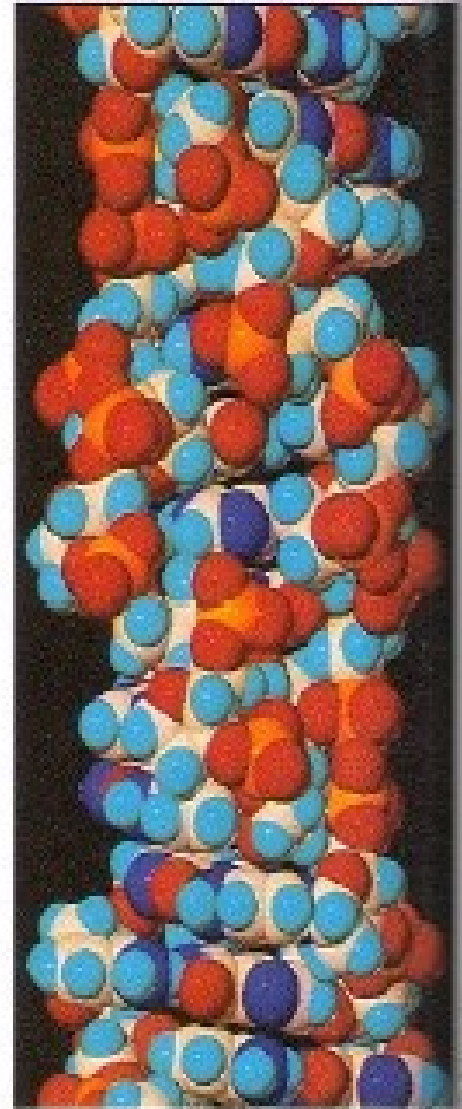
Atribut	A-DNA	B-DNA	Z-DNA
Točivá tendence šroubovice (chiralita) ^[1]	pravotočivá	pravotočivá	levotočivá
Opakování ^[2]	po každém páru	po každém páru	po každých dvou párech
Otočení po každém opakování ^[1]	32,7°	34,3°	
Průměrný počet párů na jedno otočení šroubovice ^[1]	11	10,5	12
Sklon páru k ose ^[2]	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)



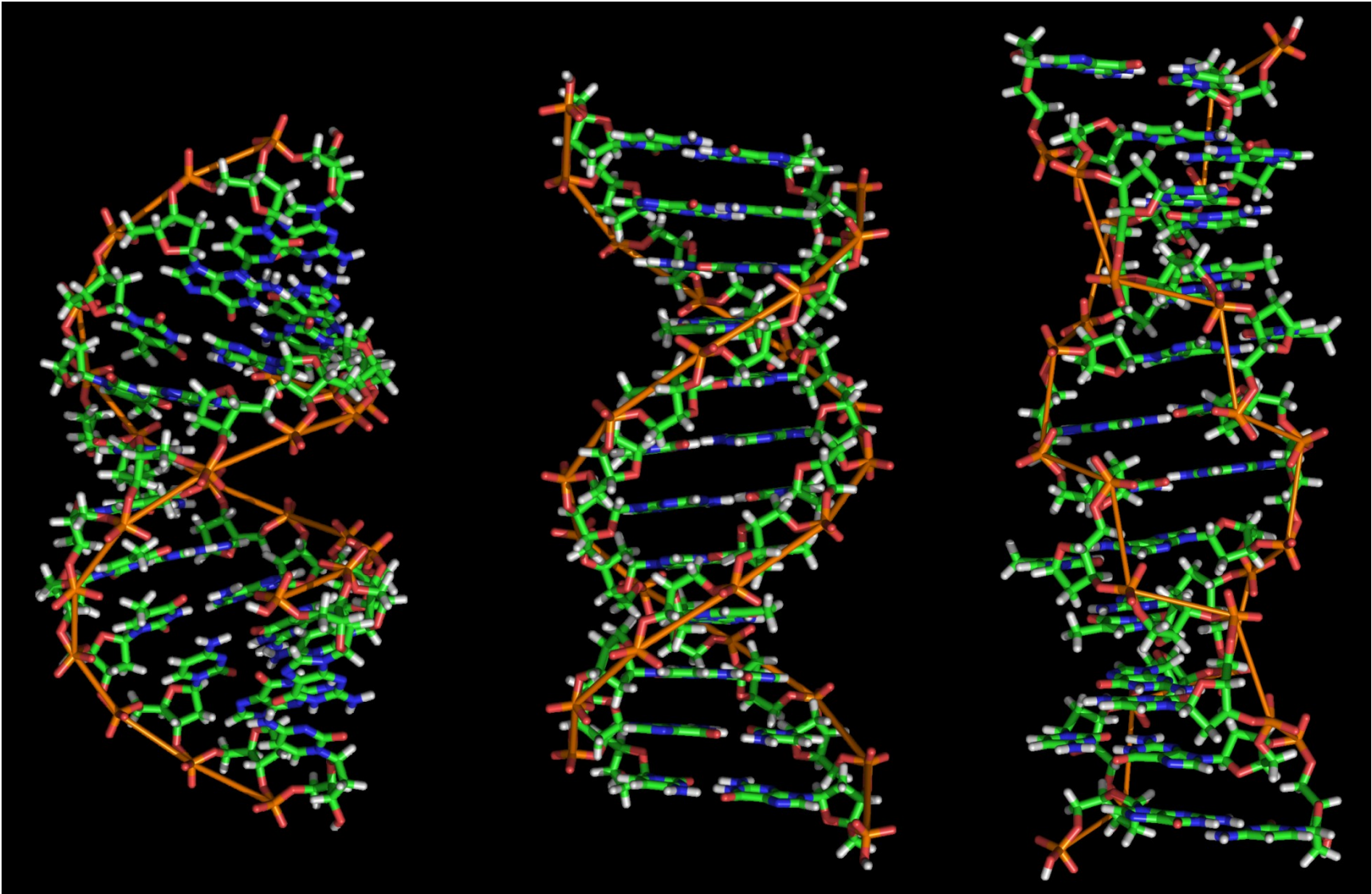
A-DNA



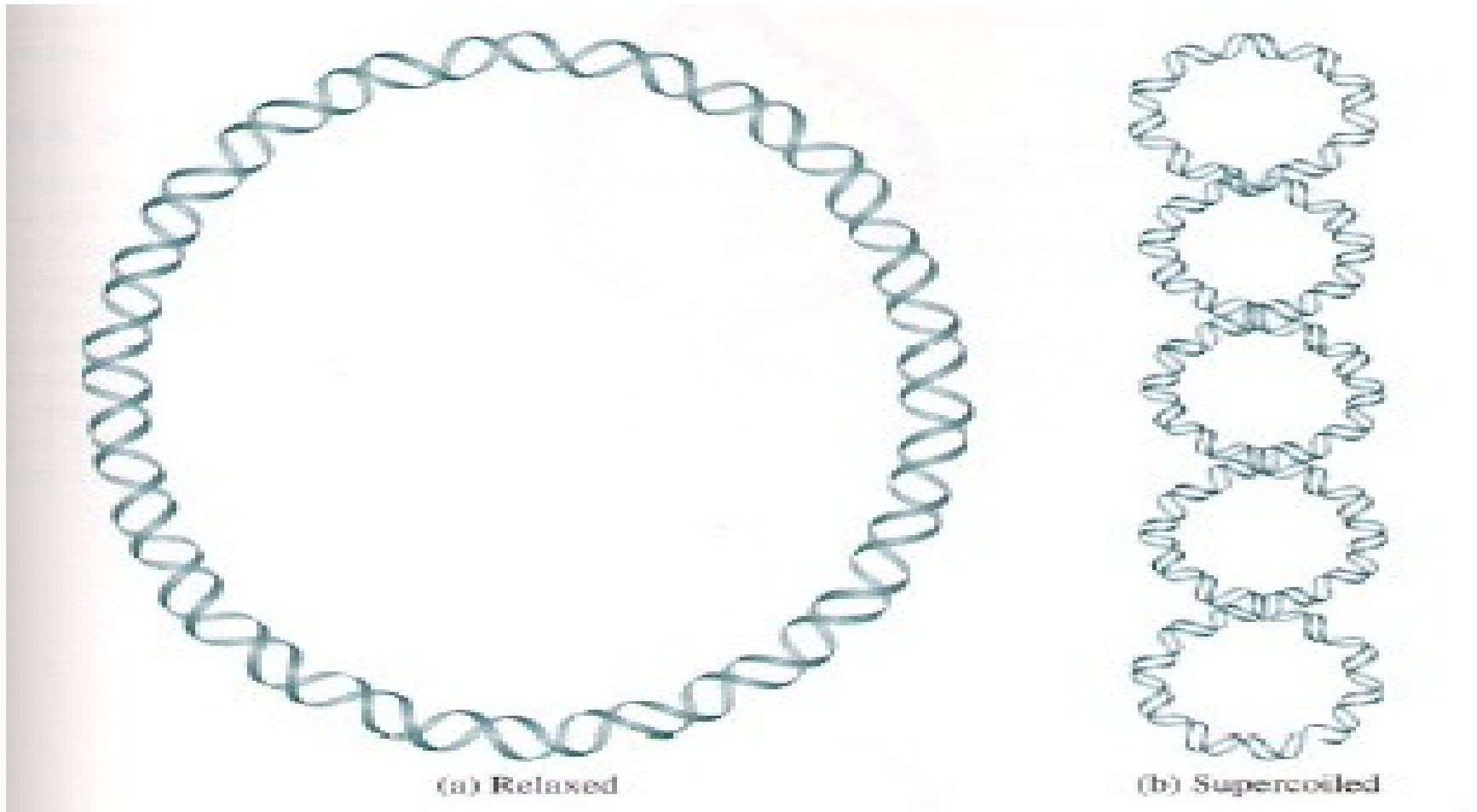
B-DNA



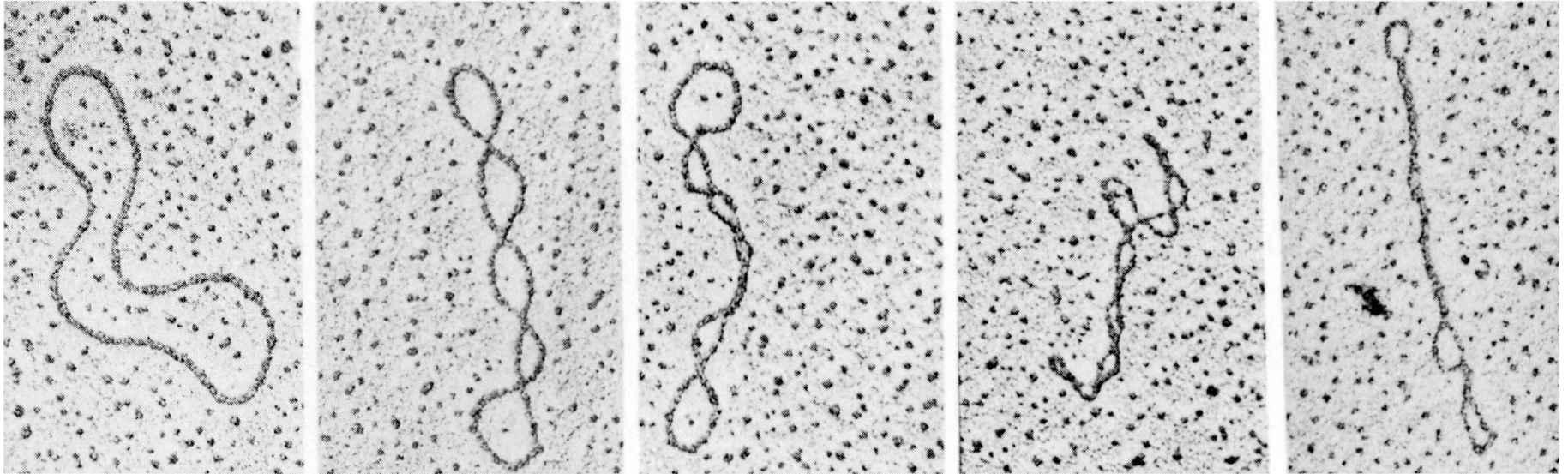
Z-DNA



Bakteriální DNA

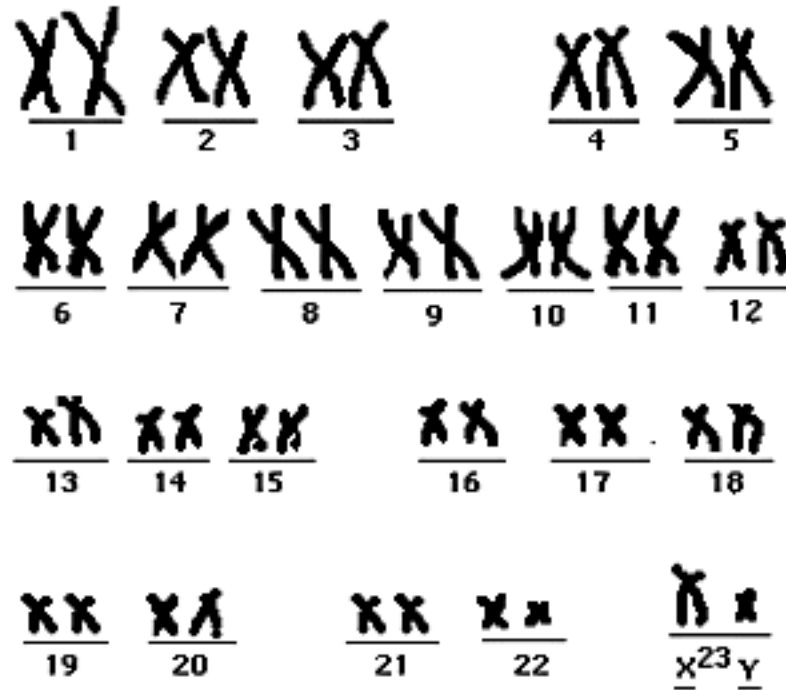


Bakteriální DNA

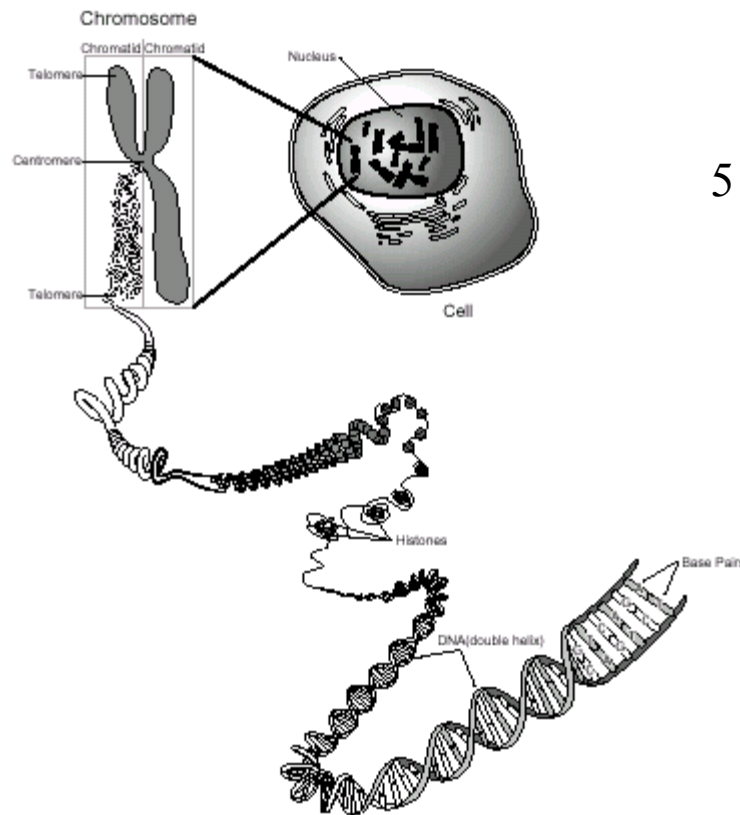


Electron micrographs by Laurien Polder. From Kornberg, A. and Baker, T.A., DNA Replication (2nd ed.), p. 36, W.H. Freeman (1992). Used with permission

Lidské chromozomy

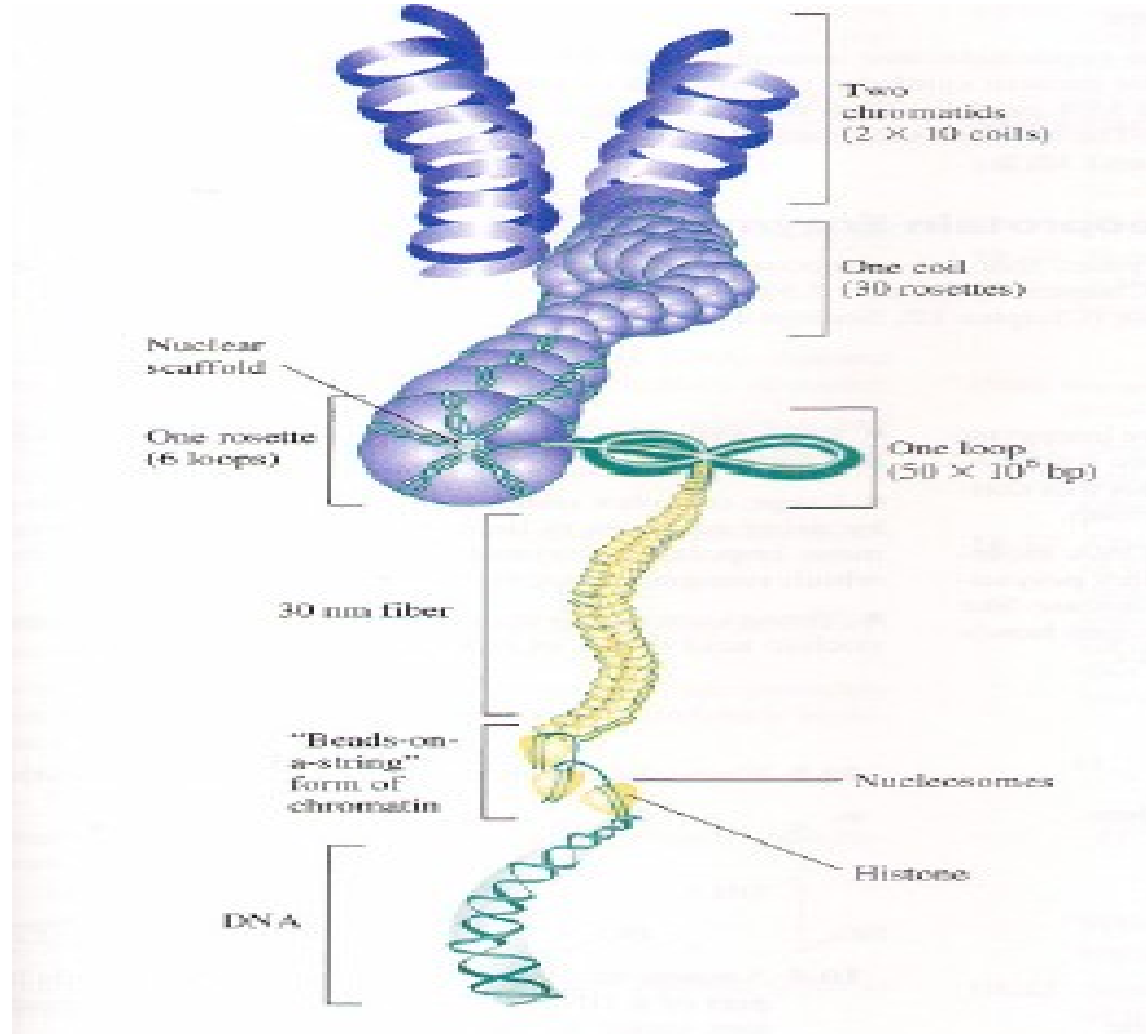


Jádro – mitotický chromozom

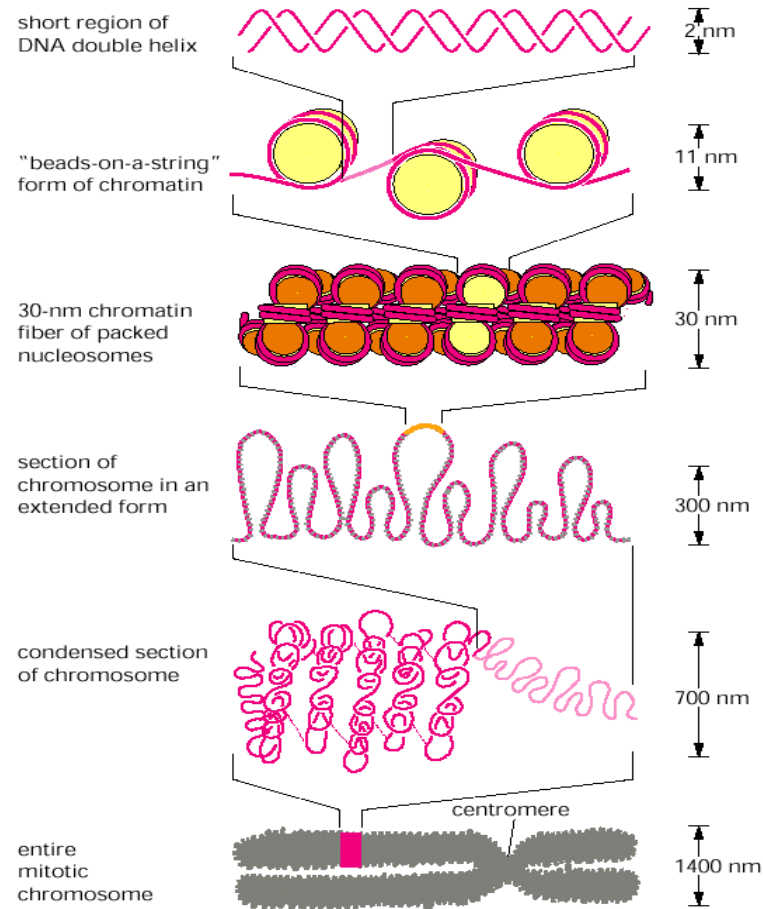


5 – 8 μm – DNA 2 metry

Chromozom



Chromatin



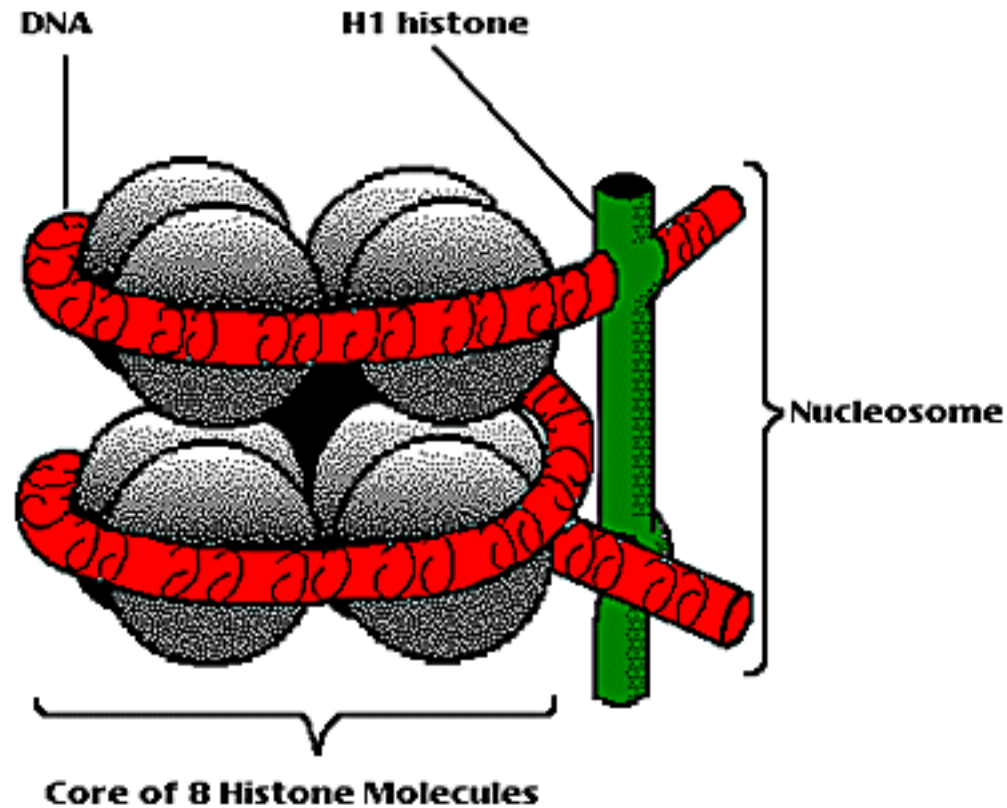
Nukleosom
(korálky)

(cívka)

(smyčka)

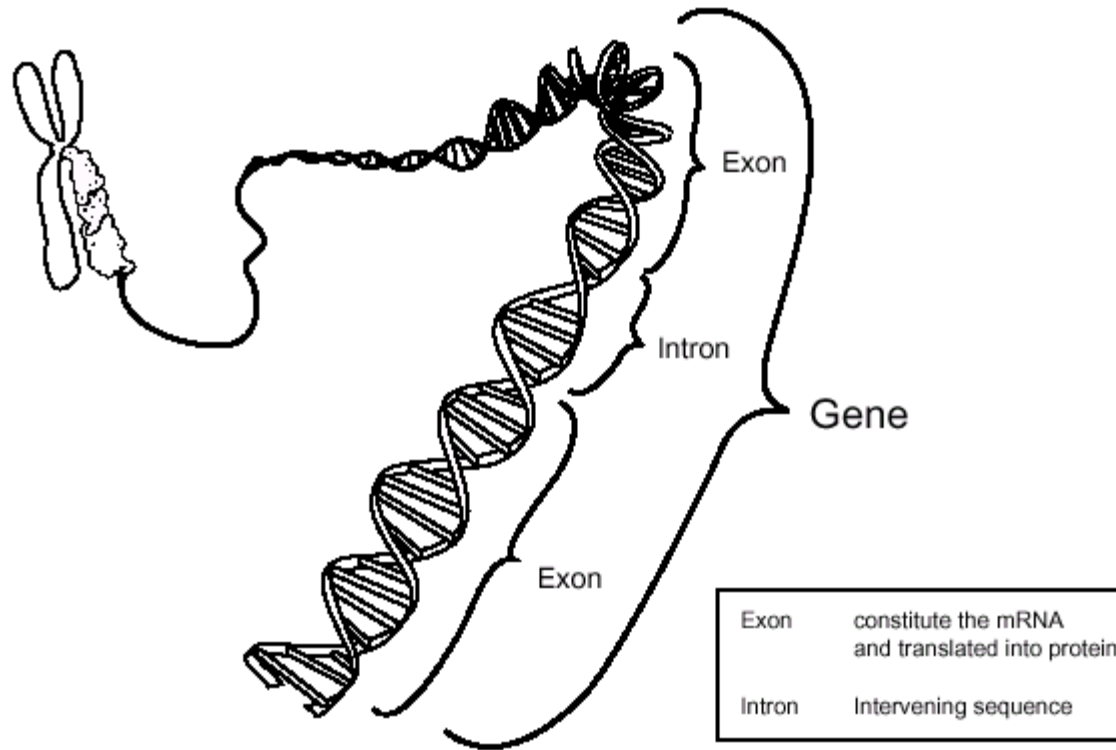
NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

Nukleosom – DNA + 5 tříd histonů

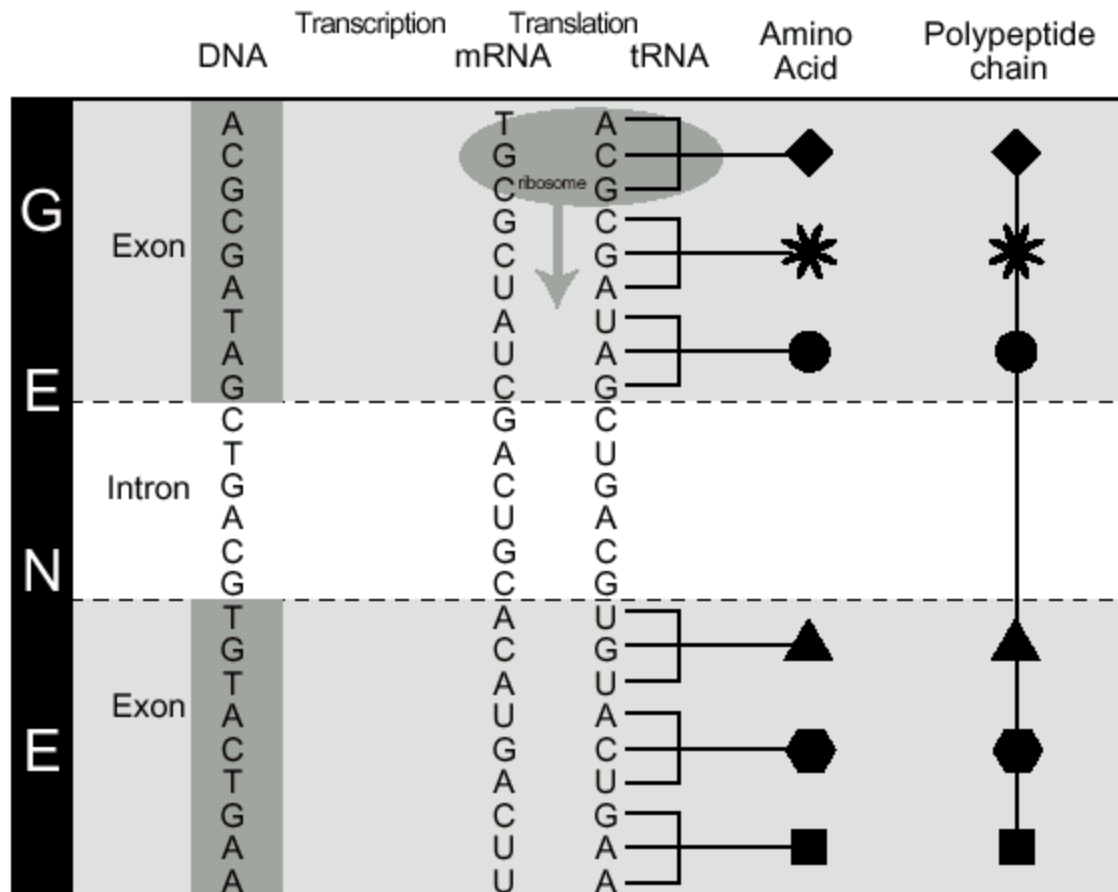


2 x H2A - 2 x H2B - 2 x H3 - 2 x H4

Exon a intron



Exon a intron



Stanovení sekvence DNA

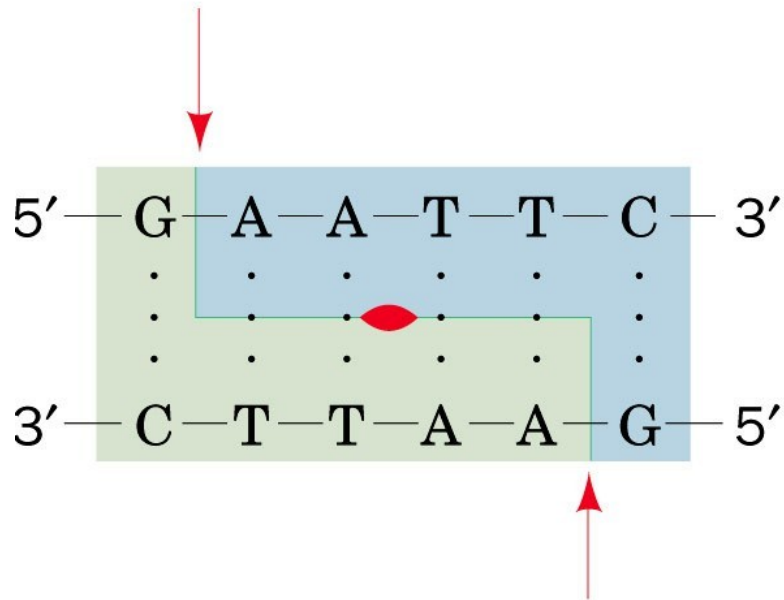
- Restrikční enzymy
- Chemické štěpení – Maxam Gilbertovo metoda
- Enzymová metoda
- Pyrosekvenování

Restrikční enzymy

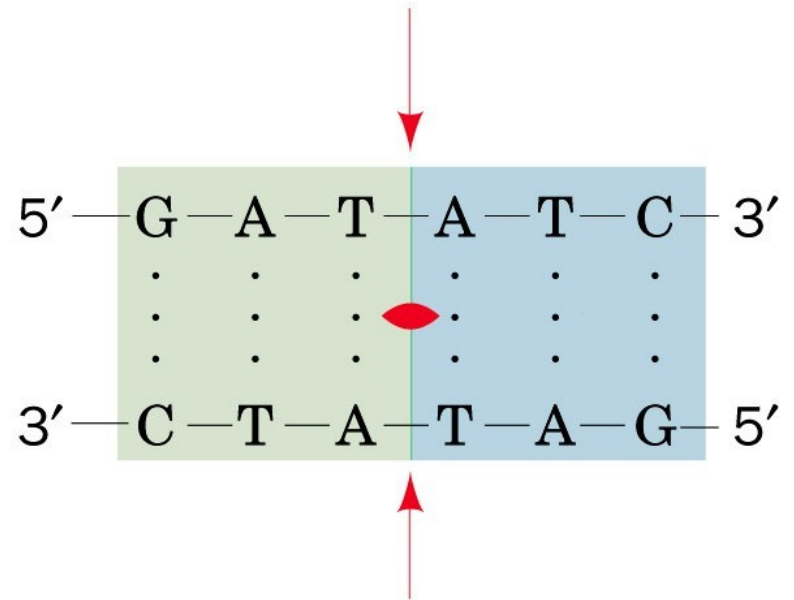
Enzyme	Recognition Sequence ^a	Microorganism
<i>AhaI</i>	AG↓C* ^a T	<i>Anthrobacter luteus</i>
<i>BamHI</i>	G↓GATC* ^a C	<i>Bacillus amyloliquefaciens</i> H
<i>BglI</i>	GCCNNNN↓NGCC	<i>Bacillus globigii</i>
<i>BglII</i>	A↓GATCT	<i>Bacillus globigii</i>
<i>EcoRI</i>	G↓AA* ^a TTC	<i>Escherichia coli</i> RY13
<i>EcoRII</i>	↓CC*(^a)GG	<i>Escherichia coli</i> R245
<i>EcoRV</i>	GA* ^a T↓ATC	<i>Escherichia coli</i> J62 pLG74
<i>HaeII</i>	RGCGC↓Y	<i>Haemophilus aegyptius</i>
<i>HaeIII</i>	GG↓C* ^a C	<i>Haemophilus aegyptius</i>
<i>HindIII</i>	A* ^a ↓AGCTT	<i>Haemophilus influenzae</i> R ₁
<i>HpaII</i>	C↓C* ^a GG	<i>Haemophilus parainfluenzae</i>
<i>MspI</i>	C* ^a ↓CGG	<i>Moraxella</i> species
<i>PstI</i>	CTGCA* ^a ↓G	<i>Providencia stuartii</i> 164
<i>PvuII</i>	CAG↓C* ^a TG	<i>Proteus vulgaris</i>
<i>SalI</i>	G↓TCGAC	<i>Streptomyces albus</i> G
<i>TaqI</i>	T↓CGA* ^a	<i>Thermus aquaticus</i>
<i>XhoI</i>	C↓TCGAG	<i>Xanthomonas holcicola</i>

Restrikční enzymy

(a) *EcoRI*



(b) *EcoRV*



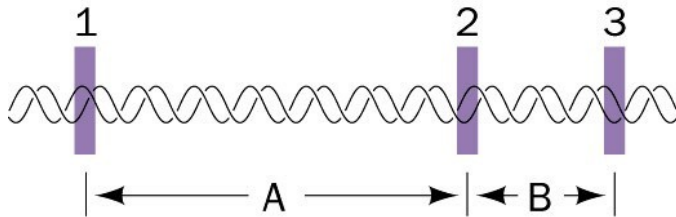
 Cleavage site

 Twofold symmetry axis

Restrikční enzymy

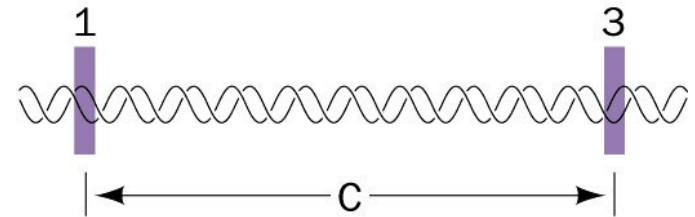
Chromosome I

DNA has
3 target sites



Chromosome II

DNA has only
2 of the target sites



Cleave with
restriction enzyme
and electrophoresis

Fragment C is
the size of
A + B combined

Restrikční enzymy

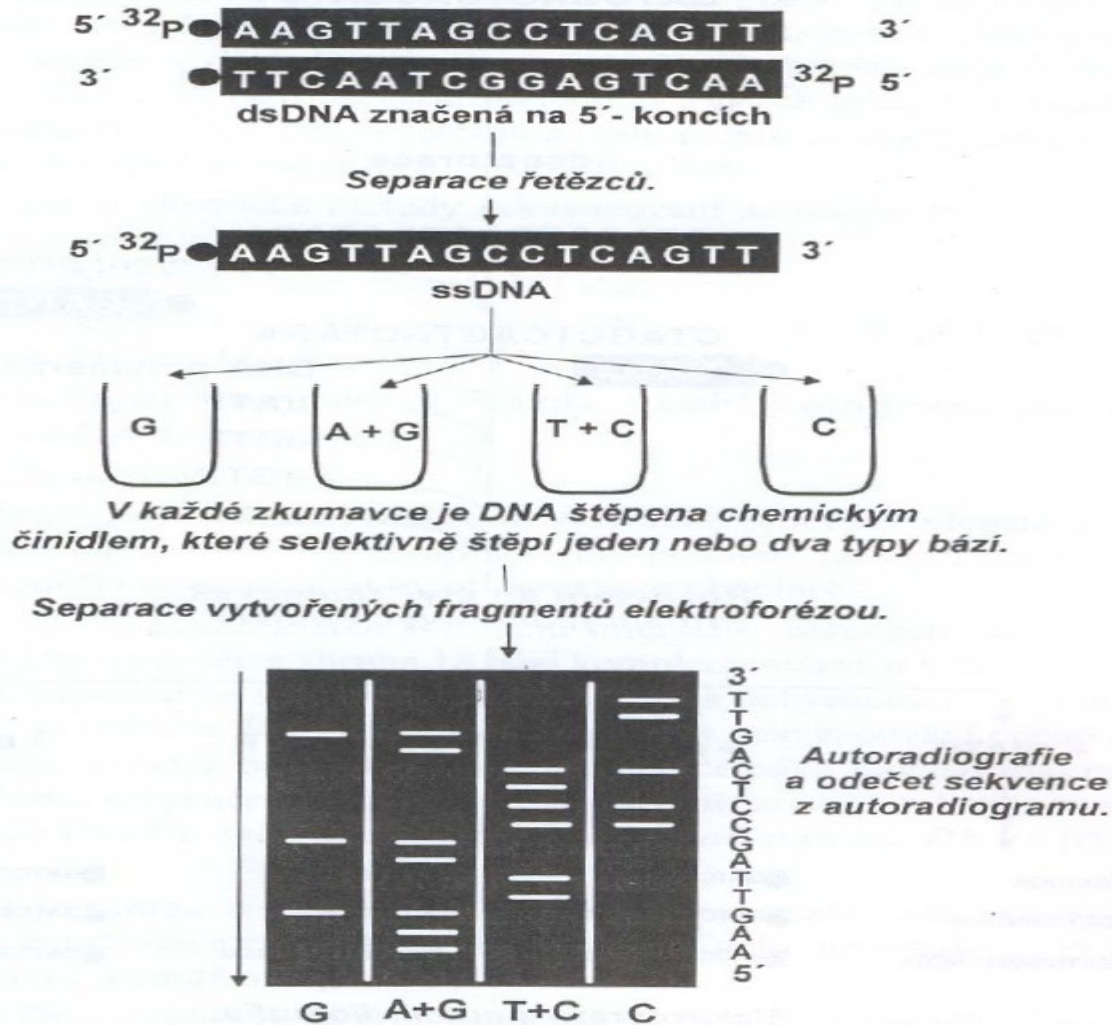


Maxam Gilbertova metoda

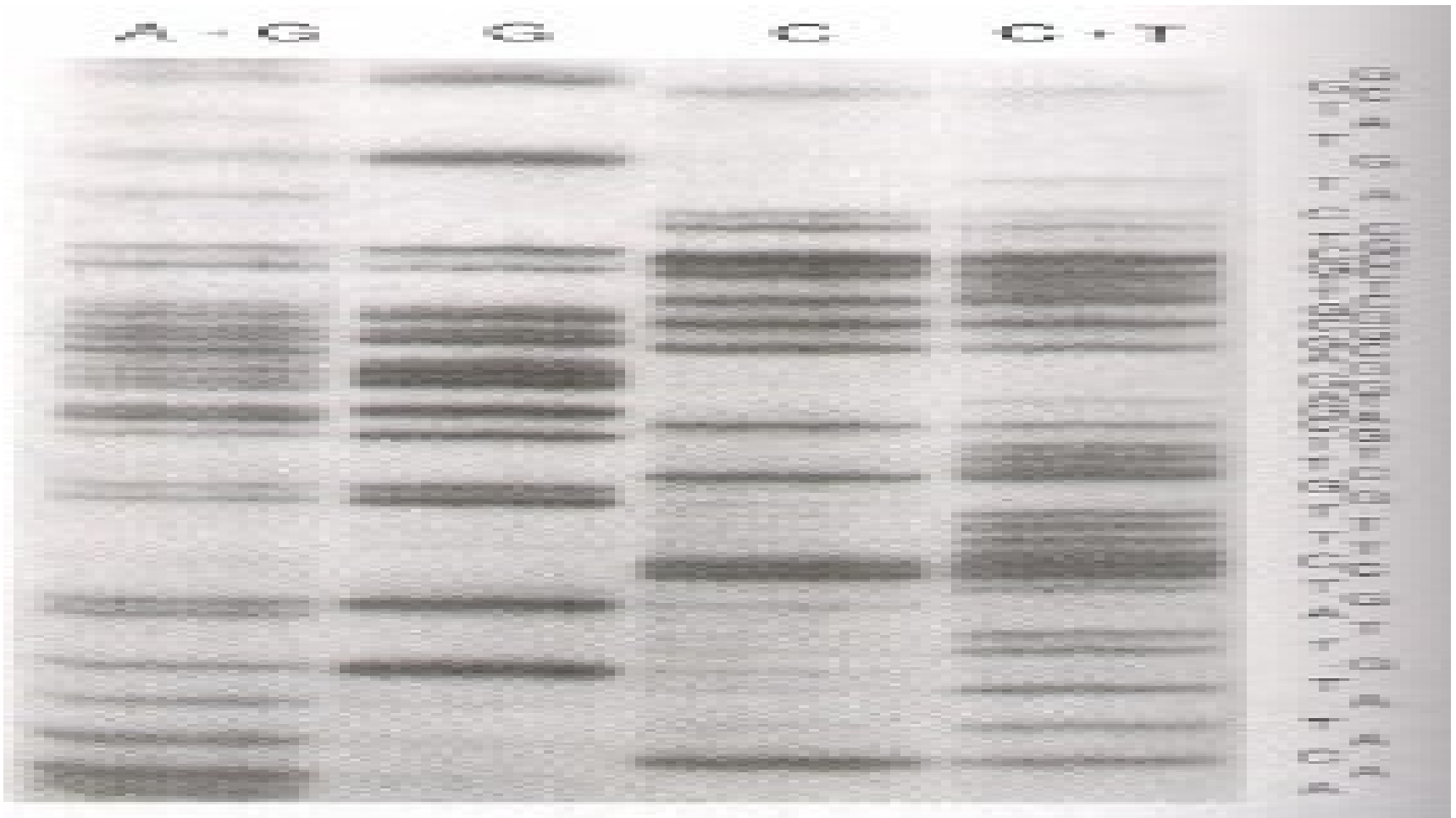
Maxam-Gilbertova metoda

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

Maxam Gilbertova metoda

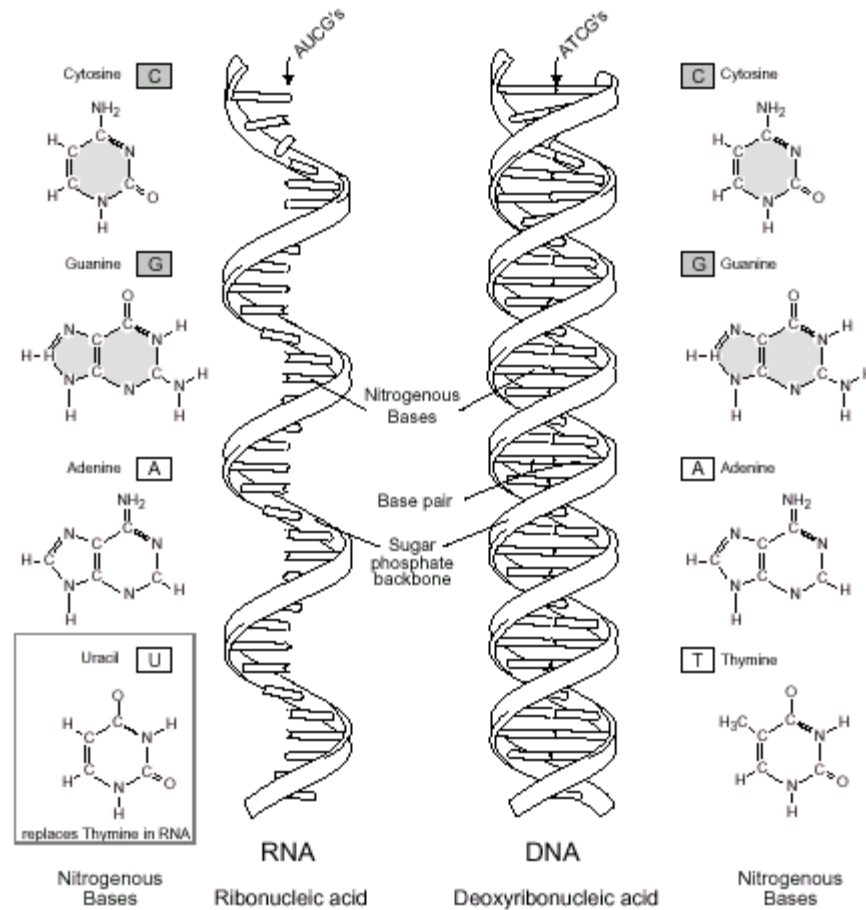


Maxam Gilbertova metoda



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

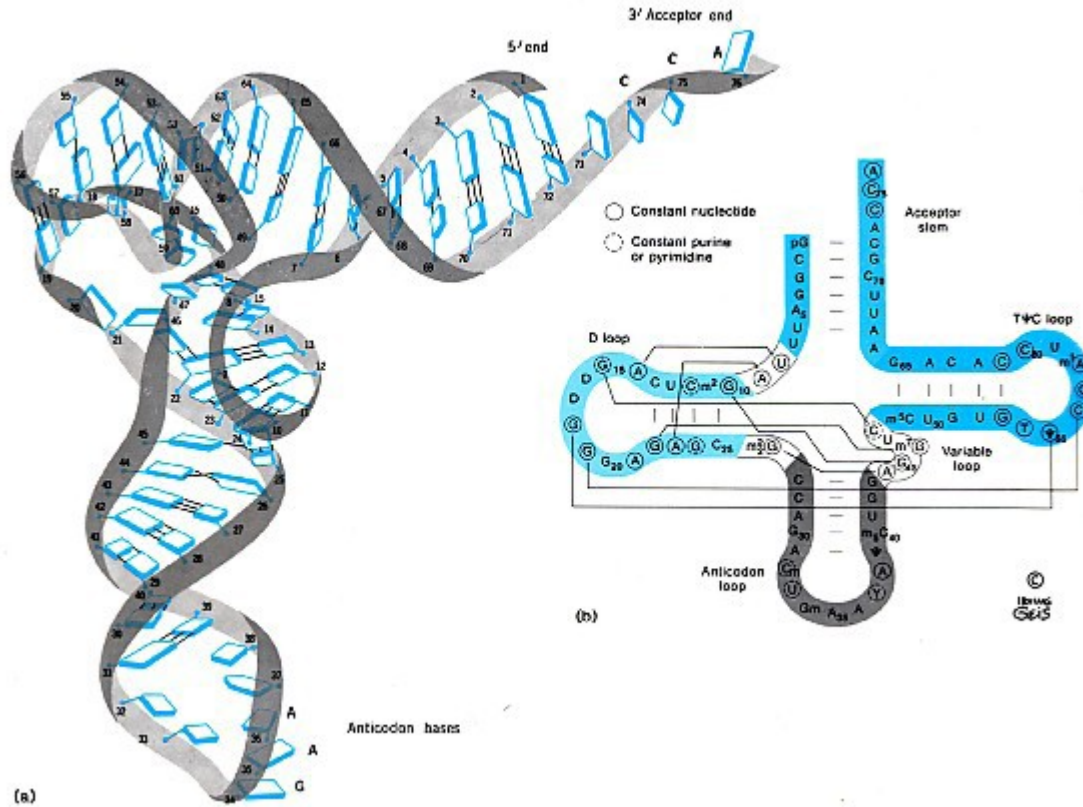
RNA versus DNA



Formy RNA

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

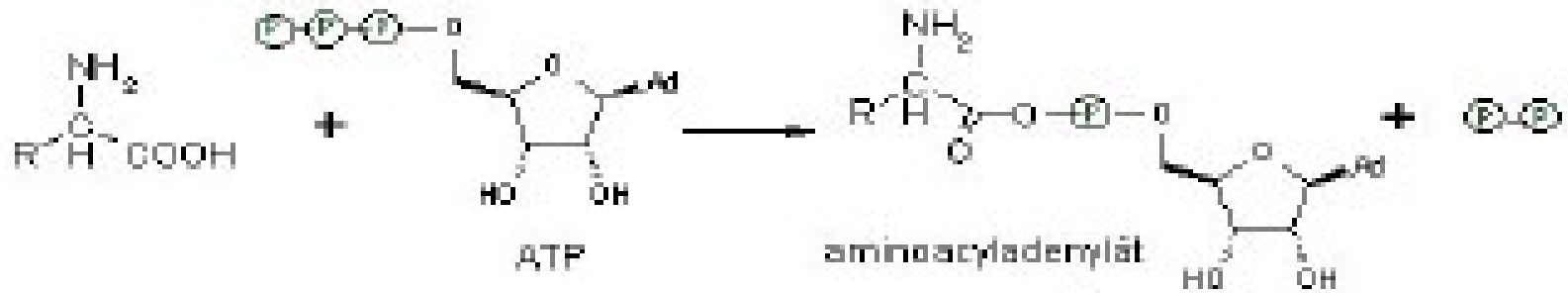
tRNA



(a)

(b)

