



# Betekintés a bioanalitikába

Dr. Percze Krisztina, Mészáros Tamás

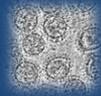
Molekuláris Biológiai Tanszék, Semmelweis Egyetem

# Biológiai eredetű anyagok azonosítása

Vírusok

Mikrobák

morfológia, fenotípus  
fertőző képesség  
nukleinsav, fehérje



Nukleinsavak

közvetlenül

Fehérjék

közvetlenül

enzim aktivitáson keresztül

Anyagcseretermékek

direkt módon

enzim aktivitáson keresztül

# A nukleinsavak kémiai tulajdonságai hasonlók

### 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.

### PHOSPHATES

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

The phosphate makes a nucleotide negatively charged.

### BASIC SUGAR LINKAGE

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

### BASES

The bases are nitrogen-containing ring compounds, either pyrimidines or purines.

Uracil, Cytosine, Thymine are **PYRIMIDINE**. Adenine, Guanine are **PURINE**.

### SUGARS

**PENTOSE**  
a five-carbon sugar

two kinds are used

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

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

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

### NOMENCLATURE

A nucleoside or nucleotide is named according to its nitrogenous base.

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

Single letter abbreviations are used variously as shorthand for (1) the base alone, (2) the nucleoside, or (3) the whole nucleotide—the context will usually make clear which of the three entities is meant. When the context is not sufficient, we will add the terms "base", "nucleoside", "nucleotide", or—as in the examples below—use the full 3-letter nucleotide code.

AMP = adenosine monophosphate  
dAMP = deoxyadenosine monophosphate  
UDP = uridine diphosphate  
ATP = adenosine triphosphate

BASE + SUGAR = NUCLEOSIDE

BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

### NUCLEIC ACIDS

Nucleotides are joined together by a phosphodiester linkage between 5' and 3' carbon atoms to form nucleic acids. The linear sequence of nucleotides in a nucleic acid chain is commonly abbreviated by a one-letter code, A-G-C-T-T-A-C-A, with the 5' end of the chain at the left.

example: **DNA**

### NUCLEOTIDES HAVE MANY OTHER FUNCTIONS

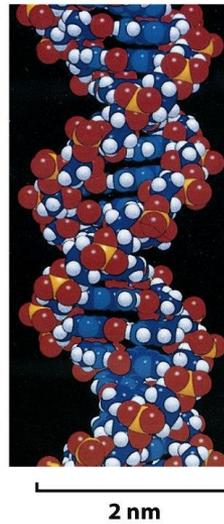
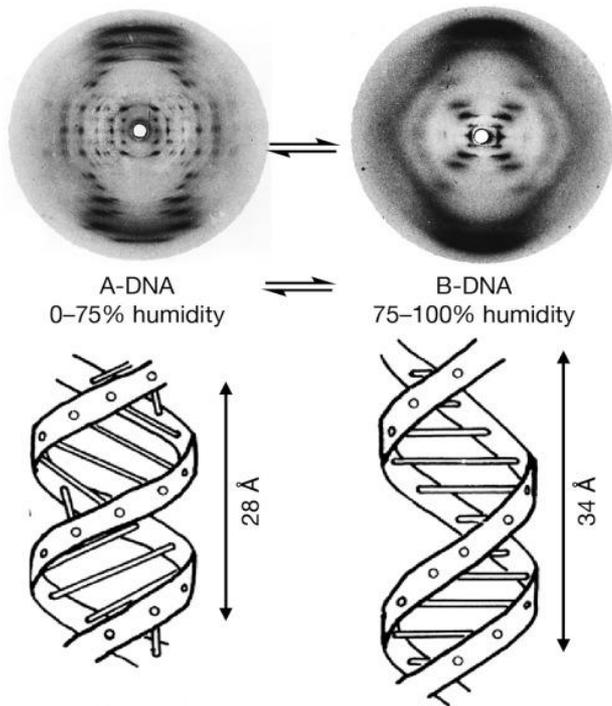
- They carry chemical energy in their easily hydrolyzed phosphoanhydride bonds.
 

example: **ATP** (or **ATP**)
- They combine with other groups to form coenzymes.
 