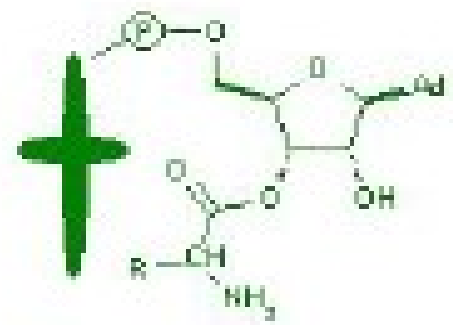
Anticodon bases



tRNA

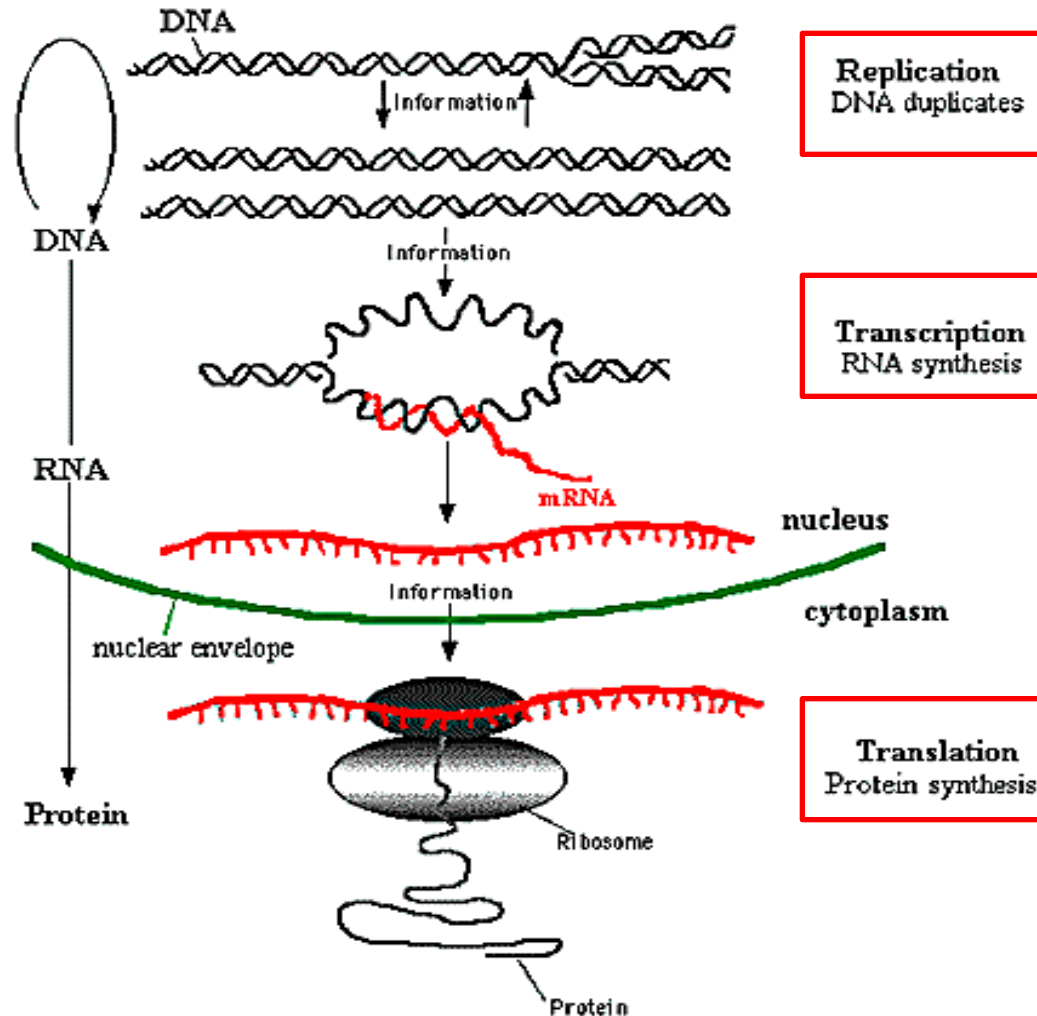


aminoacyl-tRNA + AMP

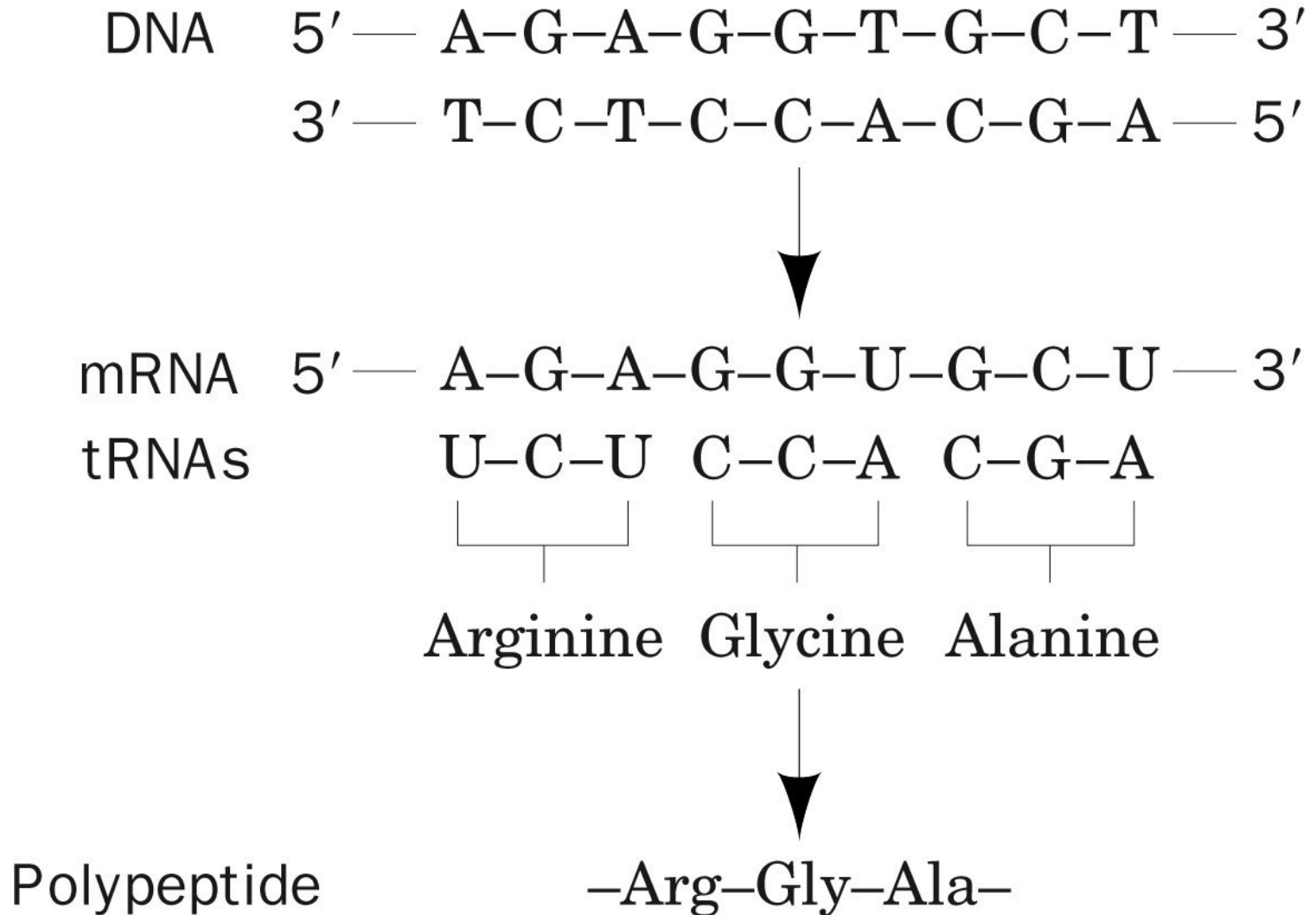


Centrální dogma mol.biologie

Crick 1958 - cesta od NK k proteinu

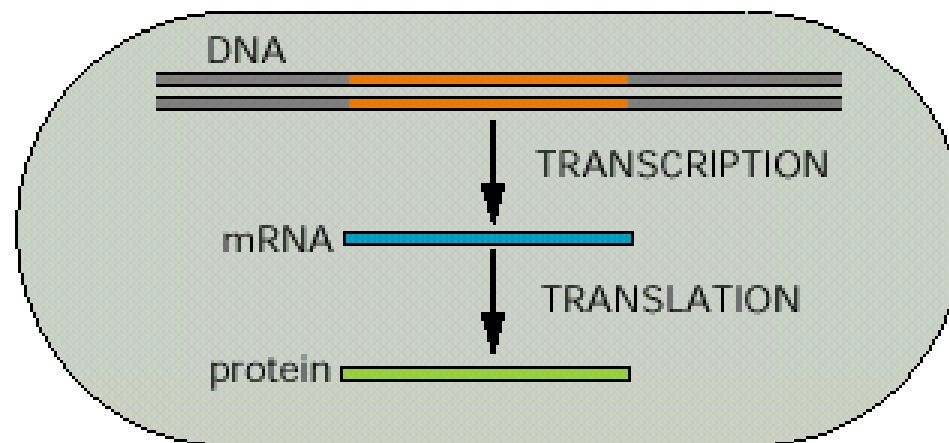


Centrální dogma mol.biologie



Prokaryota

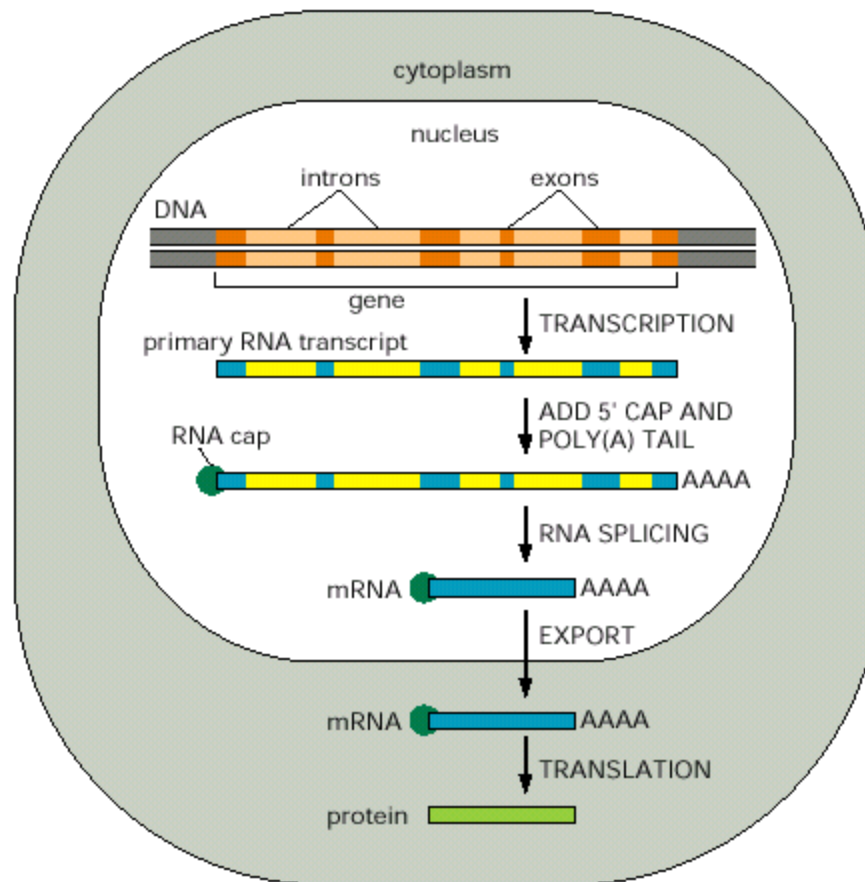
(B) PROCARYOTES



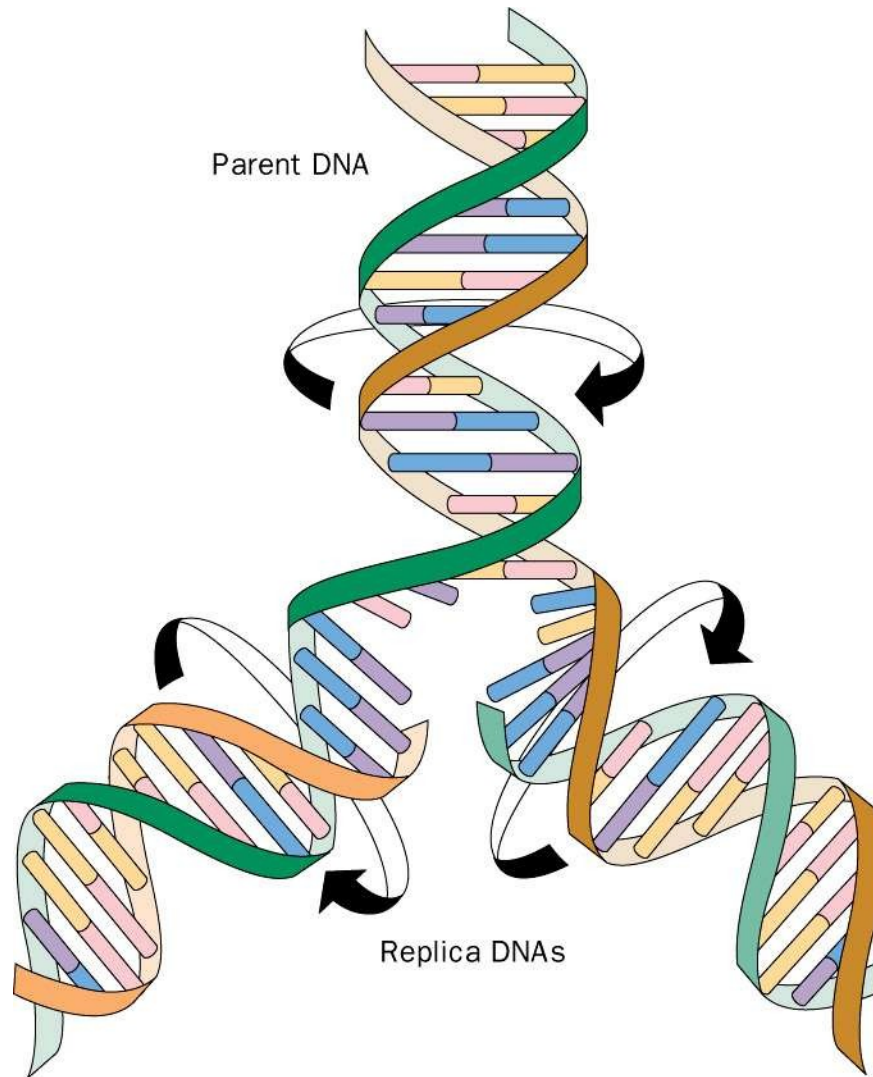
Eukaryota

(A) EUCARYOTES

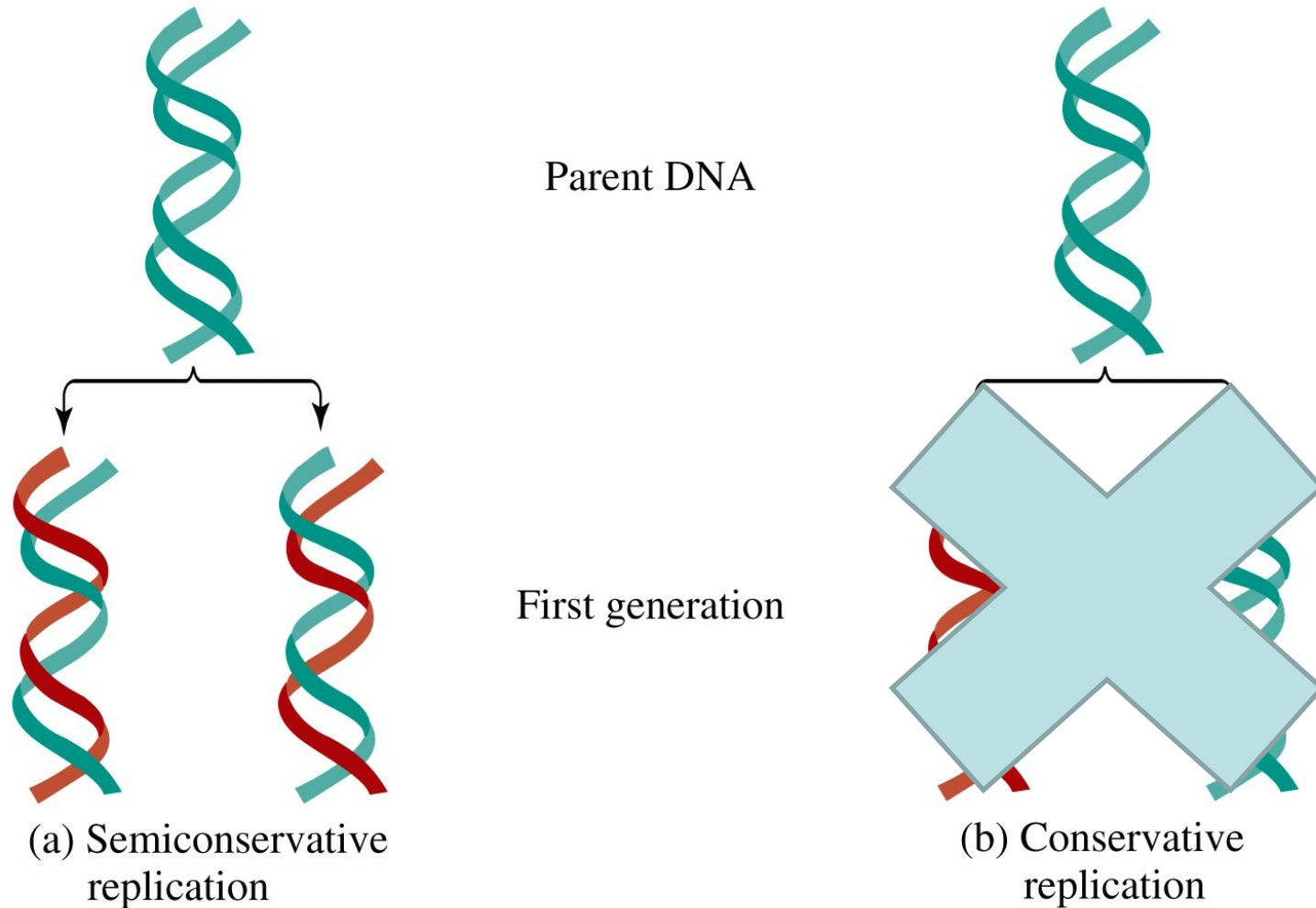
(B)



Replikace DNA



Replikace DNA



Meselsonův a Stahlův experiment

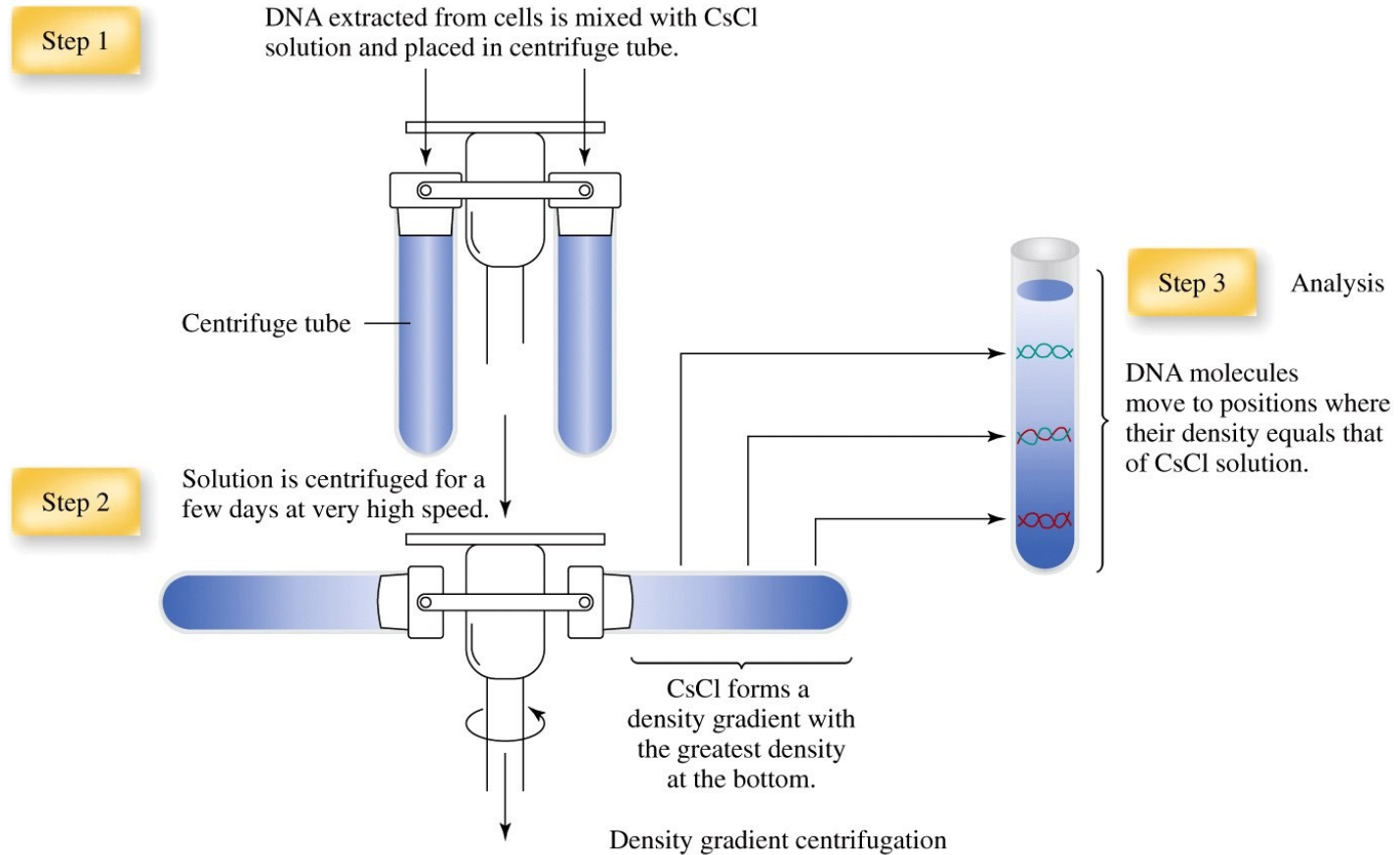


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Ultracentrifugace

Preparativní ultracentrifuga

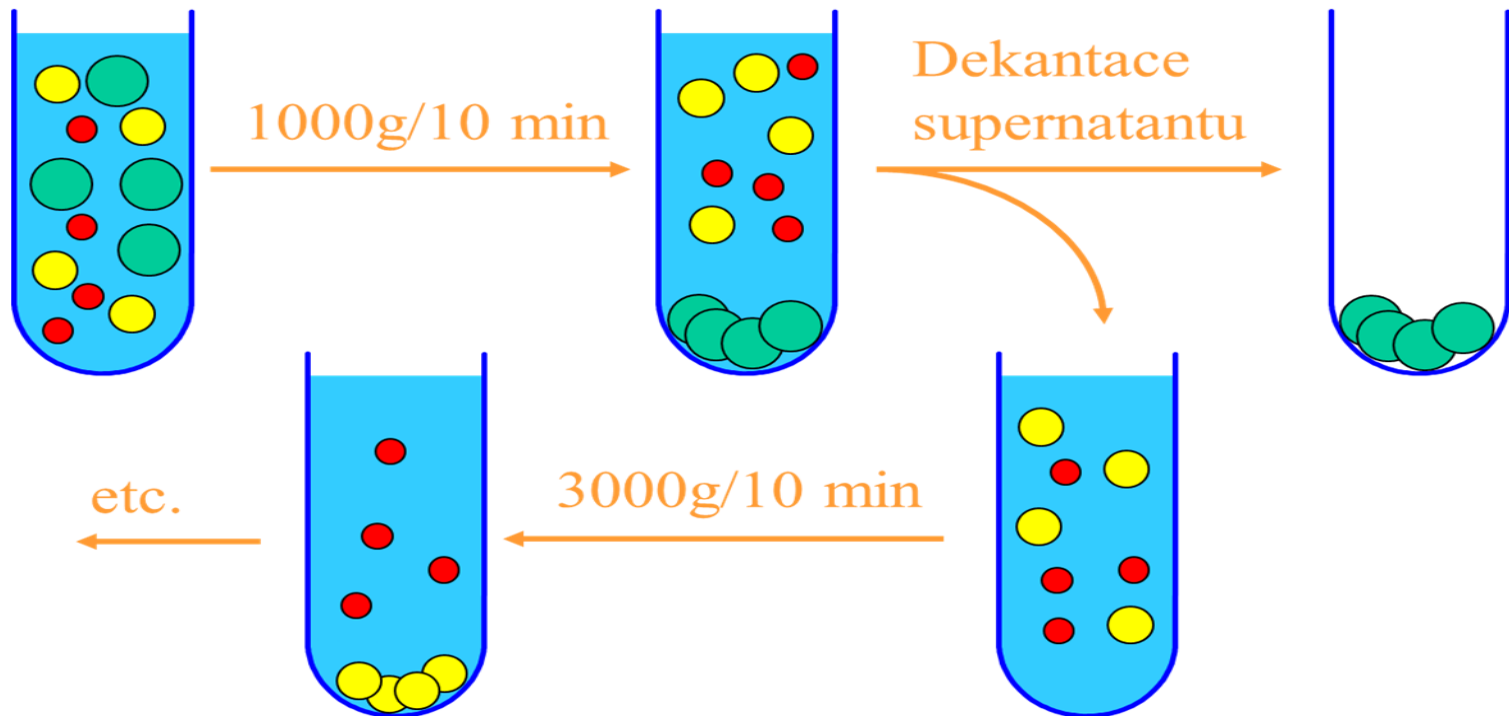


Rotory

- Úhlový – diferenciální centrifugace
- Výkyvné – zonální centrifugace

Diferenciální centrifugace

- opakovaná centrifugace se zvyšující se rychlostí otáček = gravitací



Úhlový rotor

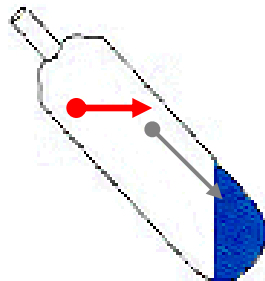
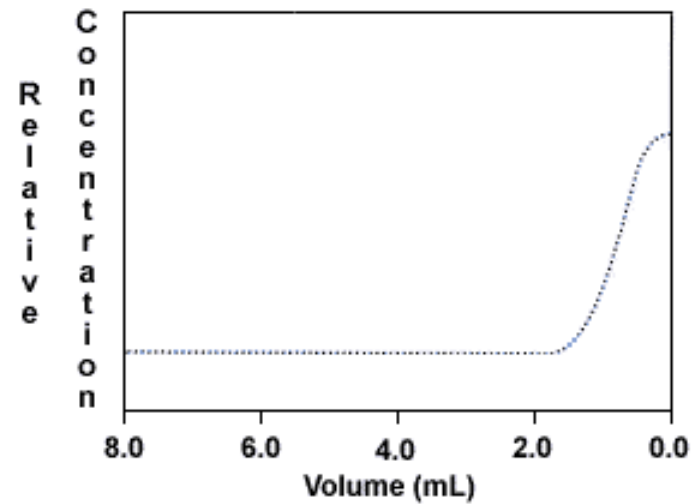
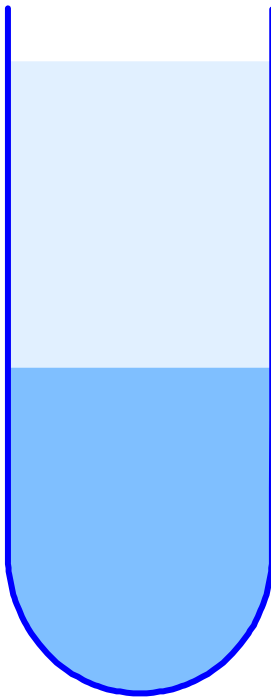


Figure 4

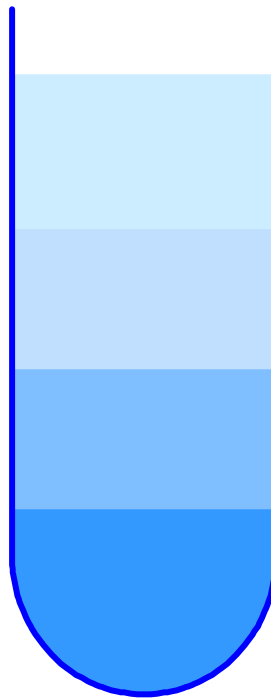


Gradientová centrifugace

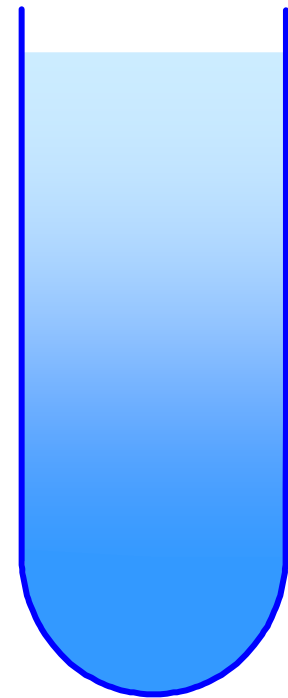
Hustotní bariera



Diskontinuální

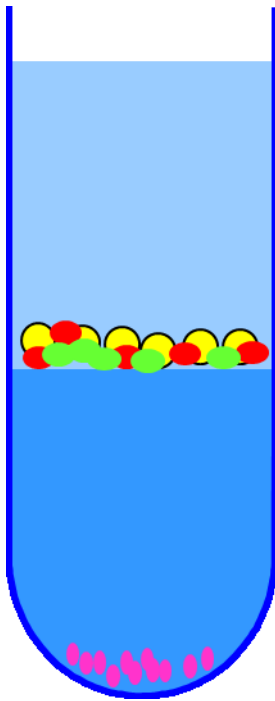


Kontinuální

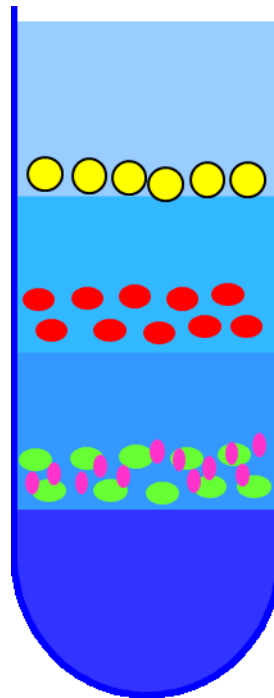


Gradientová centrifugace

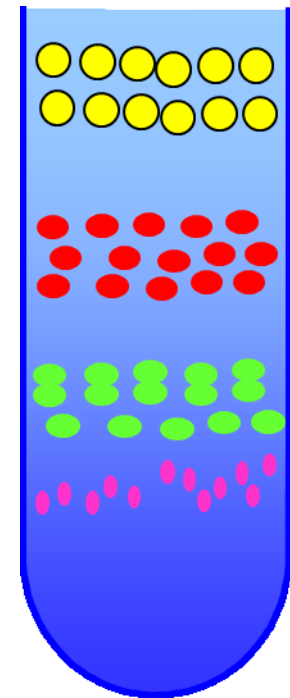
Hustotní bariera



Diskontinuální



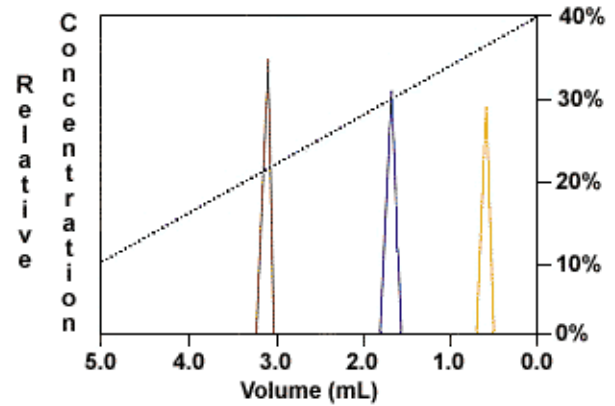
Kontinuální



Výkyvný rotor



Figure 2



Gradientová centrifugace média

- Sacharosa

- Glycerol

- Ficoll - dextran

- Percoll – SiO₂

Hypertonické prostředí

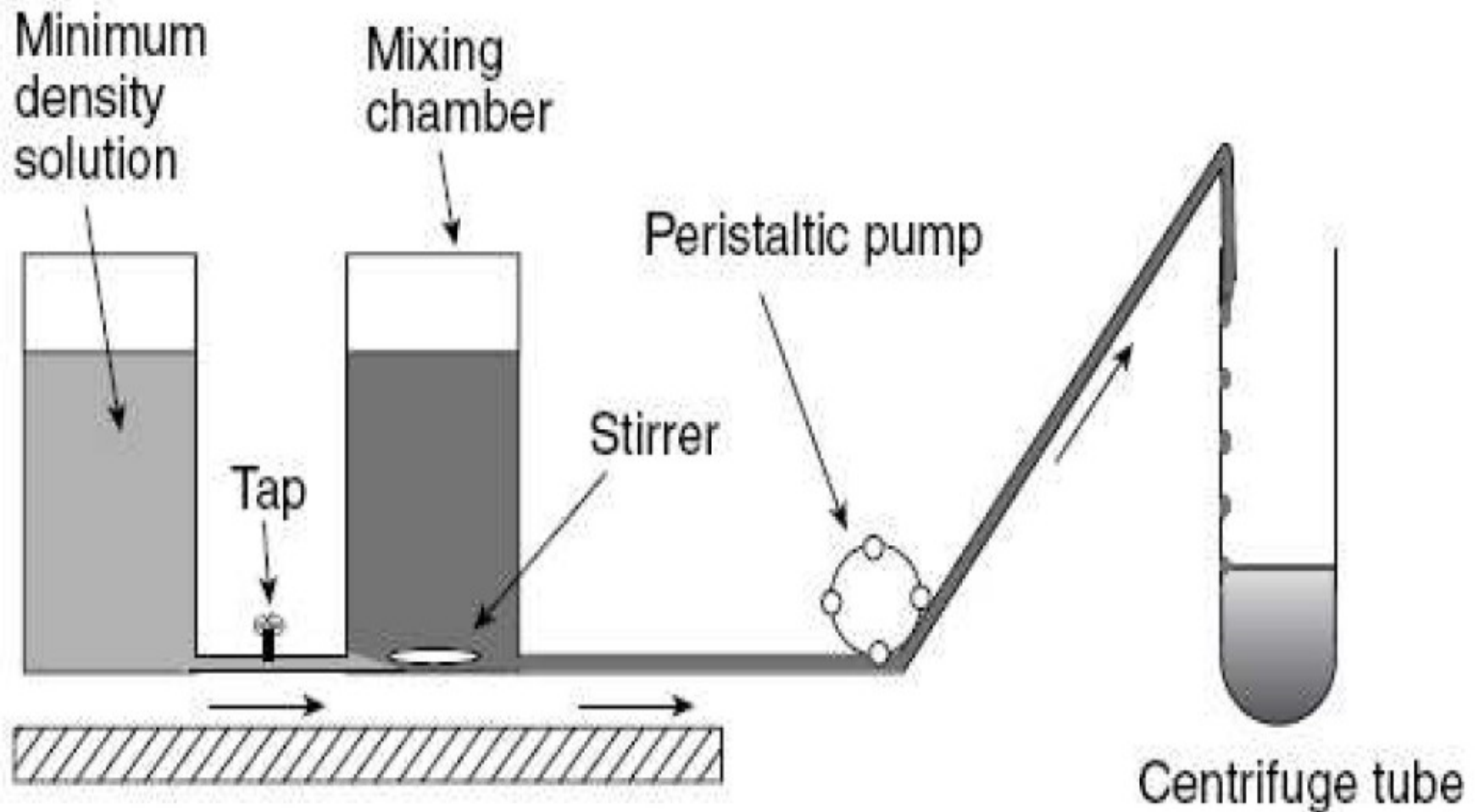
Nutno připravit gradient

- CsCl

- Cs₂SO₄

Gradient vzniká během centrifugace

Gradientová centrifugace



Gradientová centrifugace

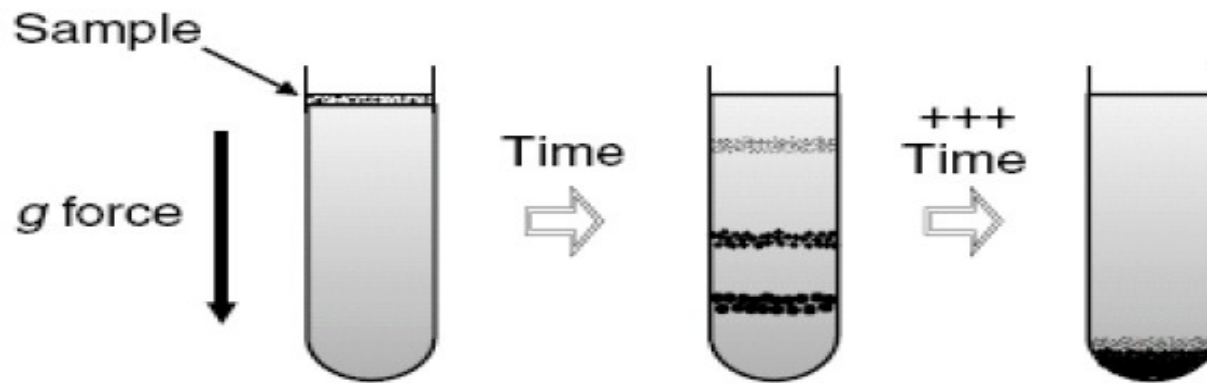
Metoda

Izopyknickeá

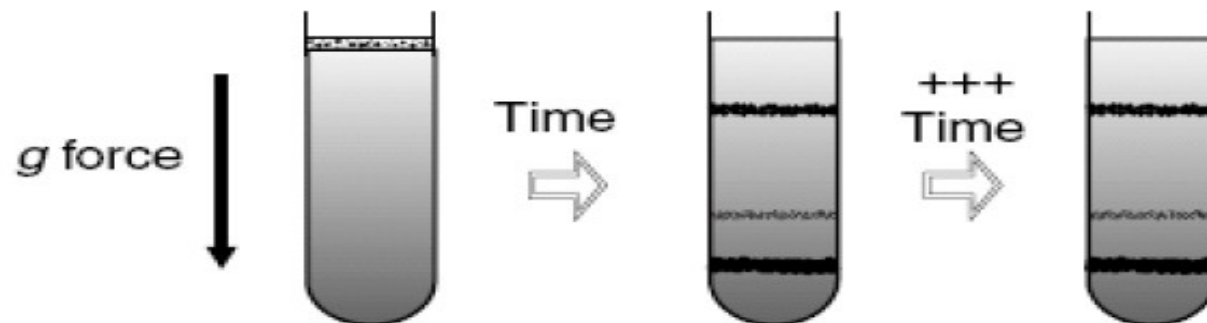
Neomovážná

Gradientová centrifugace

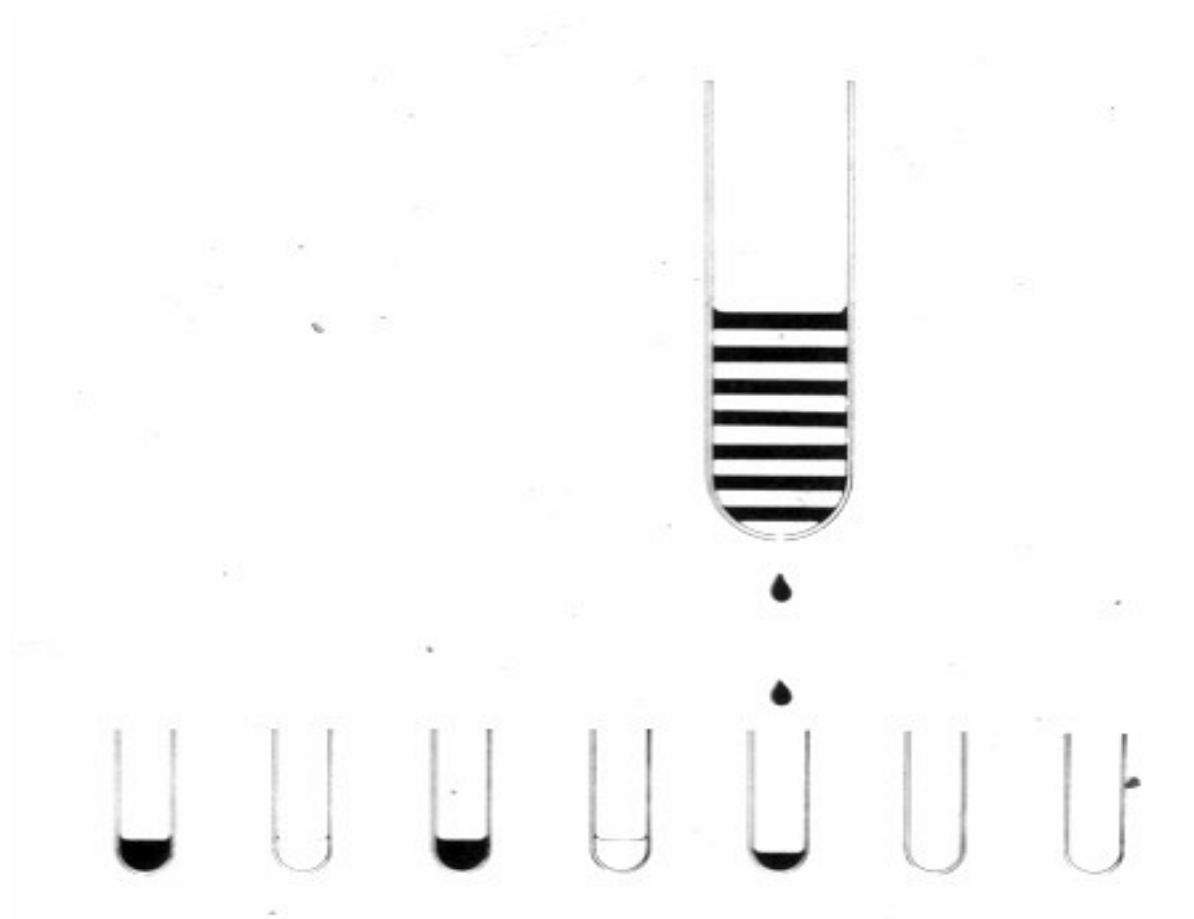
Rate-zonal centrifugation



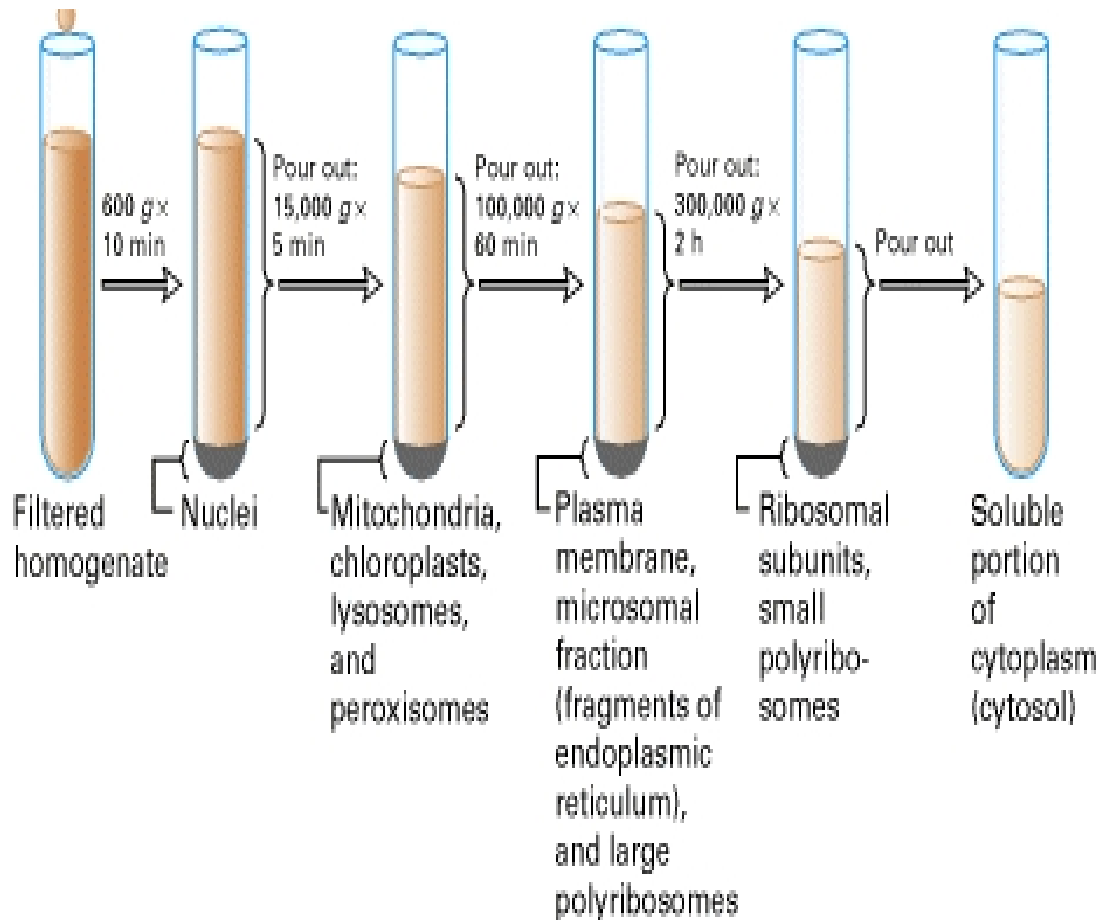
Isopycnic centrifugation



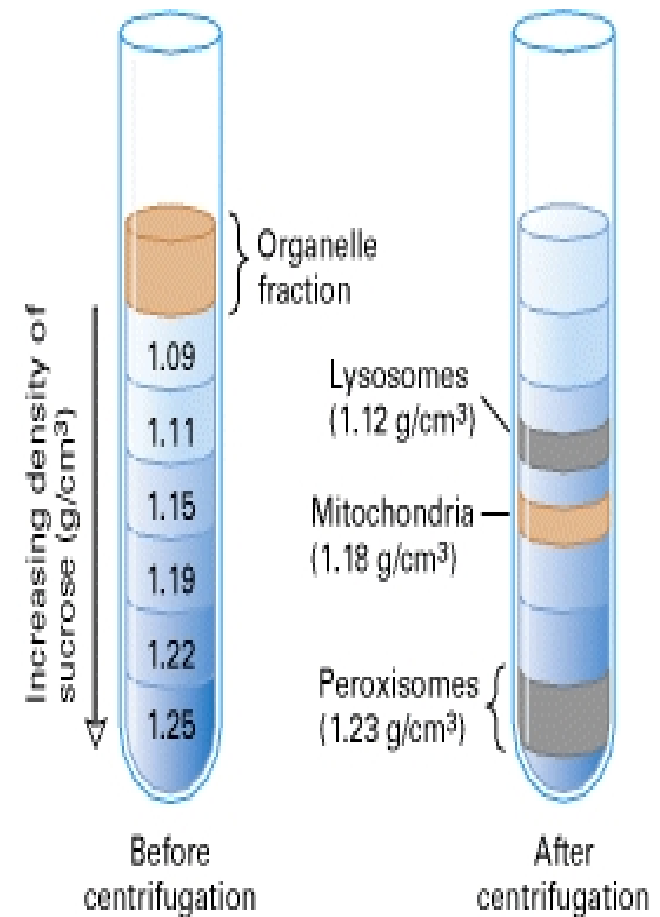
Gradientová centrifugace



Diferenciální versus gradientová centrifugace



4 x



1 x

Meselsonův a Stahlův experiment

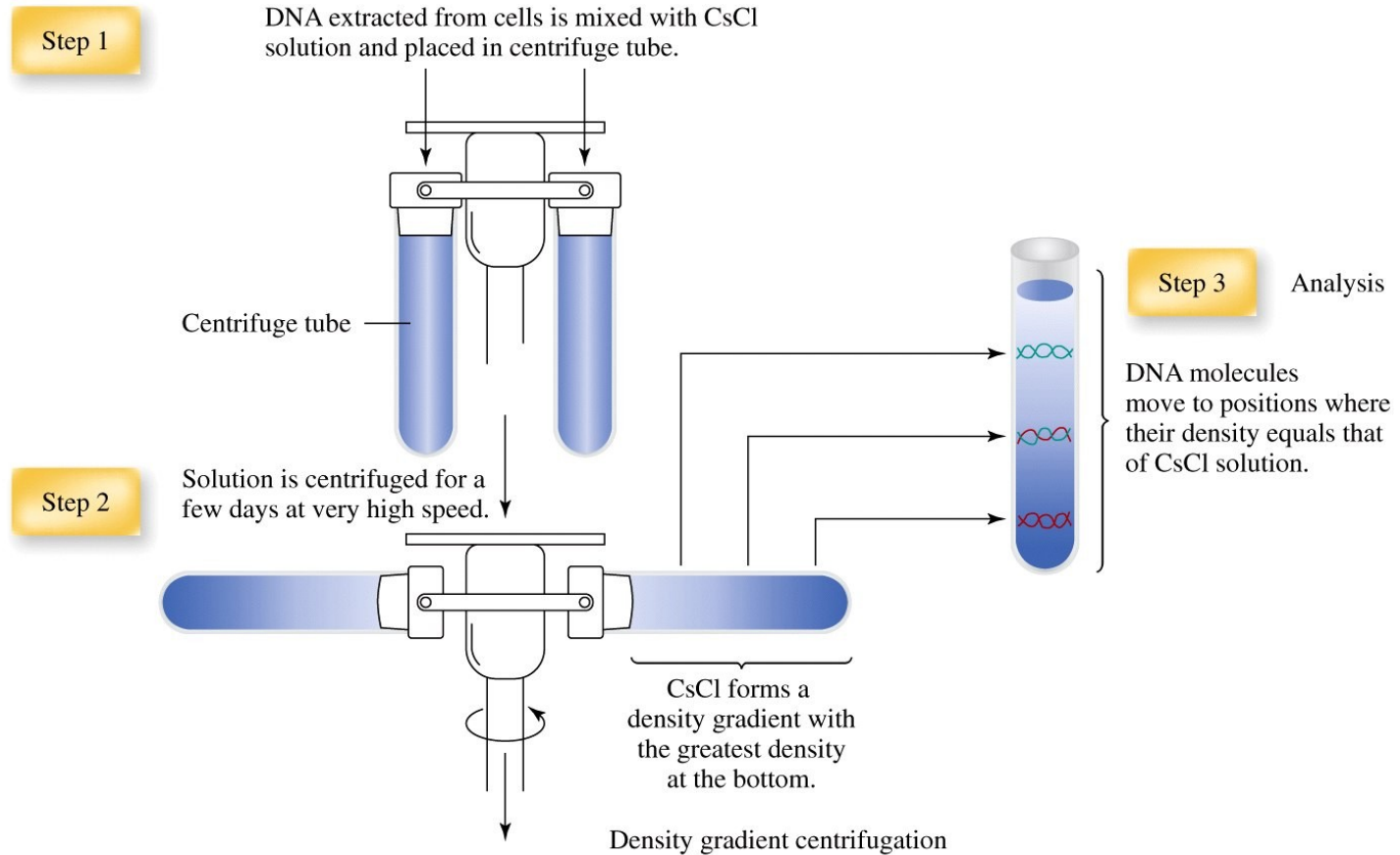


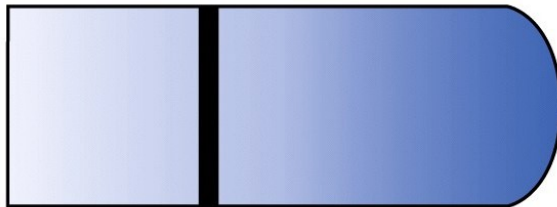
Figure 11-2a Concepts in Biochemistry, 3/e
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Meselsonův a Stahlův experiment

Direction of sedimentation



Heavy DNA



Light DNA



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

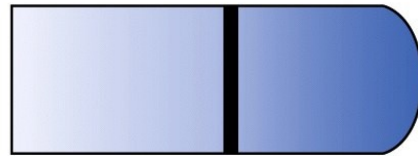


Normal ^{14}N -DNA
(with two light
strands)

Preliminary experiment

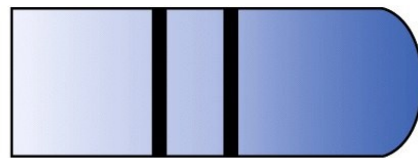
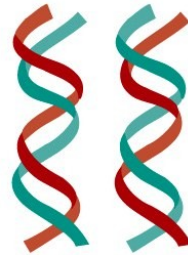
Meselsonův a Stahlův experiment

Experimental results



Hybrid DNA

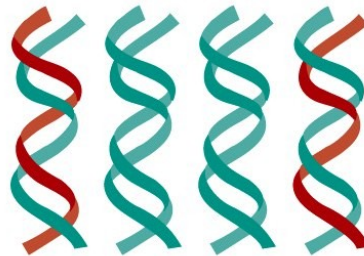
After one generation on $^{14}\text{N-NH}_4\text{Cl}$



Light
DNA

Hybrid
DNA

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



Conclusions

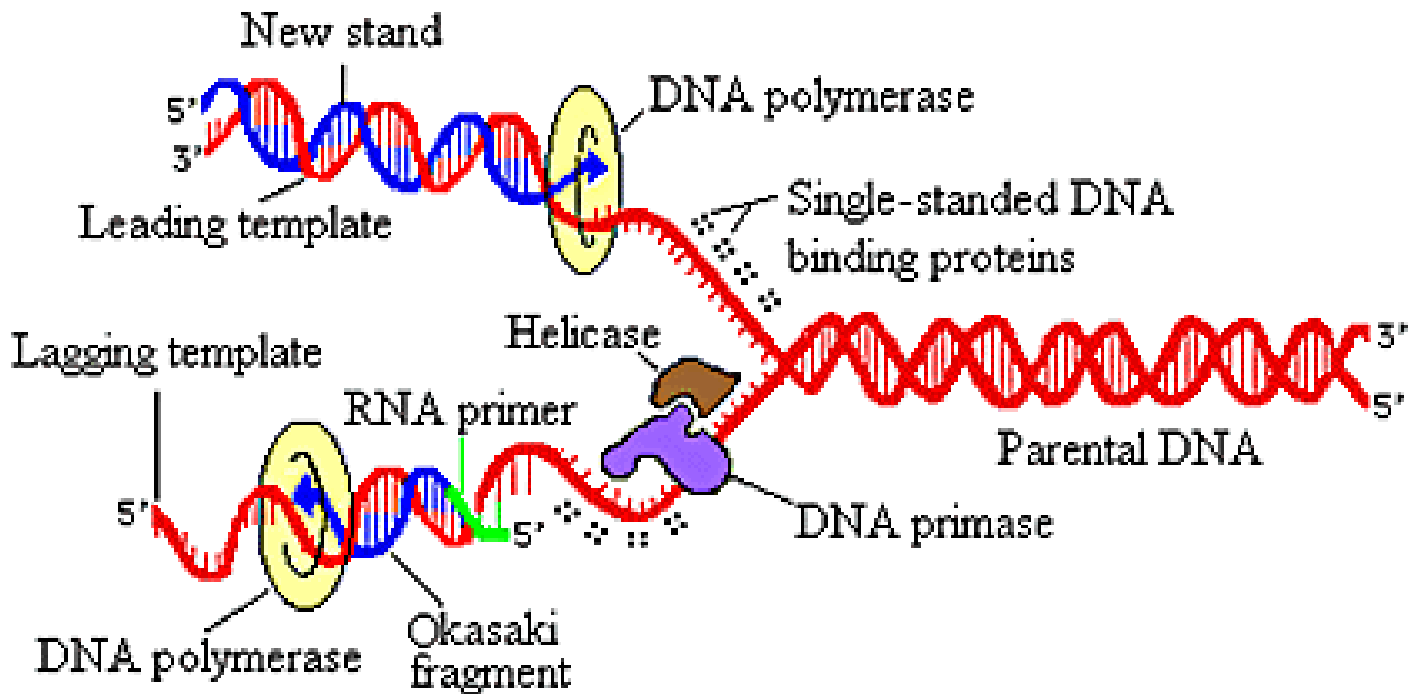
First generation:
Both DNAs contain one light and one heavy strand.

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

Prokaryontní replikace

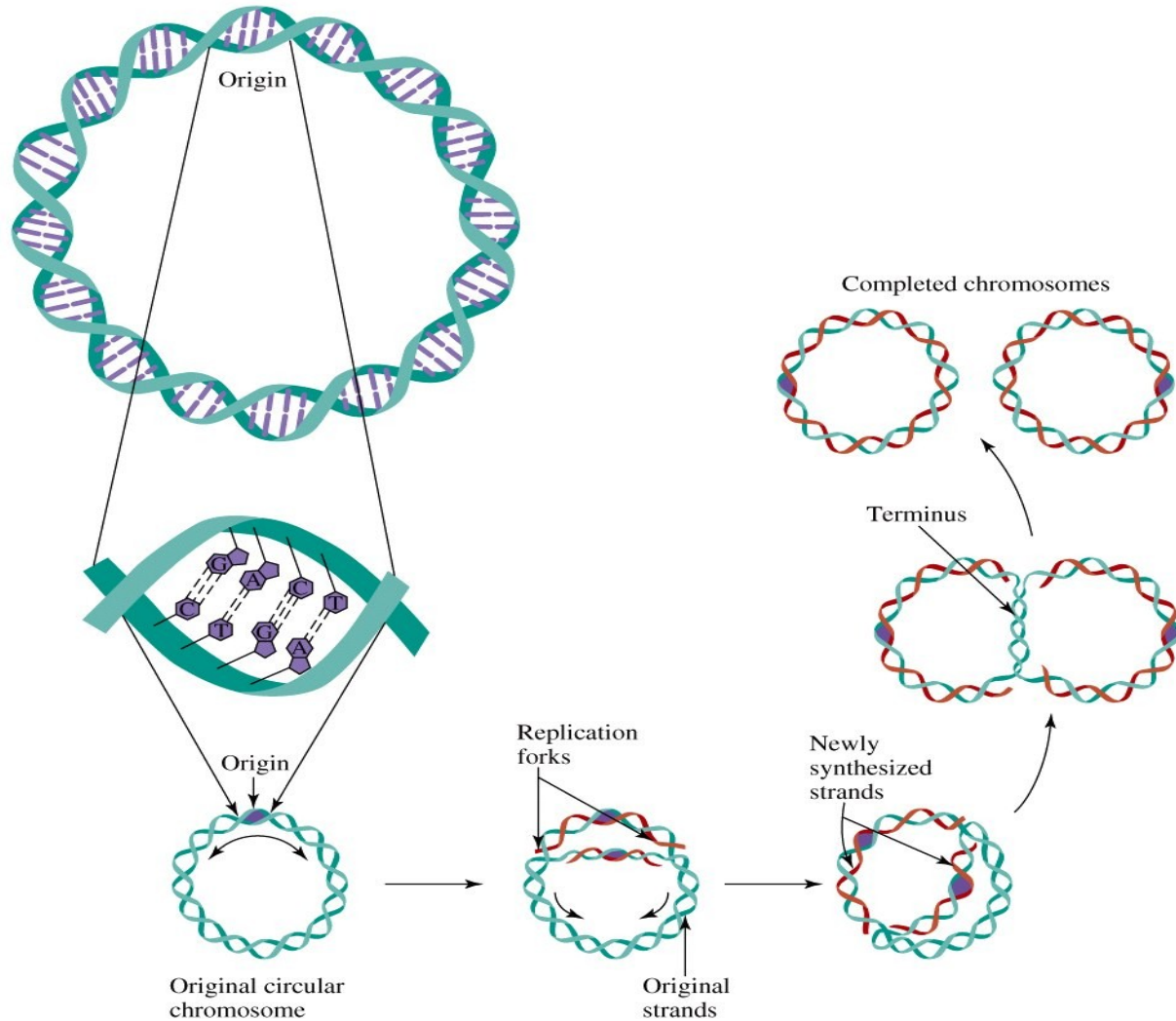
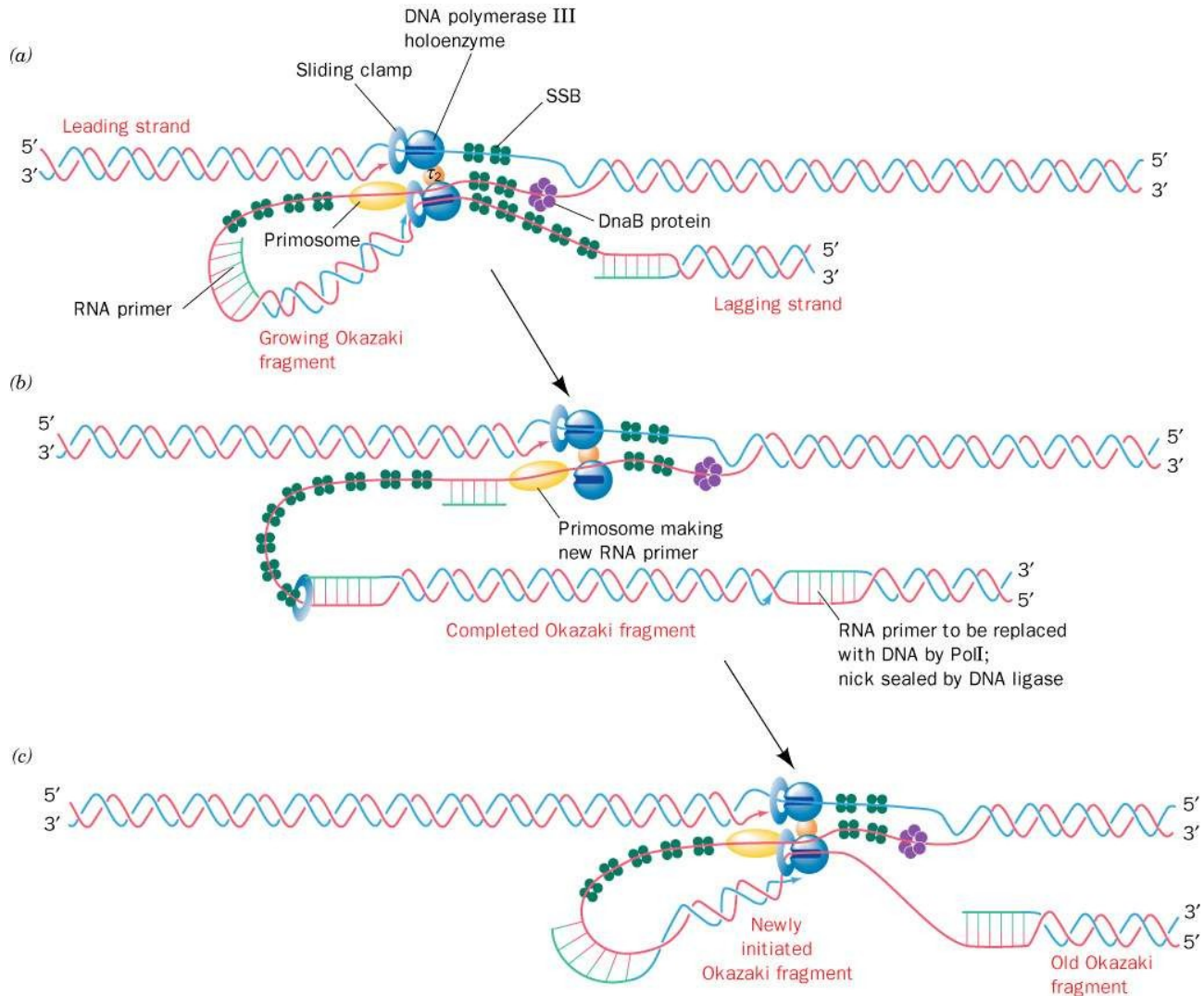


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Prokaryontní replikace



Replikace u E.coli

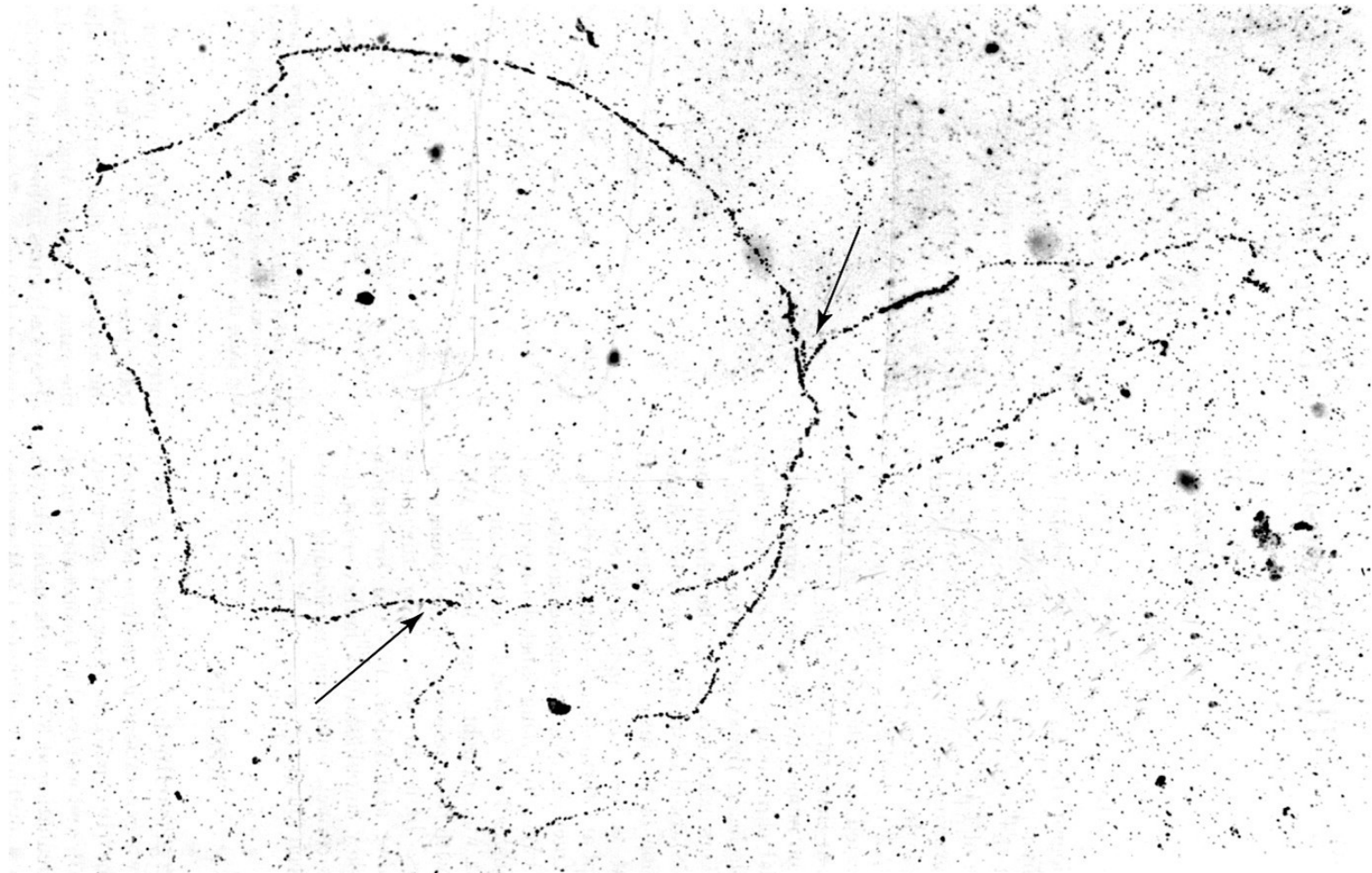


Figure 11-3 Concepts in Biochemistry, 3/e

Eukaryontní replikaci

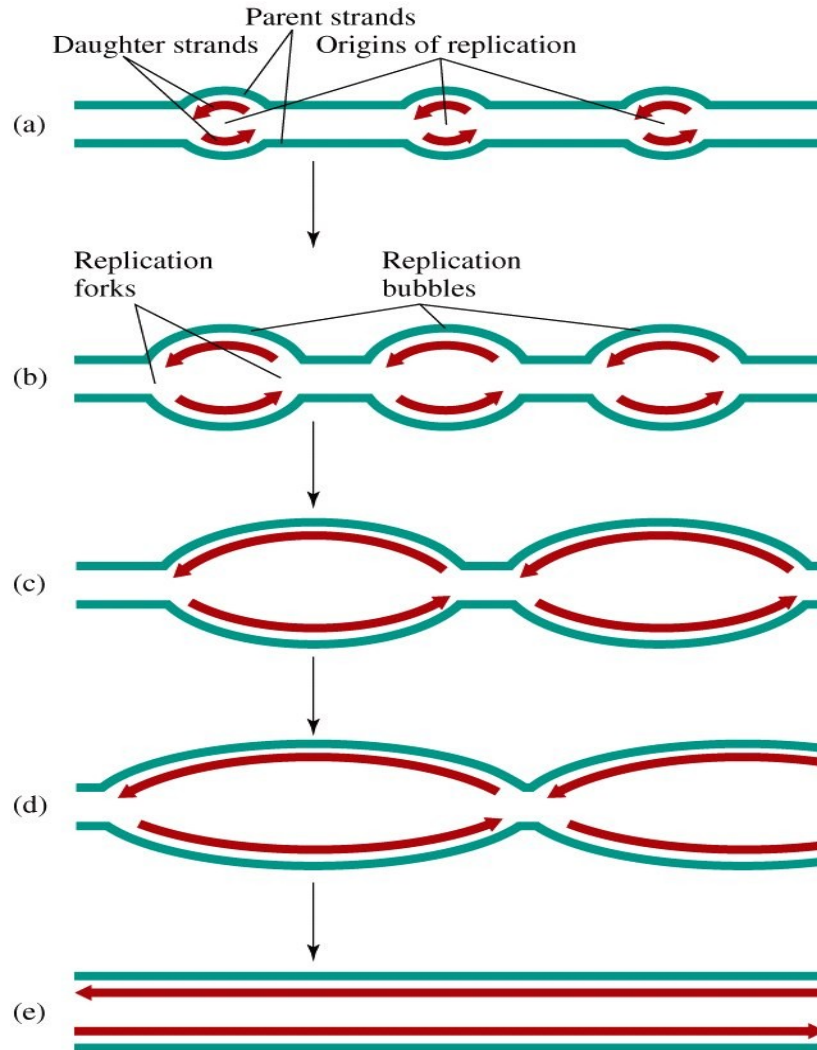
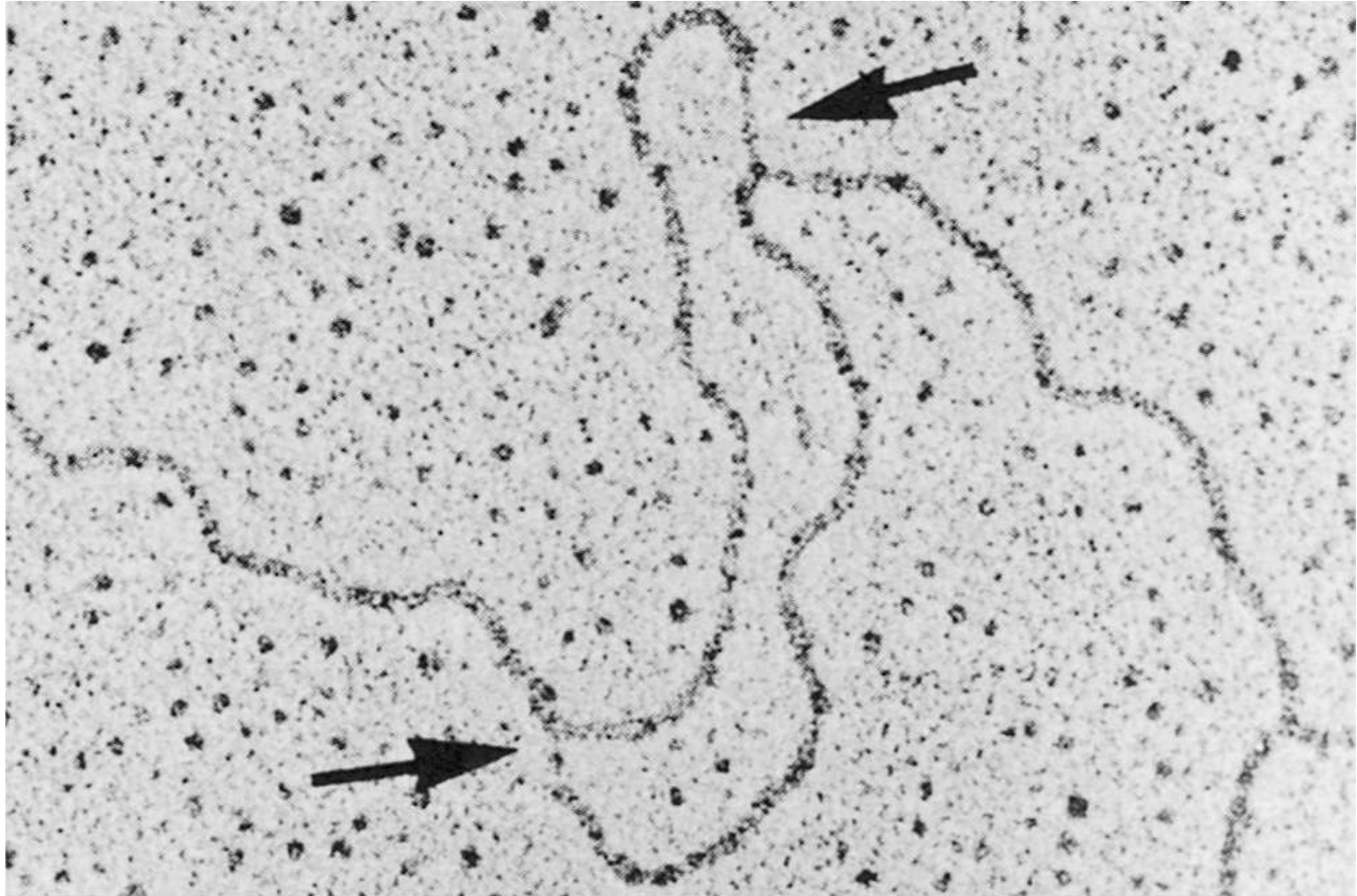


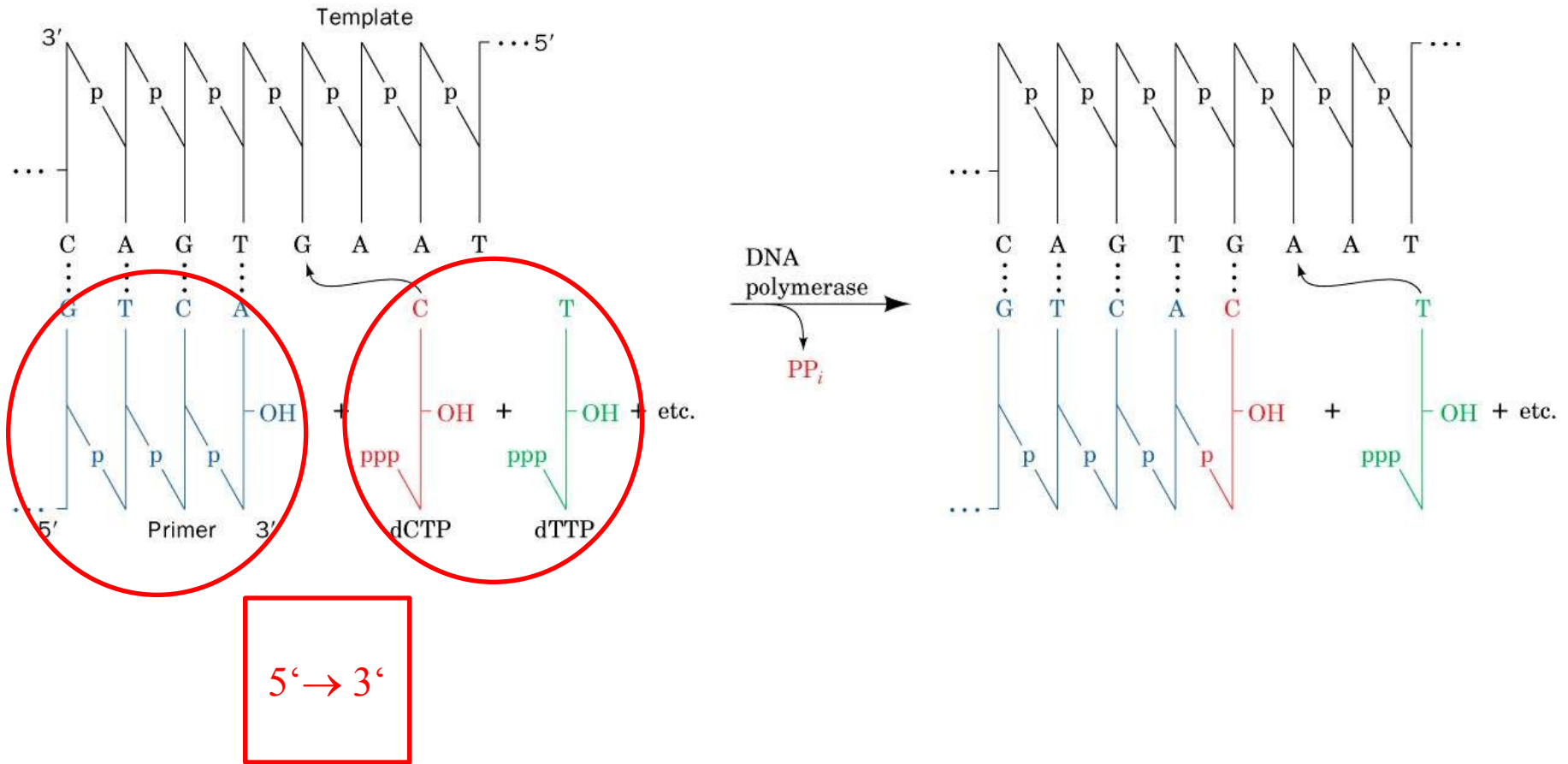
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Replikace u drosofil



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

DNA polymeráza III



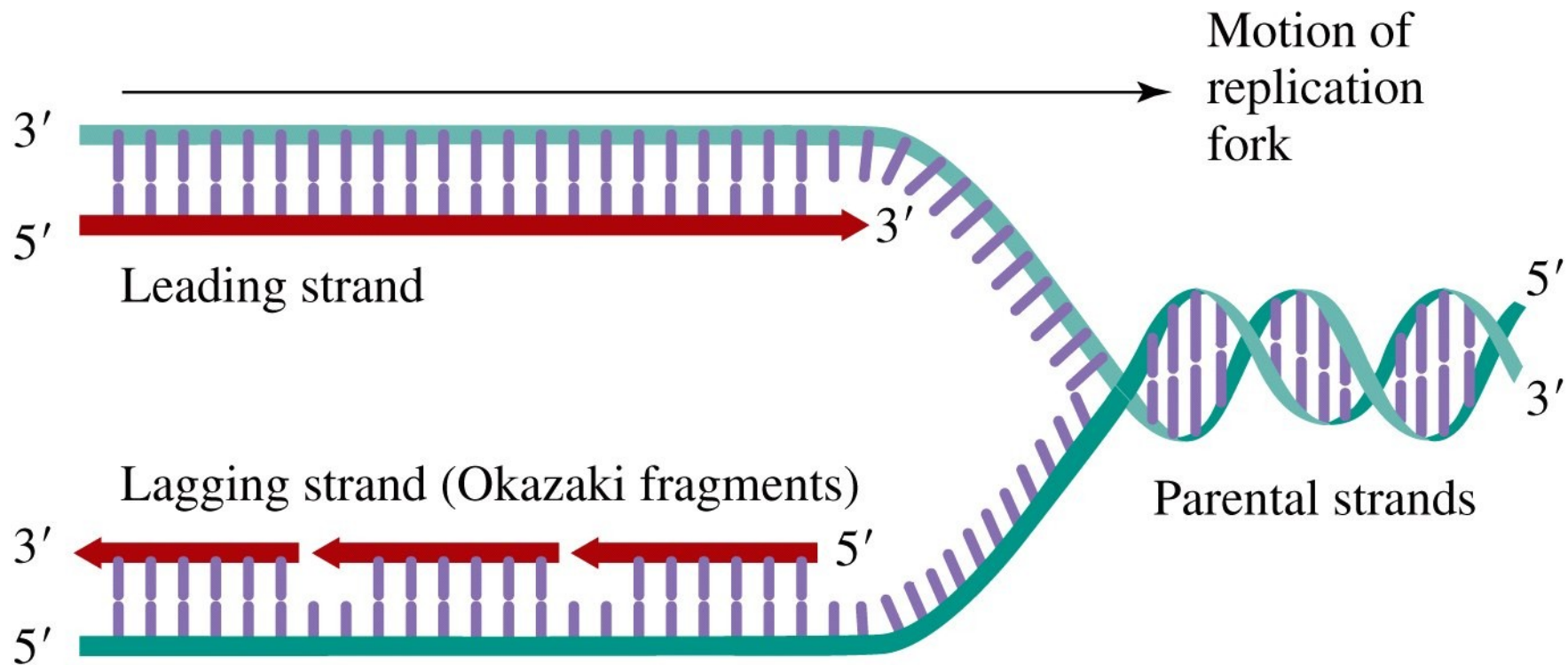


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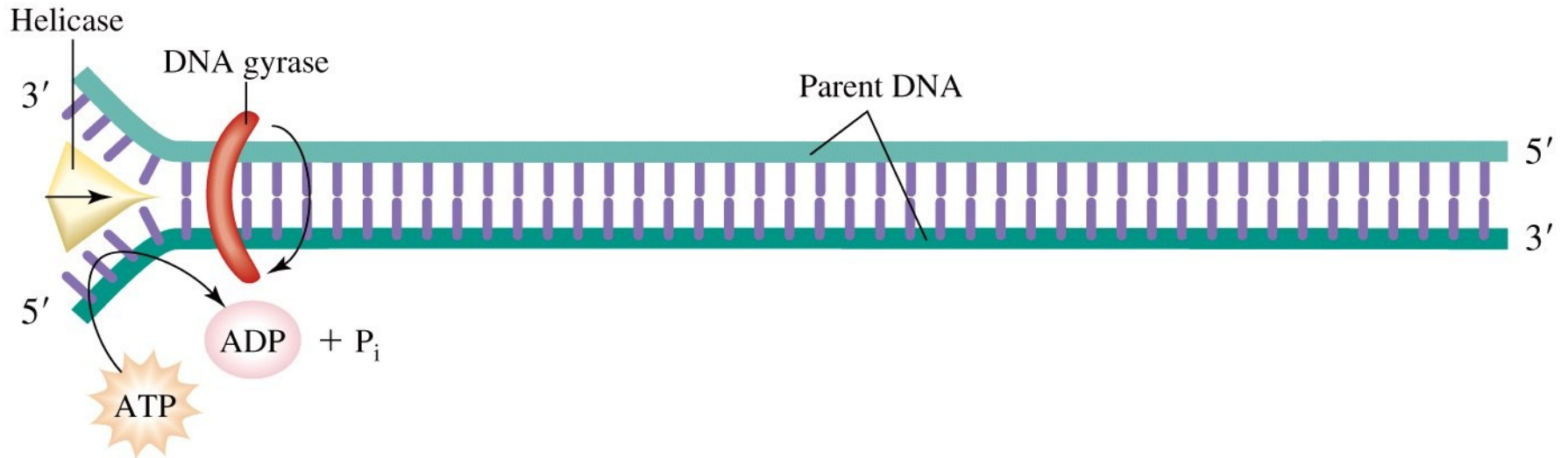


Figure 11-10a Concepts in Biochemistry, 3/e
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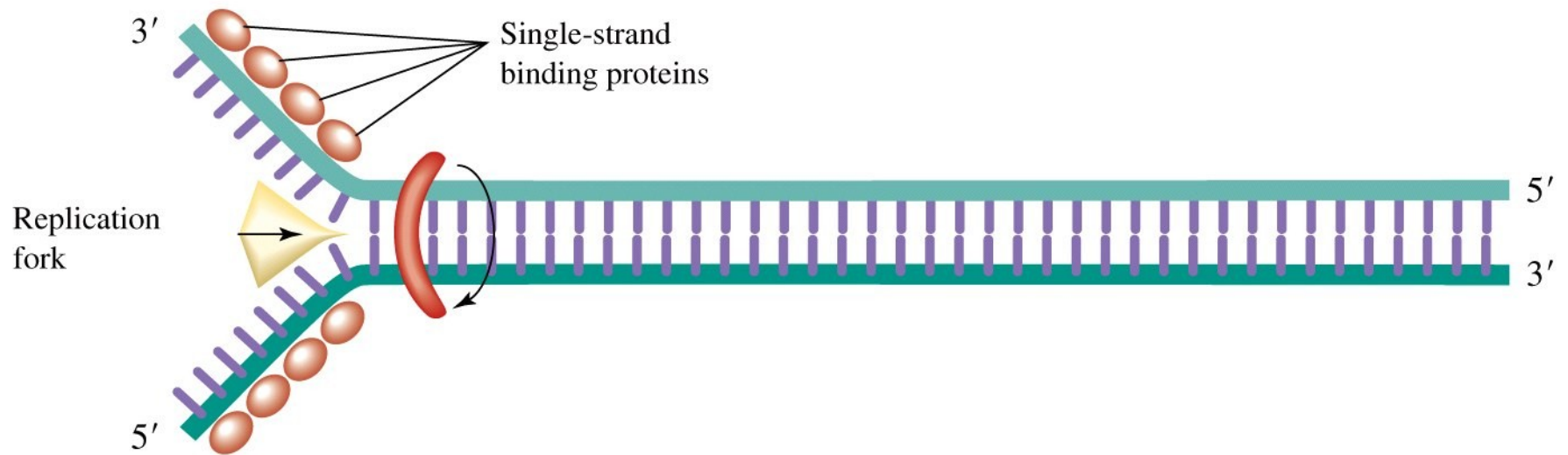


Figure 11-10b Concepts in Biochemistry, 3/e
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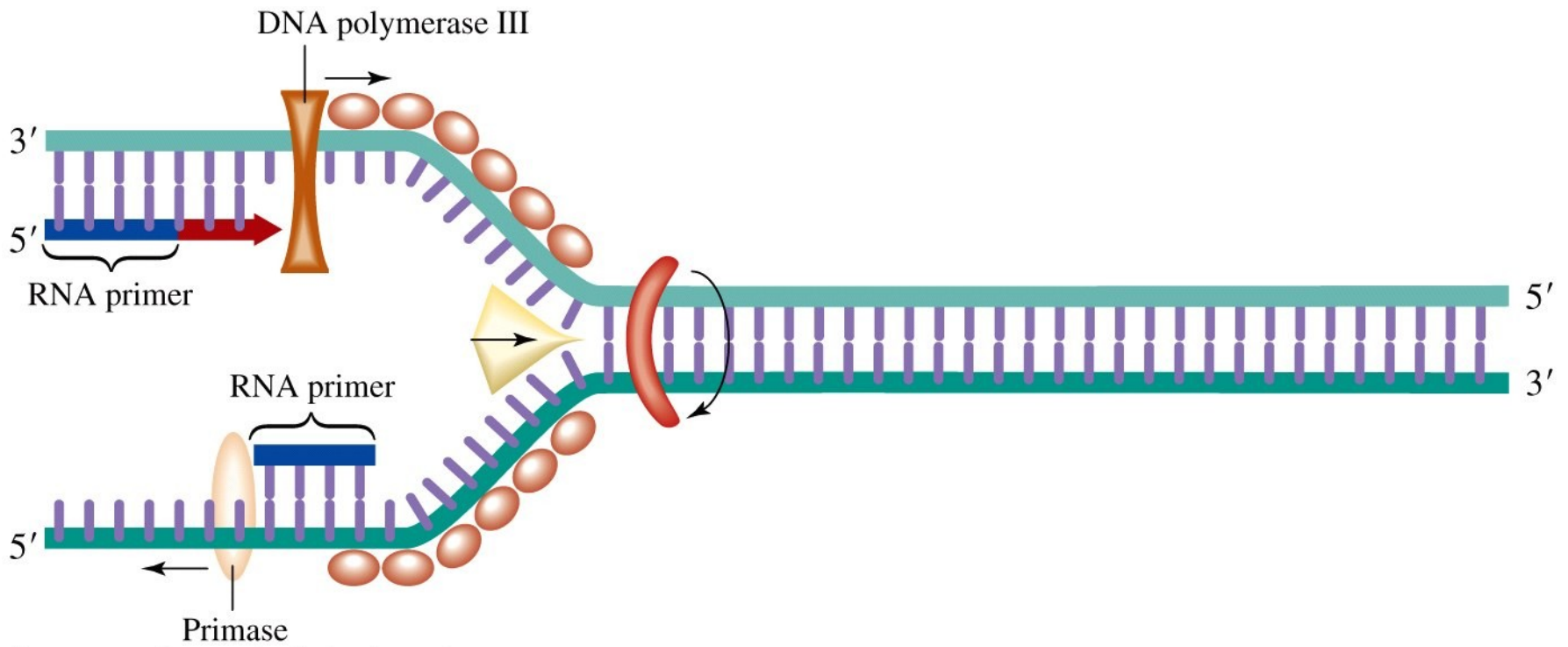


Figure 11-10c Concepts in Biochemistry, 3/e
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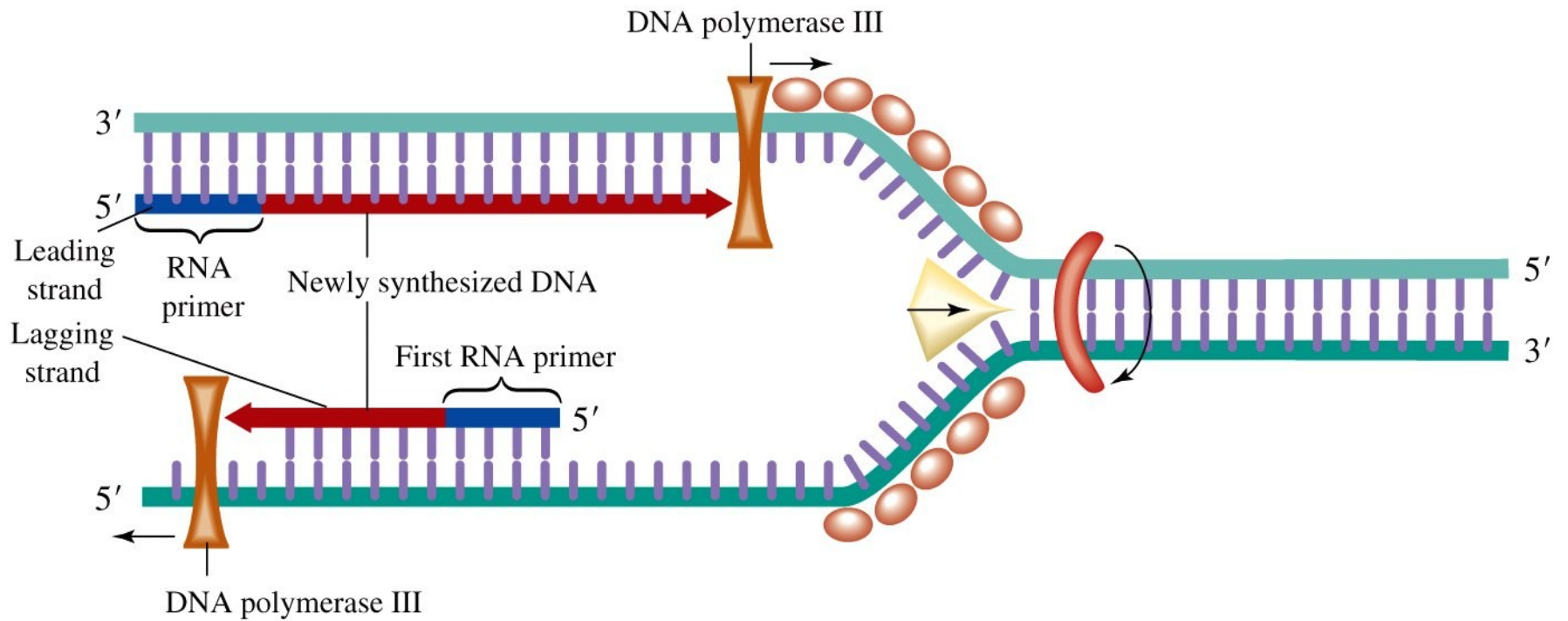


Figure 11-10d Concepts in Biochemistry, 3/e
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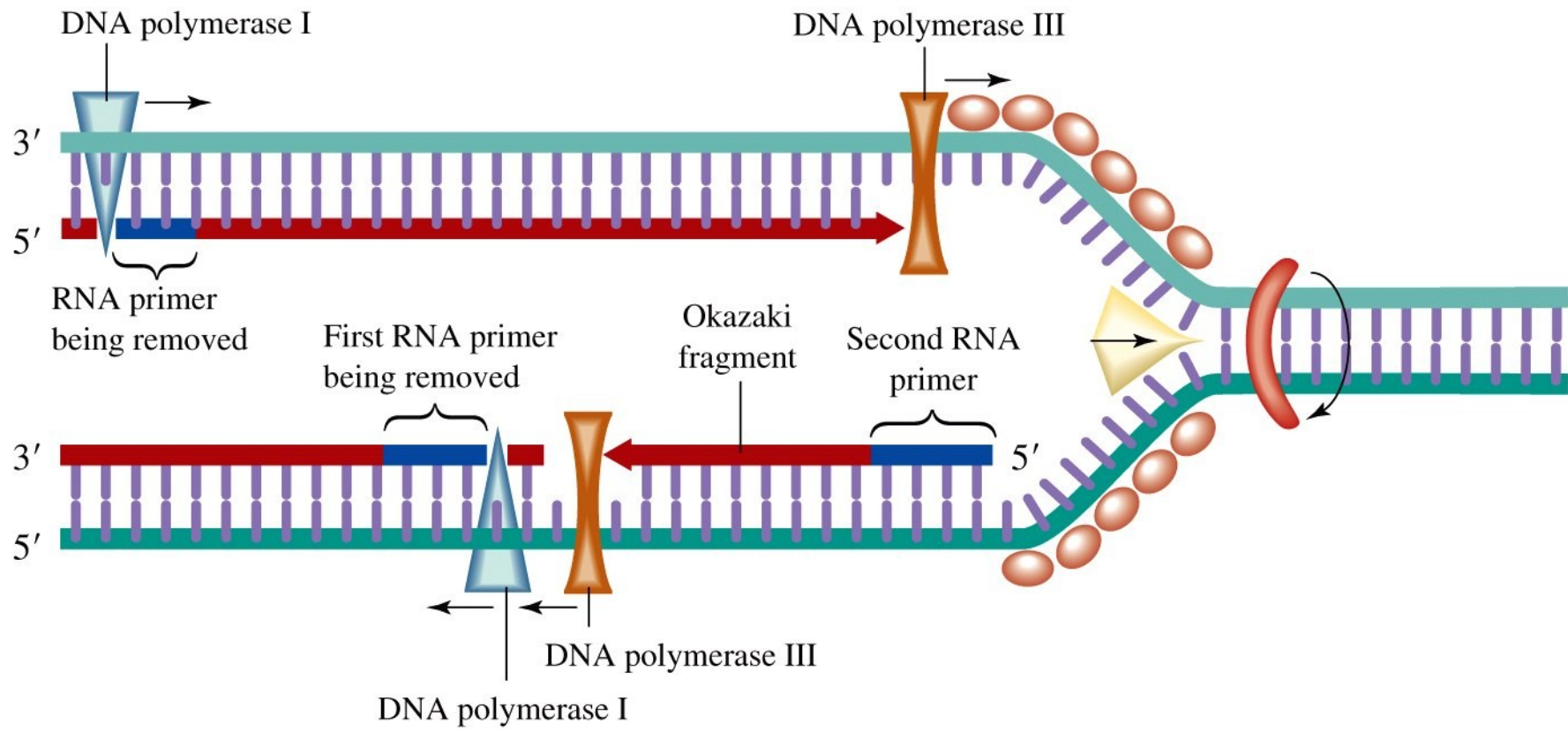


Figure 11-10e Concepts in Biochemistry, 3/e
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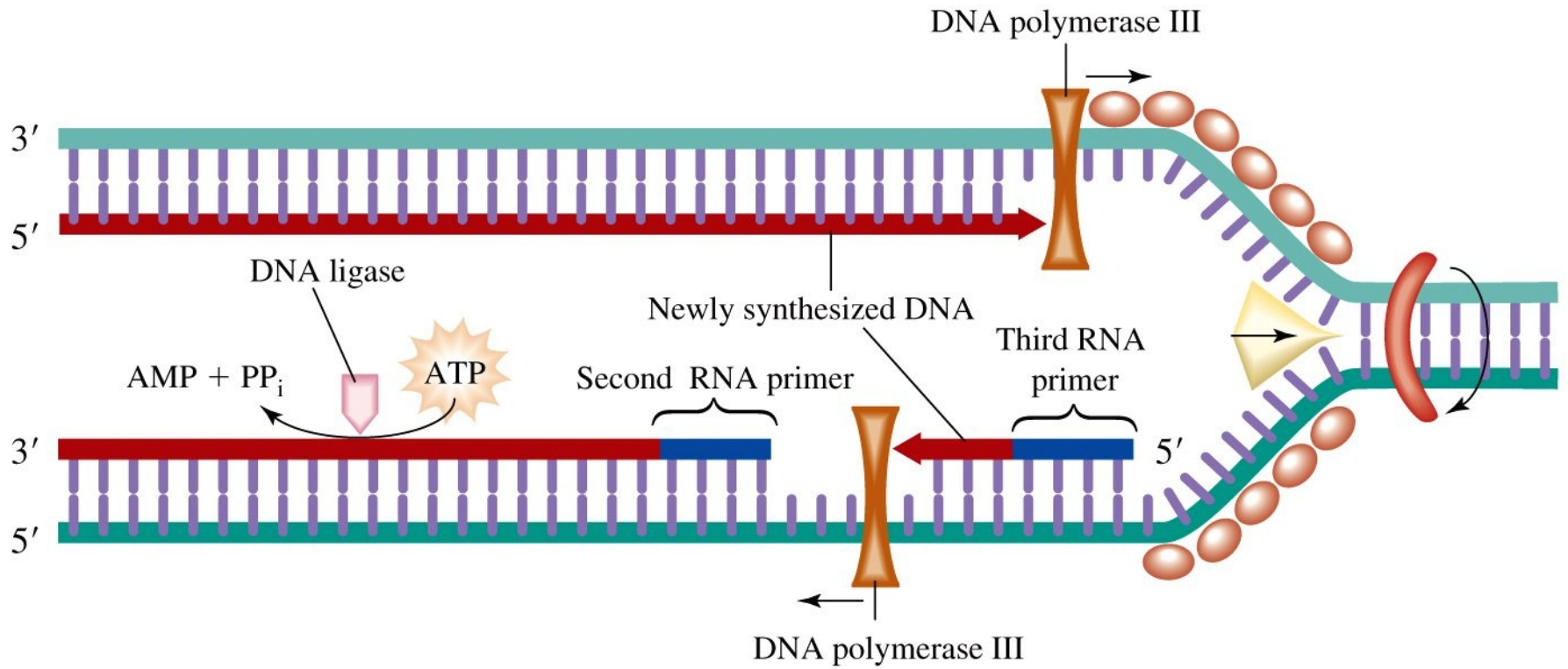
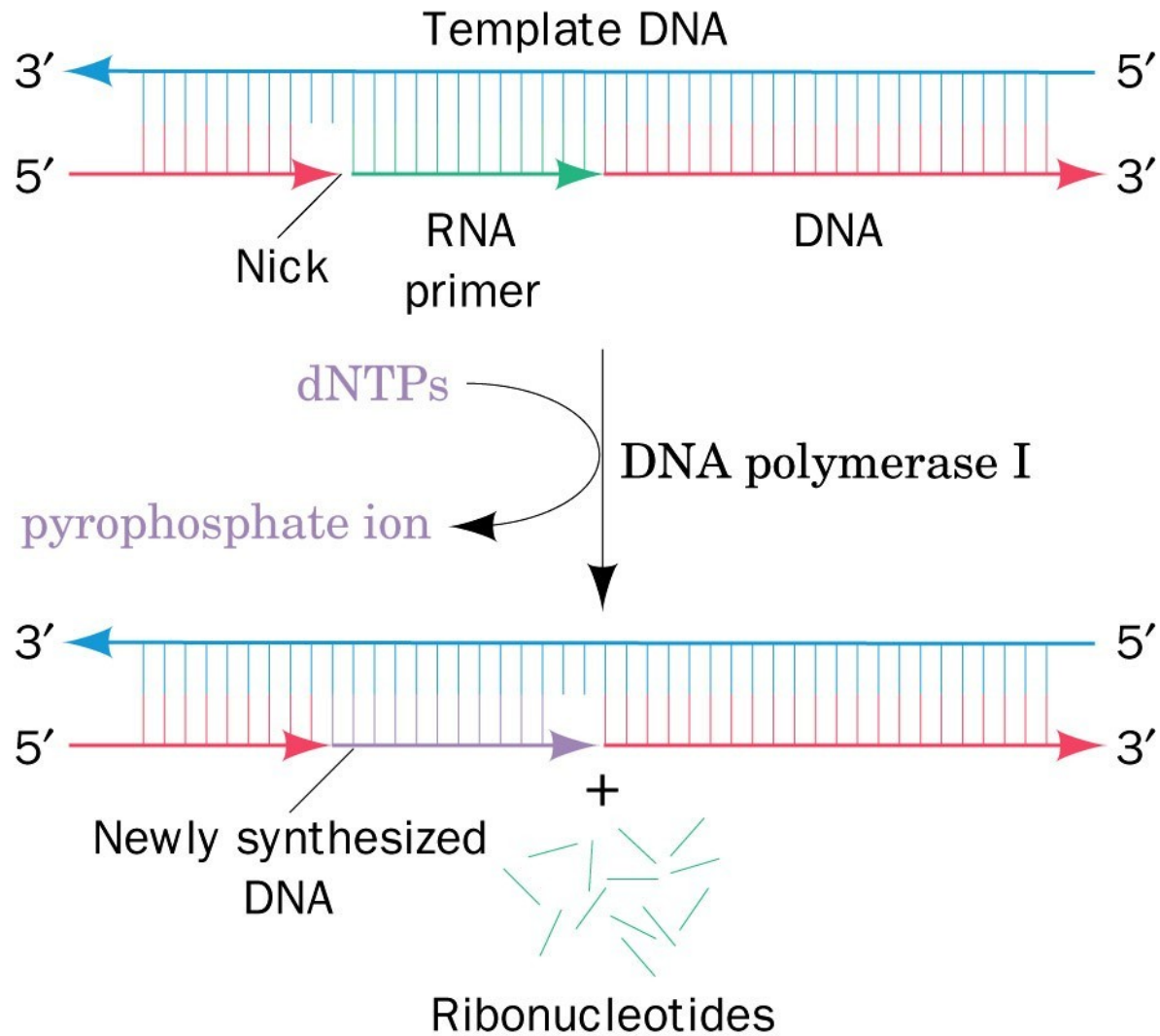
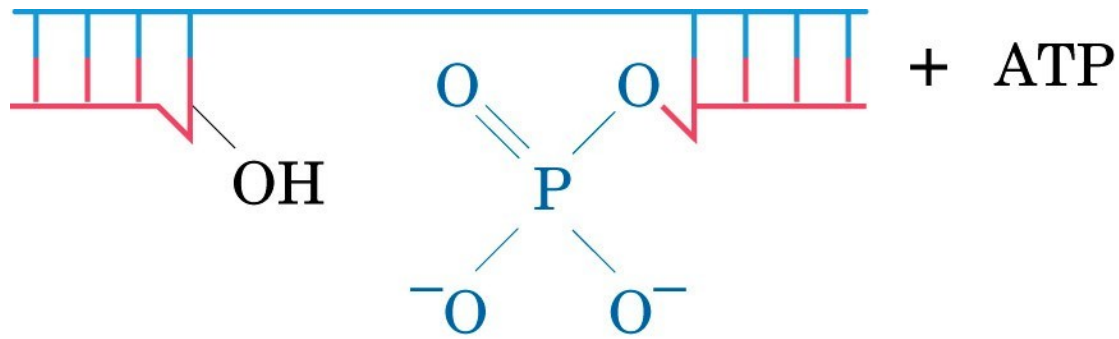
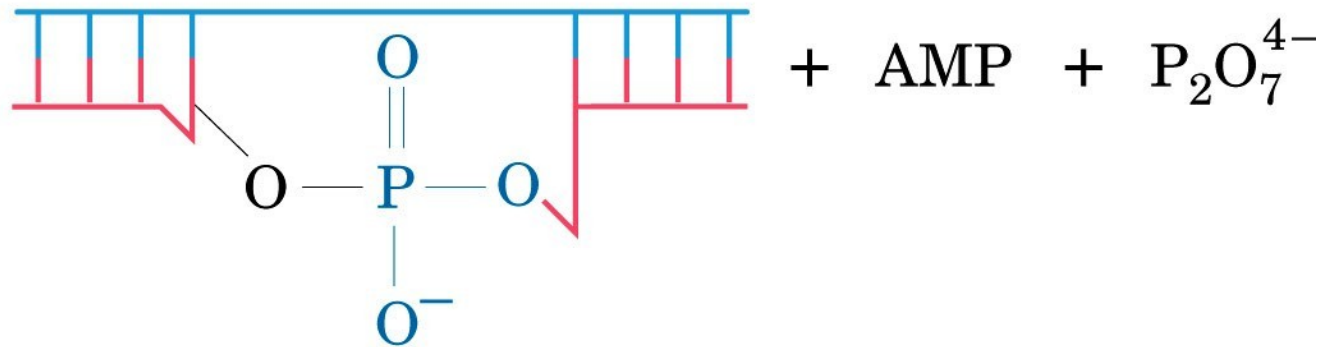


Figure 11-10f Concepts in Biochemistry, 3/e
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DNA ligase