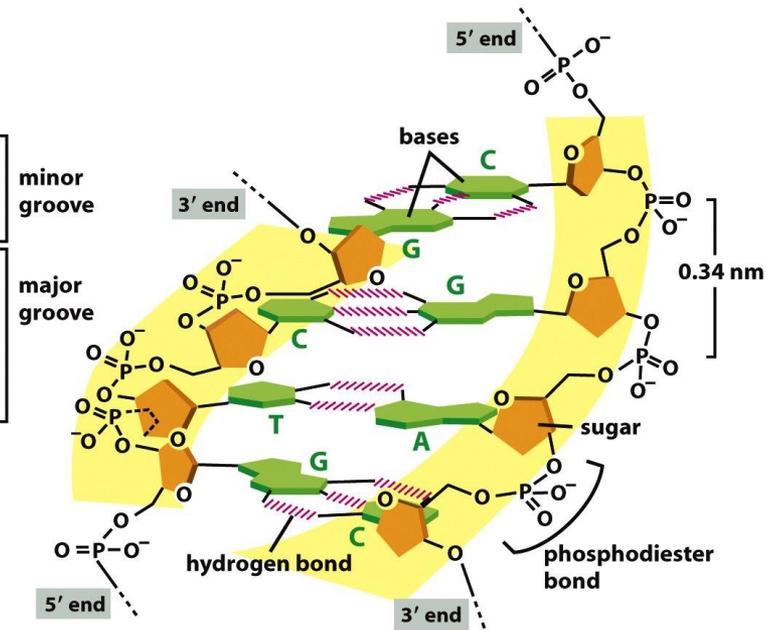
example: **coenzyme A (CoA)**
- They are used as specific signaling molecules in the cell.
 

example: **cyclic AMP (cAMP)**

# A nukleinsavak komplementer bázispárokot képeznek



(A)



(B)