Enzymová metoda

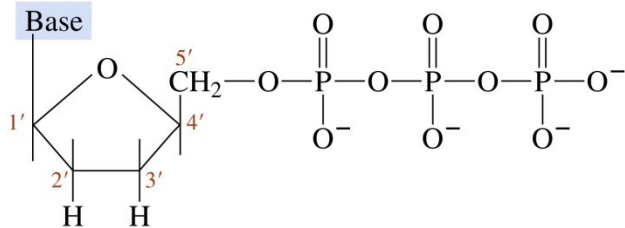


Figure 11-29 Concepts in Biochemistry, 3/e
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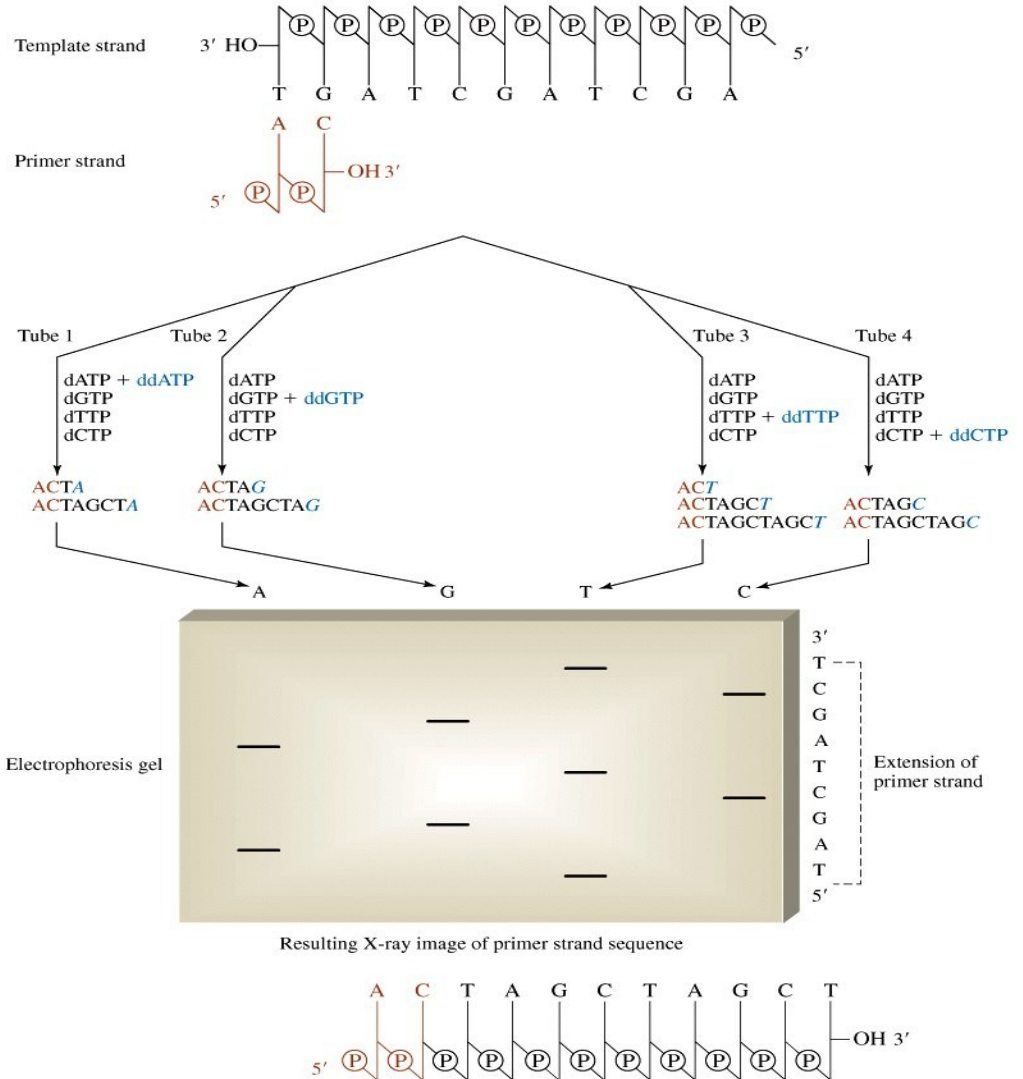
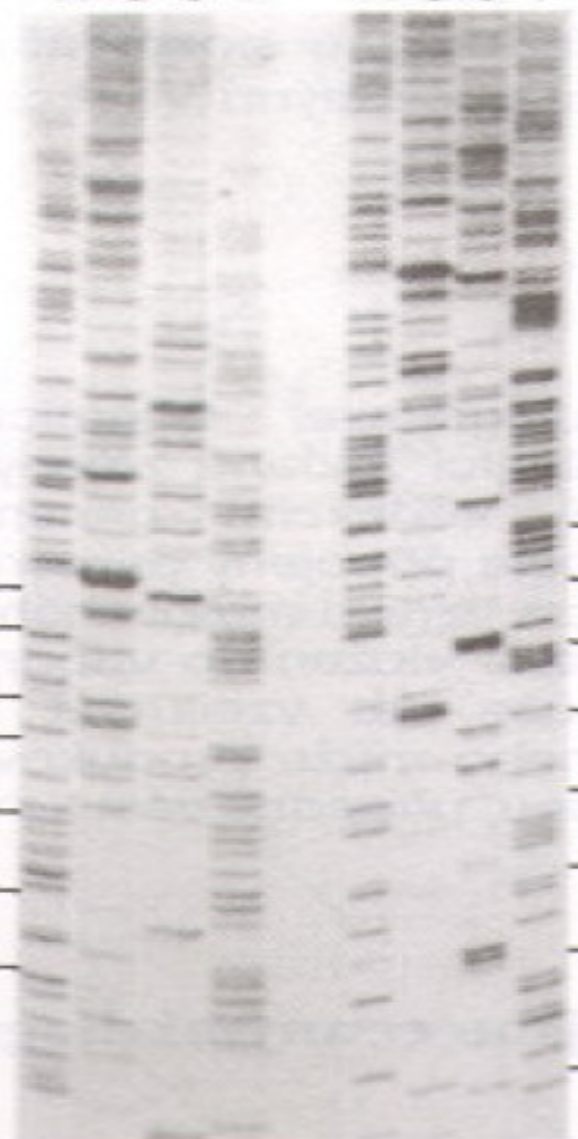


Figure 11-30 Concepts in Biochemistry, 3/e
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A G C T

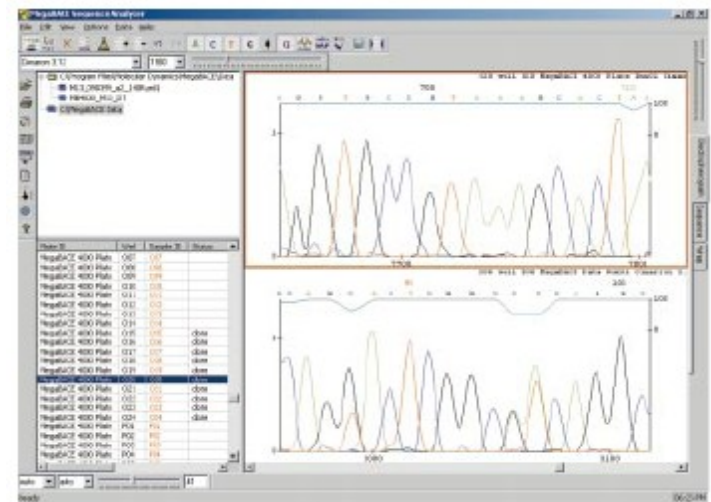
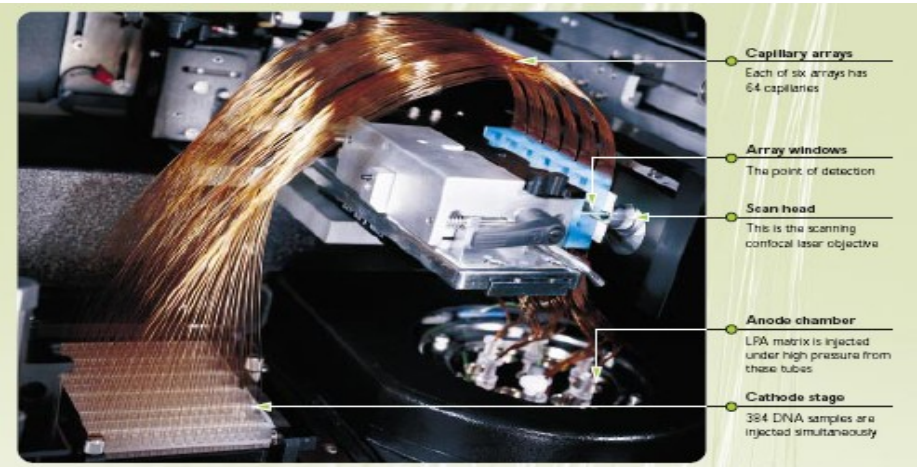
A G C T

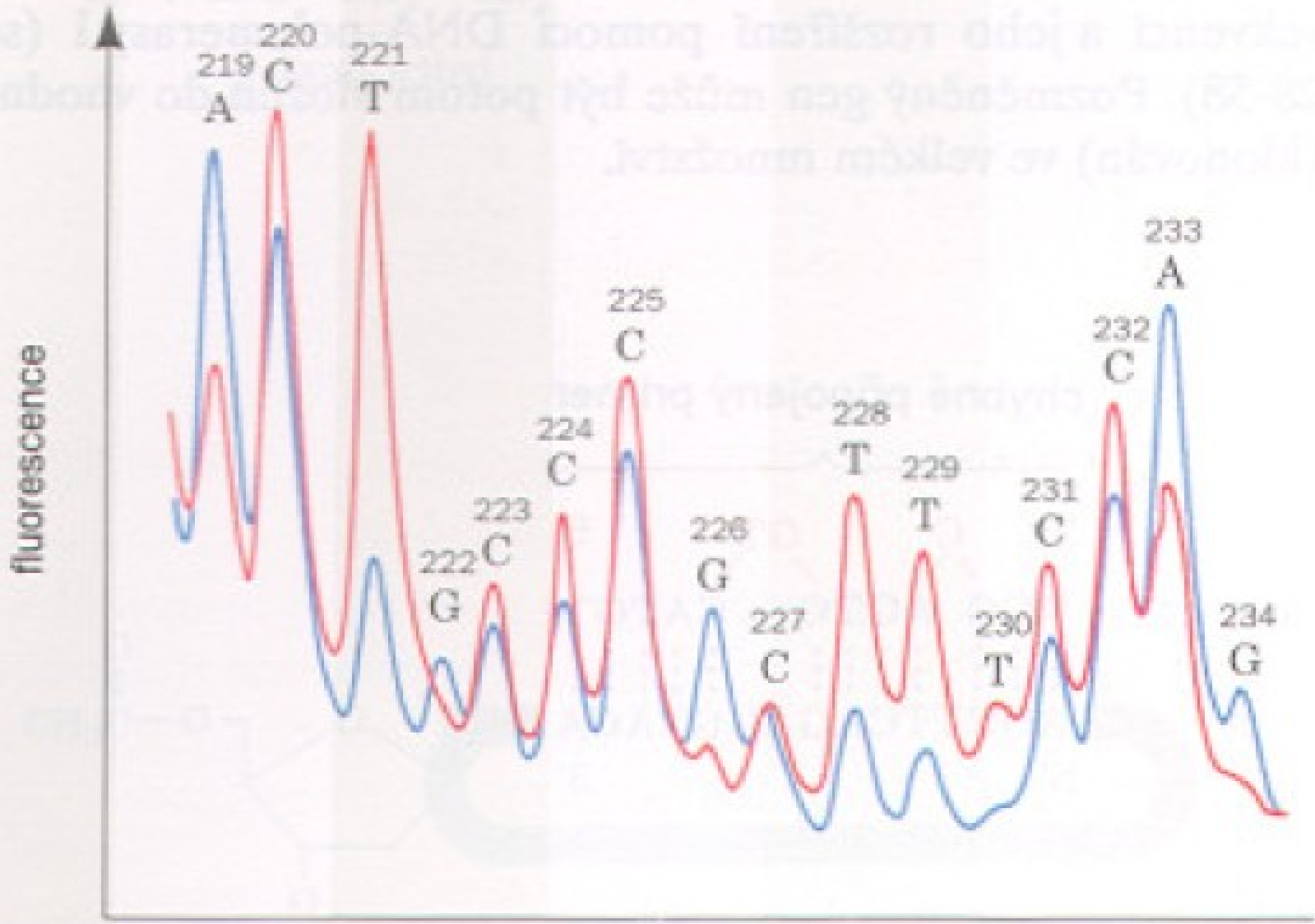
140
A G G T C C T T G G
C T T T A T G T A T
G T A A C A G A A G
G C A T T T C G C G
90 A T C T G A C T A T A
T A A T A A T A T G
T C C A A G T T T A



80
A T T T G A A C C T
C A G A T A G T A A
T T C C C T G A A T
C C T G G G A G T T
A A C A T C C G A A
T G A A C T T T T G
T A C C C A T C A T
T C G T A C T T G T

2003 - Projekt lidského genomu







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Pyrosekvenování

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



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



Pyrosekvenování

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

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

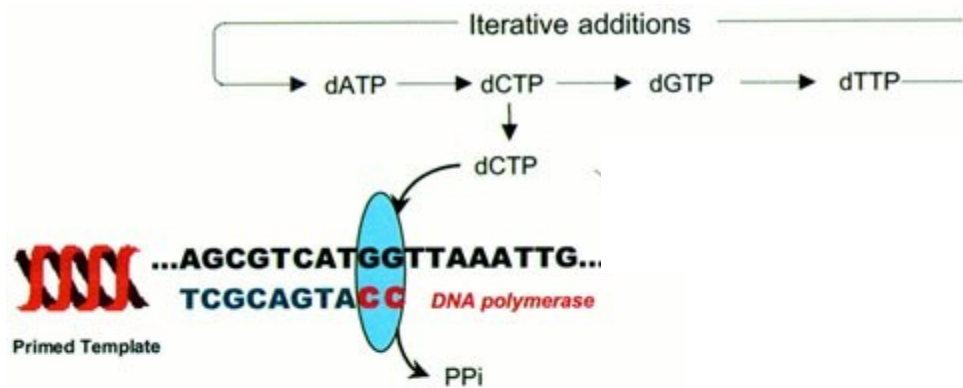
Luciferáza-luciferin-AMP + PPi + O₂ → Luciferáza + Oxyluciferin + AMP + CO₂ + světlo

Pyrosekvenování

- Poslední enzymatickou reakcí je reakce apyrázy, 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 pyrosekvenování

Technologie využívá paralelní sekvenace: více než

-2 milion sekvencí zároveň.

-Lze získat až 1GB (gigabázi) informace během jedné analýzy (cca4.5 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. ampliconové 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.

Sekvenování

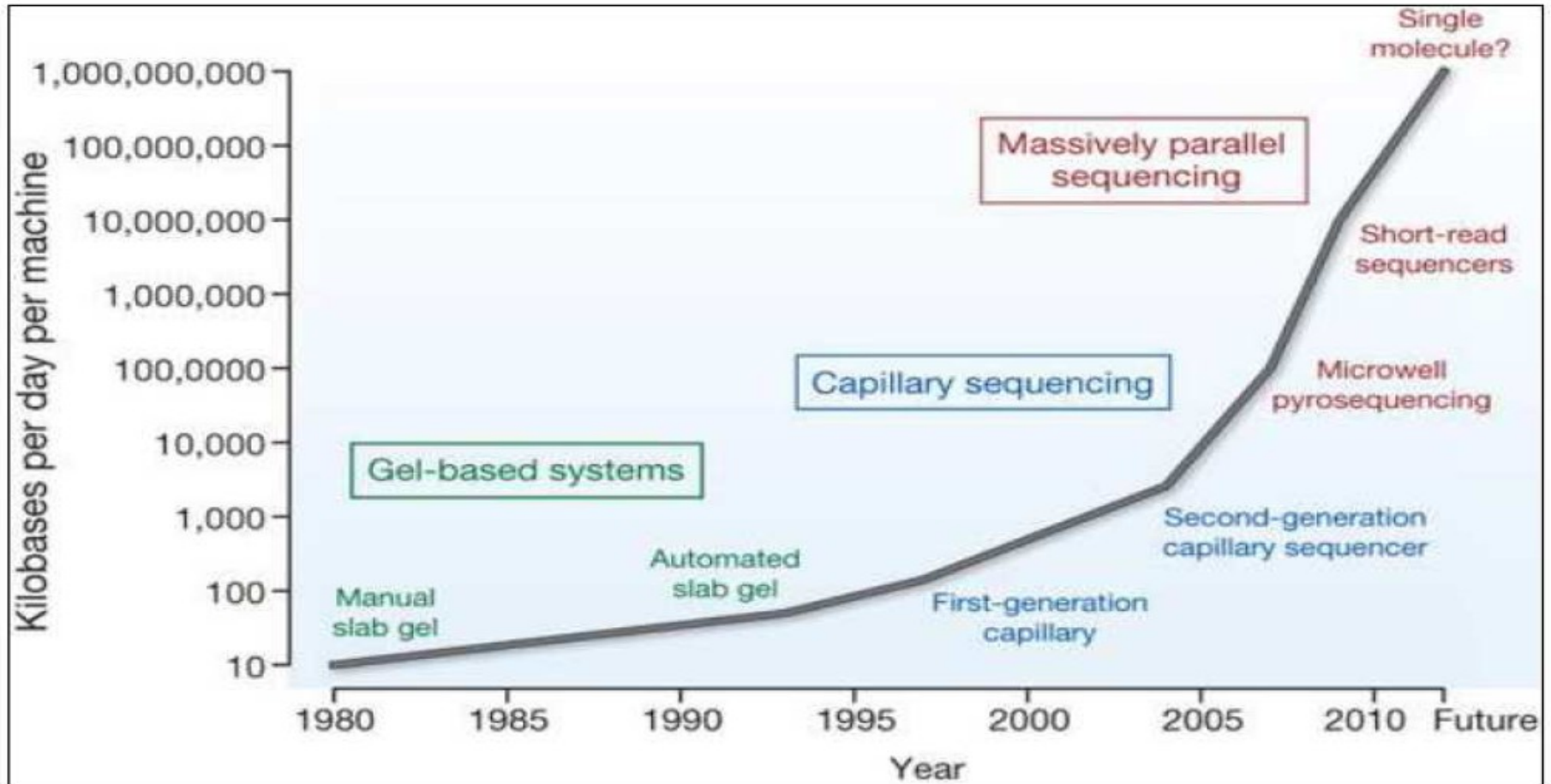


Table 13.3
Some Sequenced Genomes

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

PCR Mullis NC1993

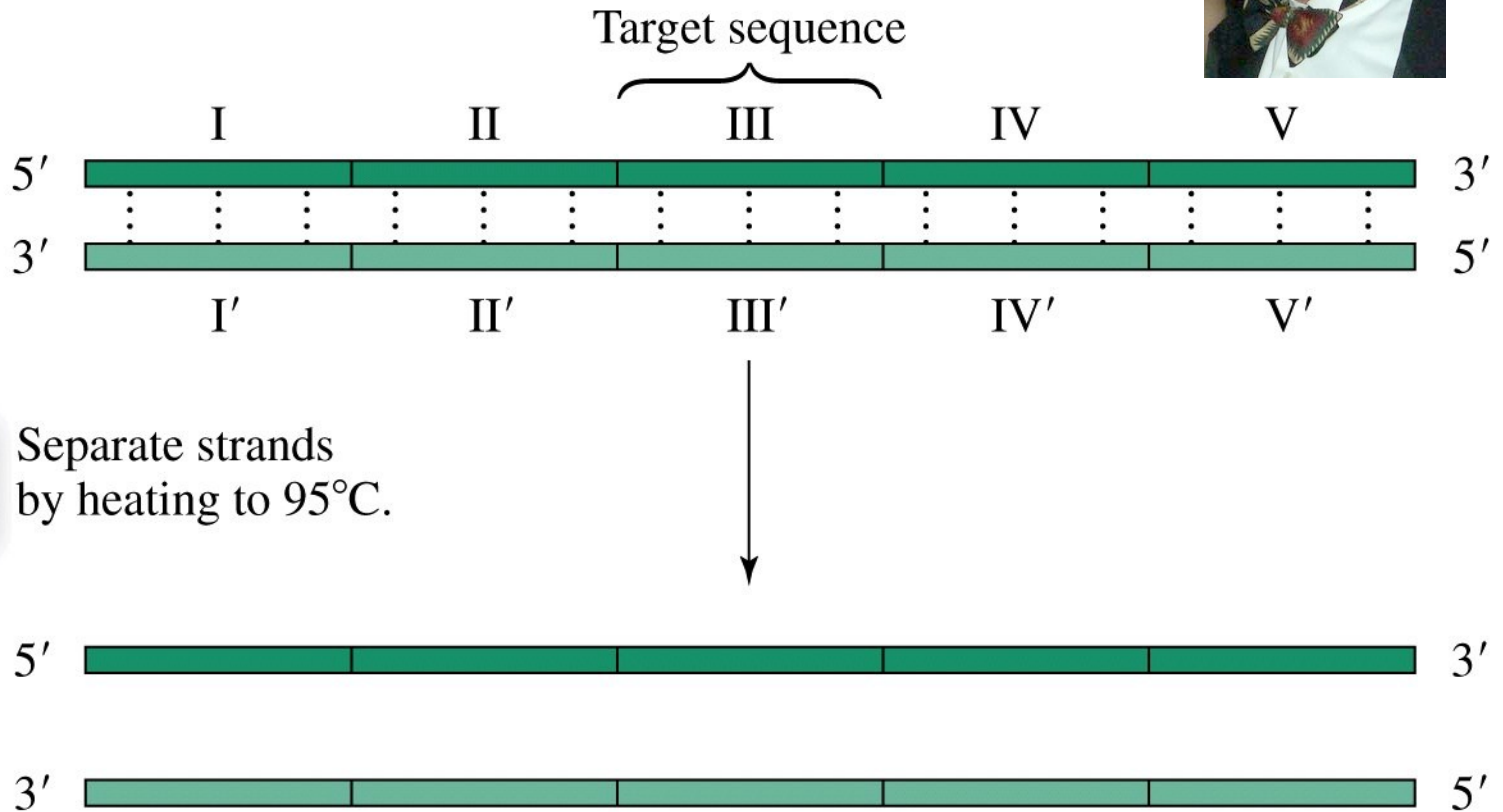
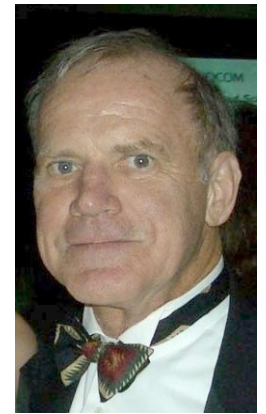


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Step 2

Hybridize primers by cooling to 50°C.

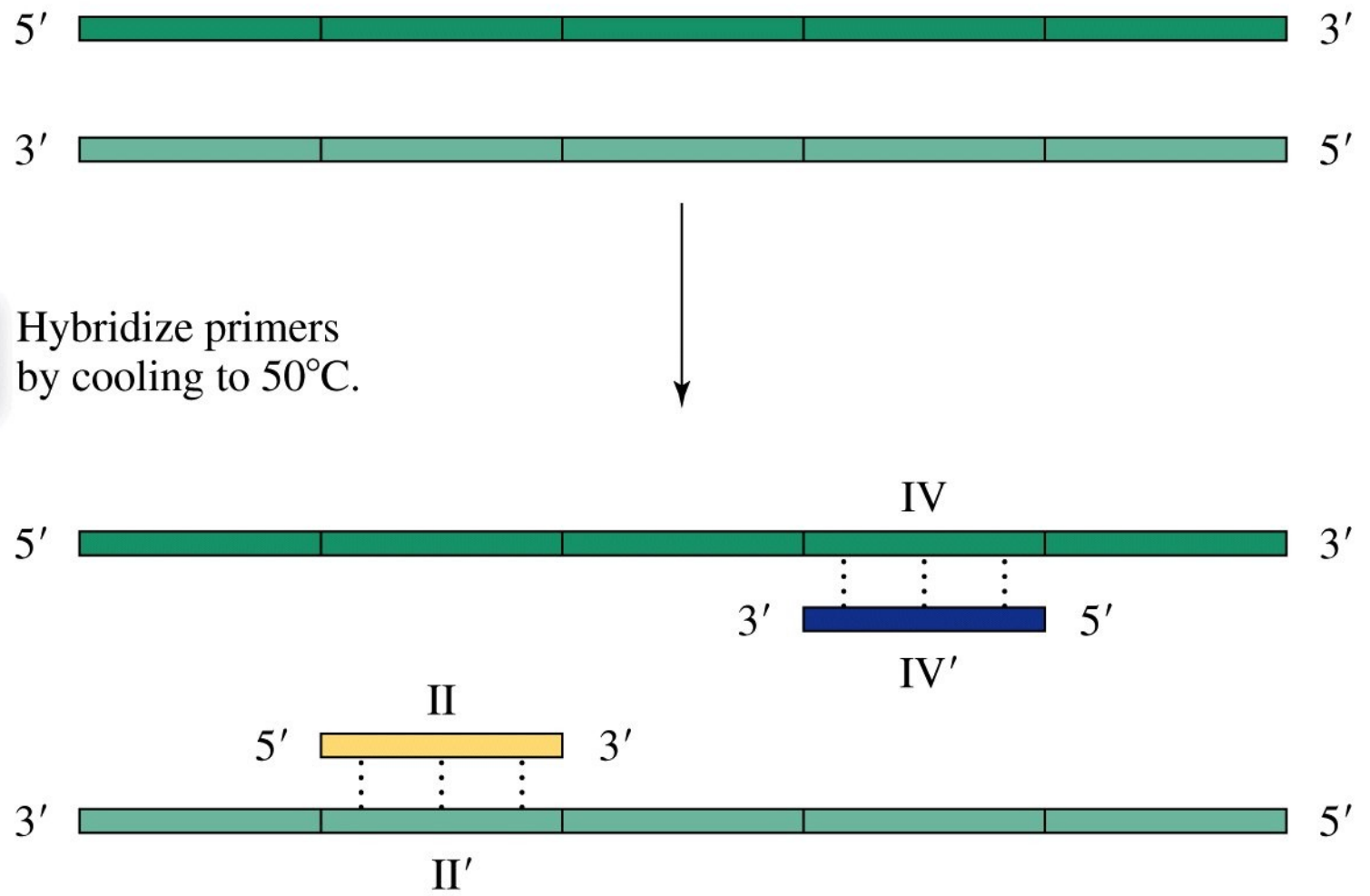
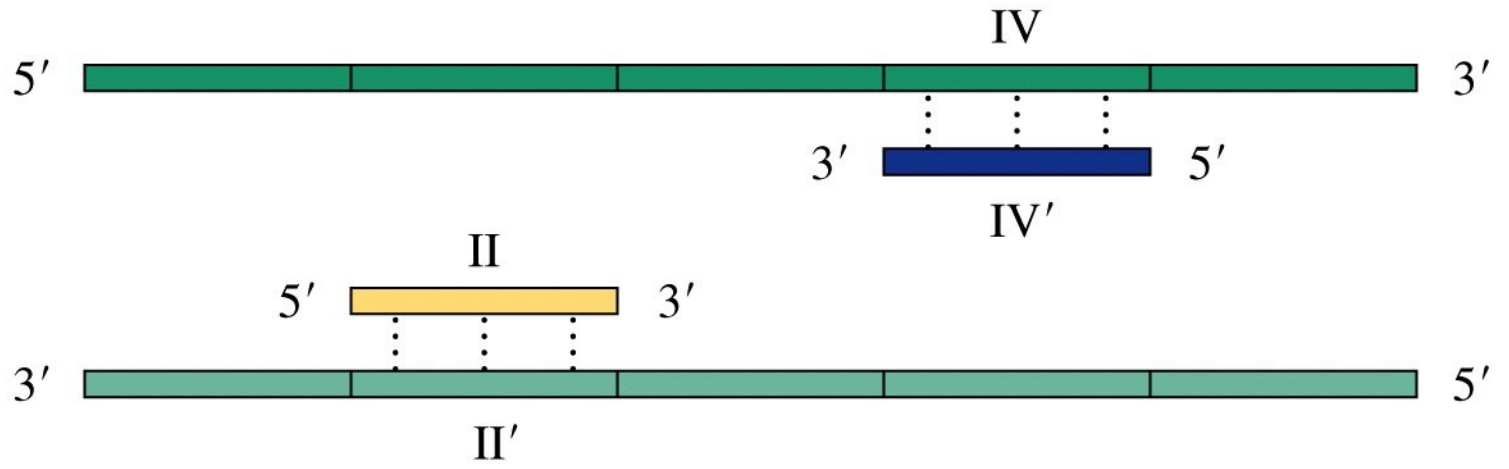


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Step 3

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

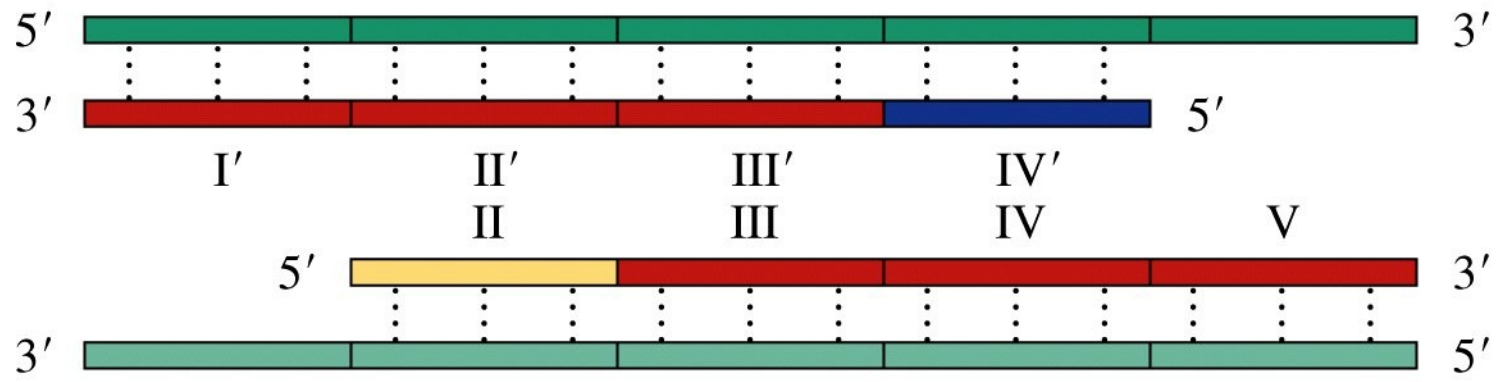
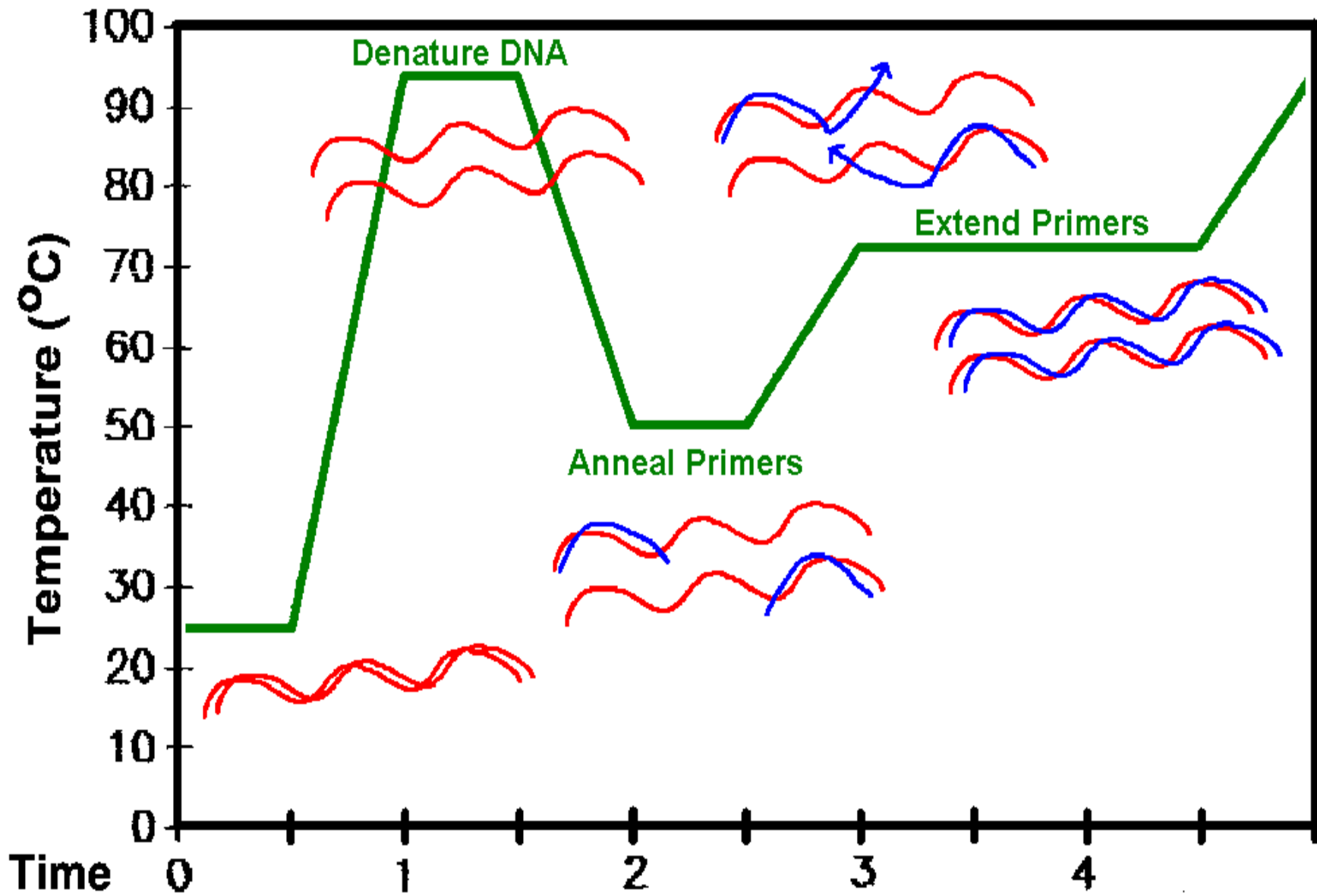
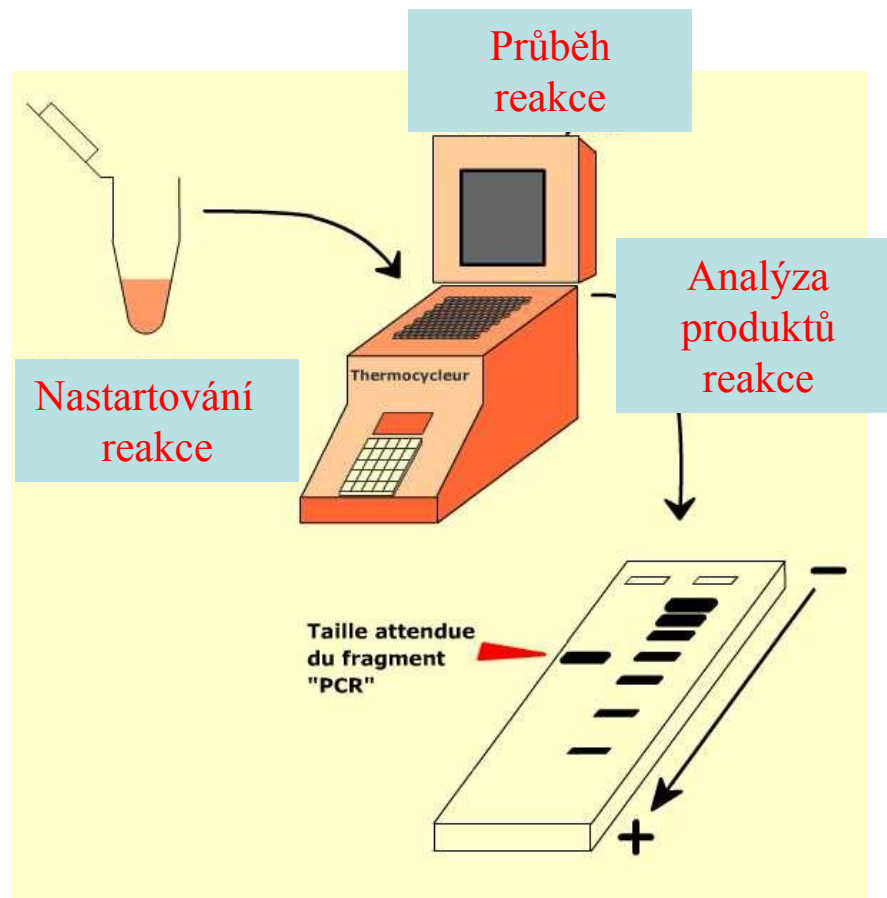


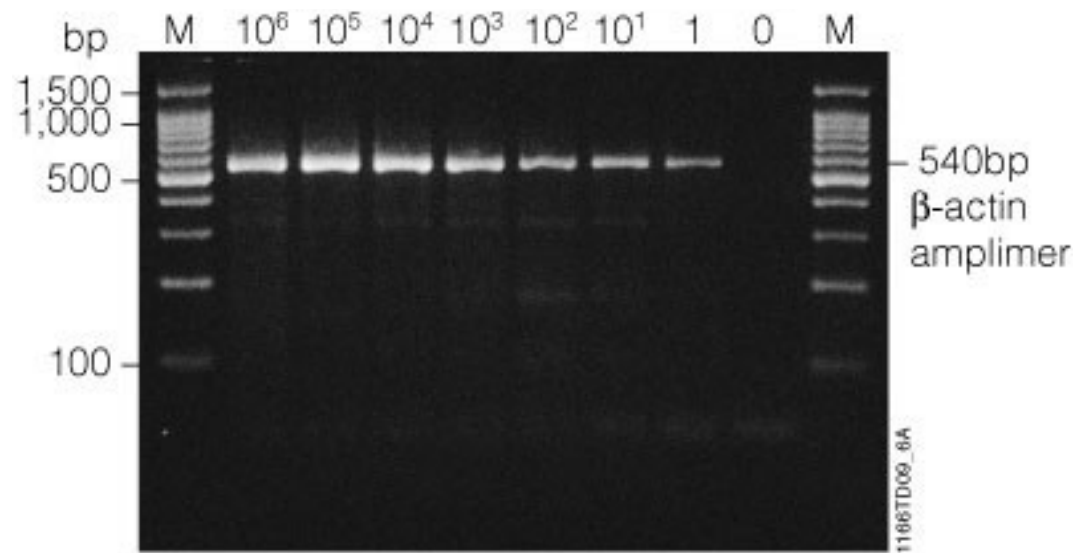
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Thermocycler





Real time PCR

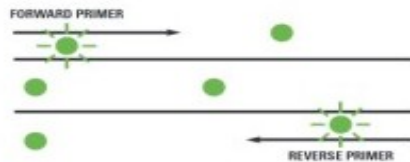
1. **Navázání:** SYBR® Green I se váže během každého cyklu na dvouvláknovou DNA.



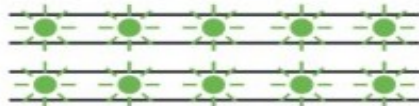
2. **Denaturace:** Ve fázi denaturace DNA je SYBR® Green I uvolněn z vazby na DNA a celková fluorescence dramaticky klesá.



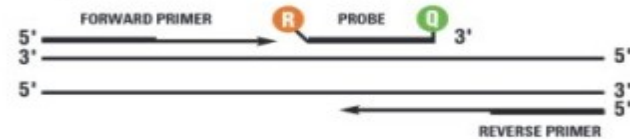
3. **Polymerizace:** Během annealingu primerů a elongace řetězce se Sybr Green opět začíná navazovat na vznikající dvouvláknovou DNA - fluorescence stoupá.



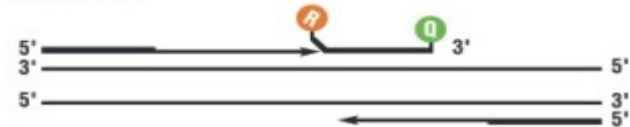
4. **Ukončení polymerizace:** Emitovaná fluorescence dosahuje maxima.



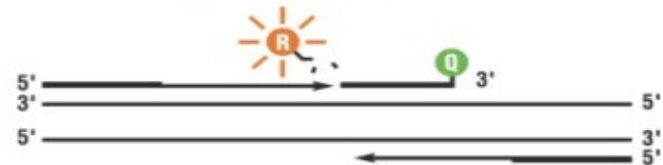
1. **Polymerizace:** Fluorescenční substrát (reporter - R) a jeho zhášec (Q) jsou navázány na 5' a 3' konce TaqMan sondy.



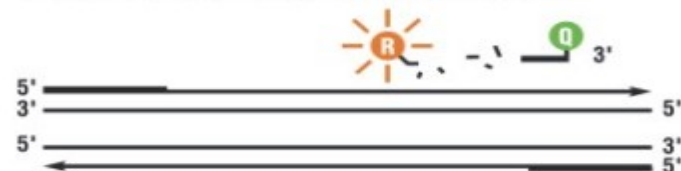
2. **Elongace řetězce:** Dokud je sonda intaktní záření emitované substrátem R je pohlcováno blízkým "zhášecem".



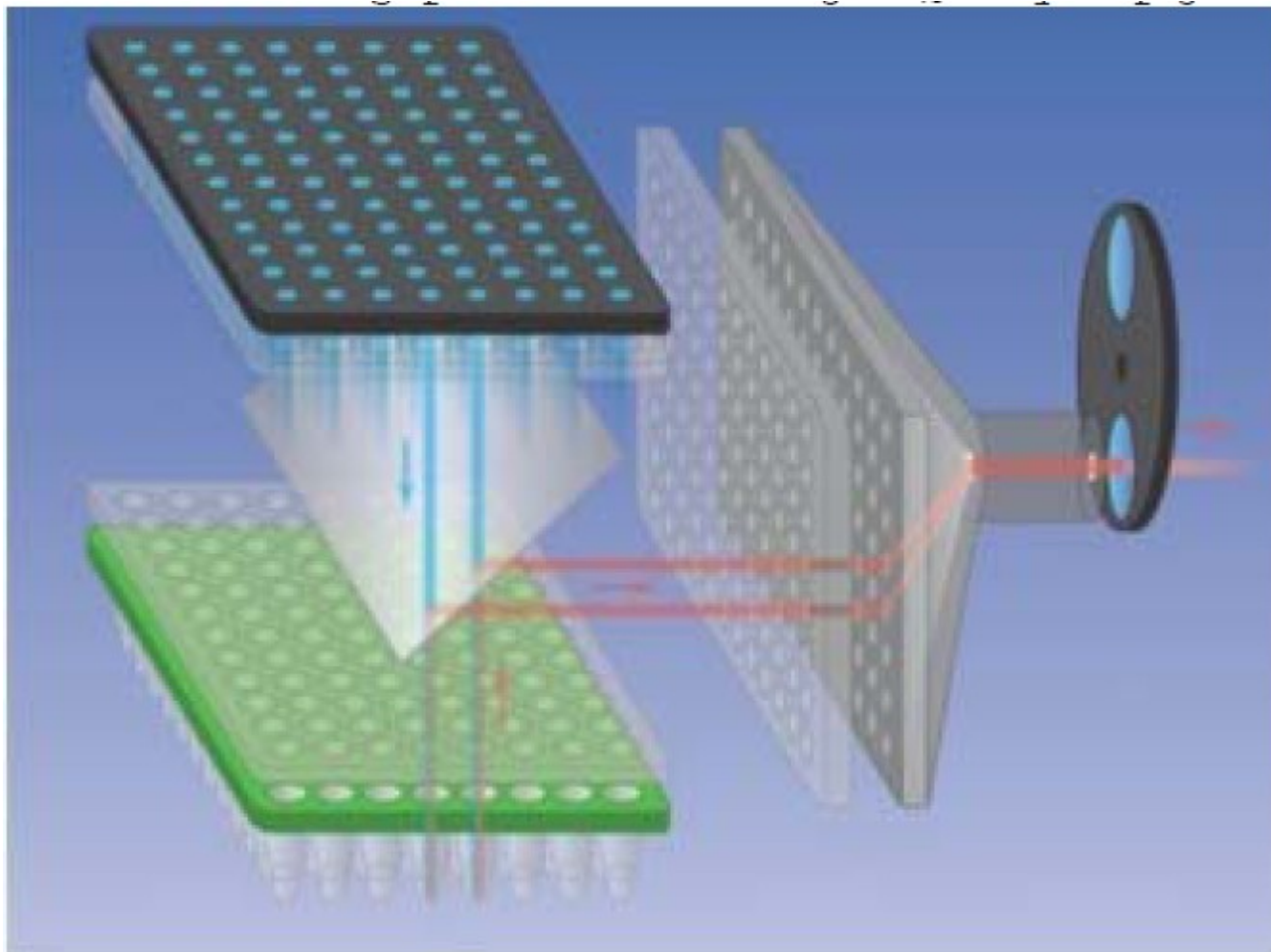
3. **Odštěpení:** když Tag polymeráza dorazí k začátku sondy, postupně ji odchlipuje až odštěpí fluorofor. Ten se tímto oddálí od zhášec a emitované světlo přestane být pohlcováno - detekovaná fluorescence stoupá.



4. **Polymerizace ukončena:** reporterová barva oddělená od zhášec emituje charakteristickou fluorescenci.



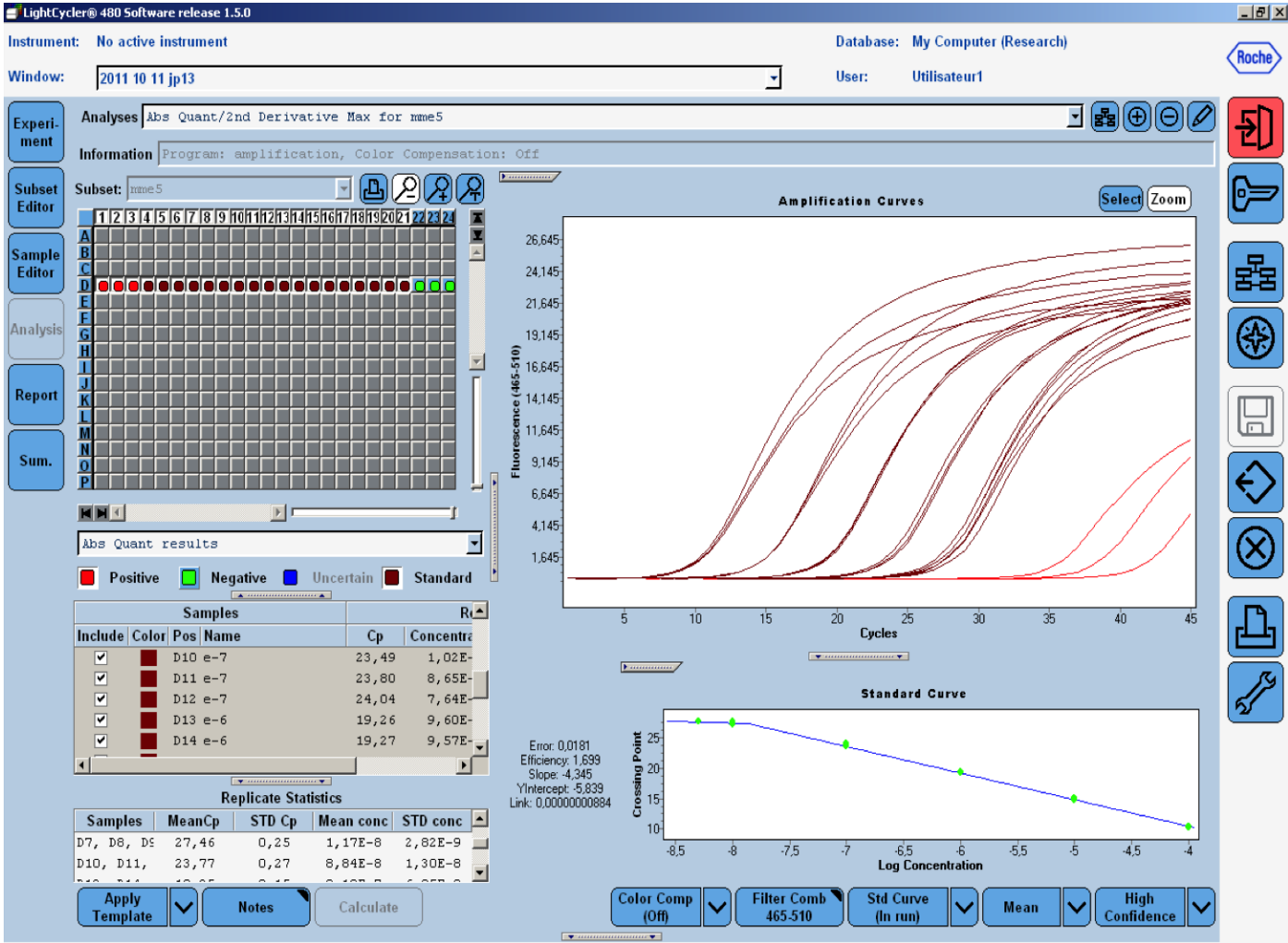
Real time PCR



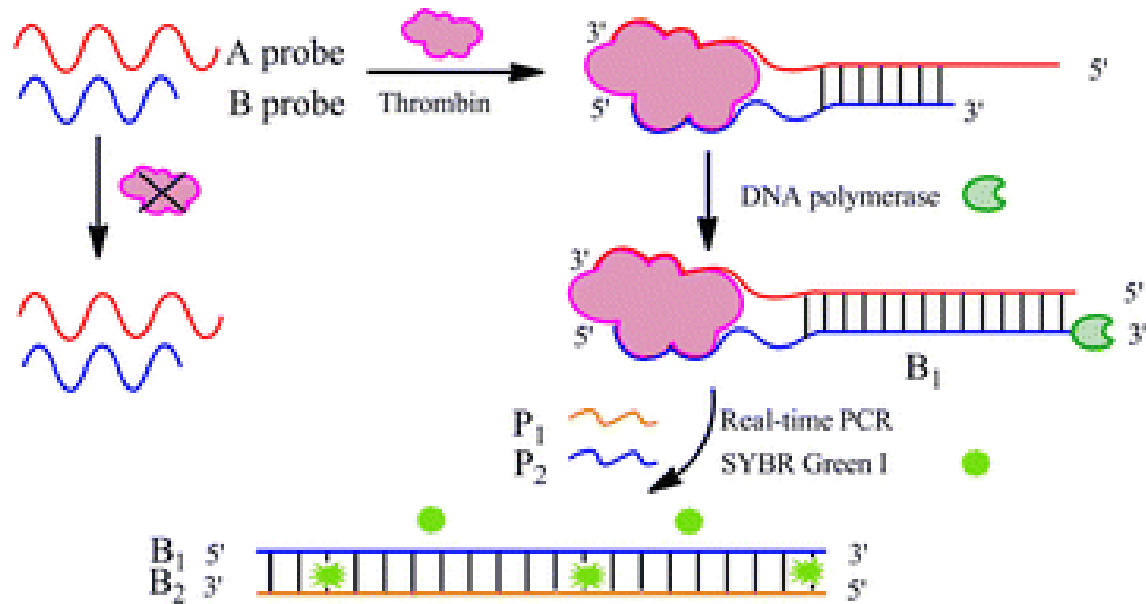
Real time PCR



Real time PCR

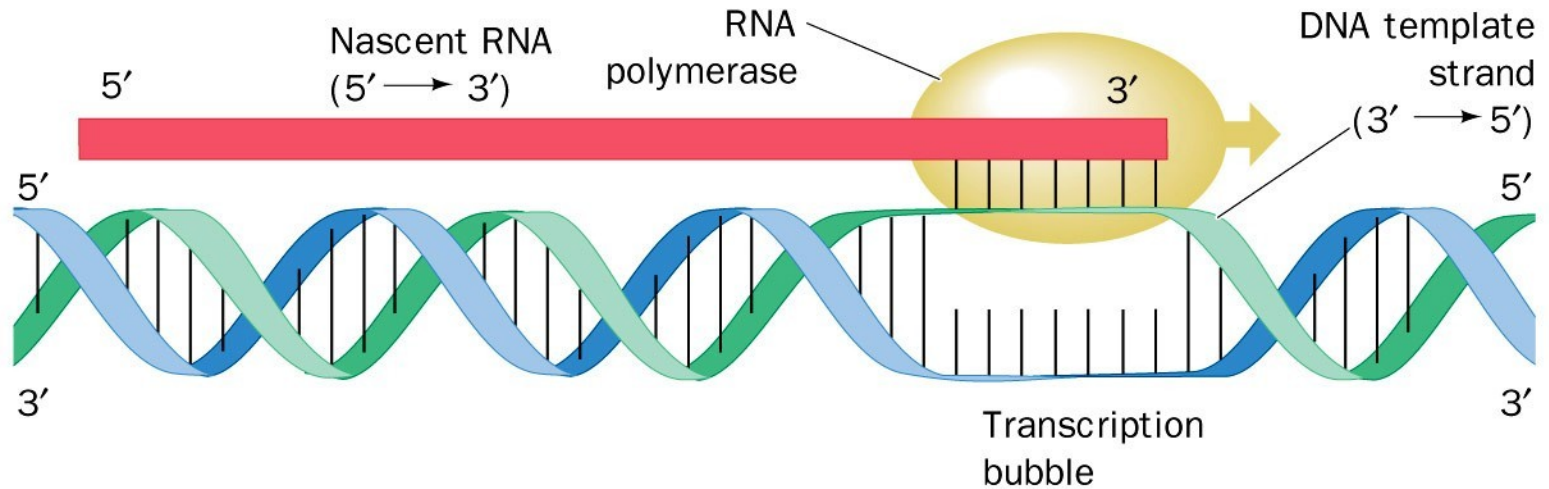


Real time PCR proteinů

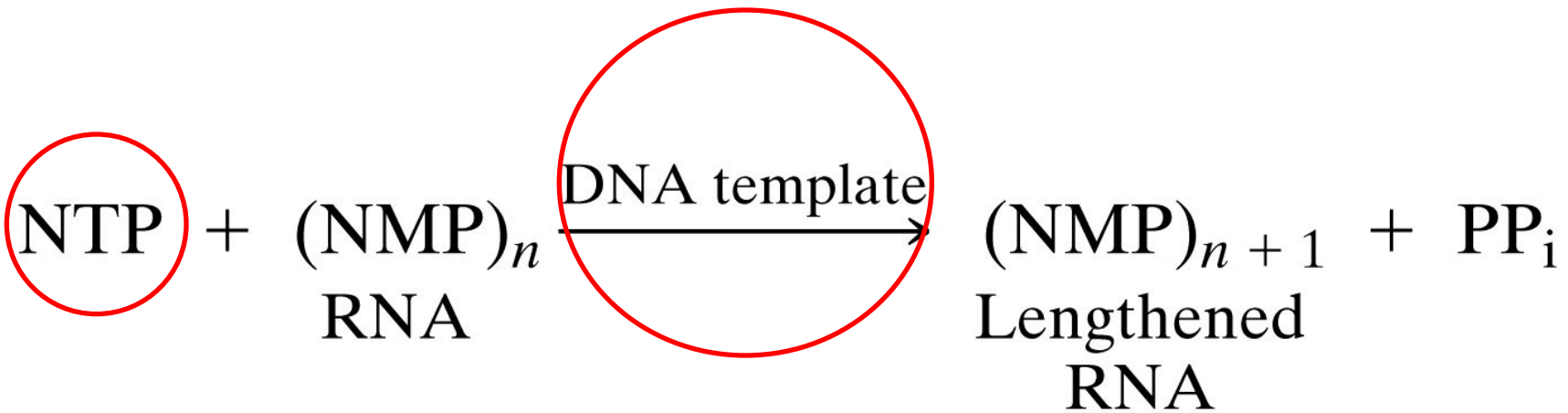


Transkripce

DNA + NTP + 1 enzym



RNA polymeráza



Unnumbered figure pg337 Concepts in Biochemistry, 3/e
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5' → 3'

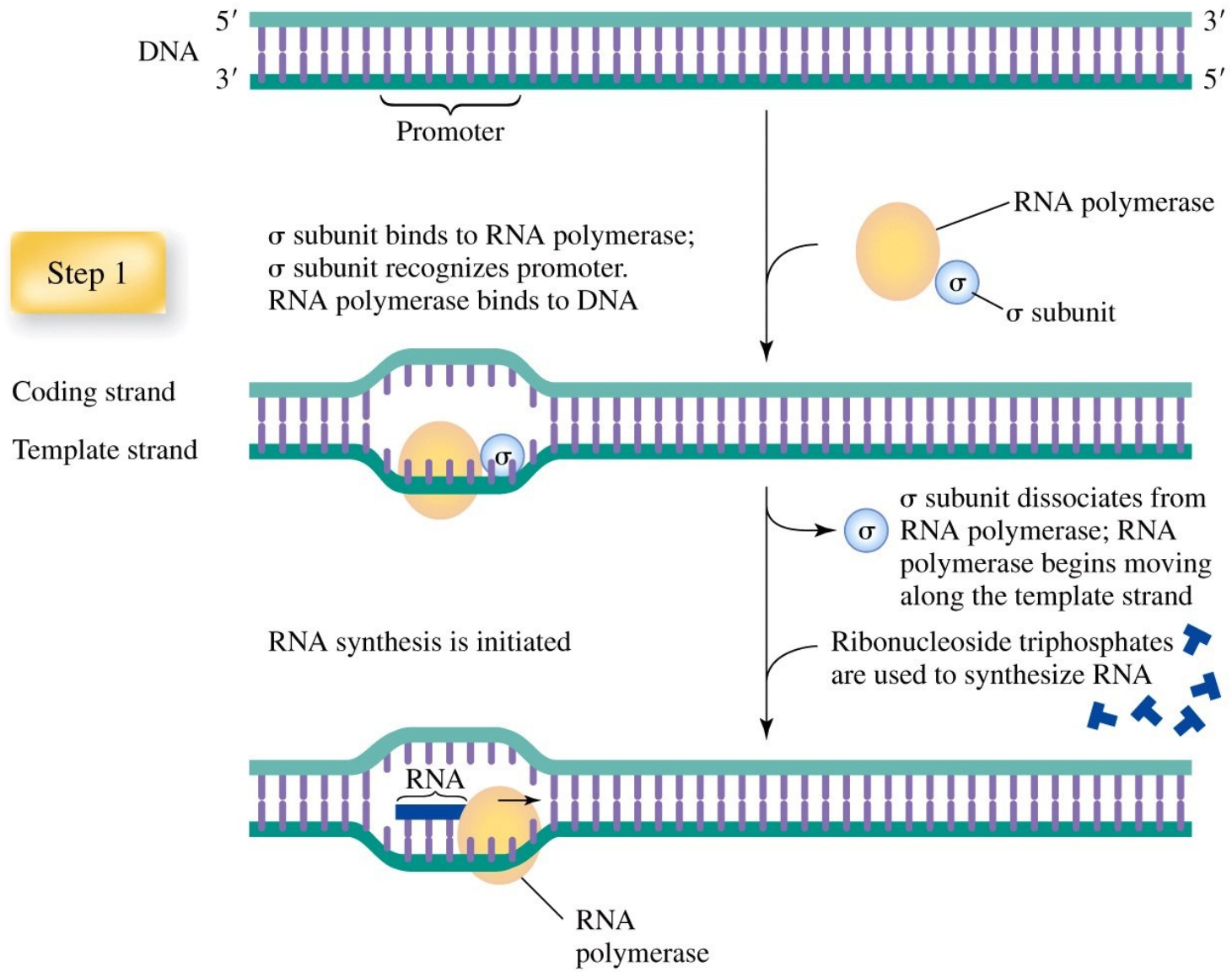


Figure 11-22 part 1 Concepts in Biochemistry, 3/e
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Step 2

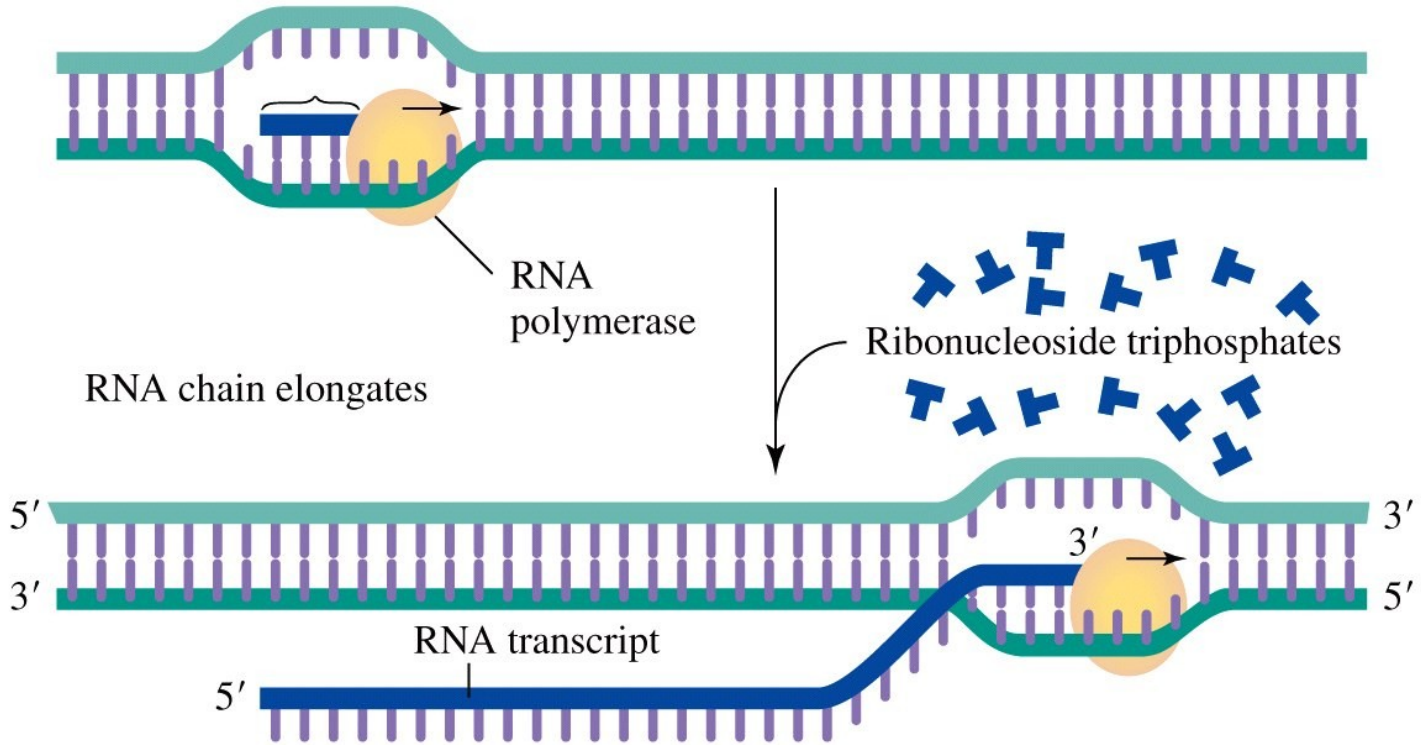


Figure 11-22 part 2 Concepts in Biochemistry, 3/e
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Step 3

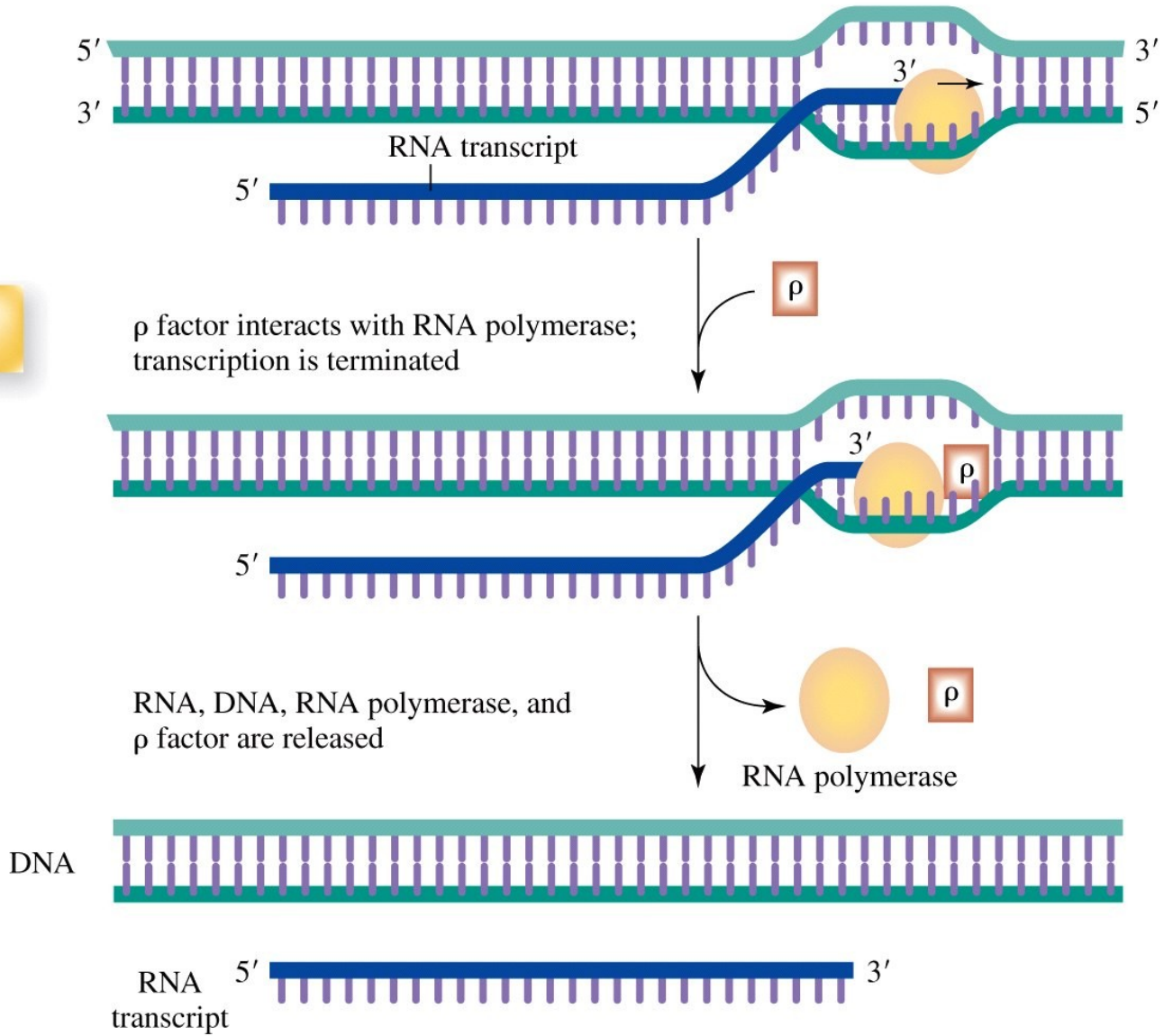
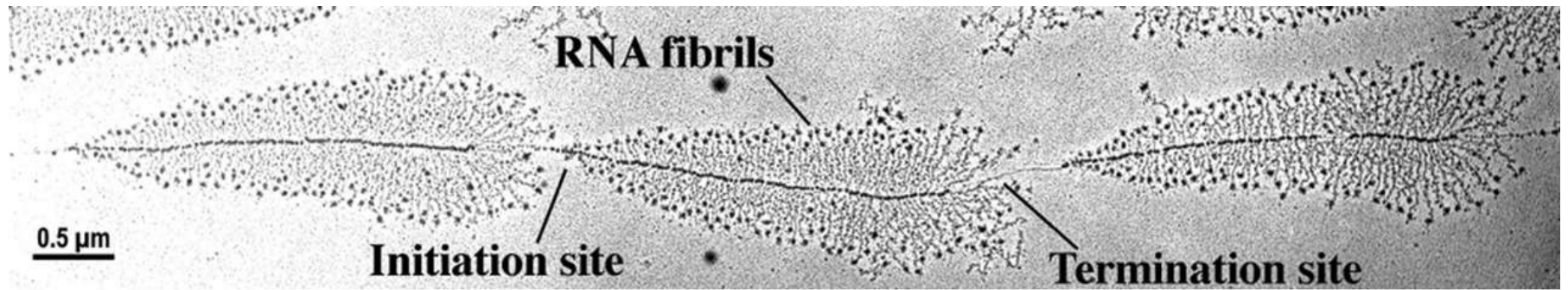
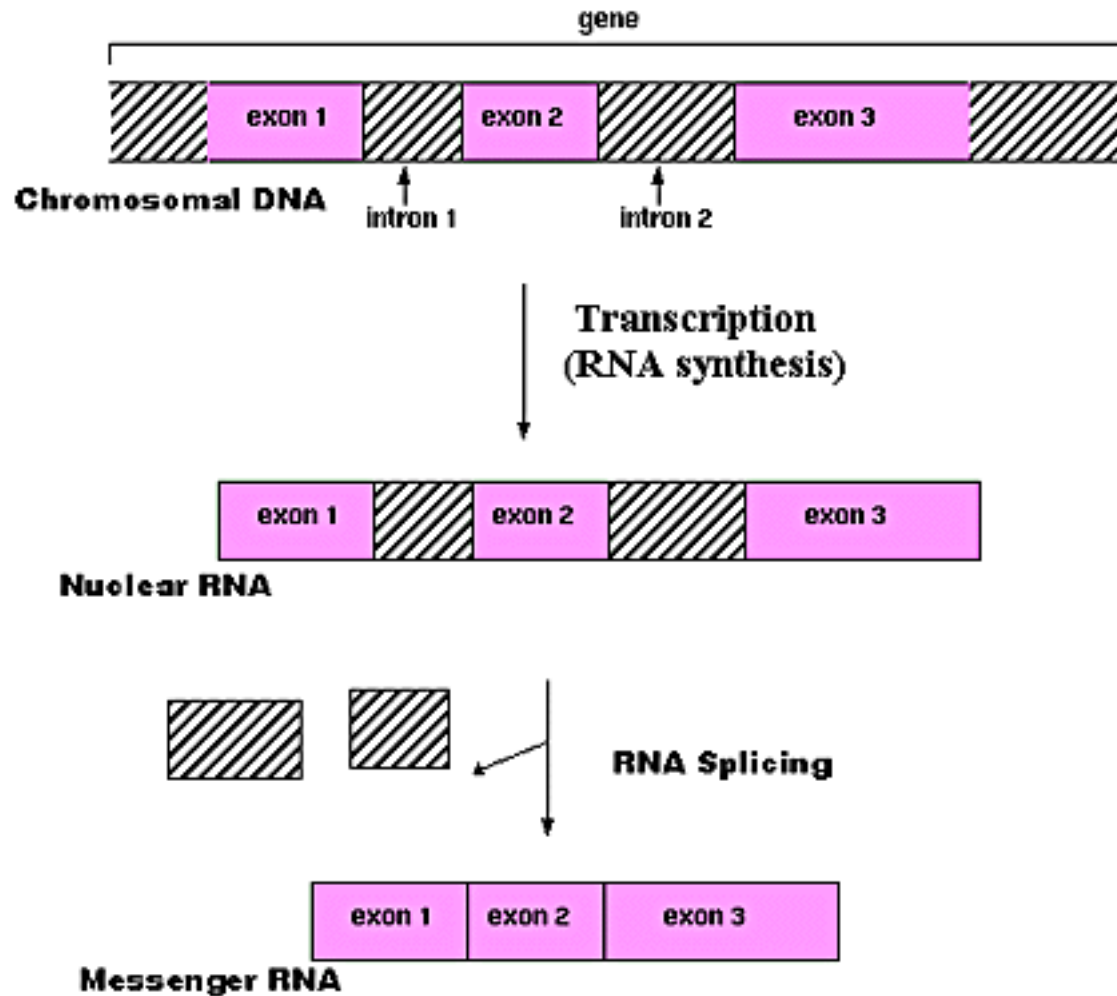


Figure 11-22 part 3 Concepts in Biochemistry, 3/e
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Courtesy of Ulrich Scheer, University of Würzburg, Germany

Syntéza eukaryontní RNA



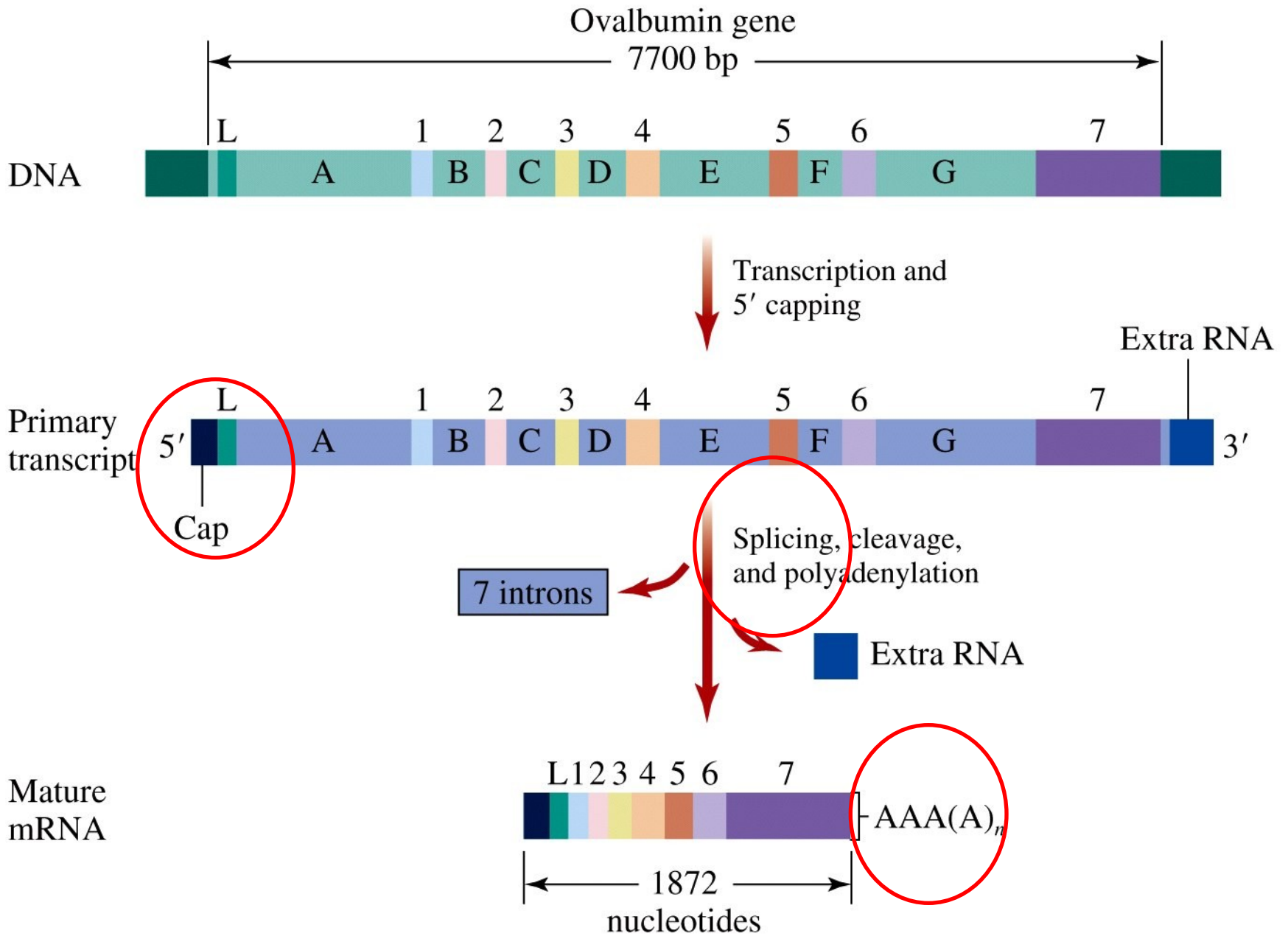
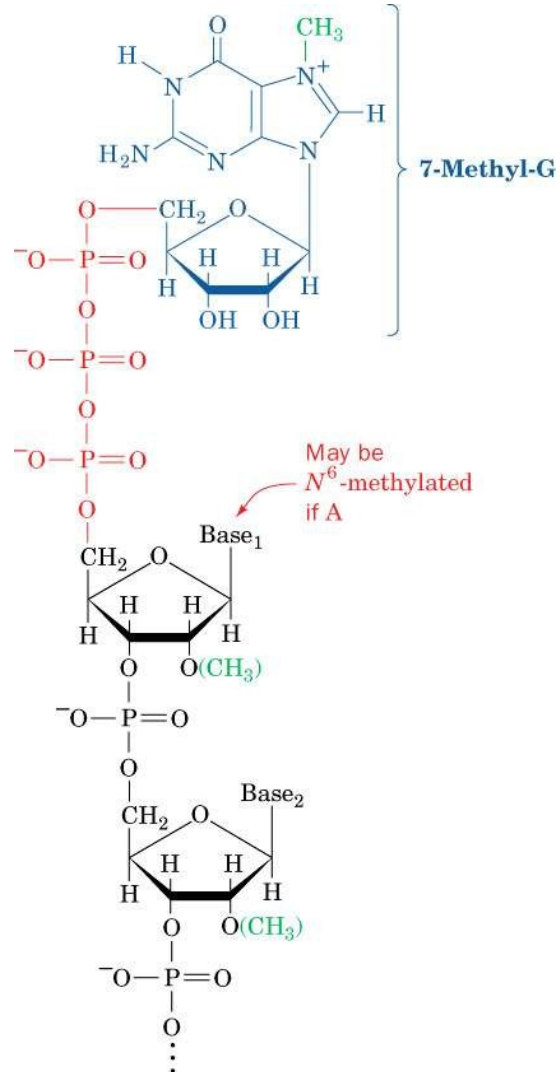


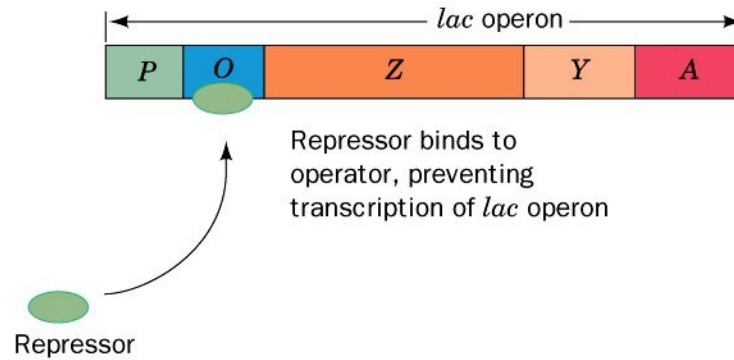
Figure 11-28 Concepts in Biochemistry, 3/e
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Cap

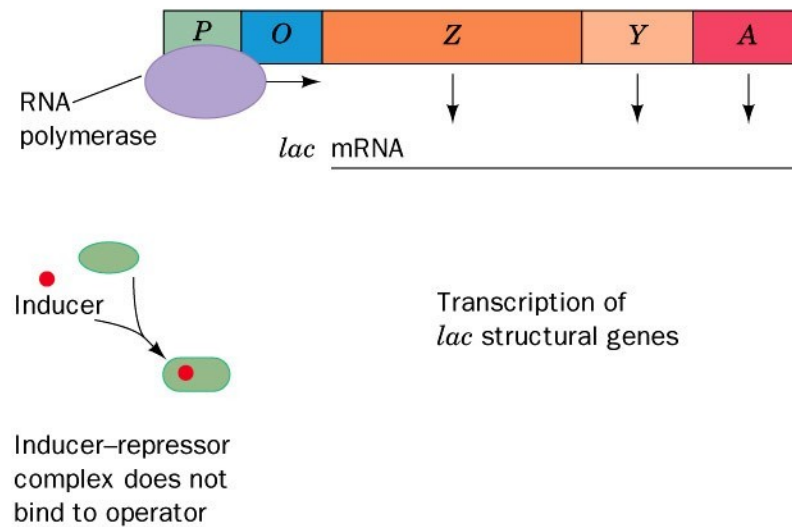


Regulate transcribe

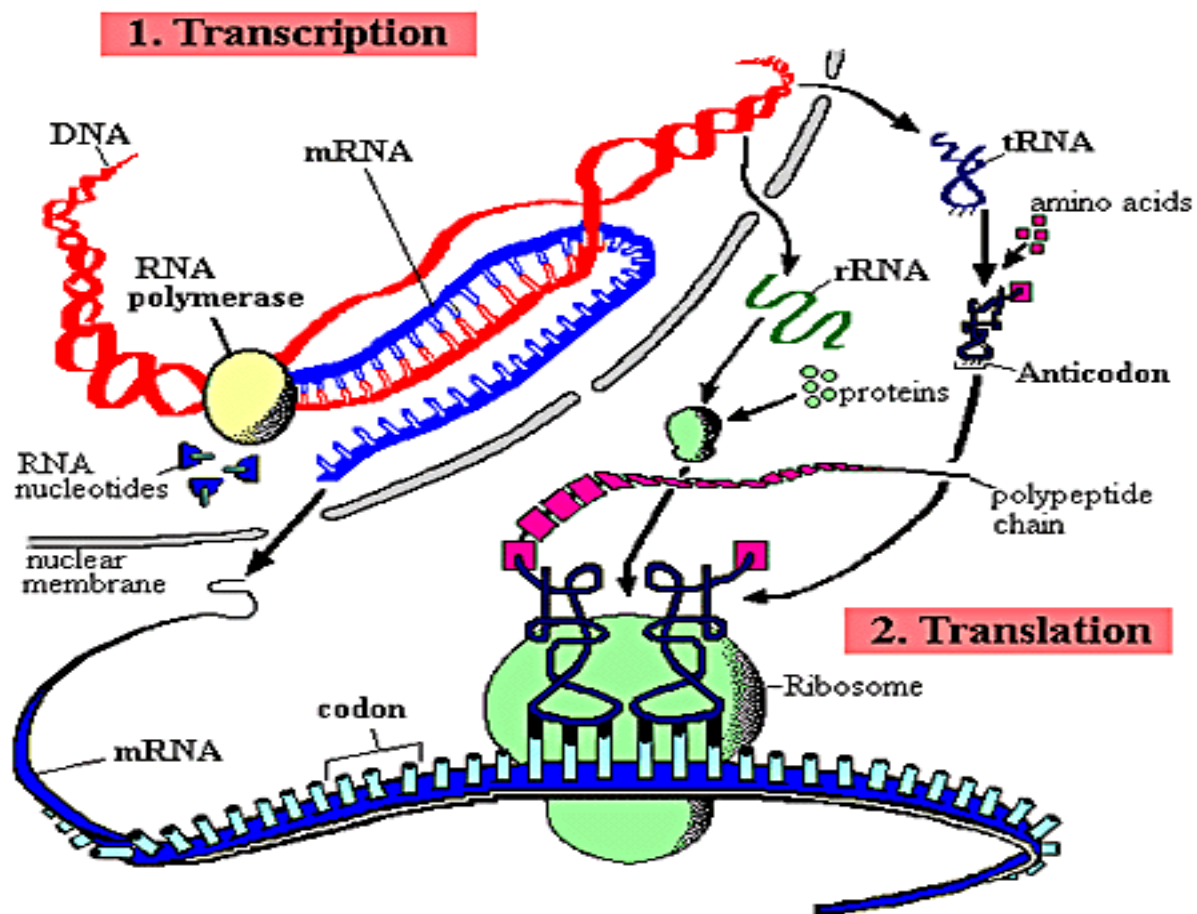
(a) Absence of inducer



(b) Presence of inducer

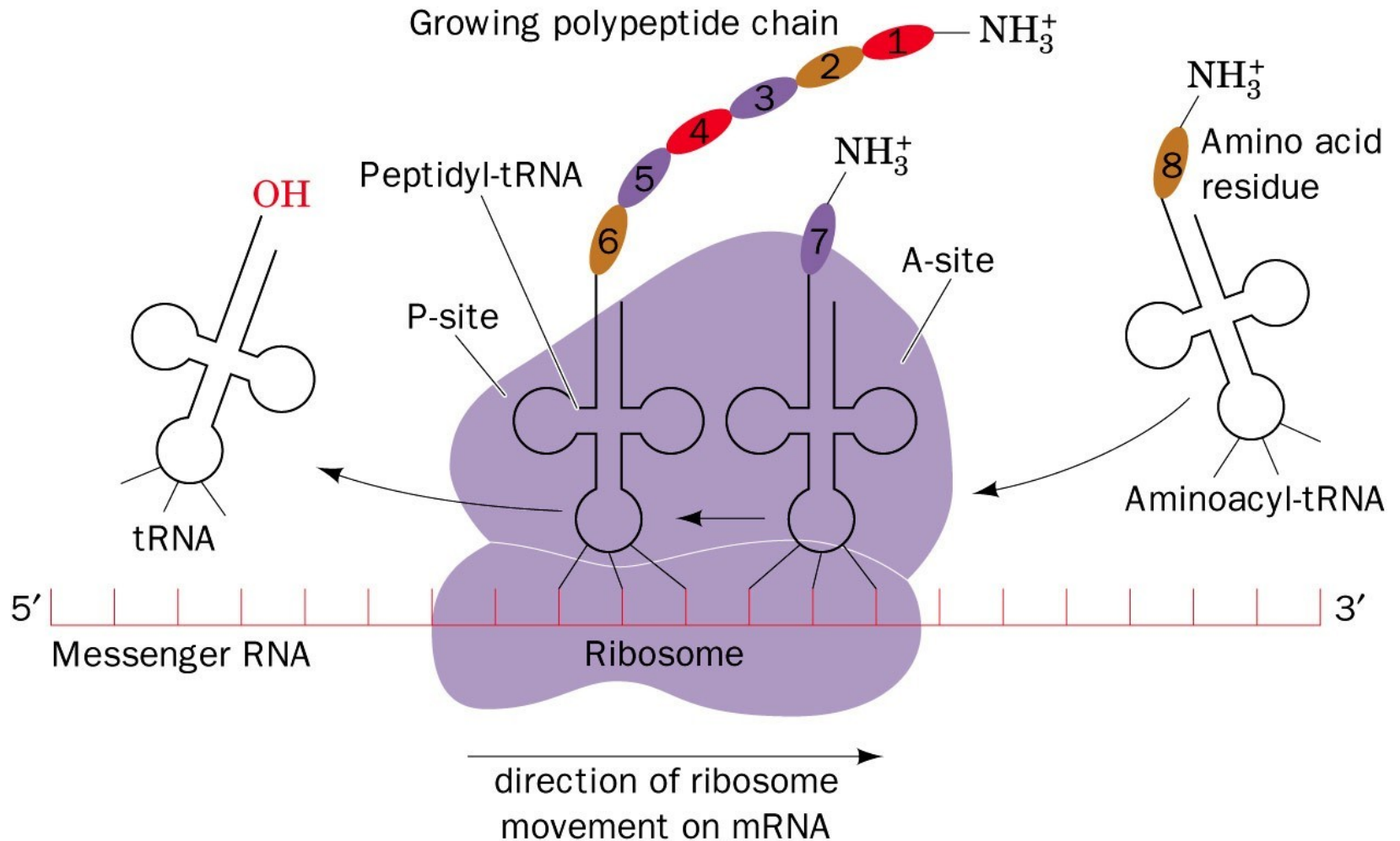


Syntéza bílkovin



Translase

mRNA + ribozomy + (tRNA-AMK) + IFn + RFn



Ribozomy

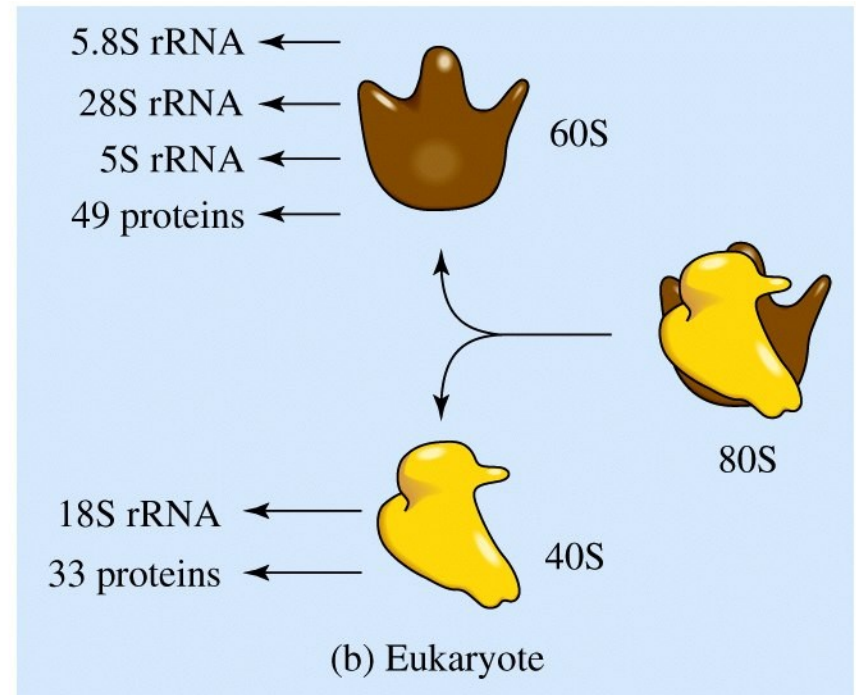
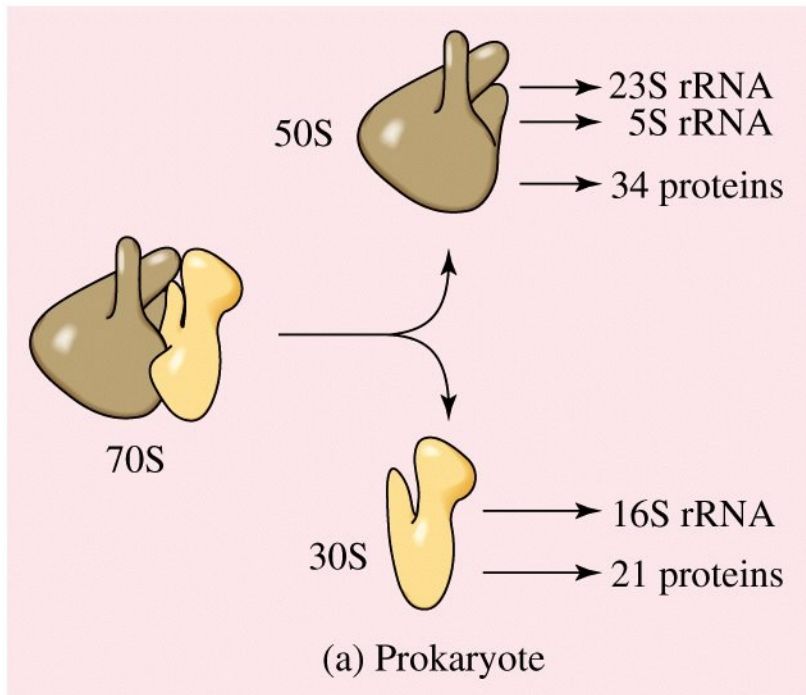
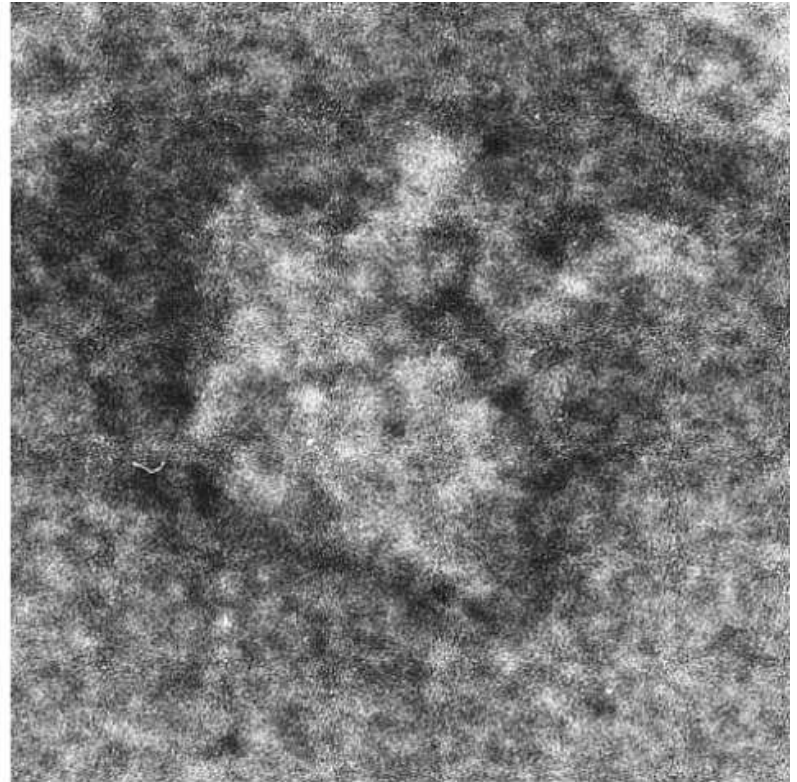
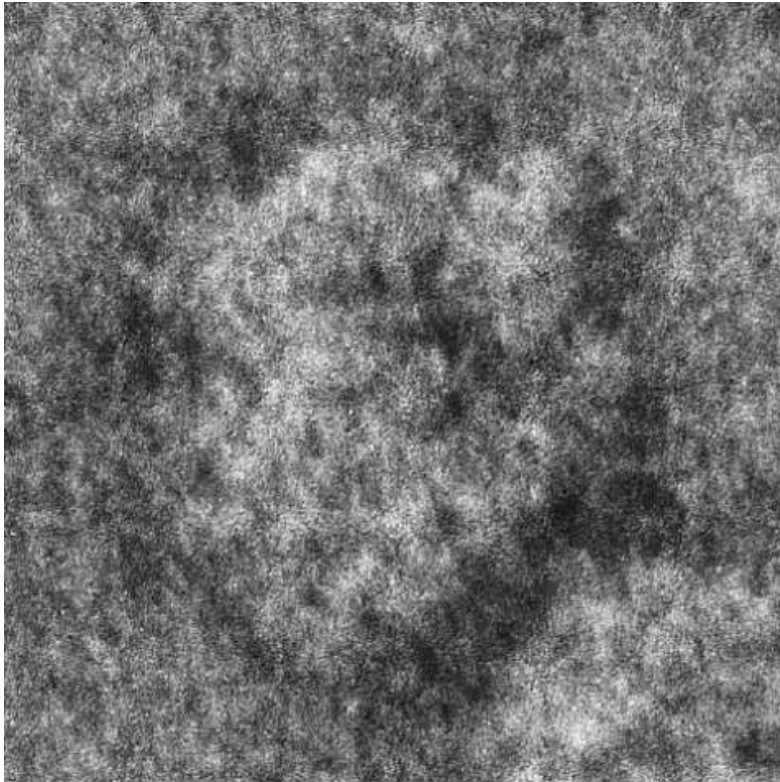


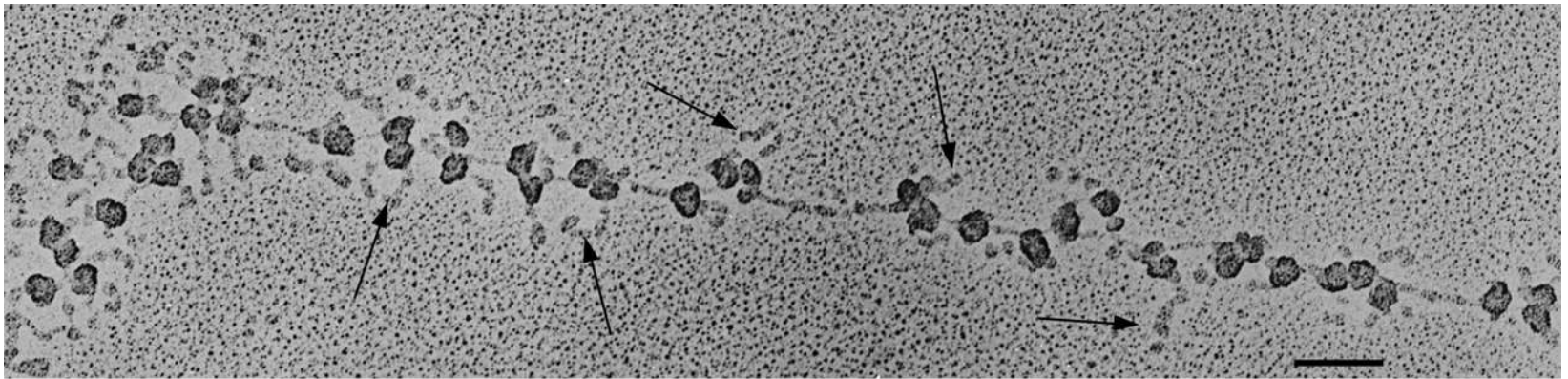
Figure 12-1 Concepts in Biochemistry, 3/e
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Ribozomy E.coli



Courtesy of James Lake, UCLA

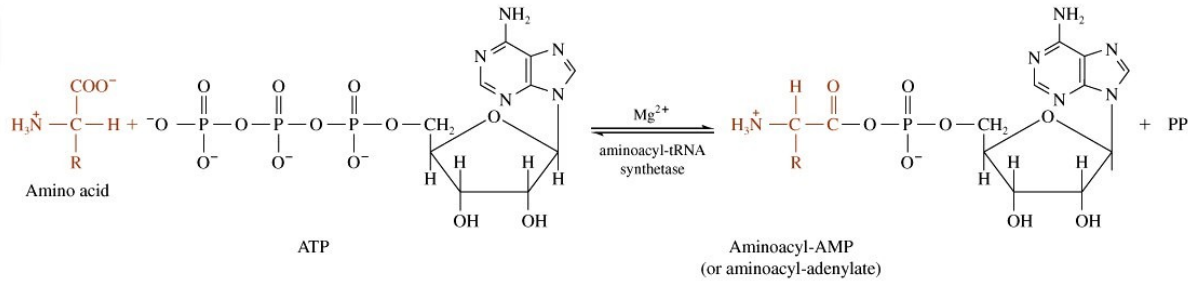
Polyribozomy *Bombyx mori*



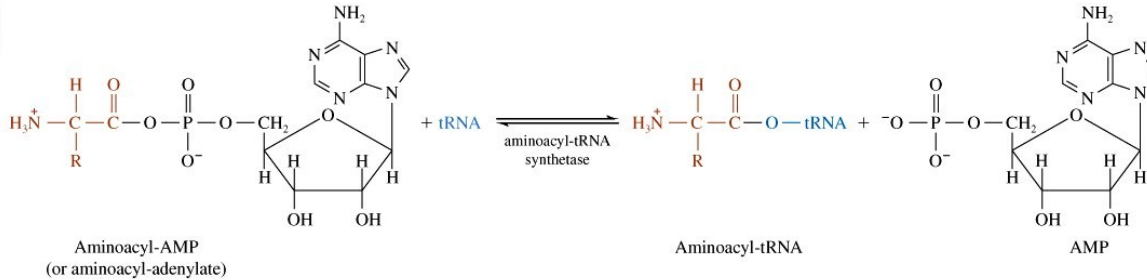
Courtesy of Oscar L. Miller, Jr. and Steven L. McKnight, University of Virginia

Aktivace AMK

Step 1



Step 2



Overall

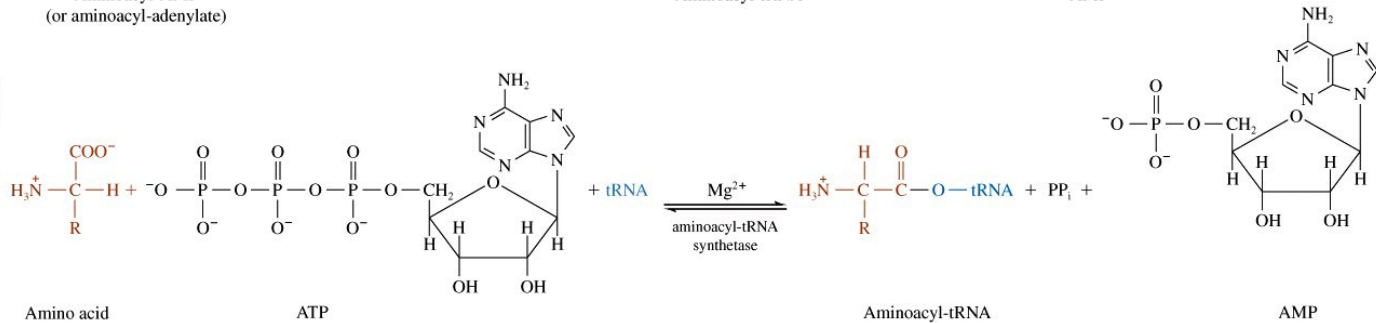


Figure 12-4 Concepts in Biochemistry, 3/e
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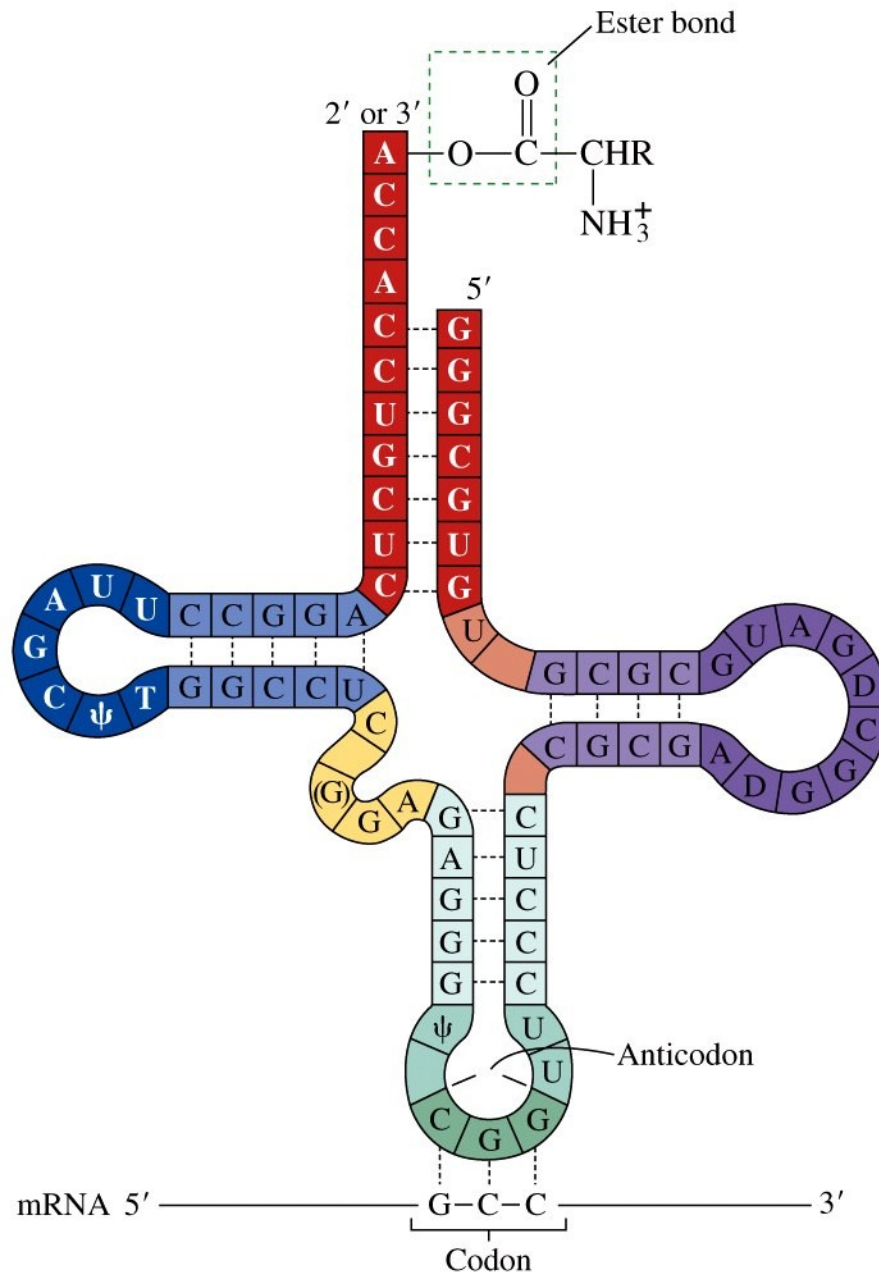
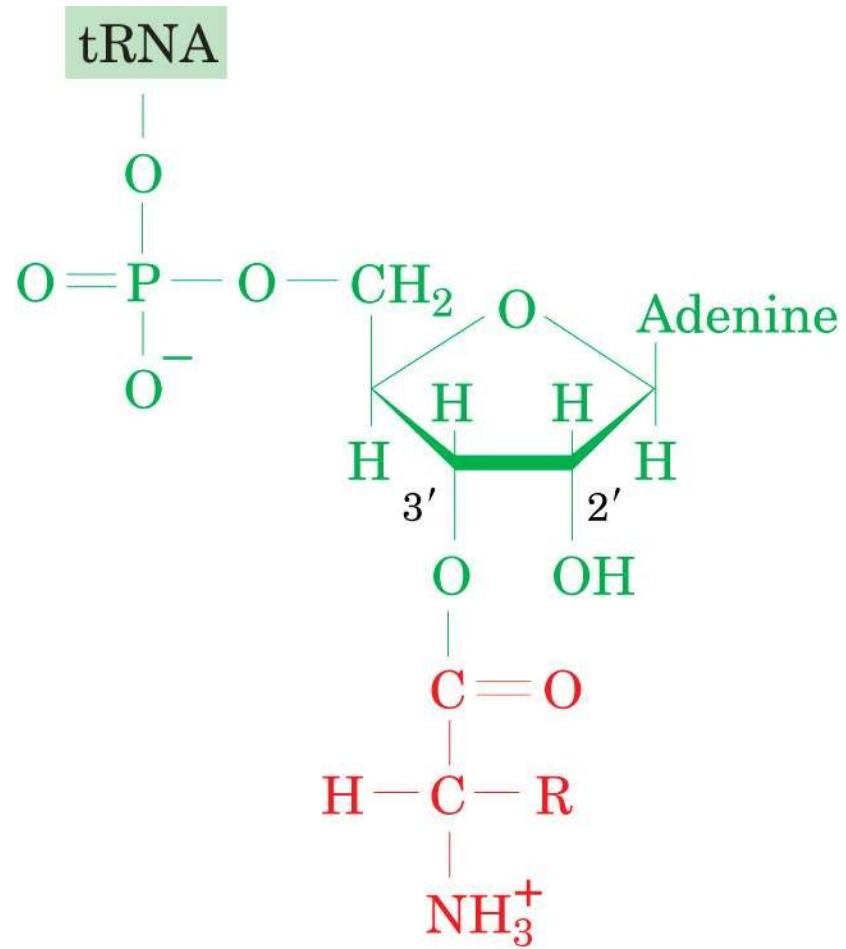