Figure 4-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

# A nukleinsav azonosítás alapvető metodikája a hibridizáció

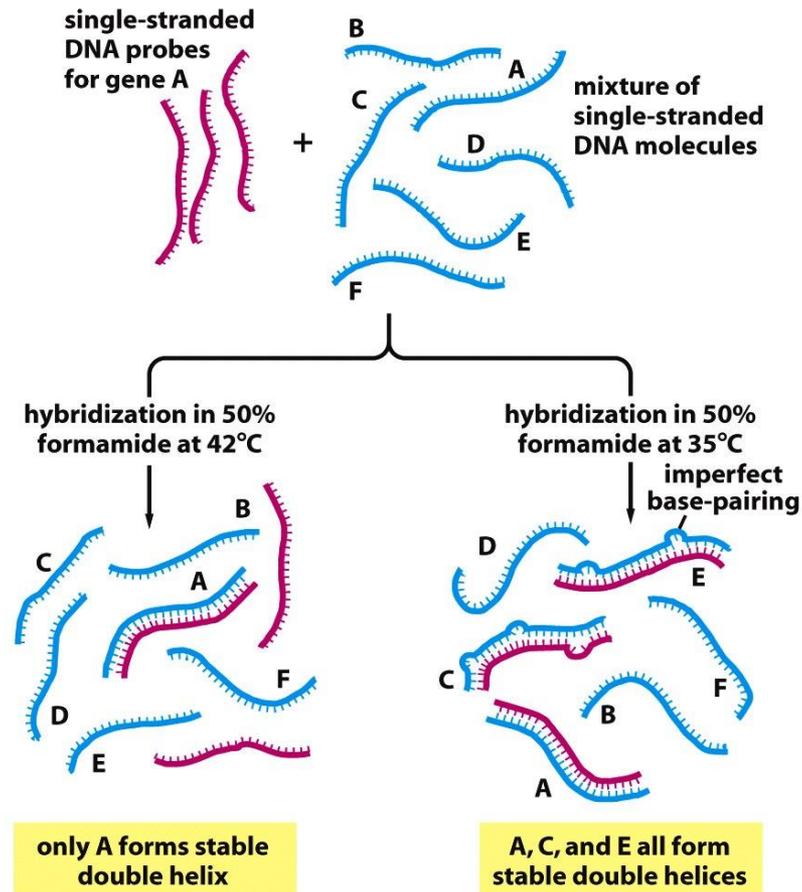
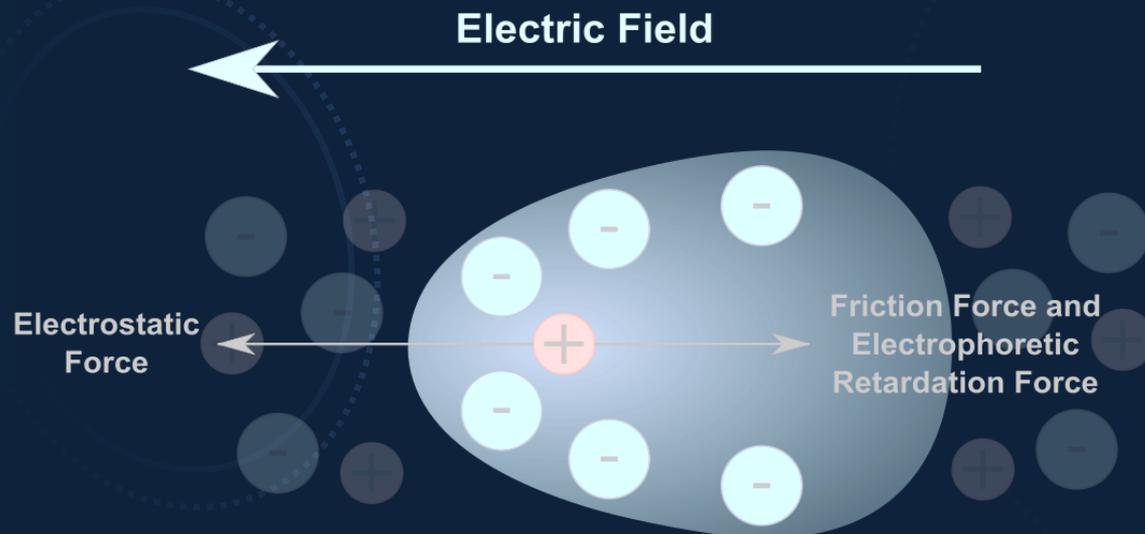


Figure 8-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

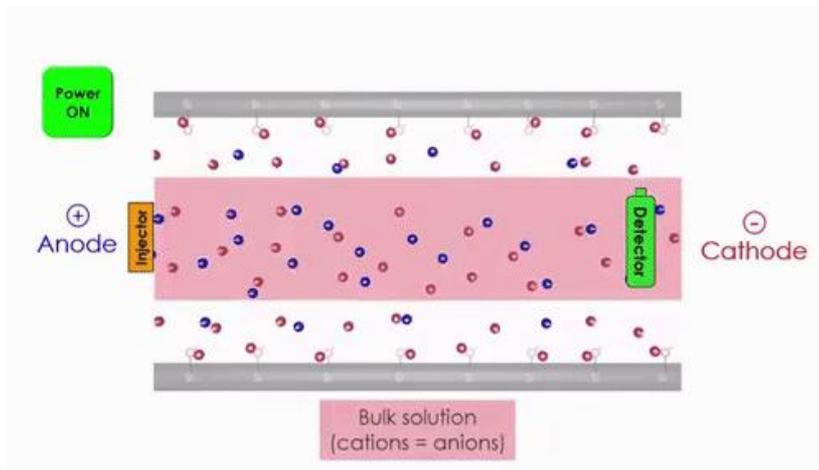
# Elektroforézis

- Elektroforézis során töltéssel rendelkező molekulák (fehérjék, nukleinsavak, szénhidrátok) elválasztása történik elektromos térerősség hatására
- Az elektroforetikus mobilitásuk (EM) a méretüktől, alakjuktól és töltésüktől függ