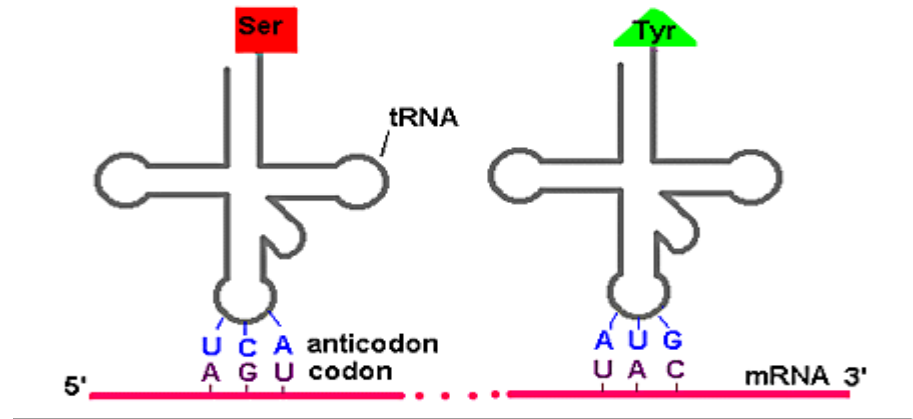
Figure 12-3 Concepts in Biochemistry, 3/e
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Aminoacyl-tRNA

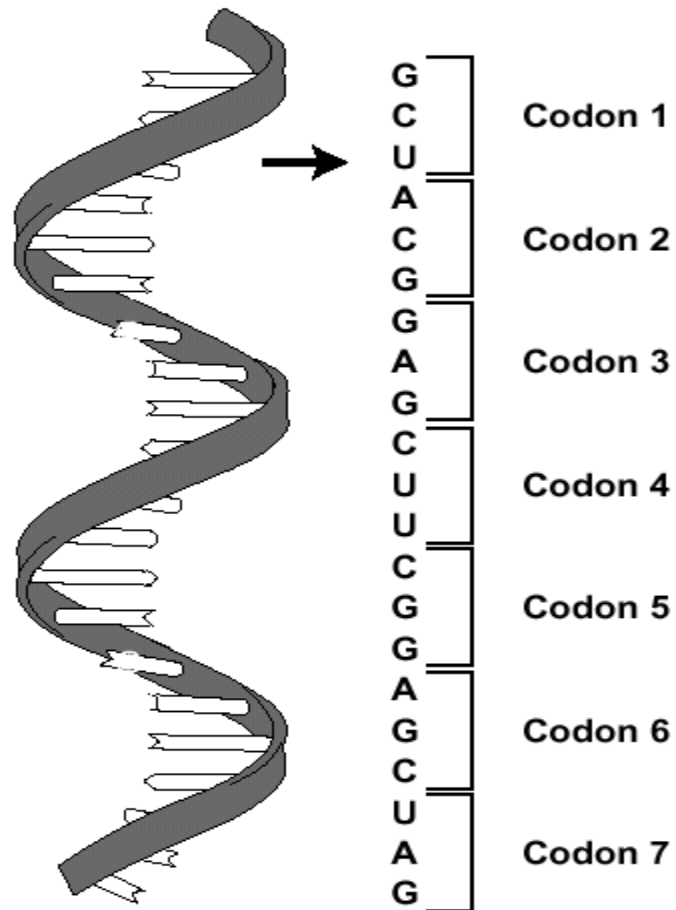
Genetický kod

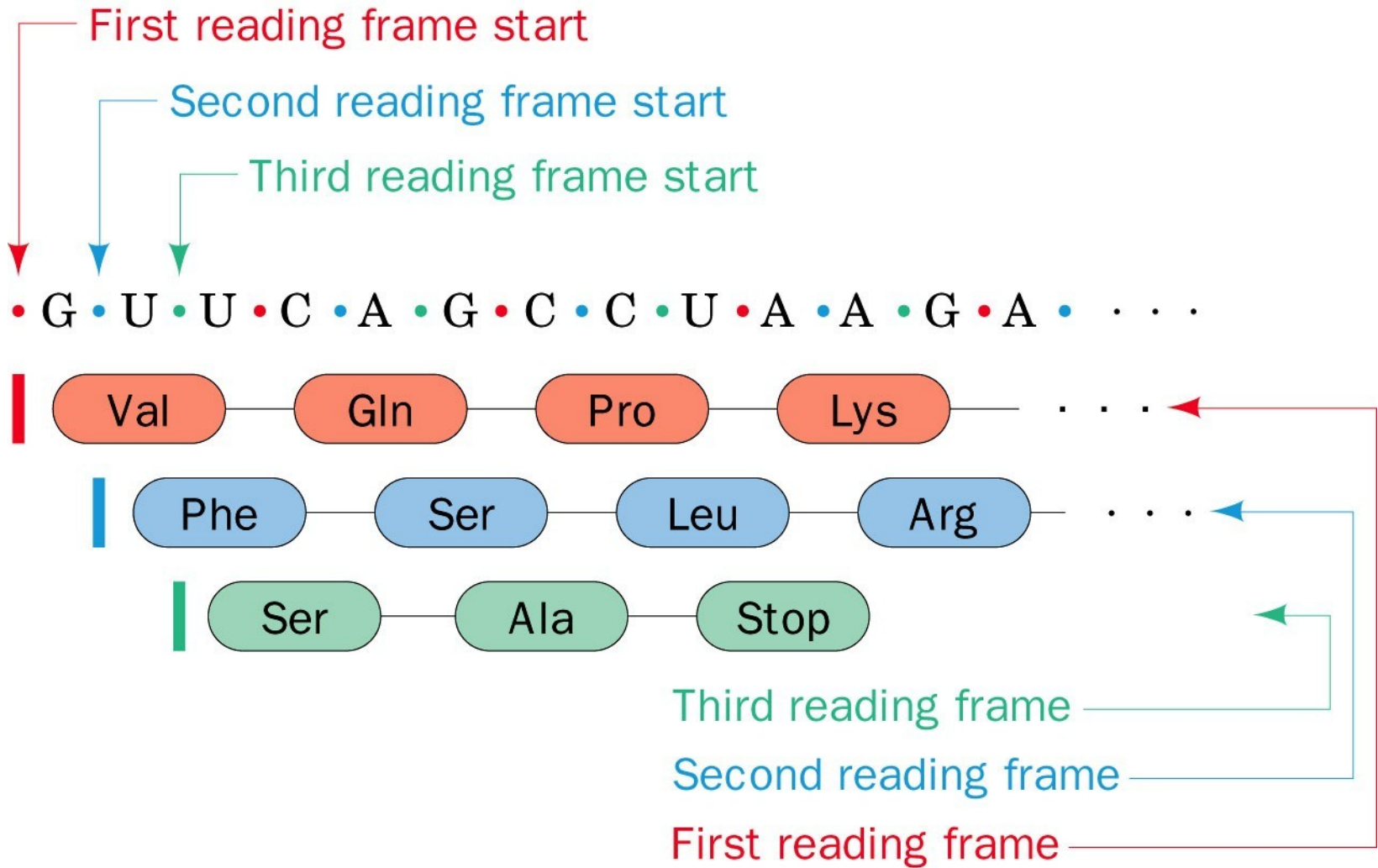
- Tripletový $4^3 = 64$
- Degenerovaný
- Nepřekrývající se
- Universální

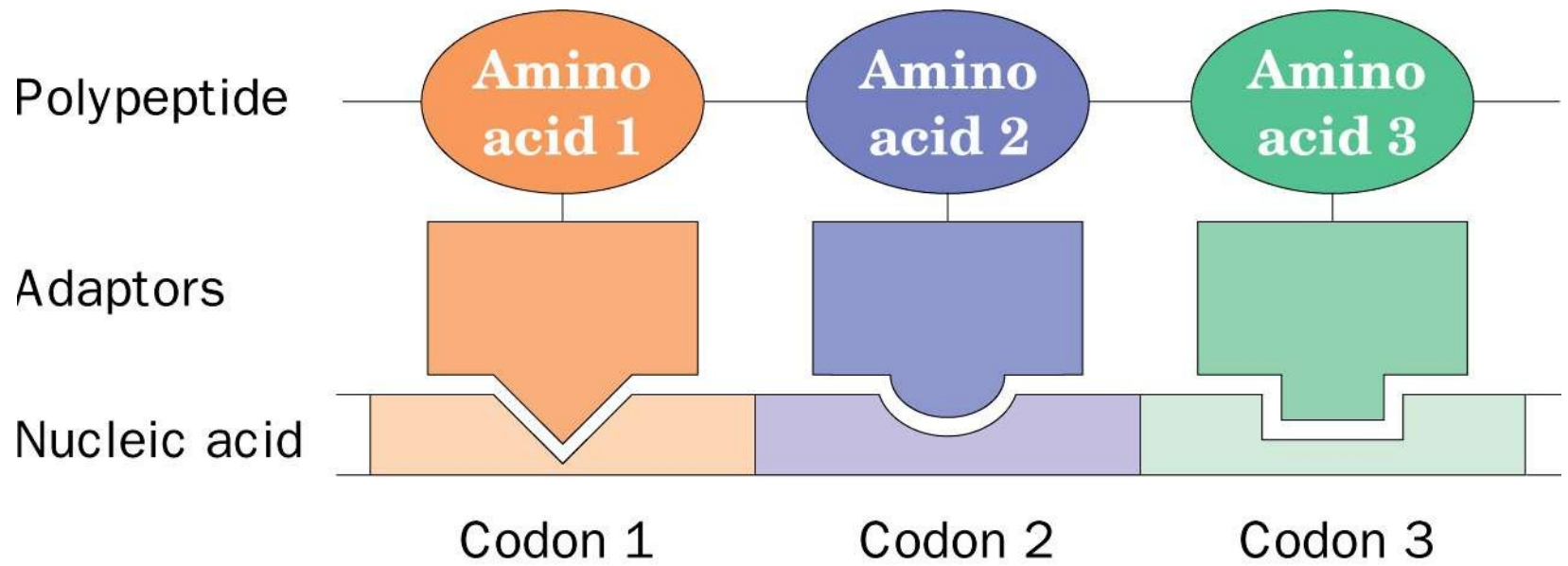


		2nd base in codon				
		U	C	A	G	
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G
						3rd base in codon

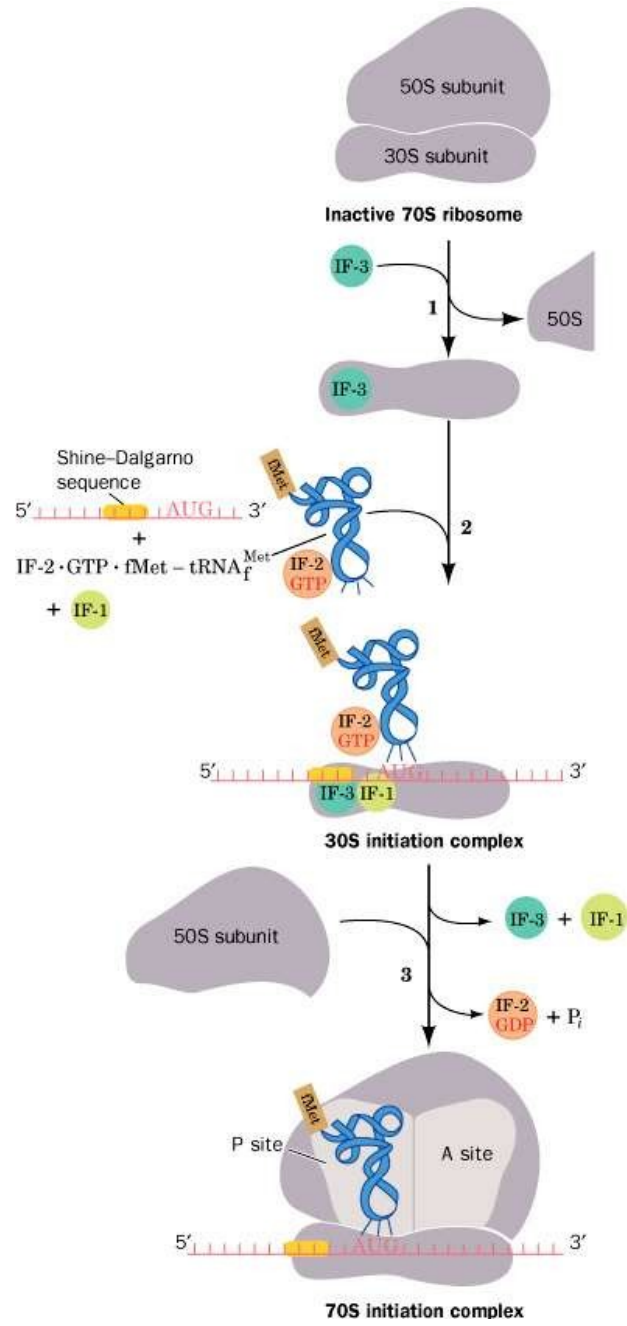
Kodón

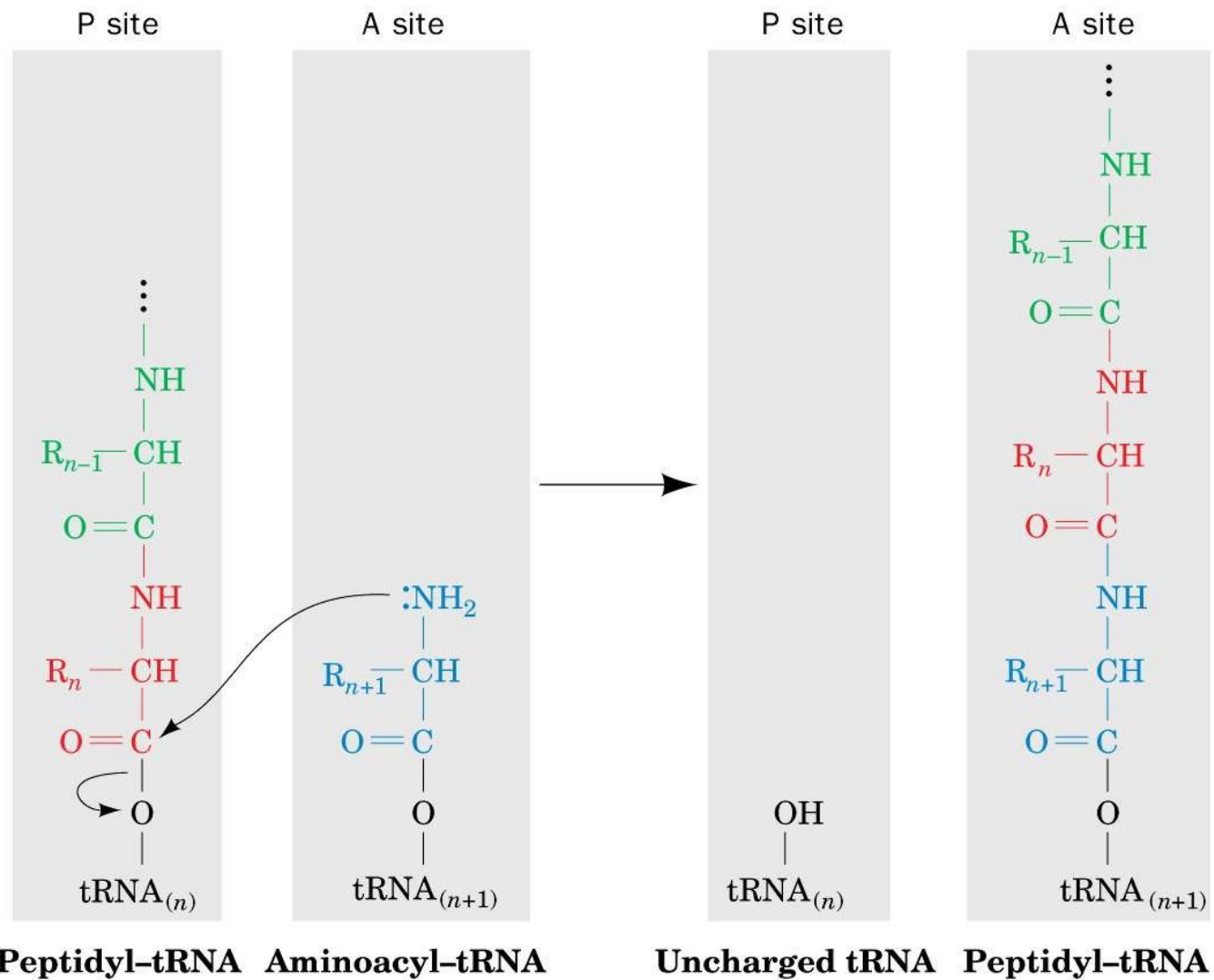




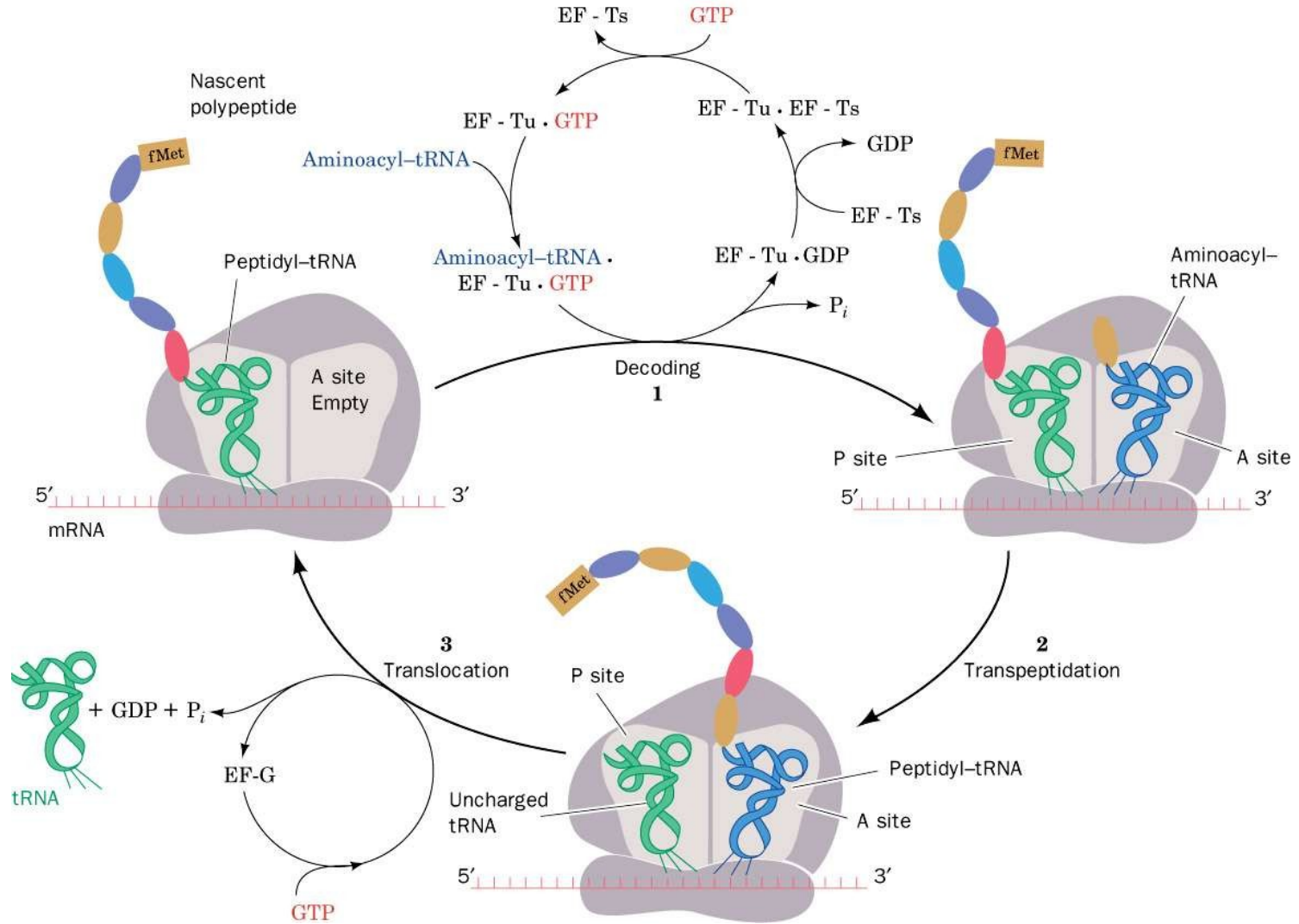


Inicializace

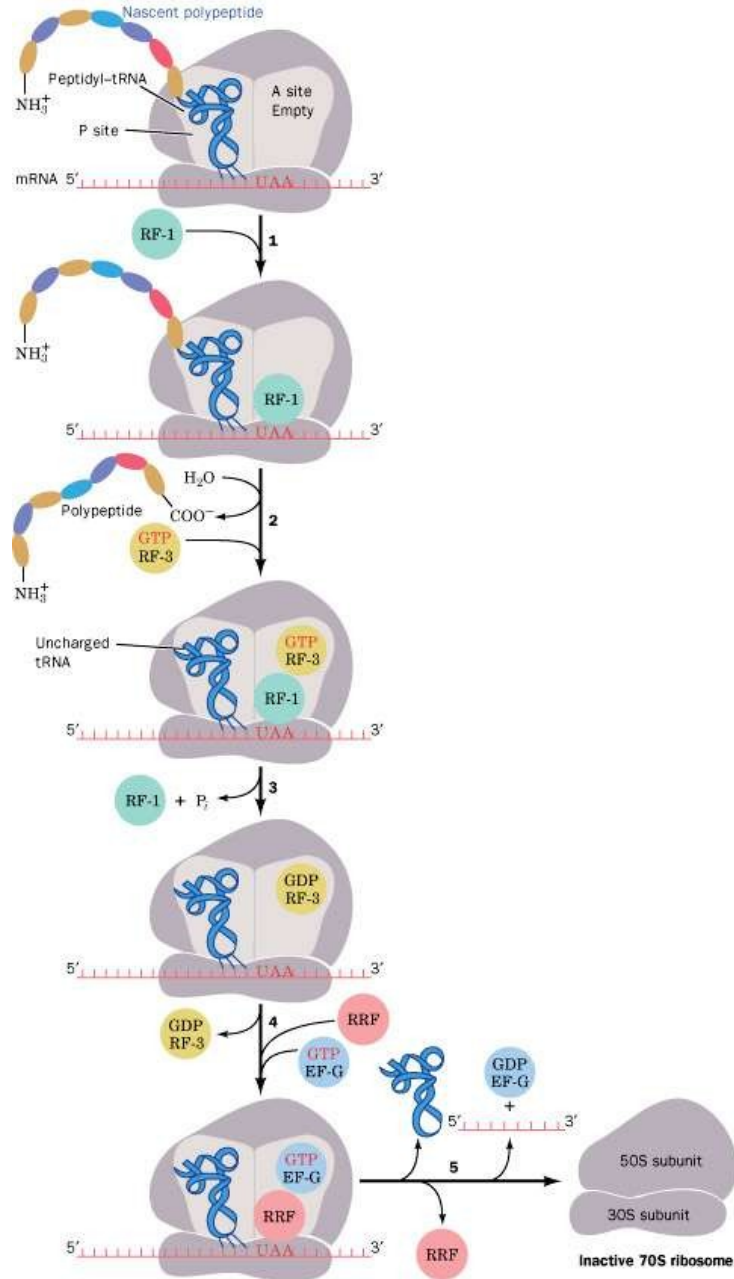




Elongate



Termination



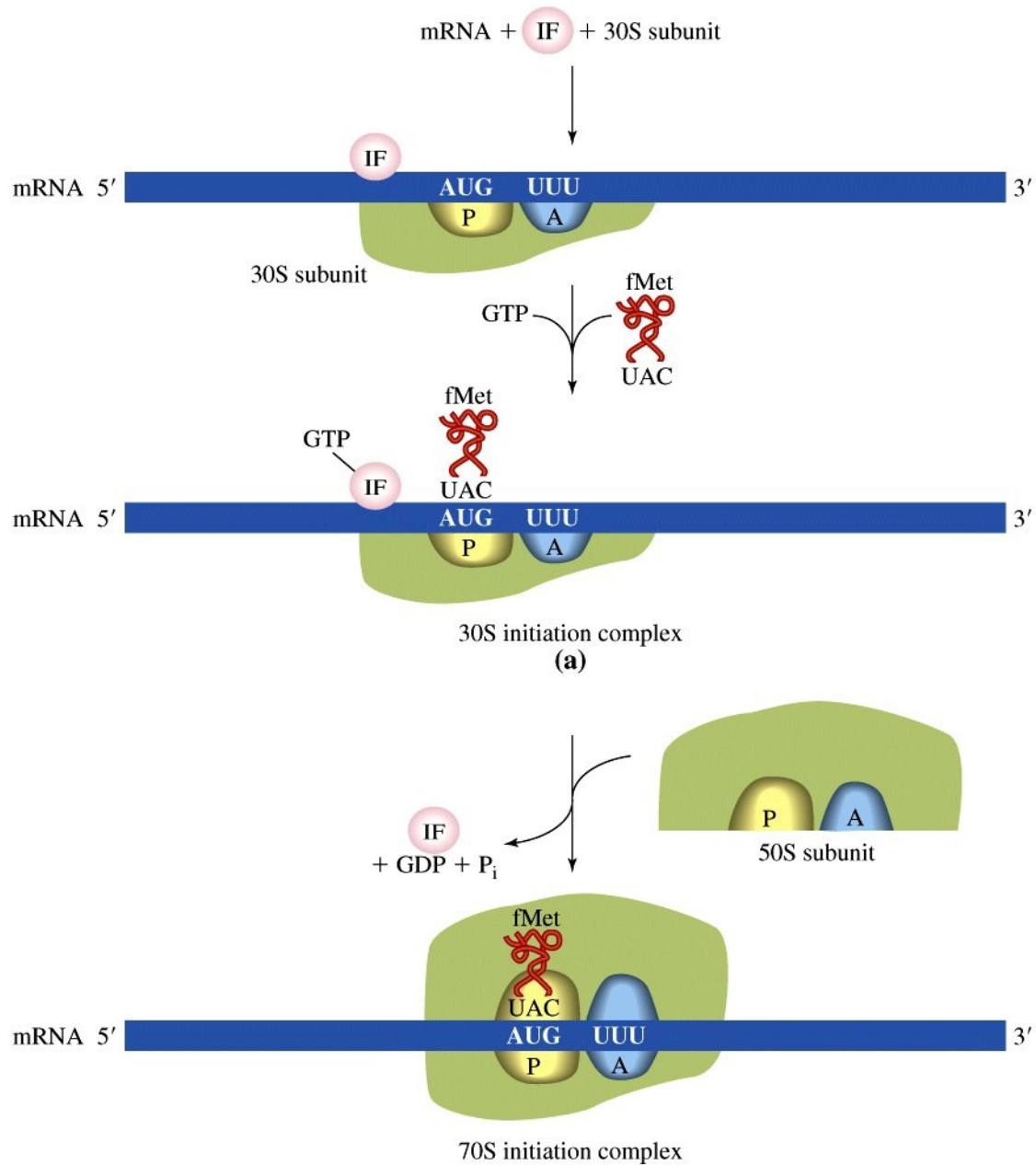


Figure 12-5a Concepts in Biochemistry, 3/e
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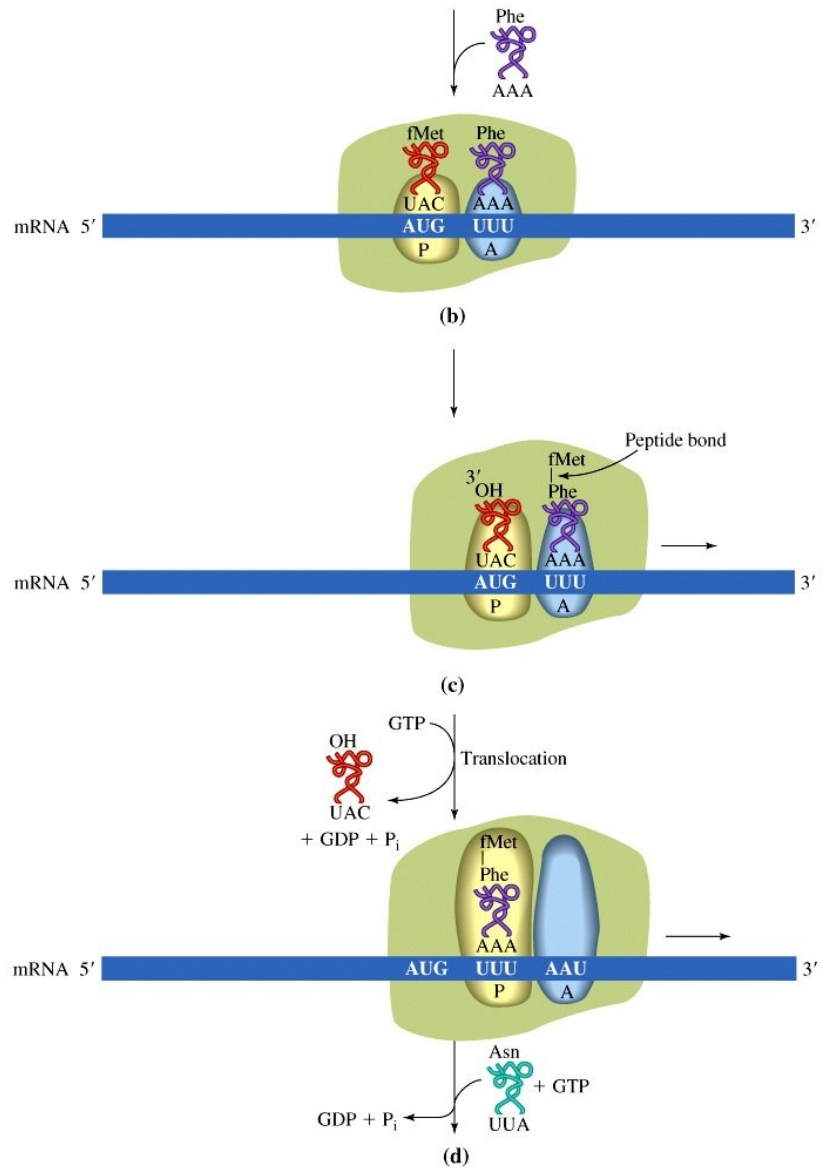


Figure 12-5b Concepts in Biochemistry, 3/e
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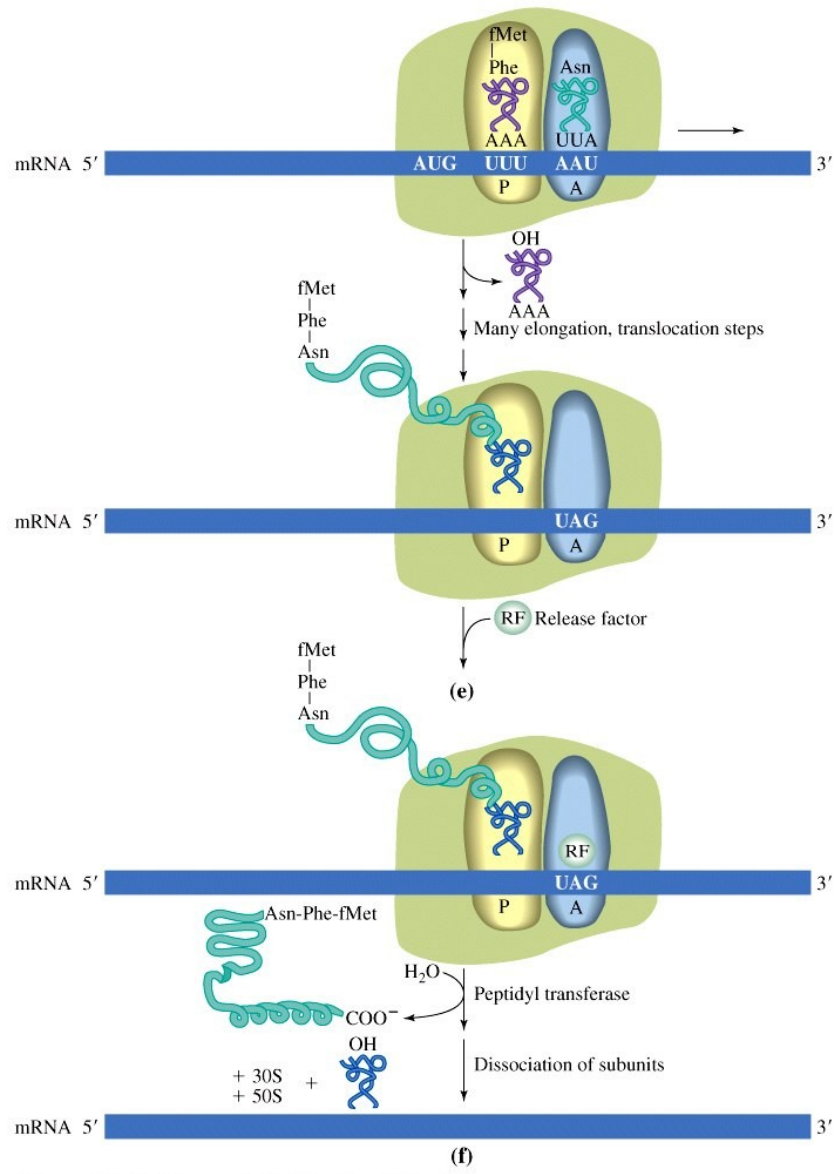


Figure 12-5c Concepts in Biochemistry, 3/e
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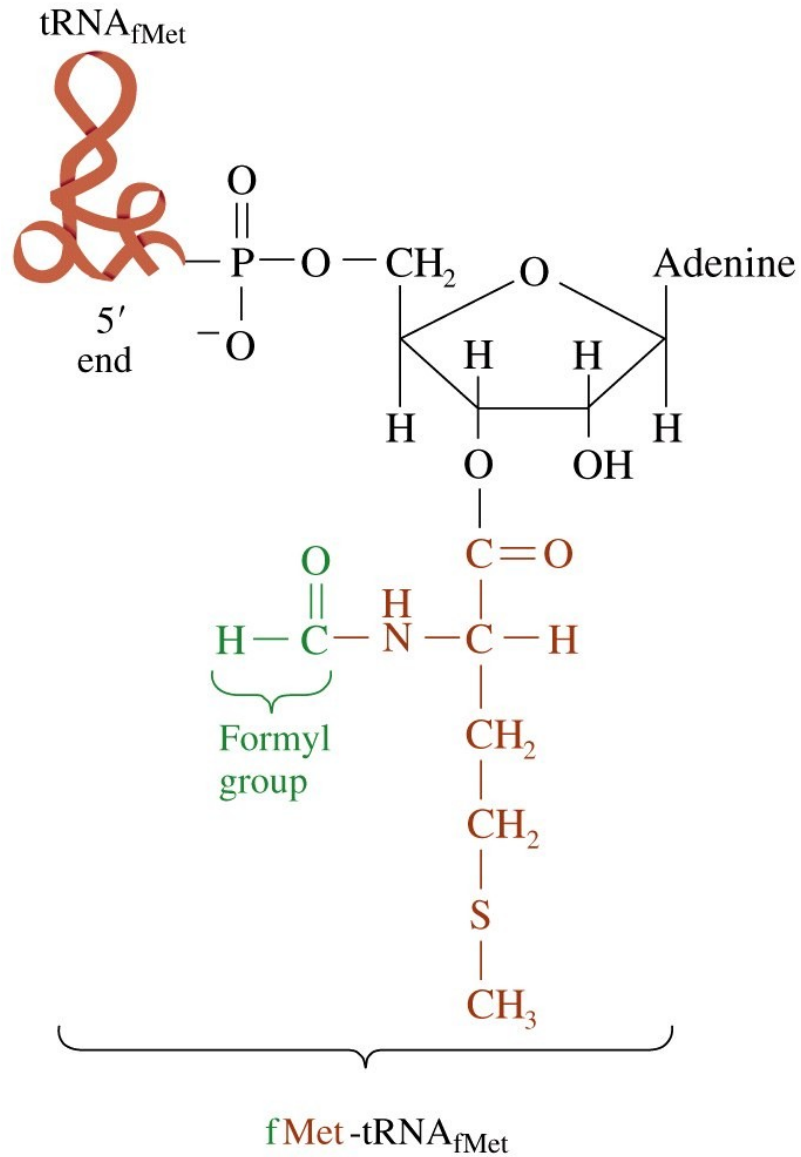


Figure 12-6 Concepts in Biochemistry, 3/e
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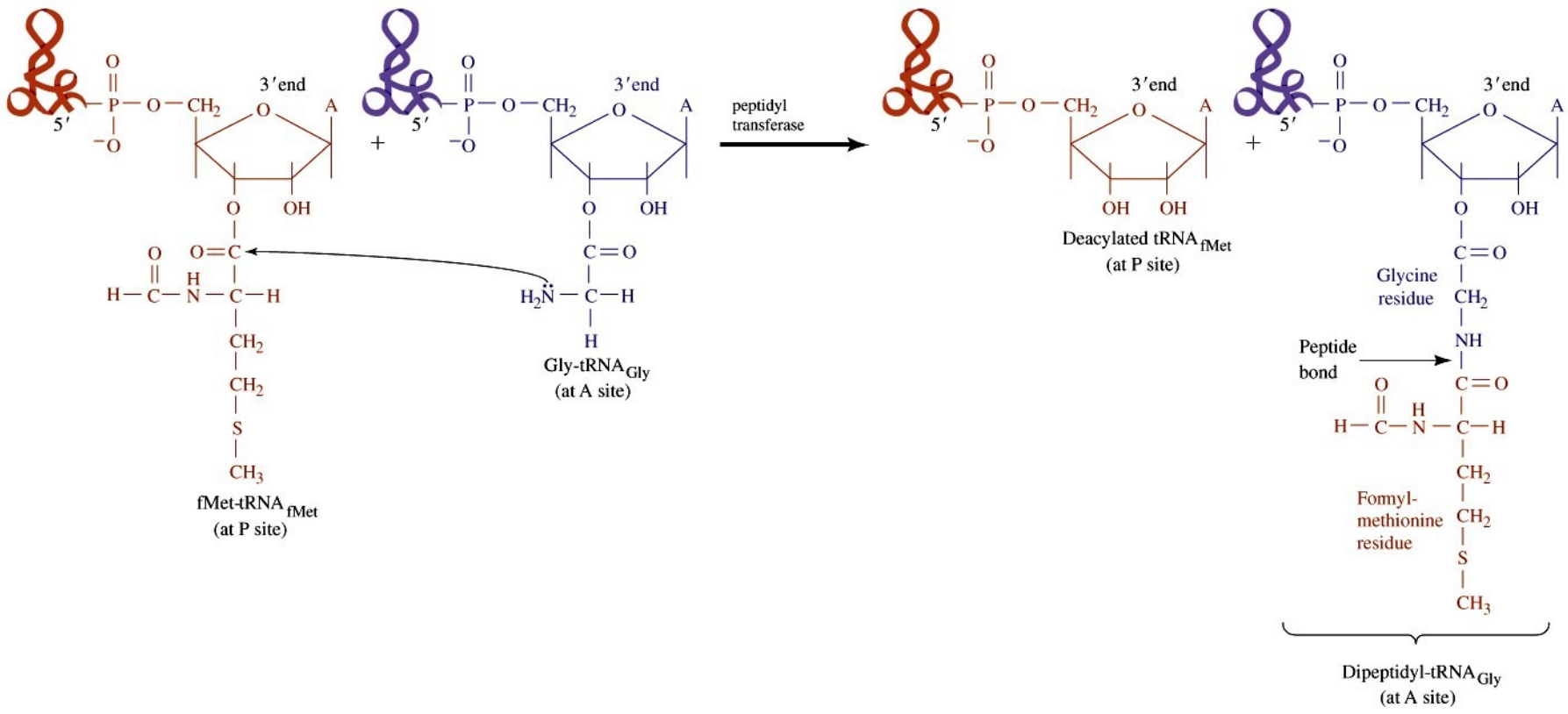
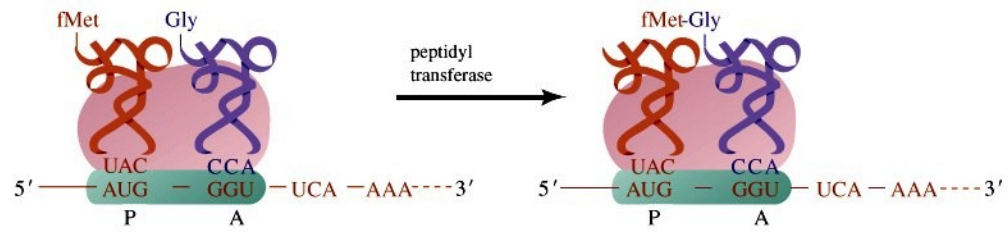


Figure 12-7 Concepts in Biochemistry, 3/e
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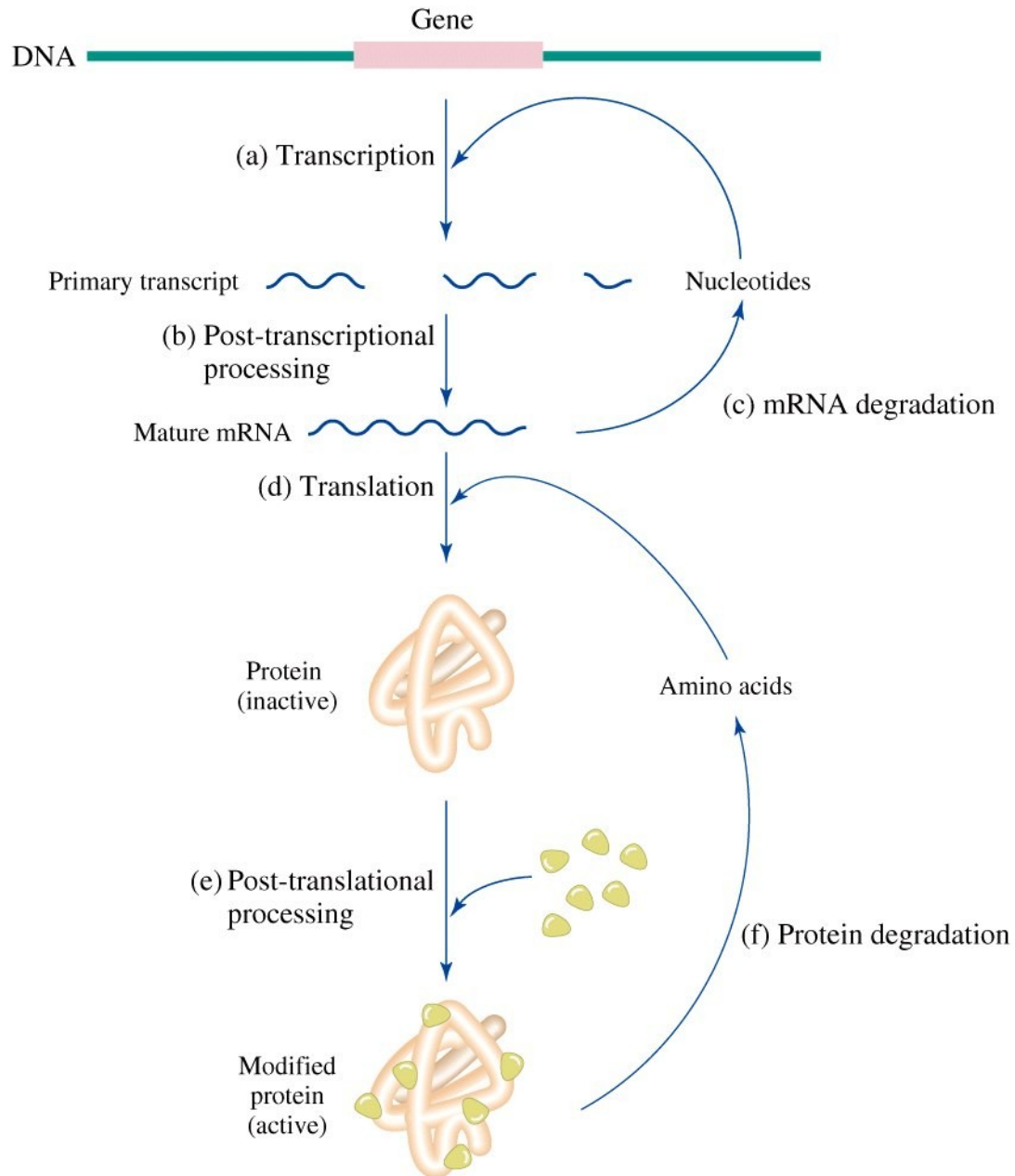


Figure 12-16 Concepts in Biochemistry, 3/e
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Kontrola exprese genu

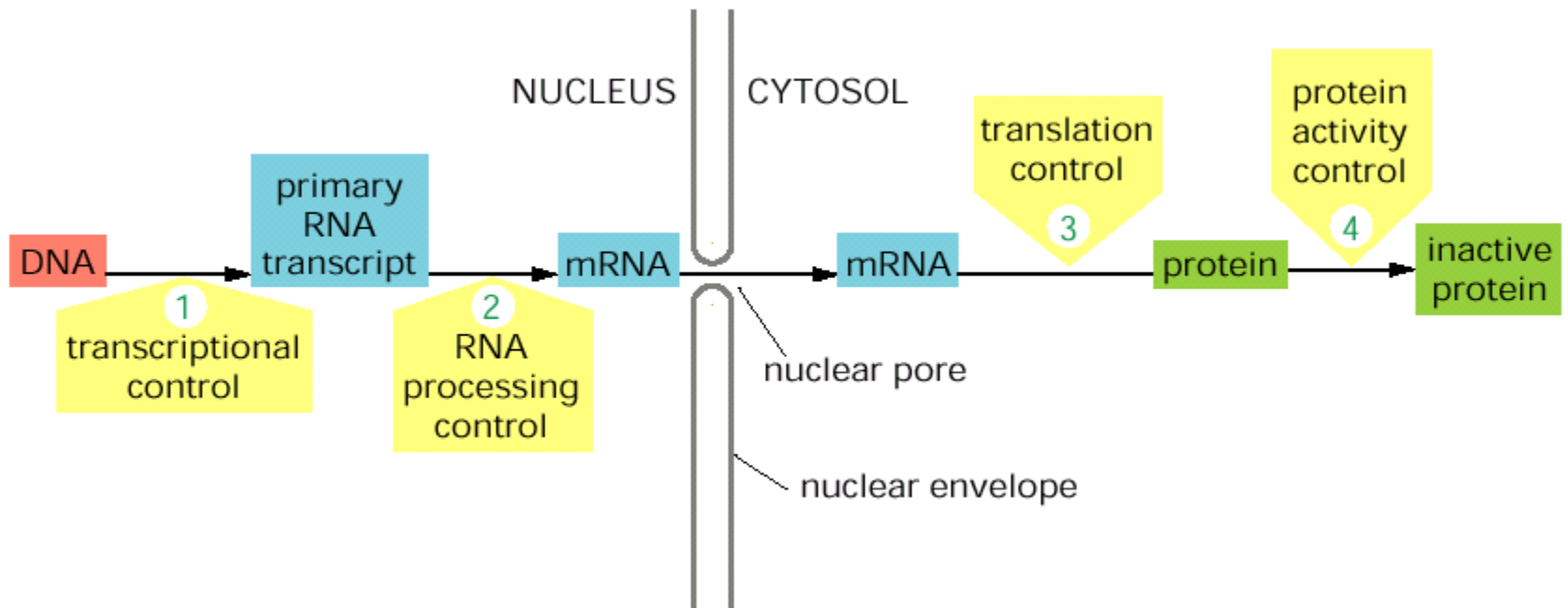


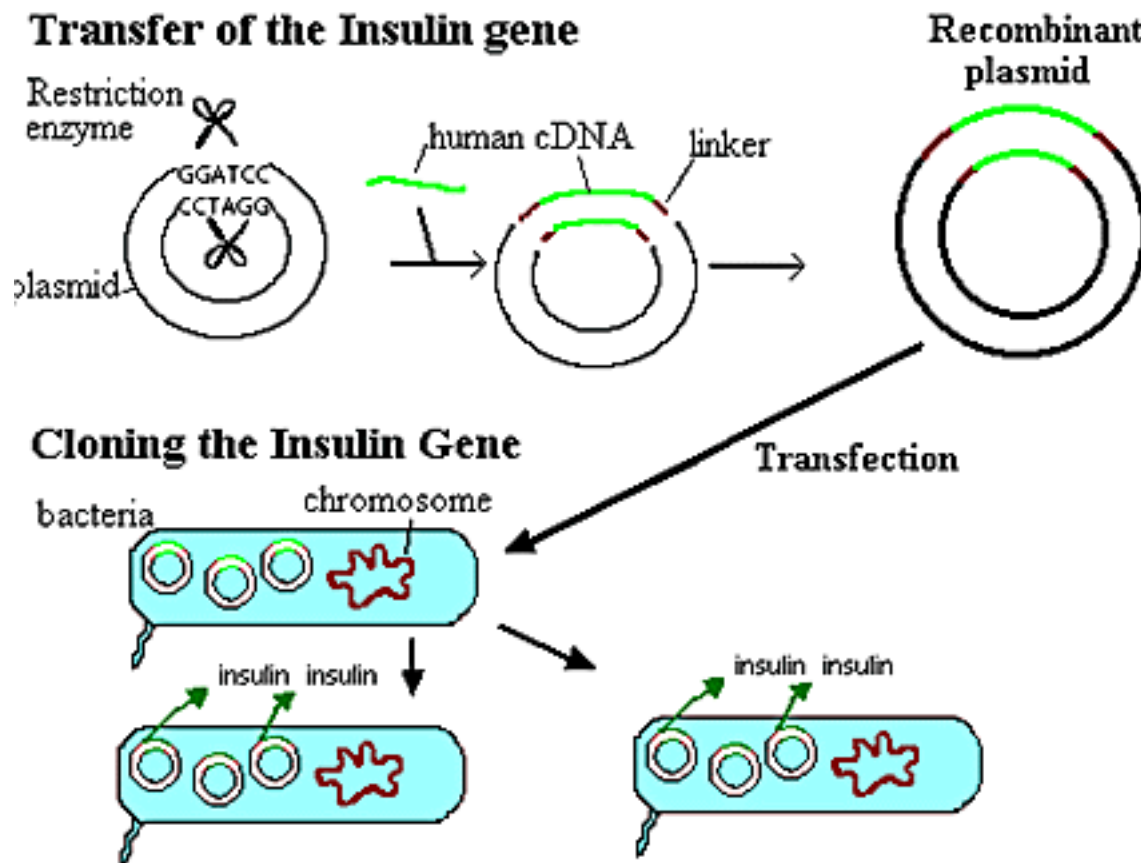
Table 12.4**Antibiotic inhibitors of protein synthesis**

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



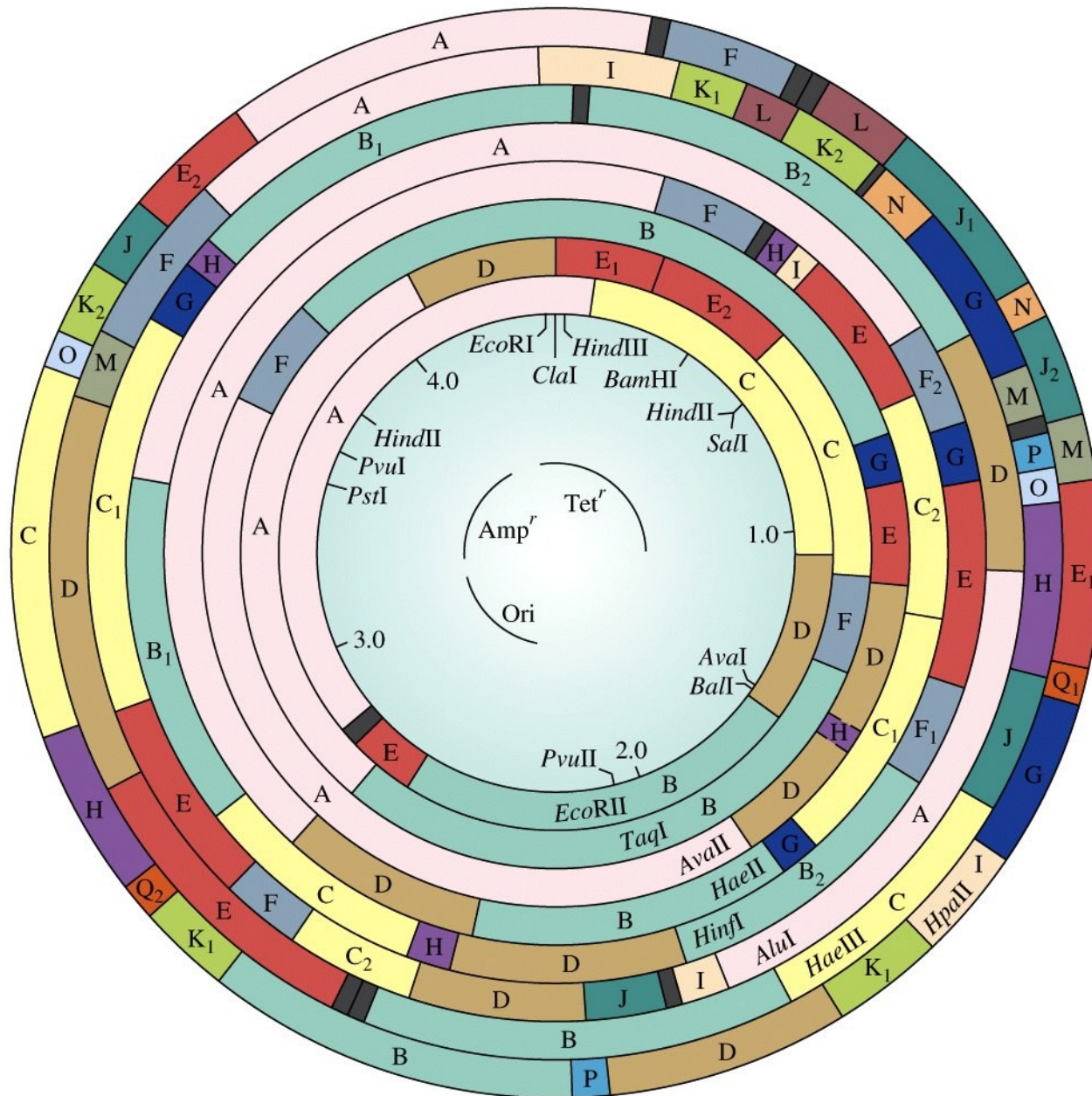


Figure 13-8 Concepts in Biochemistry, 3/e
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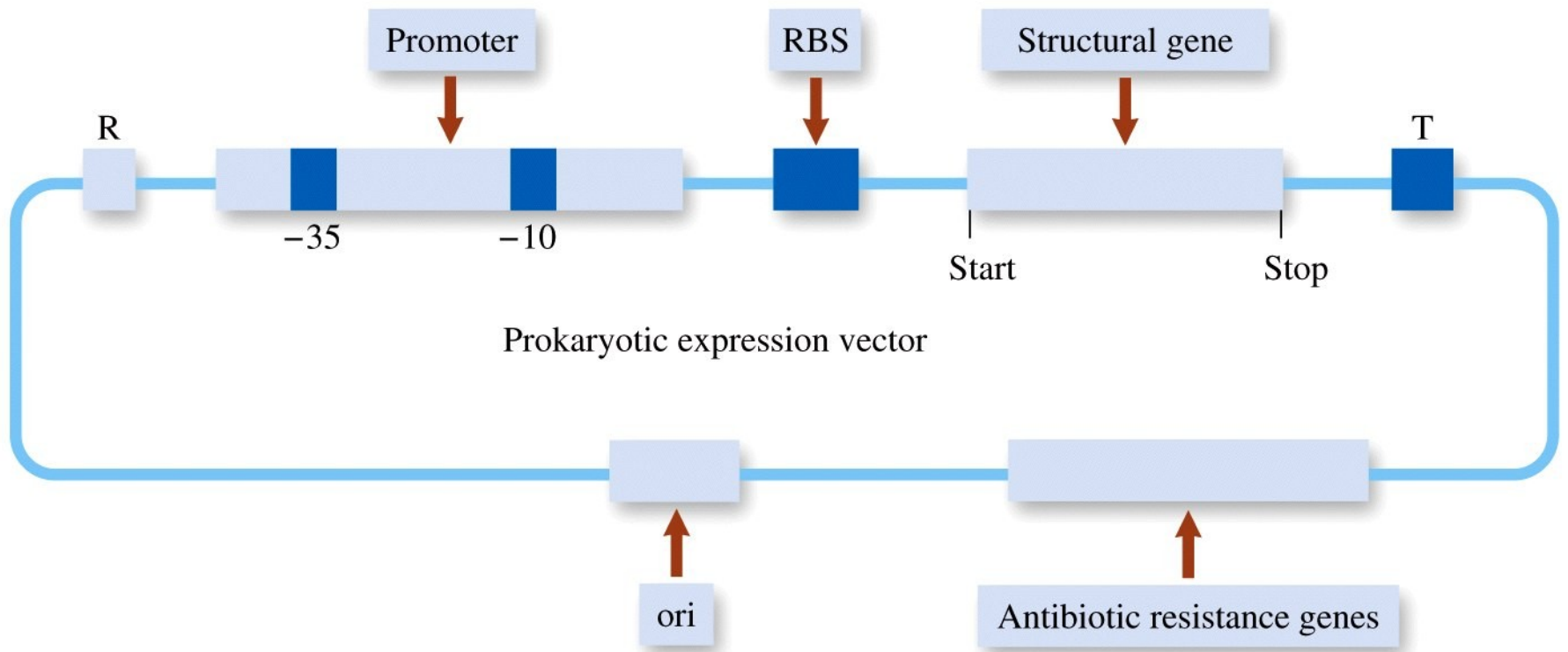
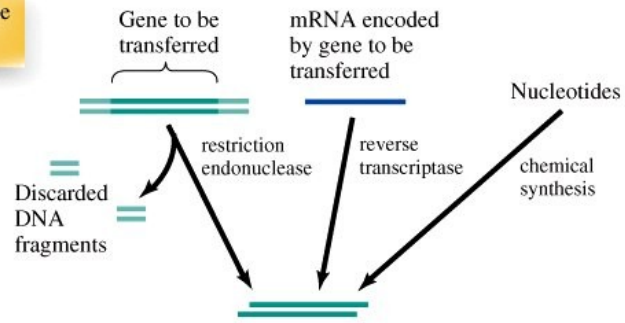
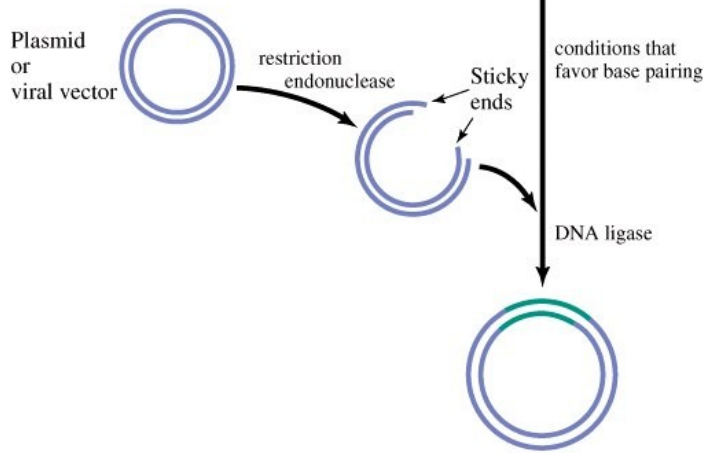


Figure 13-3 Concepts in Biochemistry, 3/e
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Step 1. Obtain gene to be transferred.



Steps 2 and 3. Attach gene to vector



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

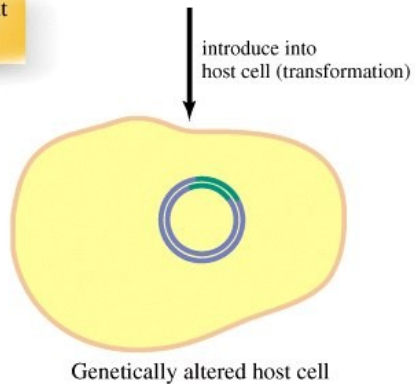


Figure 13-1 Concepts in Biochemistry, 3/e
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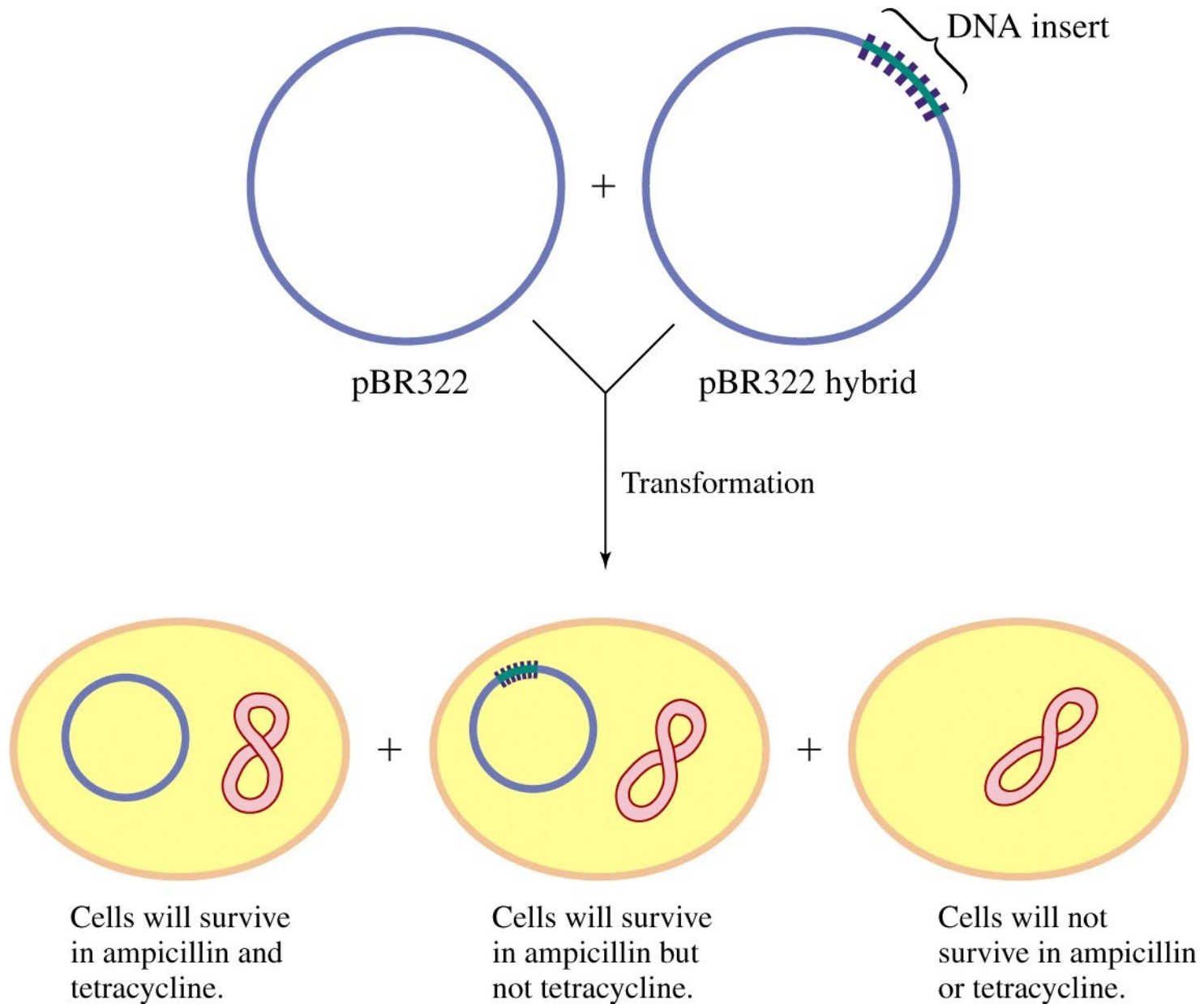


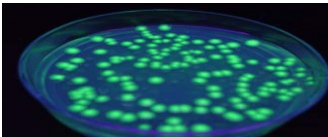
Figure 13-6 part 3 Concepts in Biochemistry, 3/e
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Inklusní tělíska

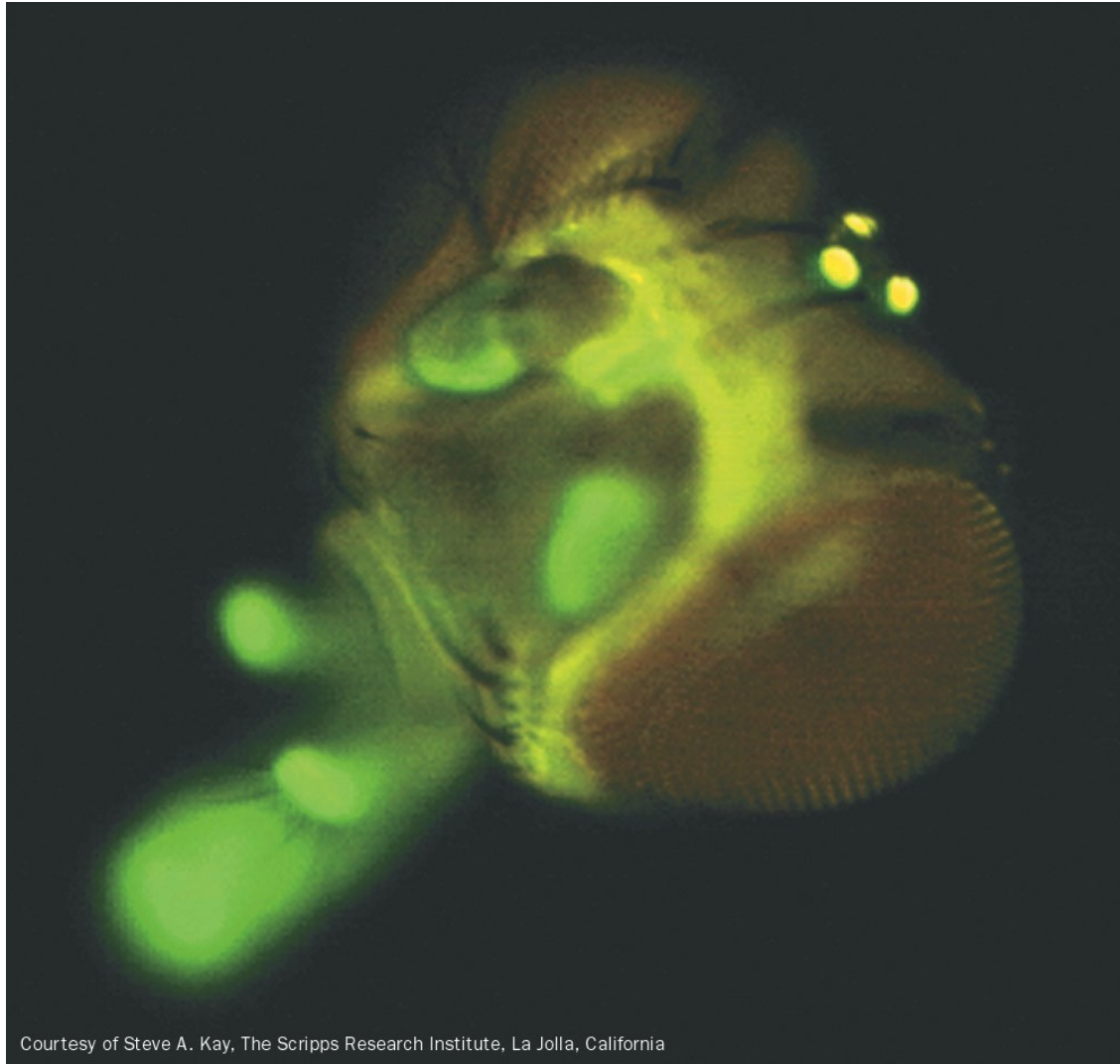


Courtesy of Teruhiko Beppu, Nihon University, Japan

Využití AC pro purifikaci rekombinantních proteinů

fúzní 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



Courtesy of Steve A. Kay, The Scripps Research Institute, La Jolla, California

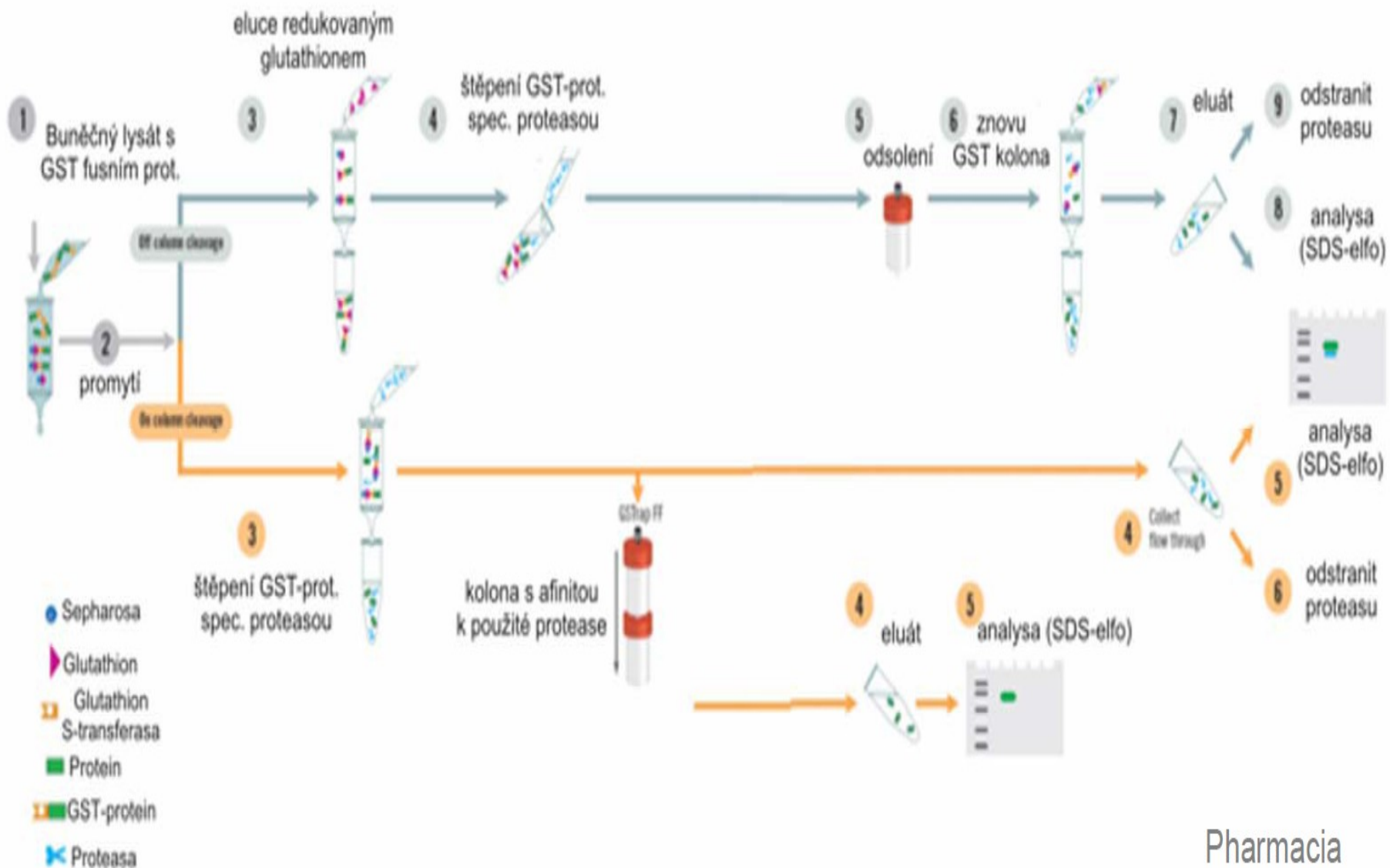


Table 13.1**Recombinant proteins and their use**

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

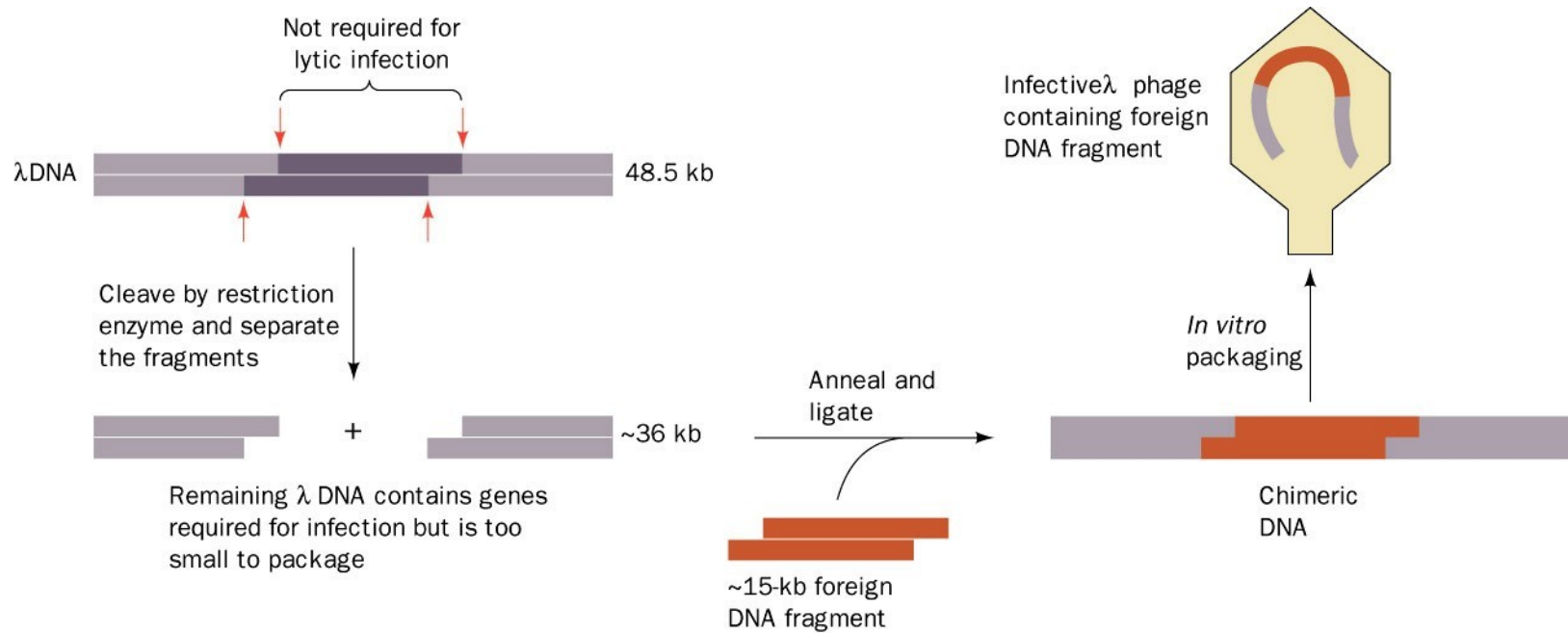
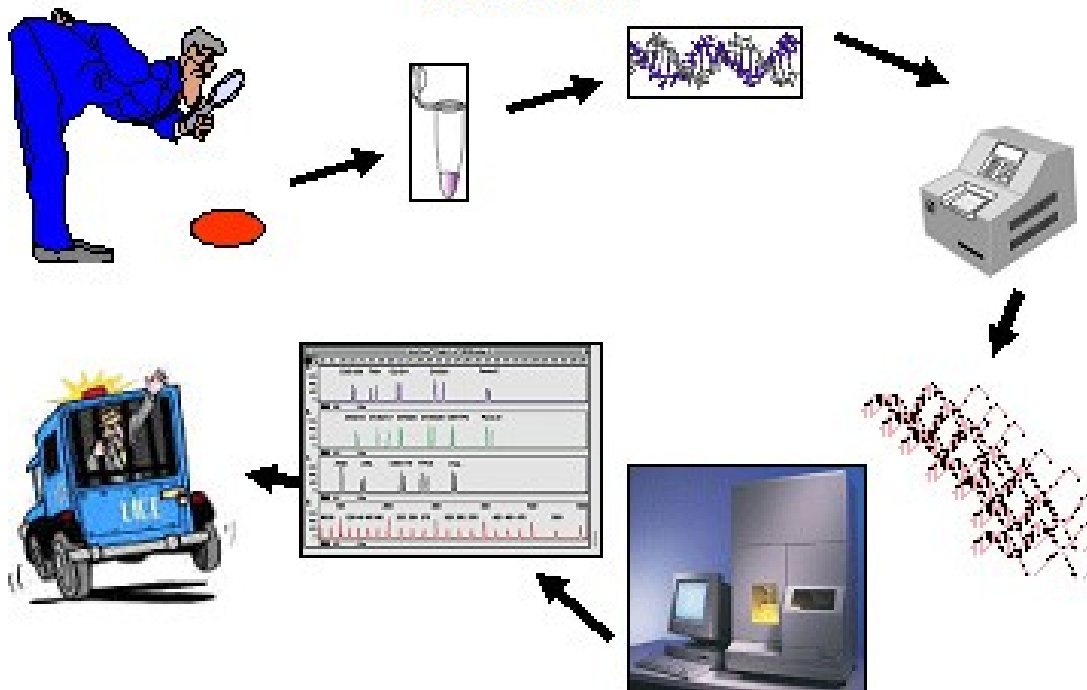


Table 13.2**Human gene therapy projects currently in or preparing for clinical trials**

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

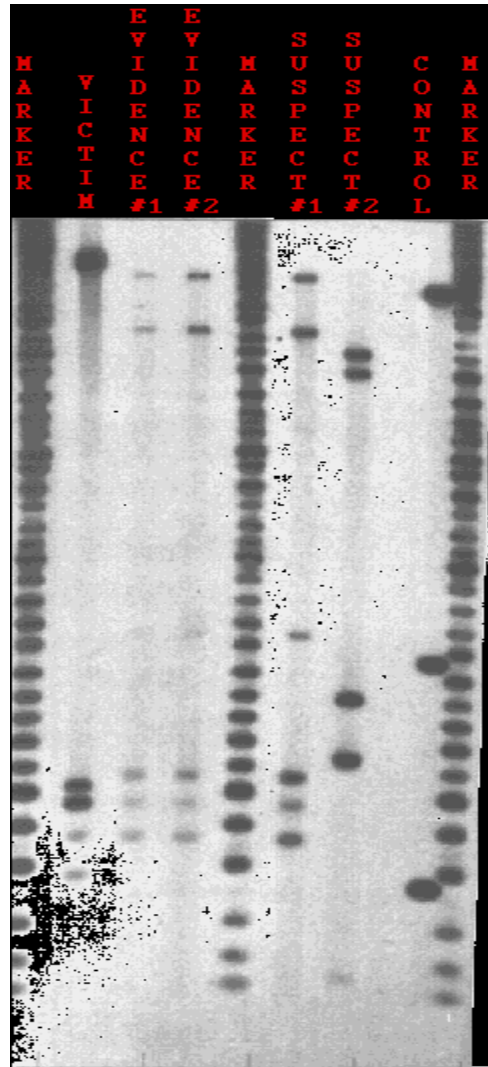
Genetická daktyloskopie

The Process of DNA Typing via PCR



Použití restričních enzymů - RFLP

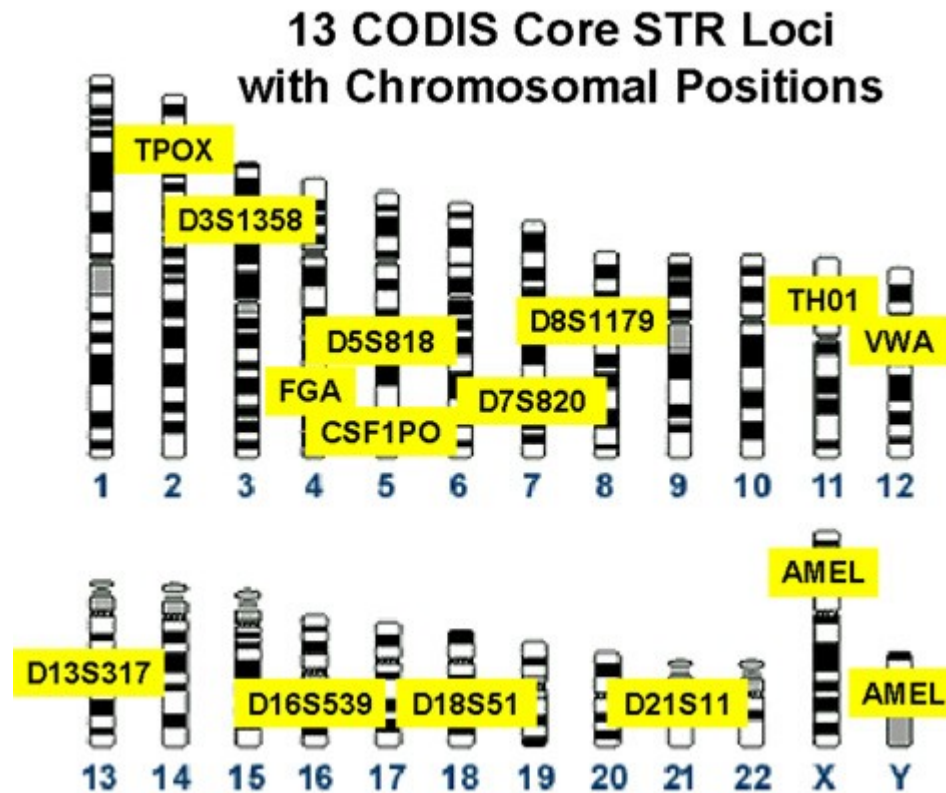
Restriction Fragment Length Polymorphism



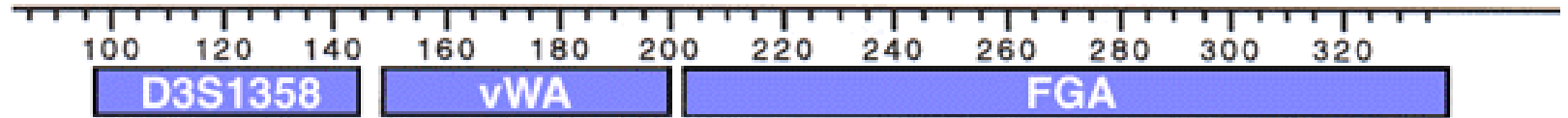
Short tandem repeats

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	

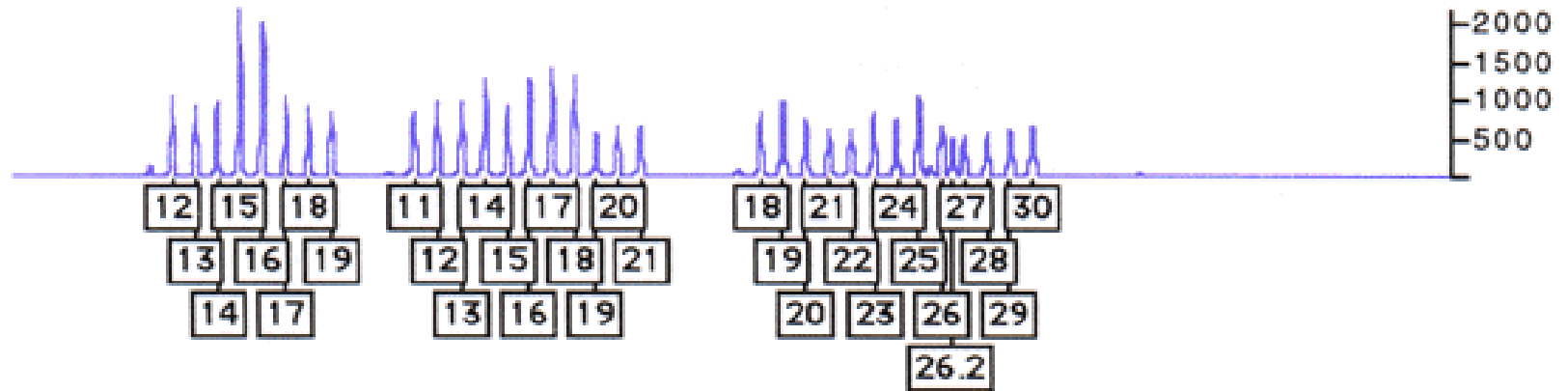
Short tandem repeats



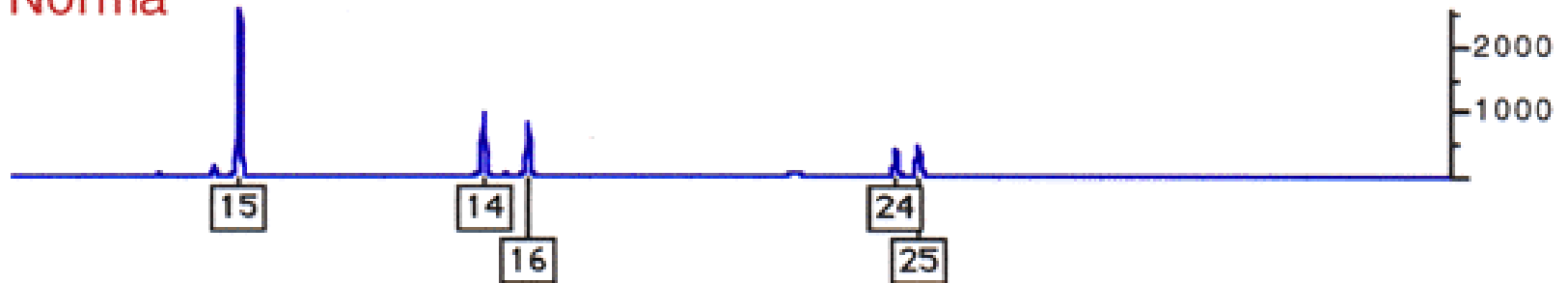
Short tandem repeats



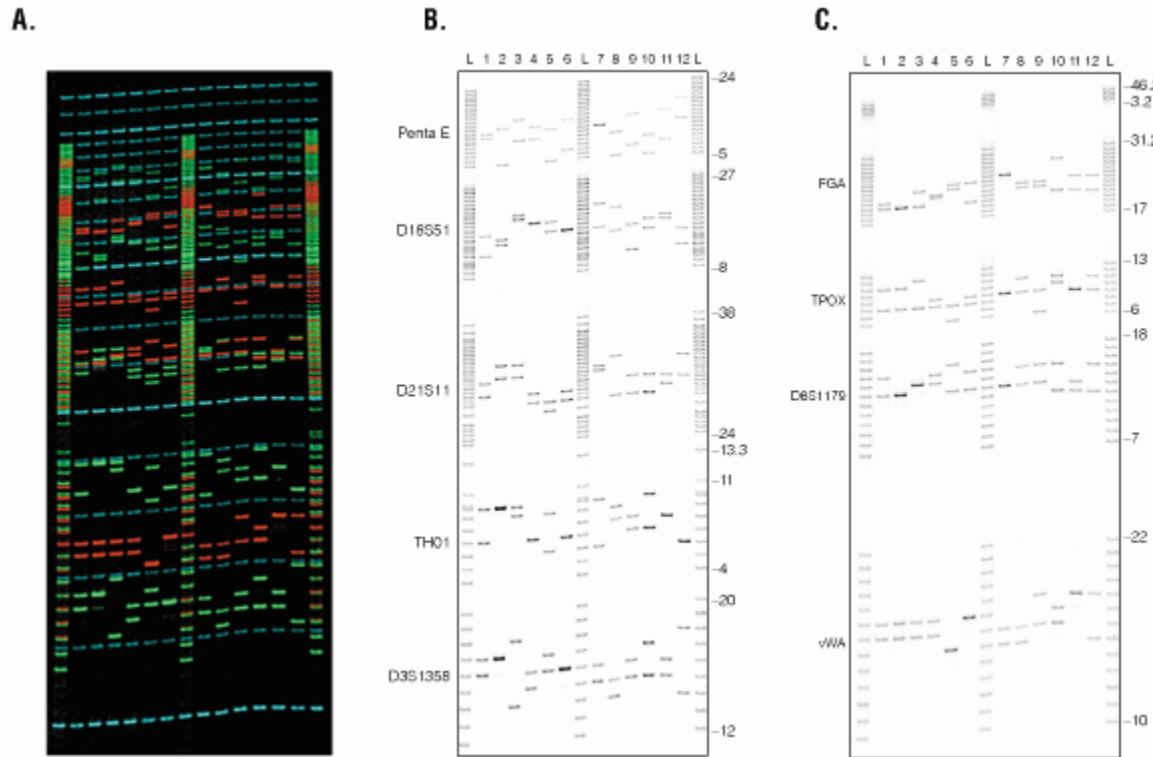
• A5*PROFI...US LADDER 4 Blue PROFILER PLUS LADDER



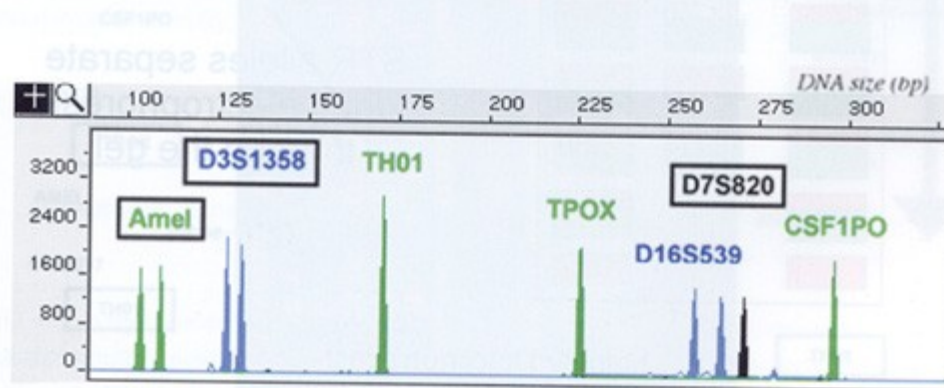
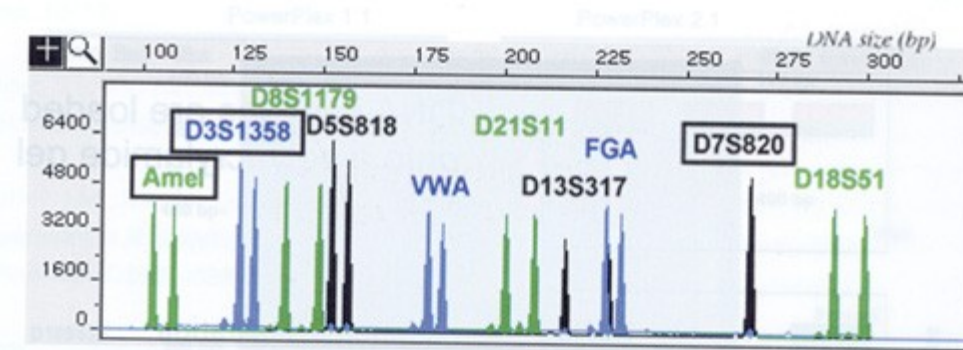
Norma



Short tandem repeats



Short tandem repeats



Testy paternity

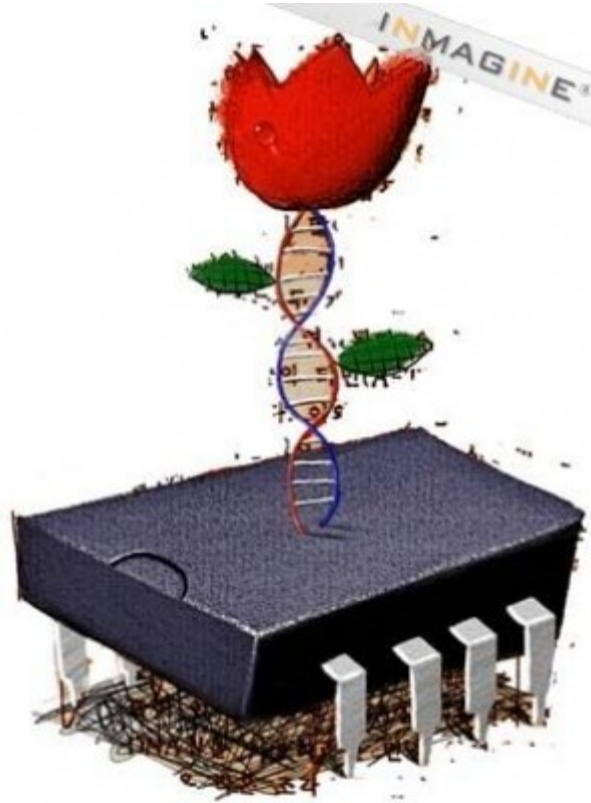
Zjednodušené testy

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

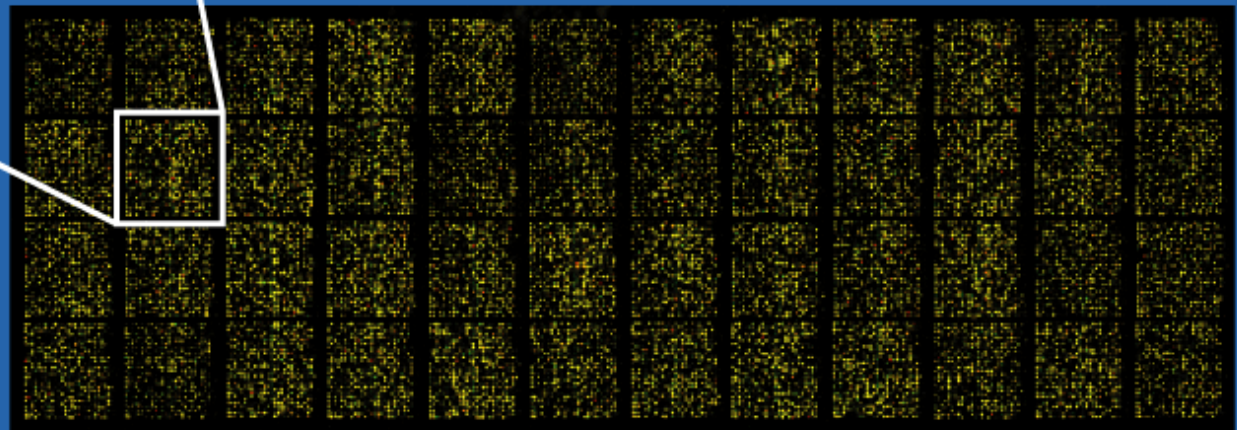
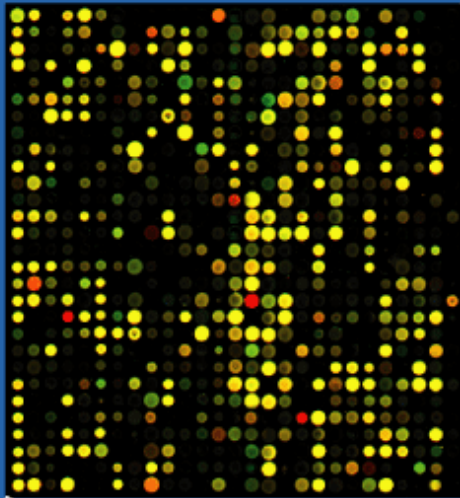
Mitochondriální Eva



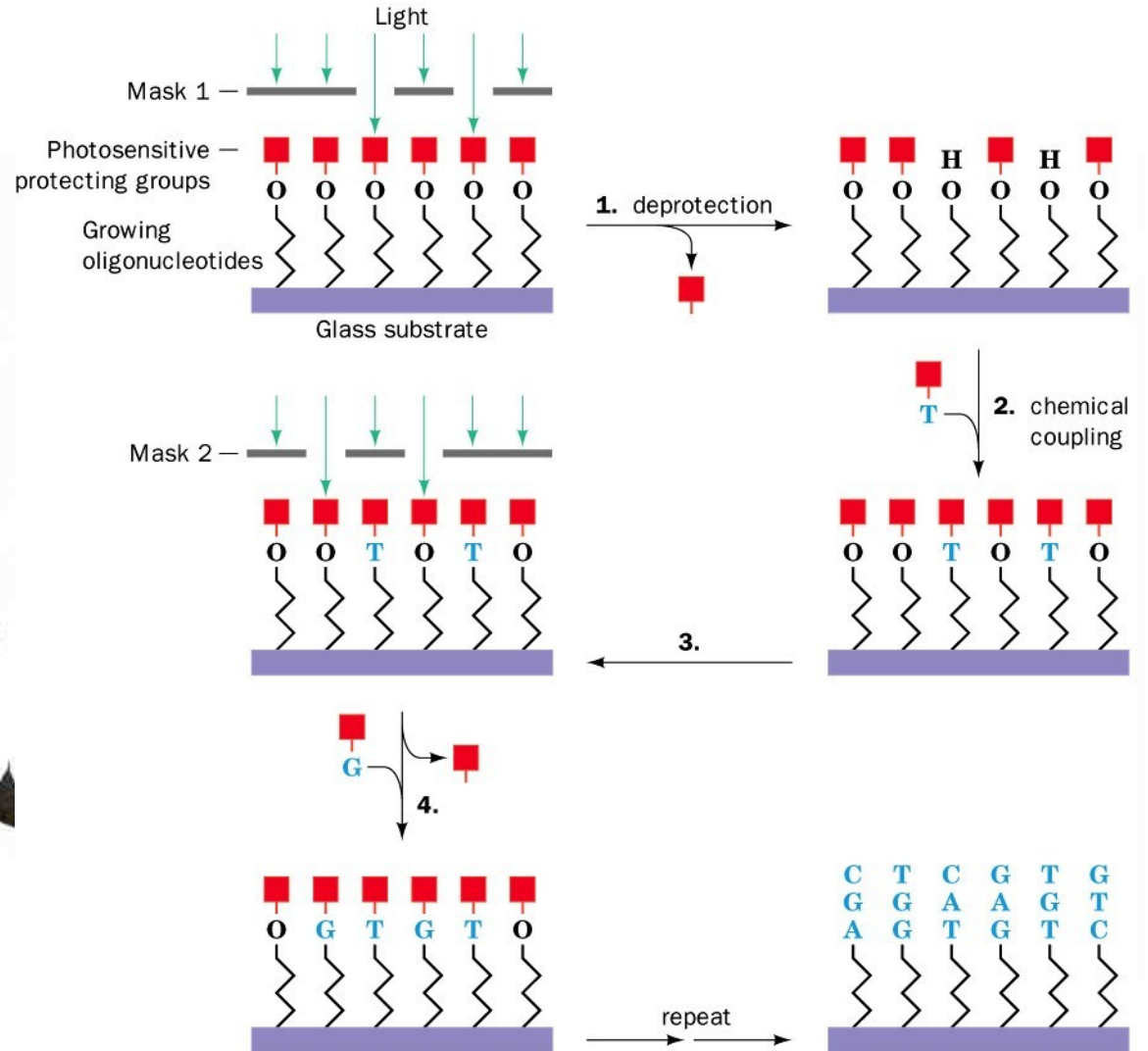
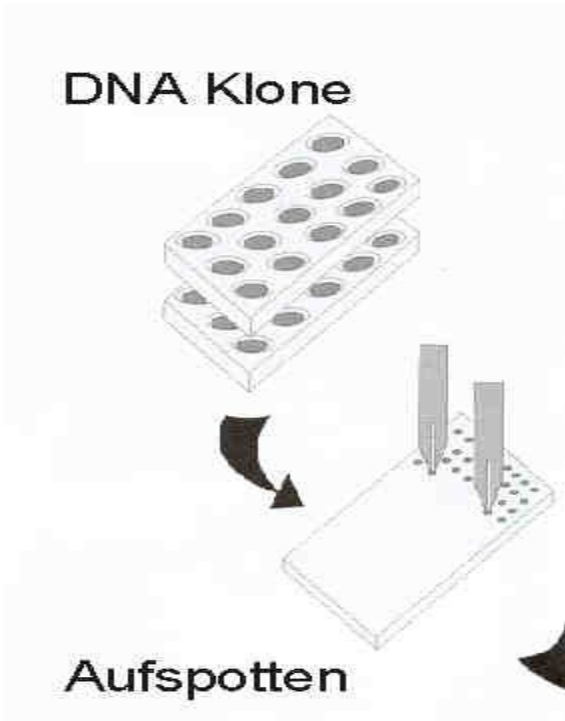
DNA chipy



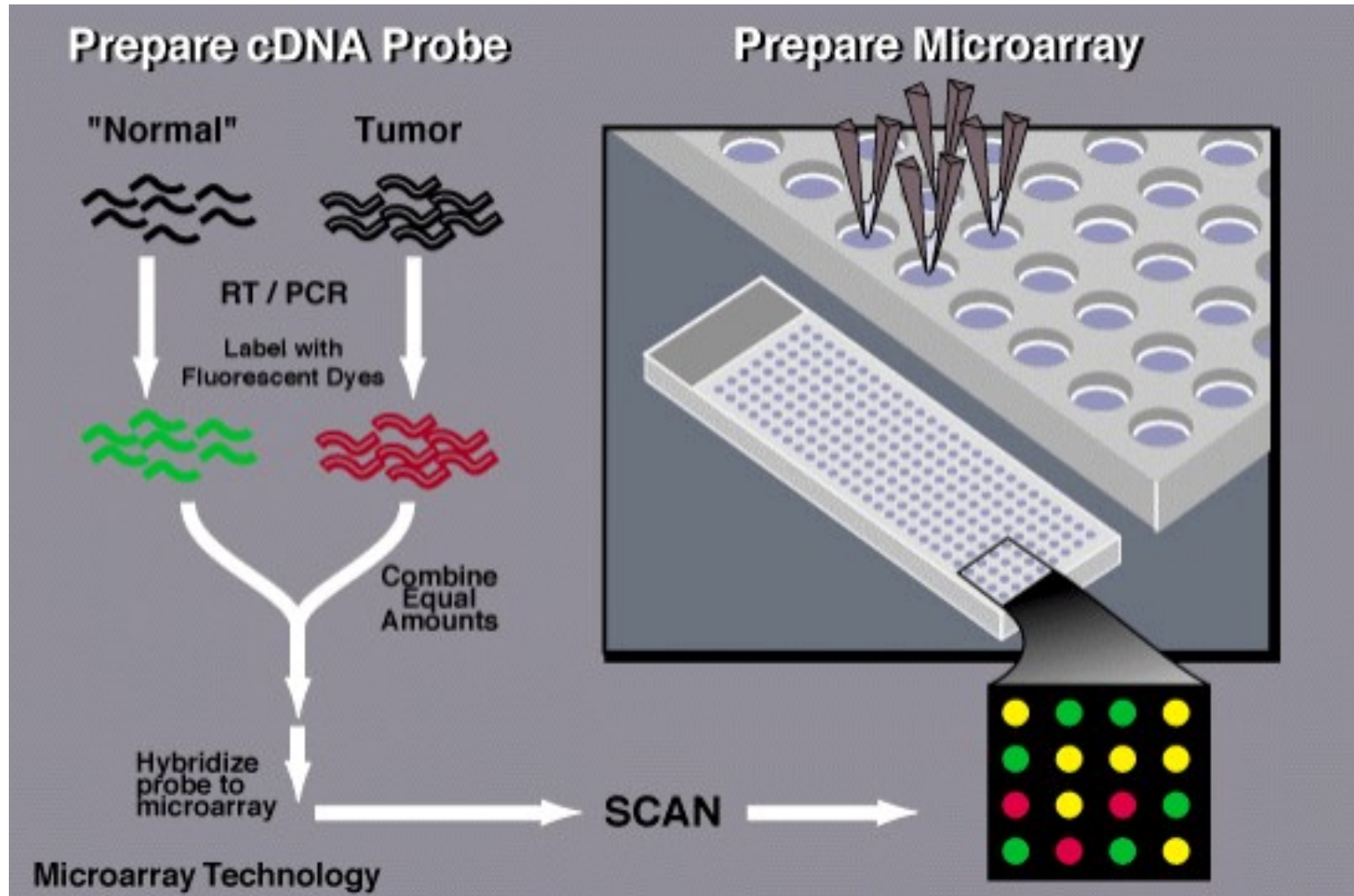
DNA chipy



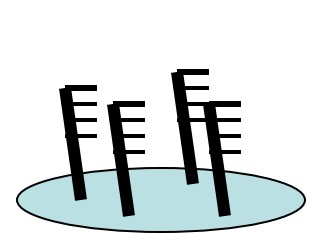
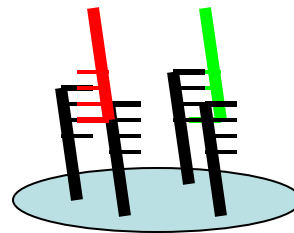
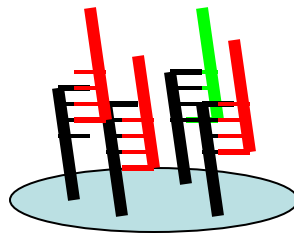
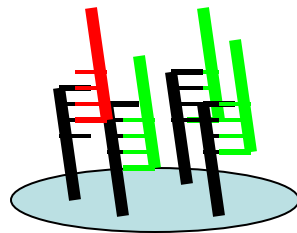
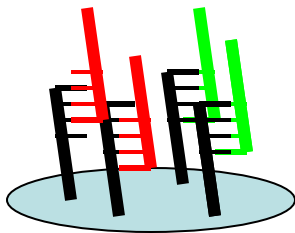
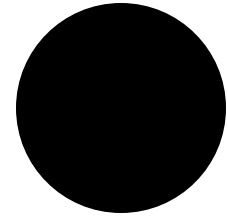
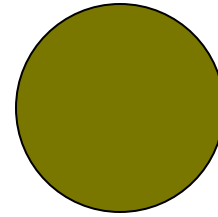
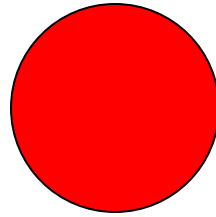
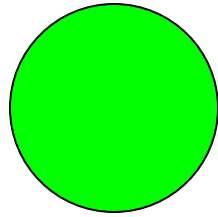
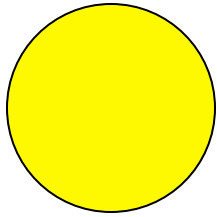
DNA chipy