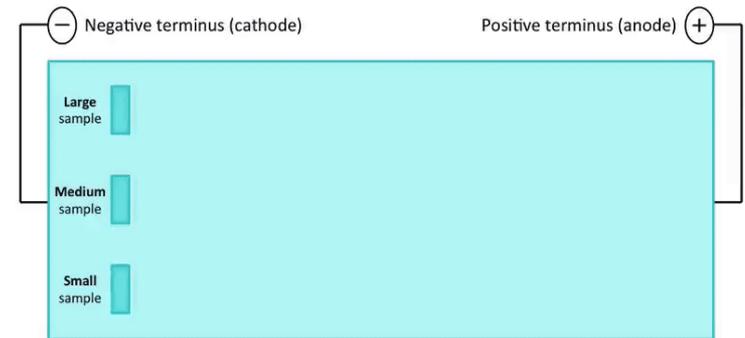
# Elektroforézis típusai

## Szabad elektroforézis (pl. kapillárisban)



- EM egyenesen arányos a töltéssel és fordítottan arányos a mérettel

## Gélelektroforézis



- A gél molekuláris szűrőként funkcionál
- Az elválasztás csak a molekulák méretétől függ
- Gél típusok:
  - agaróz (RNS/DNS)
  - akrilamid (protein)

# Southern és Northern blot analízis

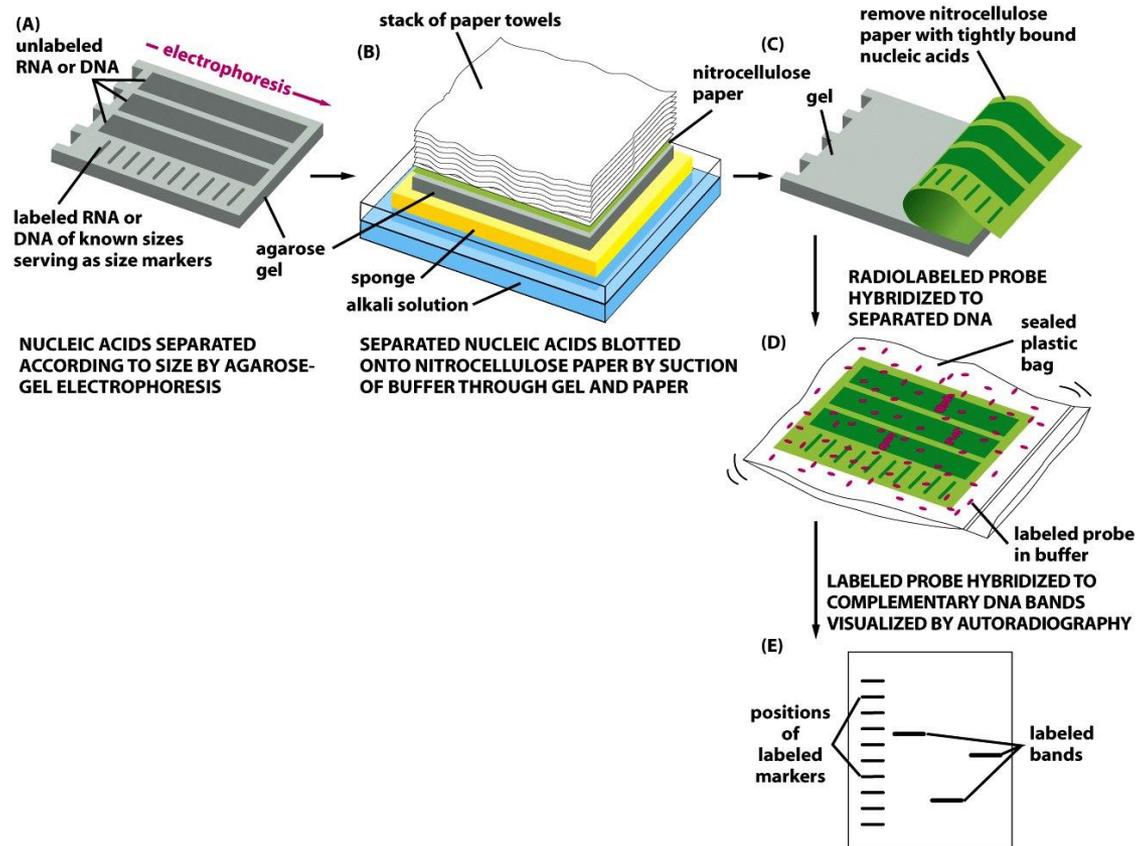
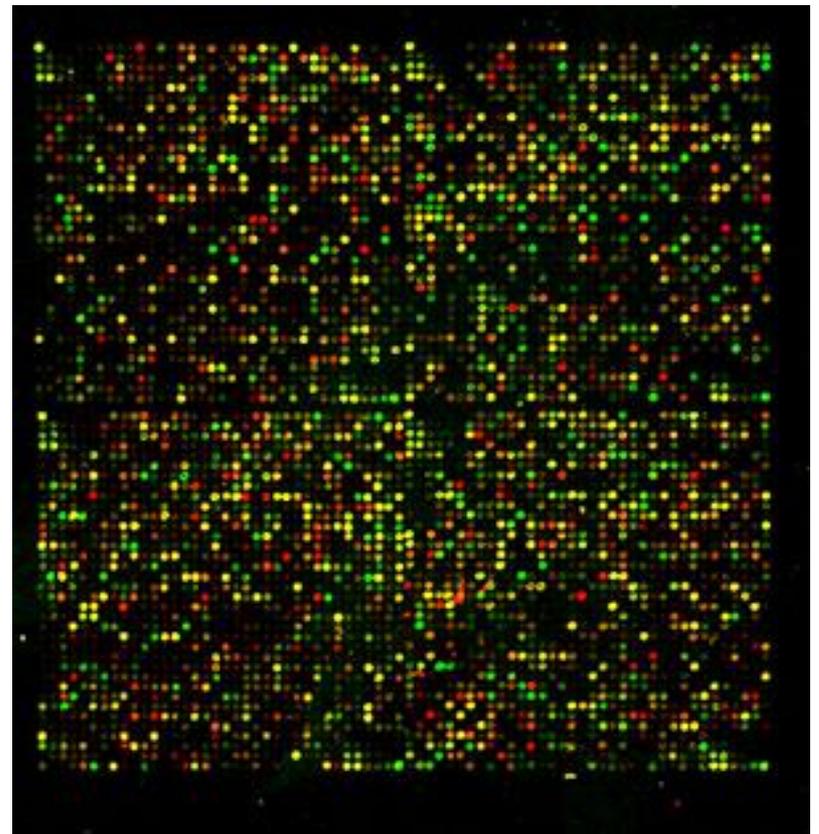
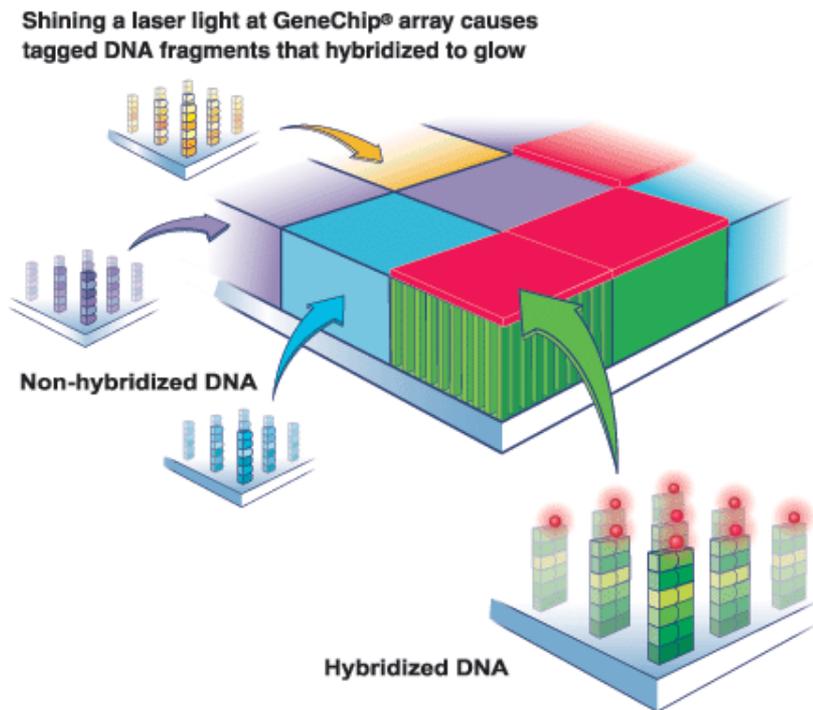
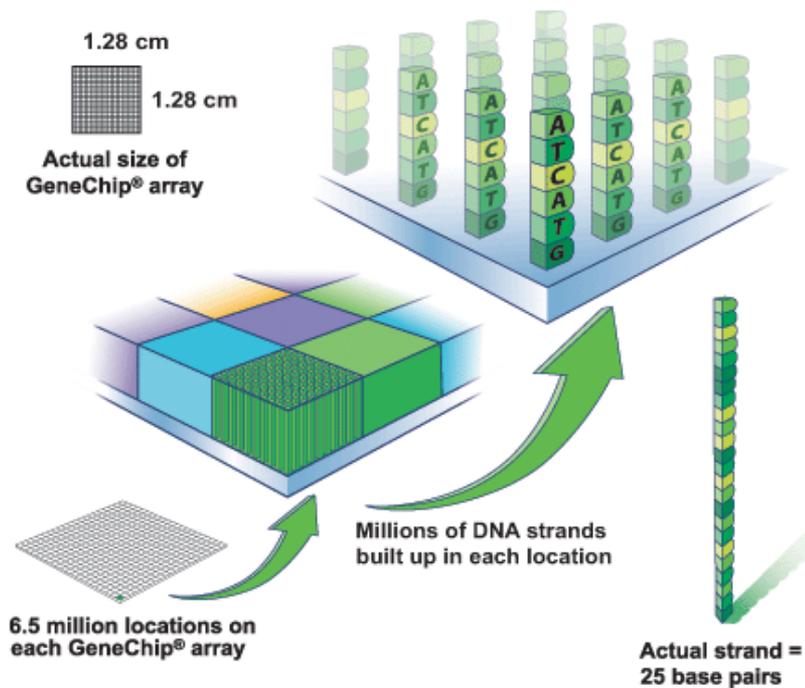