DNA chipy



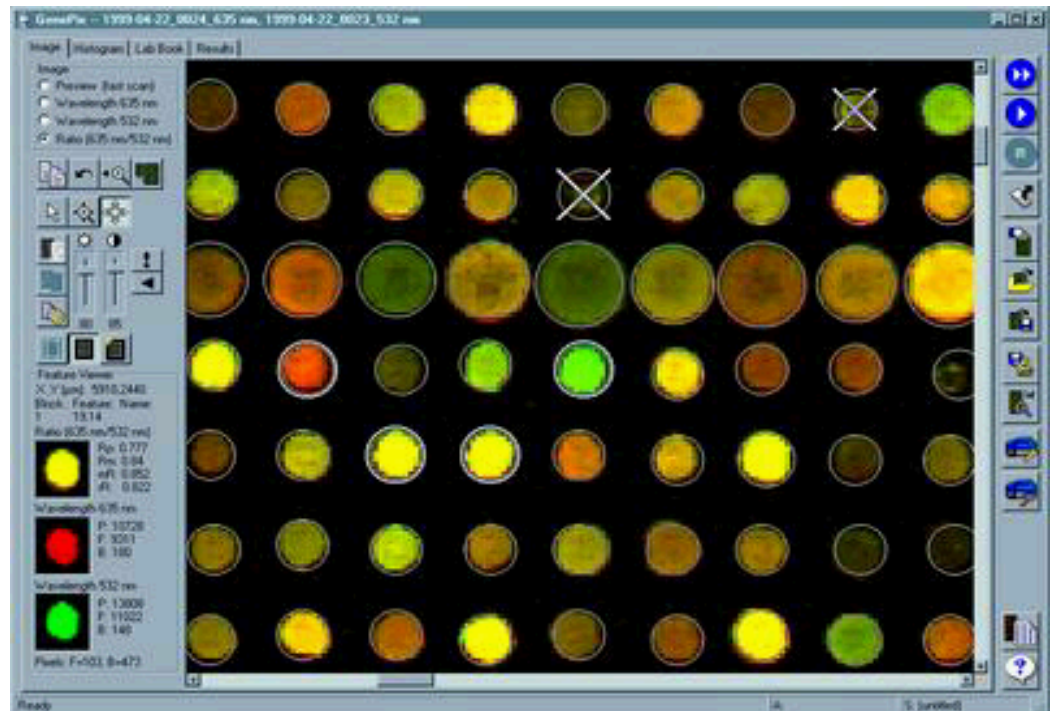
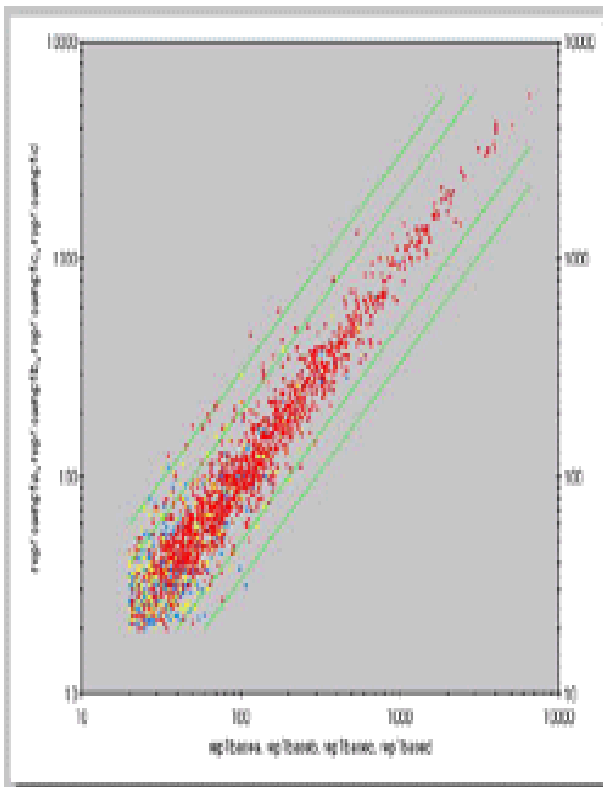
DNA chipy - barva skvrn



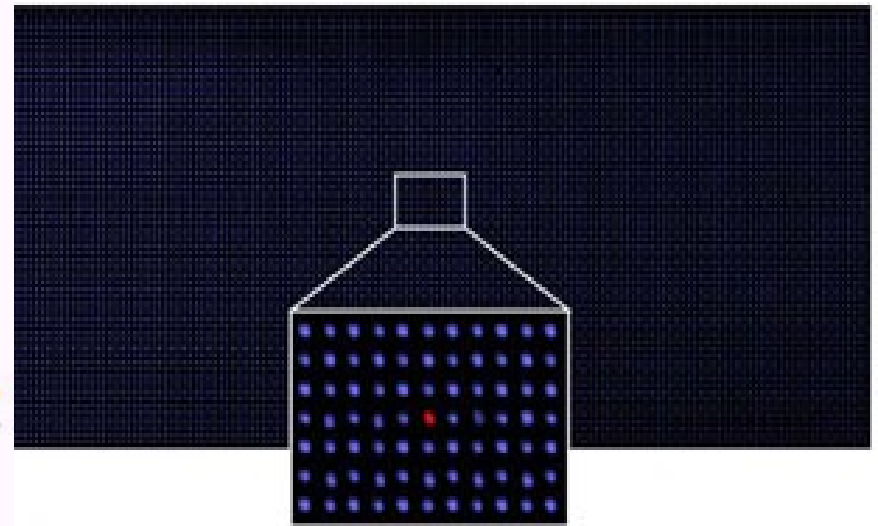
DNA chipy - vybavení



DNA chip software

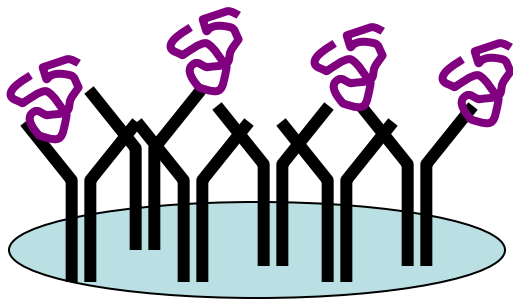


Proteinové chipy

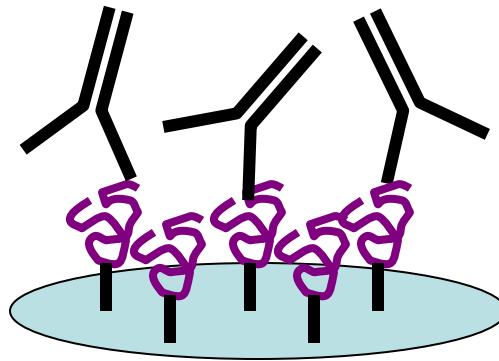


Proteinové chipy – typy interakcí

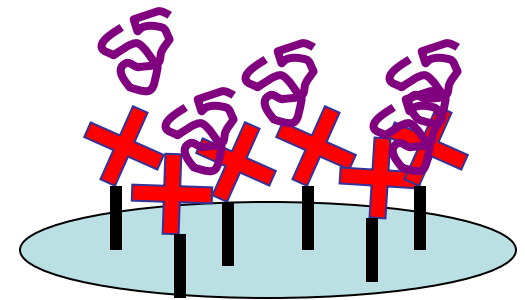
Protilátka



Antigen

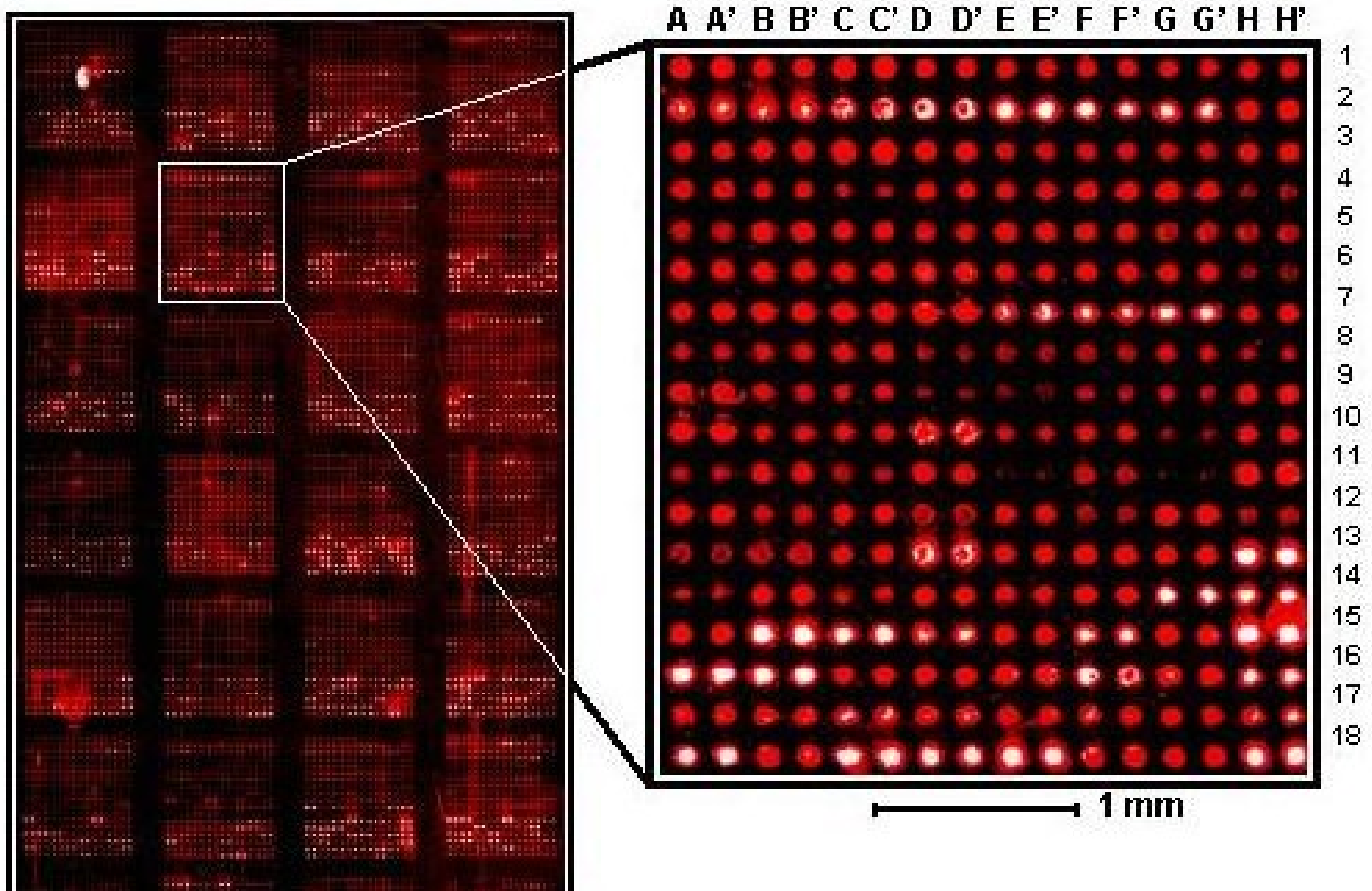


Ligand



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

Anti-GST Probe



Blotting

Side View:

Before Transfer:

(+) electrode



(-) electrode

bands in gel

nitrocellulose sheet



Direction of
Transfer
(electric field)

After Transfer:

(+) electrode



(-) electrode

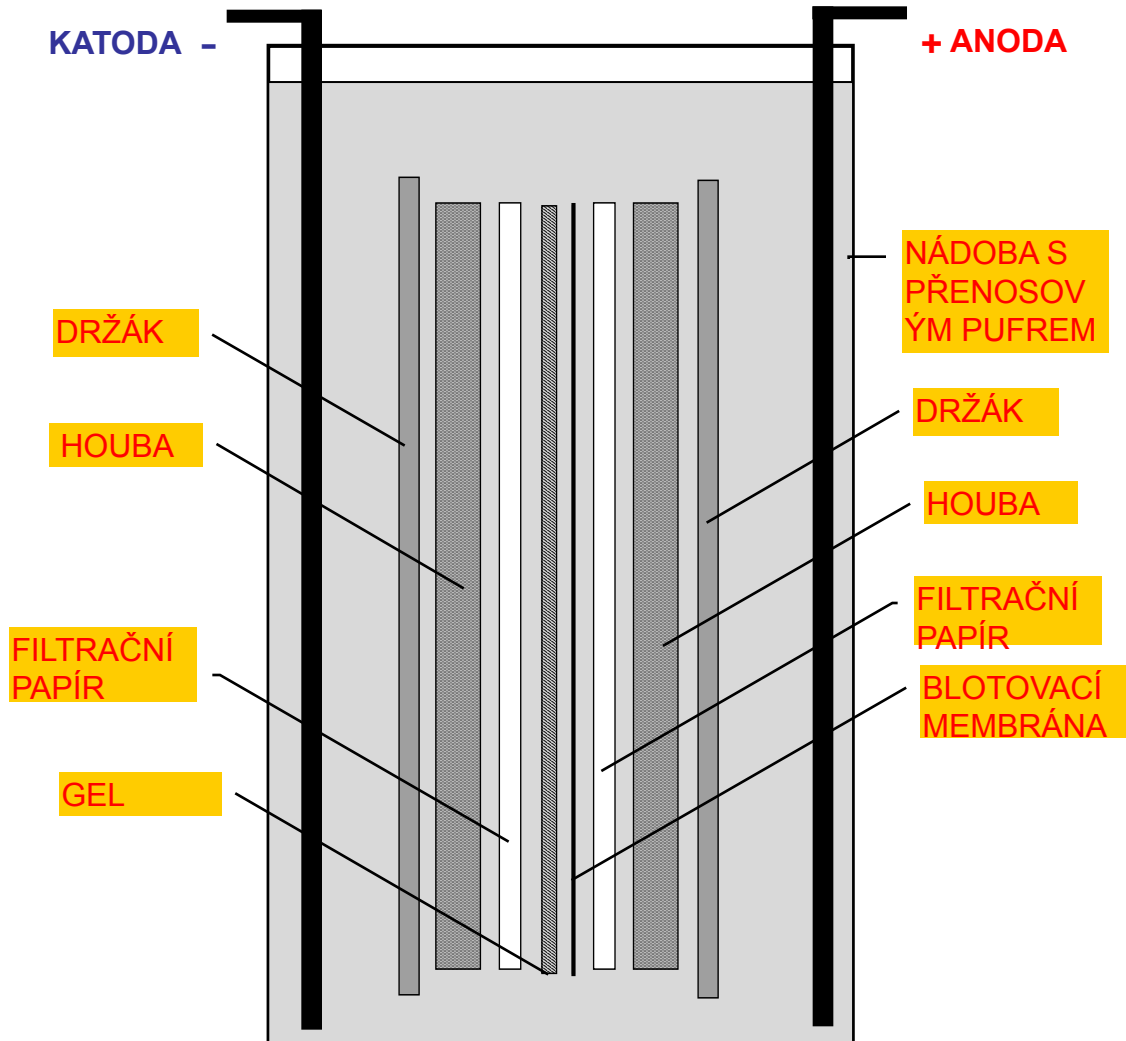
bands on
nitrocellulose

Note: All the layers are pressed tightly together.

Blotting

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

Tankový elektroblotting



Tankový elektroblotting

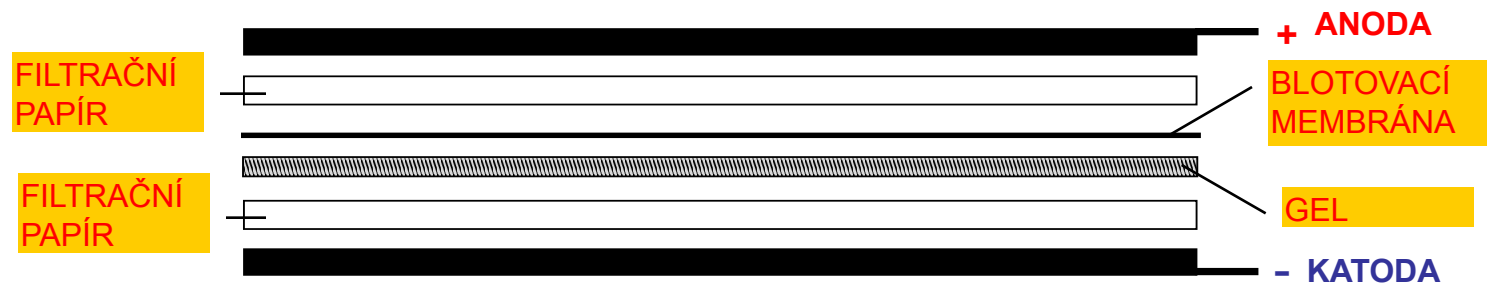


Tankový elektroblotting

Mini Protean Trans Blot Cell



„Semi dry“ blotting



„Semi dry“ blotting



Membrány

- nitrocelulosa - nejběžnější
- polyviniliden difluorid – vysoká vazebná kapacita
- diazobenzoyloxymethyl – chemická aktivace
- ionexové membrány - preparativní
- aktivovaná skleněná vlákna – pro přímou sekvenaci

Detekce

DNA

HYBRIDIZACE

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

Proteiny

REAKCE SE SUBSTRÁTEM nativní enzym, nedifundující substrát

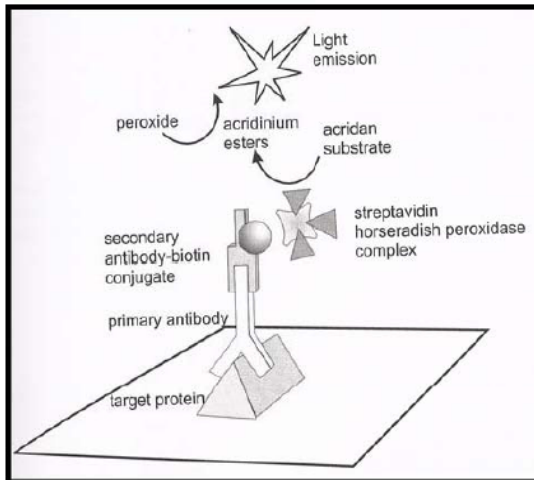
“IMUNOBLOTTING“

^{125}I -protein A

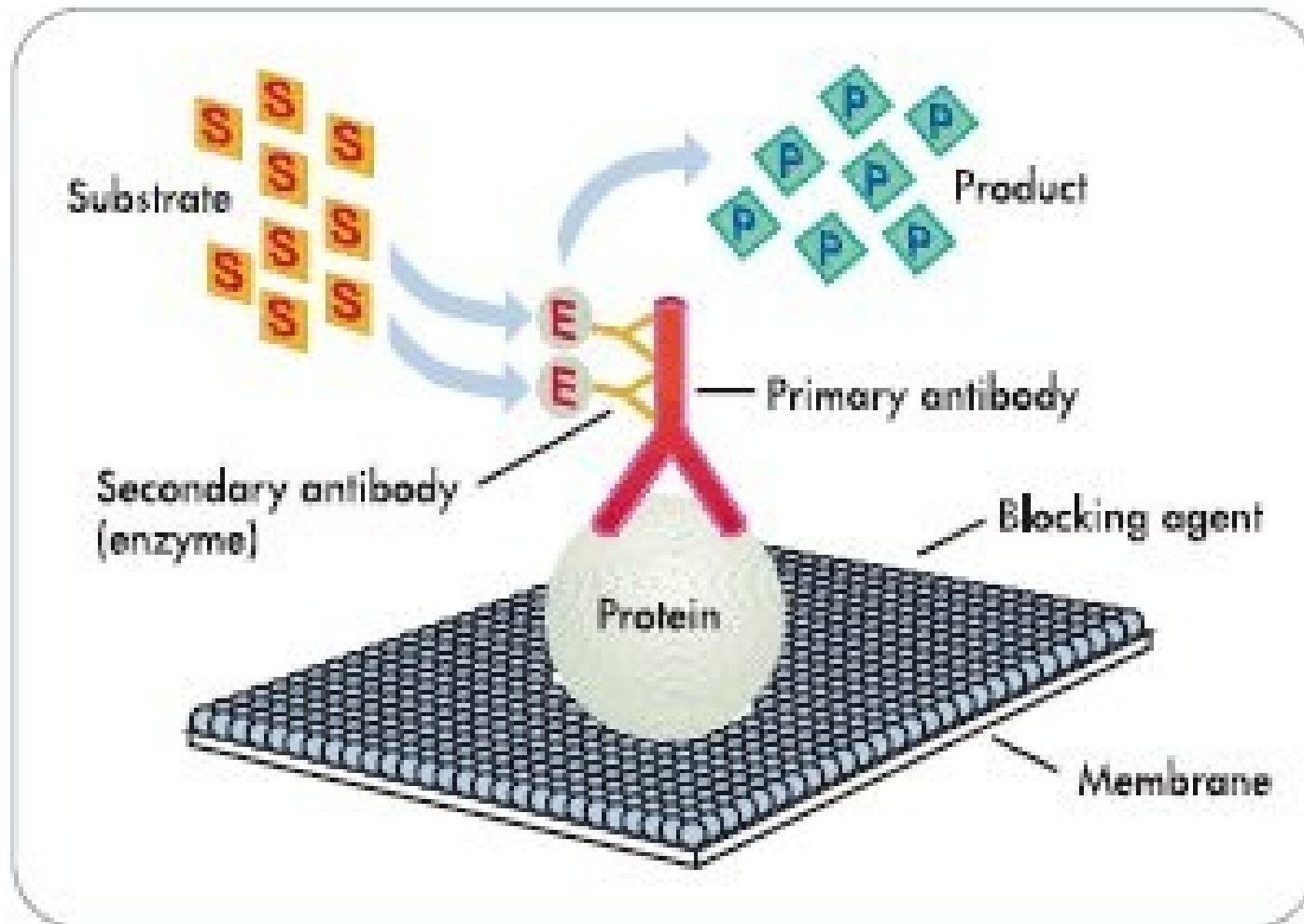
enzymem značená sekundární protilátka – konjugace s peroxidasou (tetrazoliová sůl), **alkalickou** fosfatasou

zlatem značená sekundární protilátka (100 pg)

chemiluminiscence – nejcitlivější



Detekce



Izolace nukleových kyselin

Cíl izolace

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

Typy NK

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

Nejpoužívanější metody

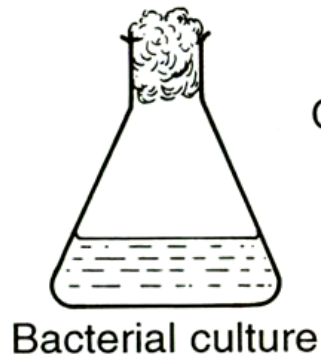
- na základě rozdílné rozpustnosti – extrakce, srážení
- na základě vlastností - chromatografie – polarita-adsorpční, náboj-ionexová
- sedimentace - gradientová ultracentrifugace

Postup

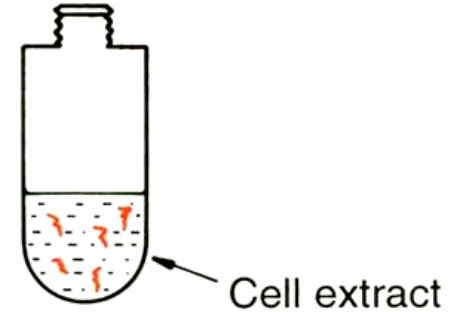
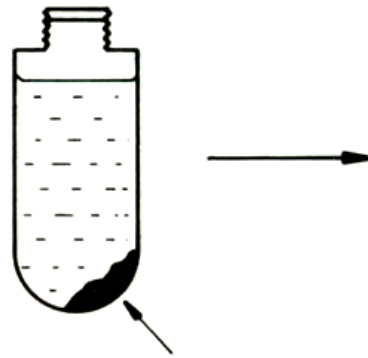
1. Rozbití buněk a membrán pro uvolnění NK
2. Inaktivace DNA- nebo RNA-degradujících enzymů (DNasy, RNasy).
3. Separace NK od dalších komponent uvolněných z buňky.
 - Extrakce/Precipitace
 - Chromatografie
 - Ultracentrifugace

Extrakce/Precipitace

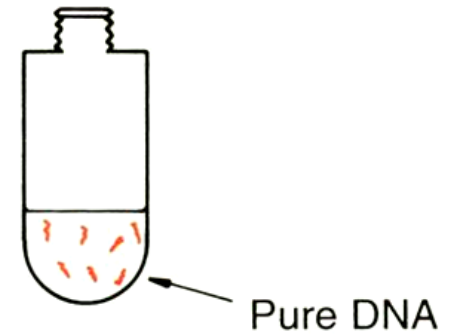
1 A culture of bacteria is grown and then harvested



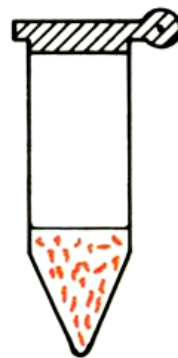
Centrifugation



Organic extraction



4 The DNA is concentrated



3 The DNA is purified from the cell extract

Izolace genomické DNA

Typická procedura

1. Sklizení buněk

2. Lyse buněk

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

3. Fenolová extrakce

- Jemné třepání několik hodin
(pH 8)

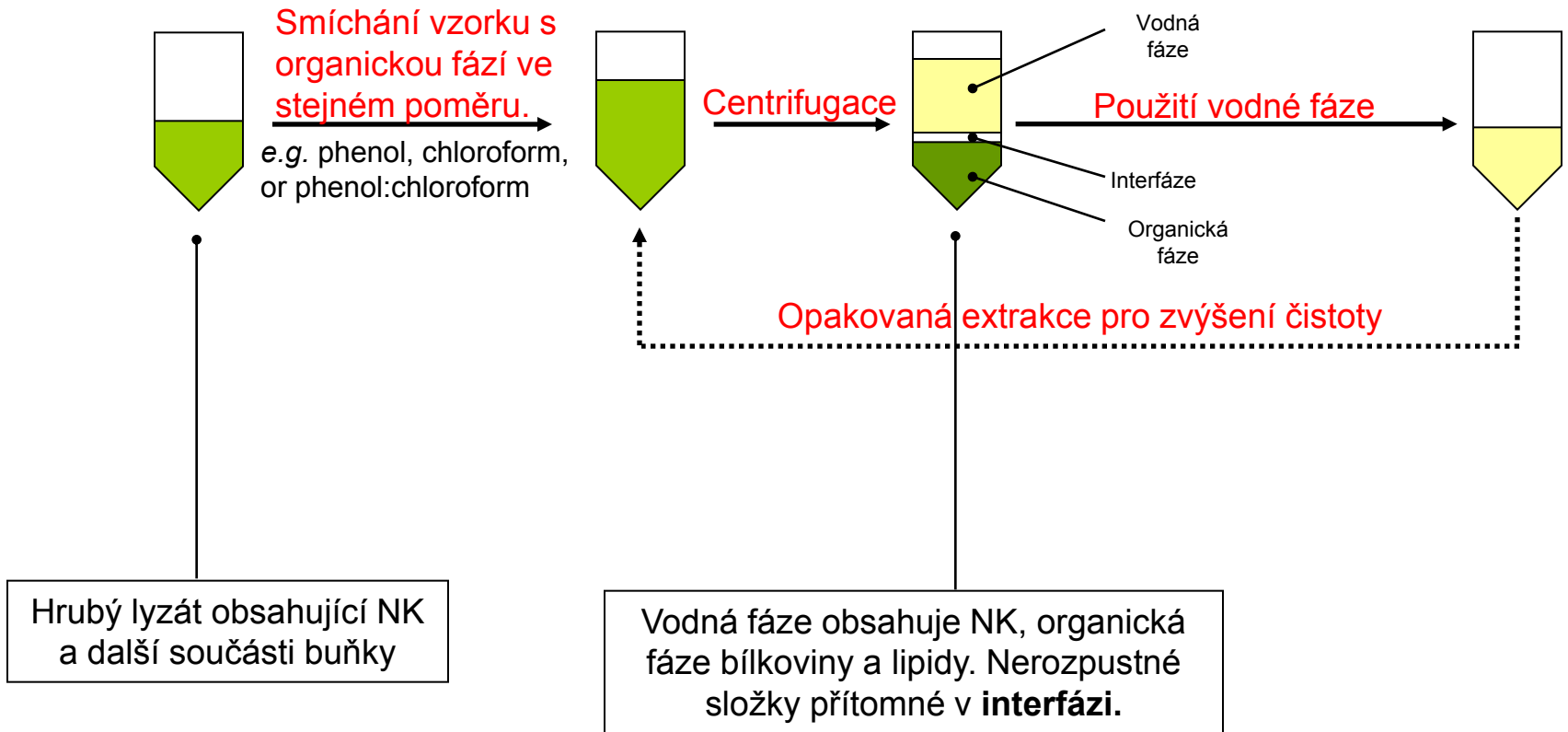
4. Ethanolová precipitace

5. Působení RNAsy a proteinasy K

6. Opakování kroku 3 a 4.

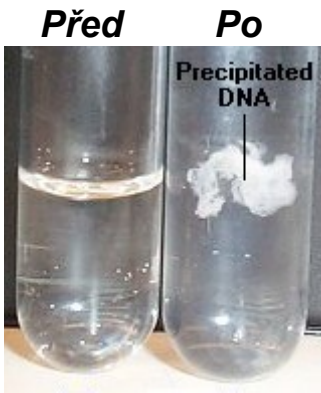
Extrakce/Precipitace

Krok 3: Organická extrakce



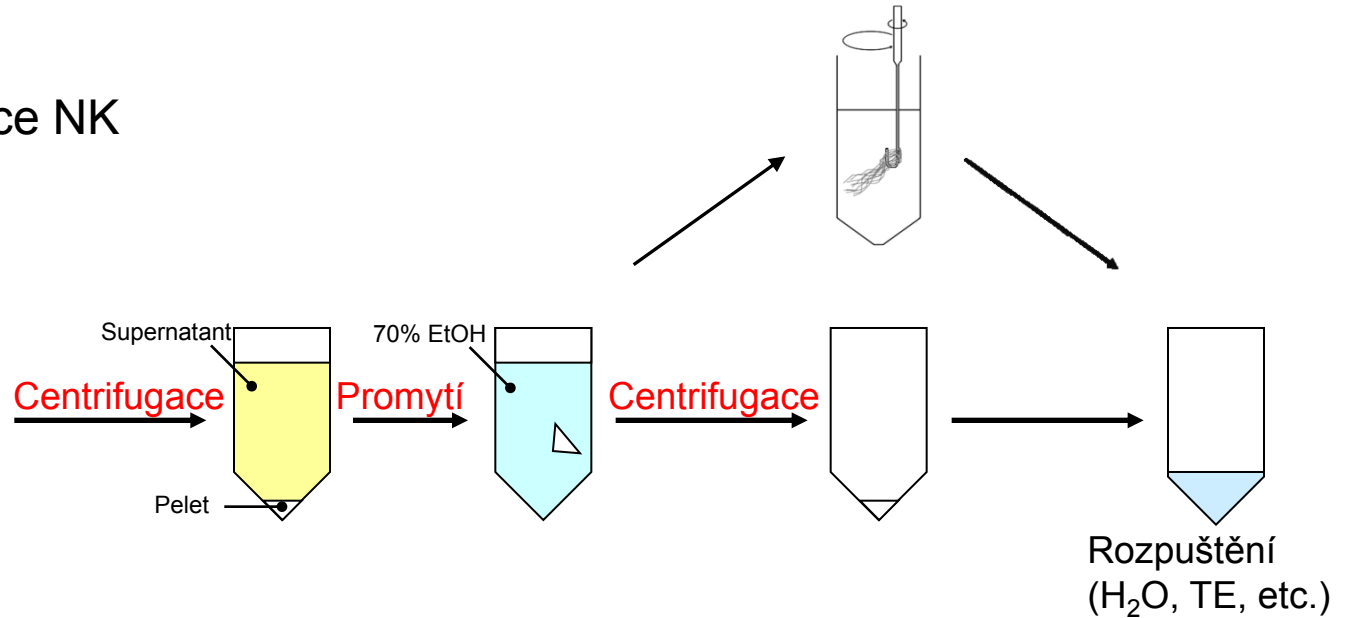
Extrakce/Precipitace

Krok 4: Precipitace NK



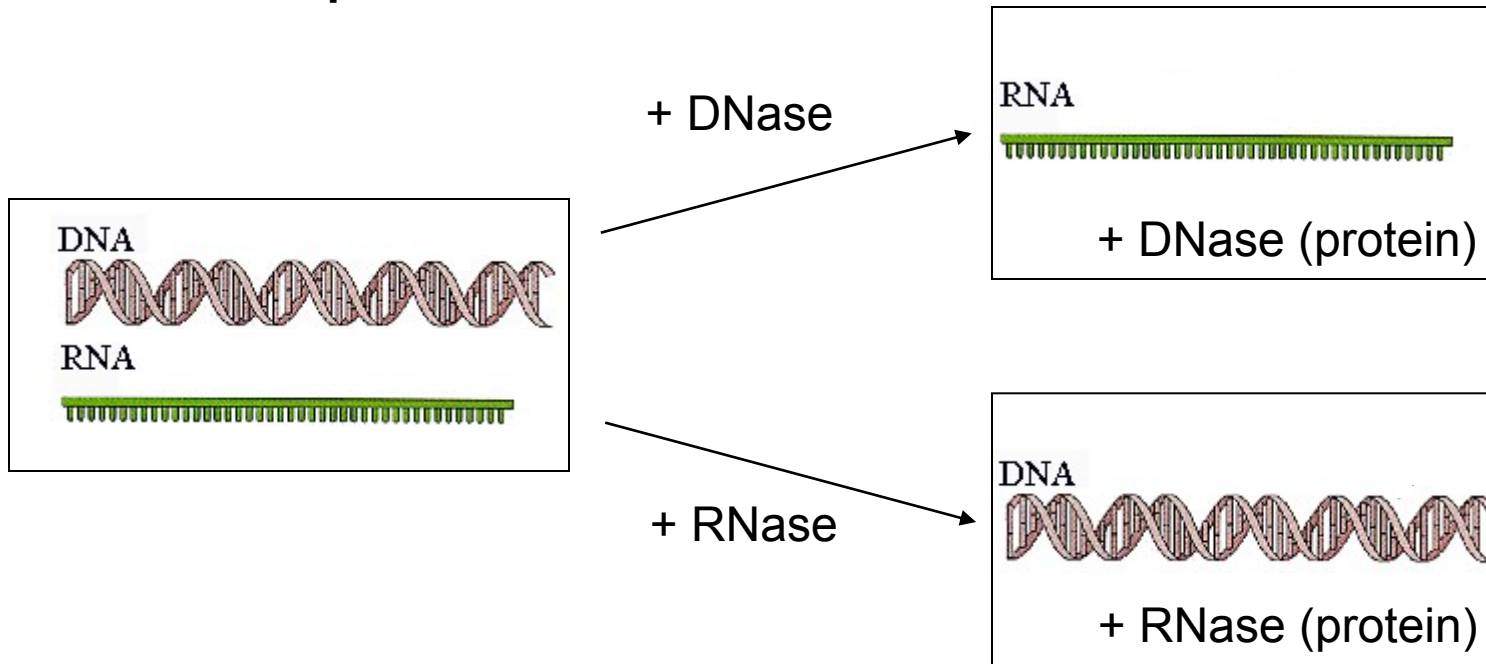
Přidání EtOH a soli

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



Detail kroku 5

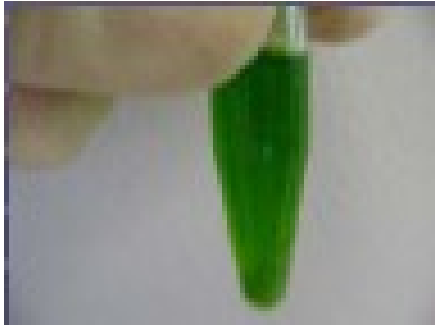
Použití nukleas pro odstranění nechtěné DNA nebo RNA



Chromatografie

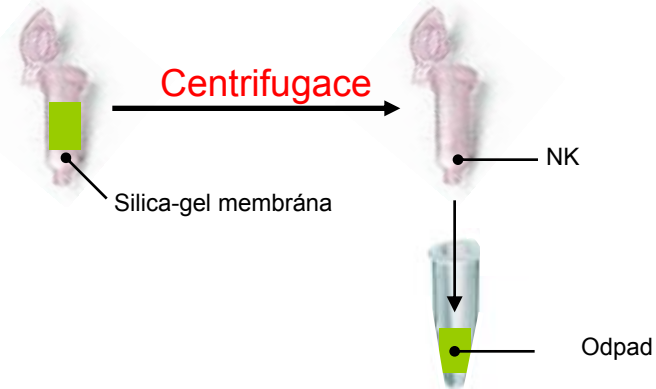
Adsorpční chromatografie

Krok 1: Příprava lyzátu



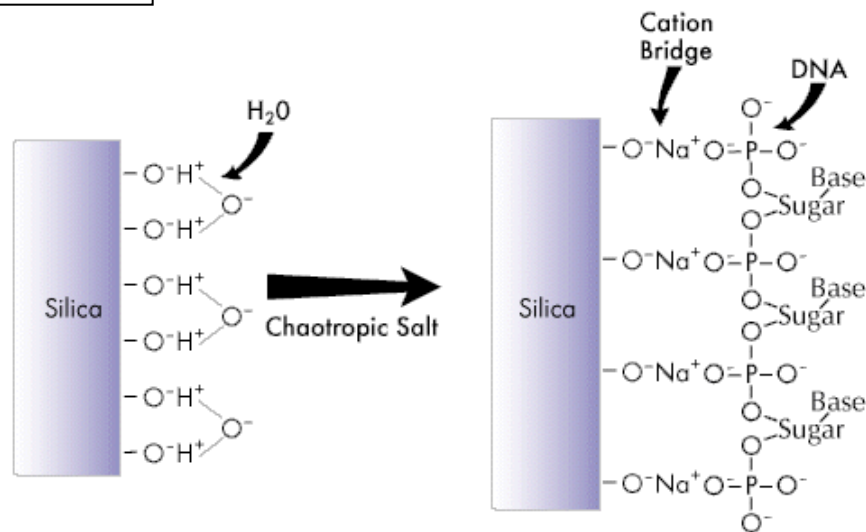
Aplikace na kolonku

Krok 2: Adsorpce na silikagel



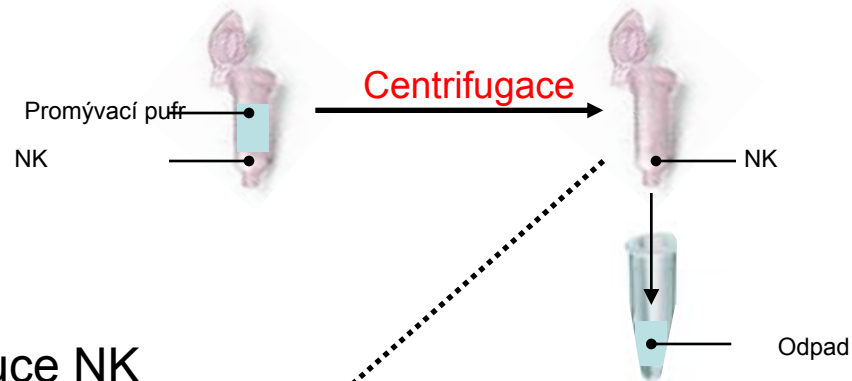
Extrakční pufr pro vazbu DNA a RNA na silikagel:

- nízké pH
- vysoká iontová síla
- chaotropní soli

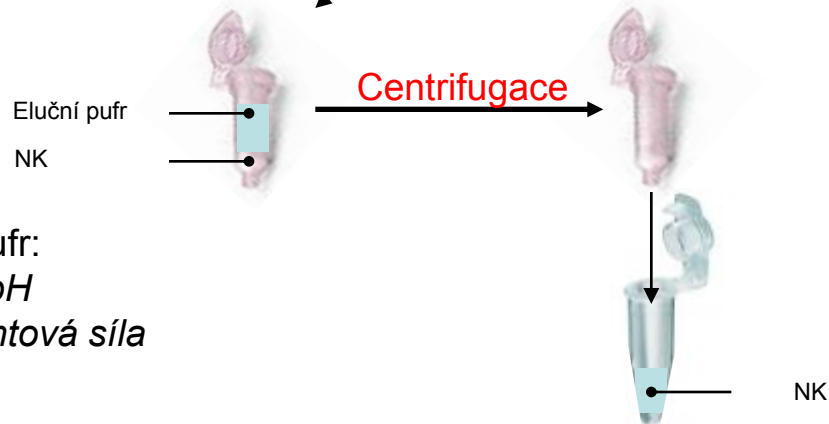


Adsorpční chromatografie

Krok 3: Vymytí kontaminant



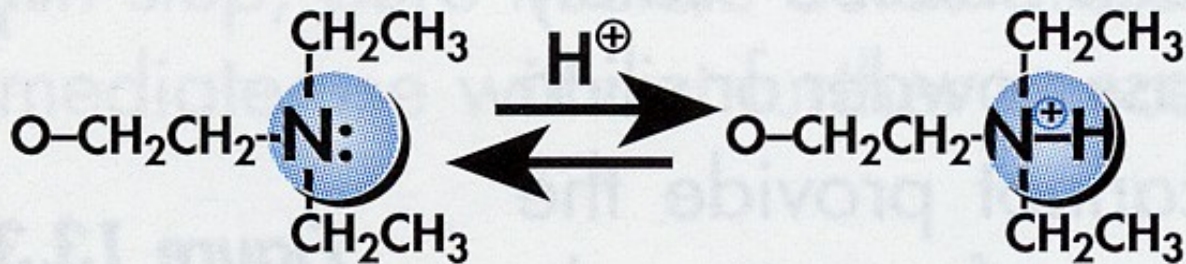
Krok 4: Eluce NK



Eluční pufr:
Vysoké pH
Nízká iontová síla

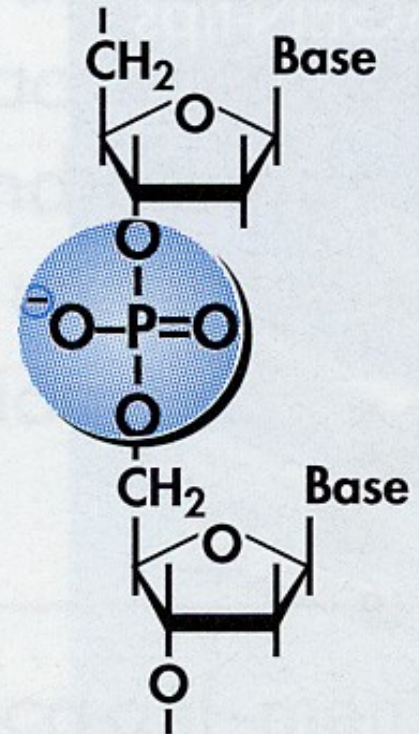
Ionexová chromatografie

Vazba při nízkém pH nízké I



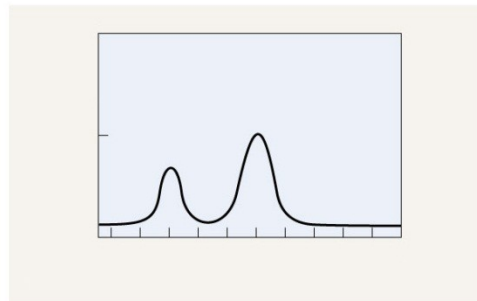
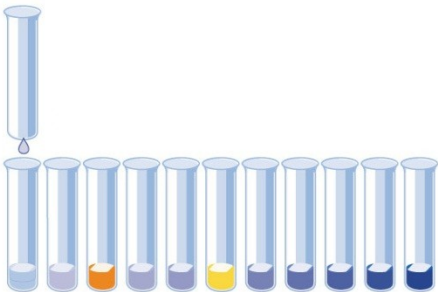
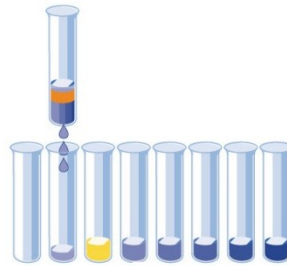
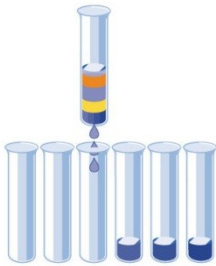
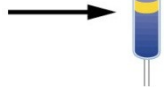
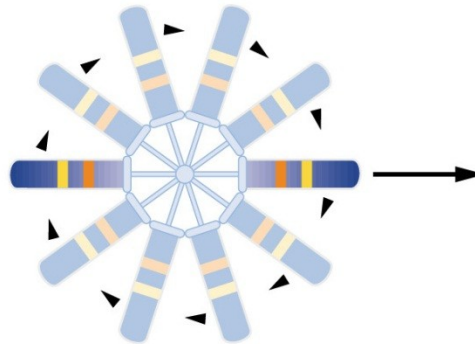
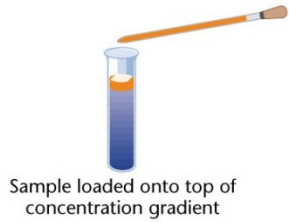
Eluce zvýšením pH nebo vysokou I

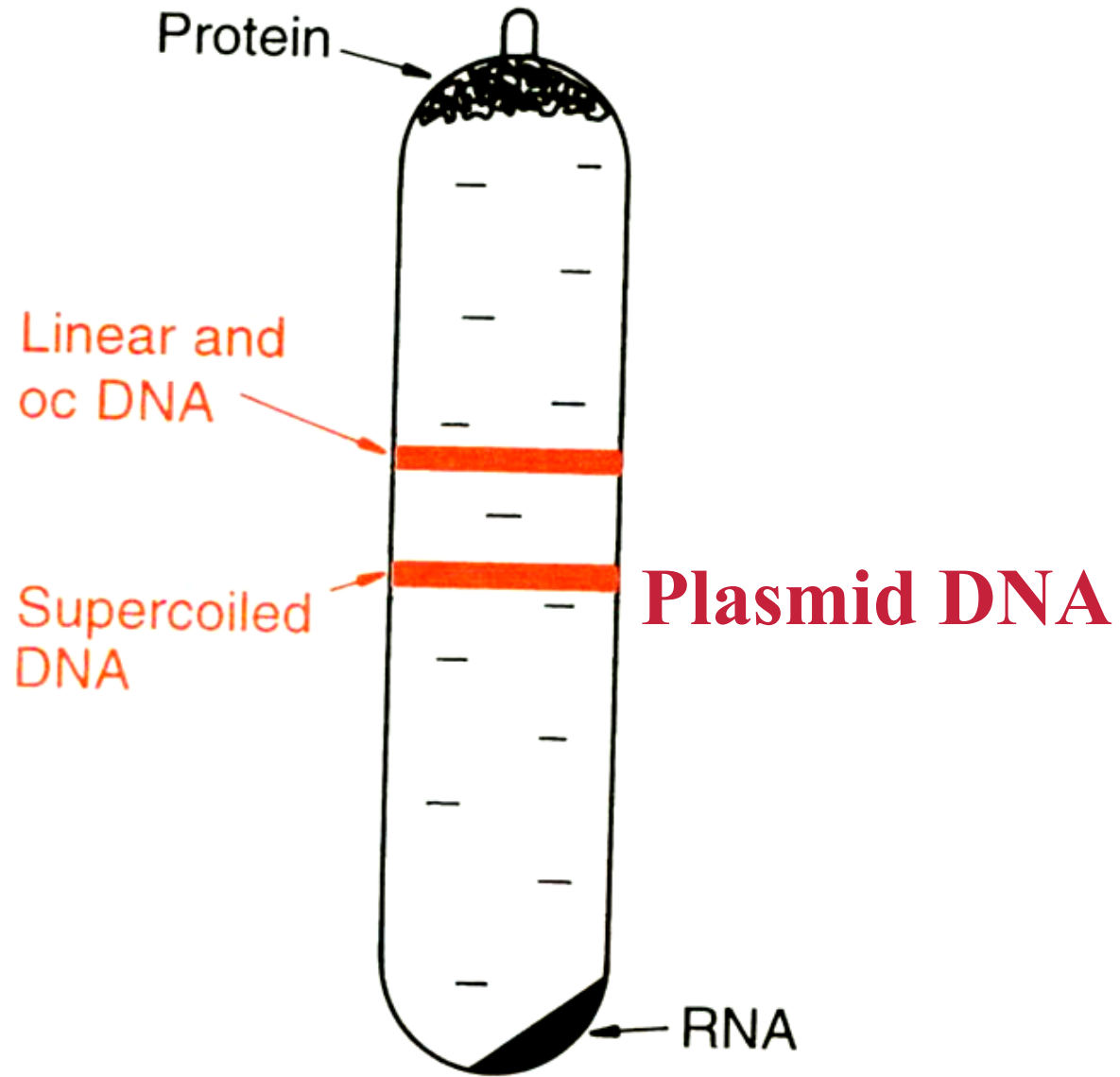
DEAE (diethylaminoethanol)



Chemical structure
of DNA

Separation of Nucleic Acids by CeCl_2 Gradient Centrifugation

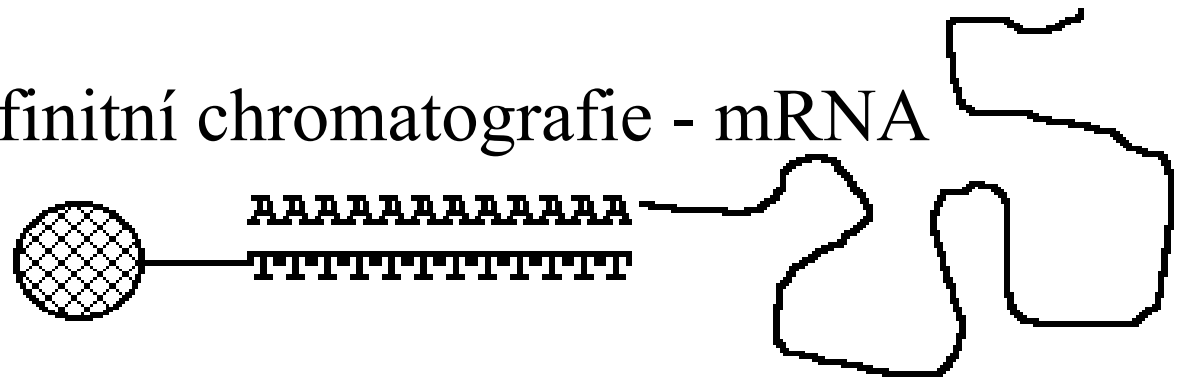




(a) An EtBr-CsCl density gradient

Izolace RNA - speciální přístupy

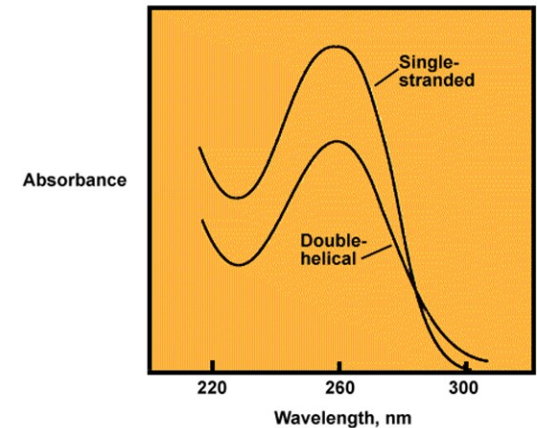
- nutno použít inhibitory RNAsy
- extrakce guanidium chloridem
- fenolová extrakce při $\text{pH} < 4$ ($\text{pH} 8$ pro DNA)
- působení RNase-free Dnase
- selektivní precipitace rRNA, mRNA s LiCl
- oligo-dT afinitní chromatografie - mRNA



Kontrola čistoty a kvantifikace NK

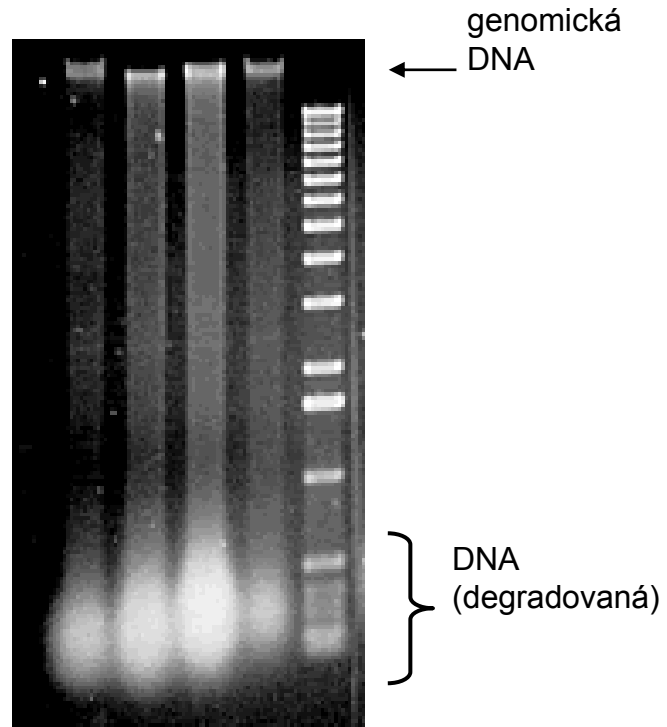
Kontrola NK

- 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