Figure 8-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

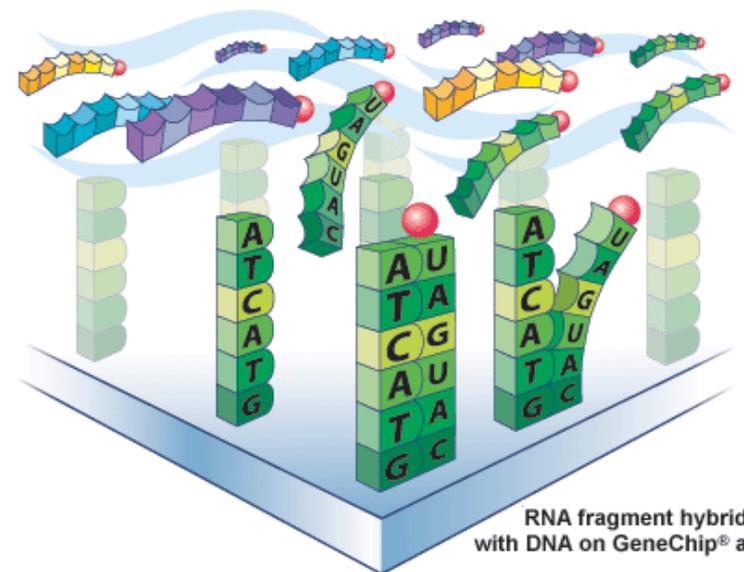
# Hibridizáció nagyléptékben, a microarray



# Hibridizáció nagyléptékben, a microarray



RNA fragments with fluorescent tags from sample to be tested



# Nukleinsavak sokszorosítása

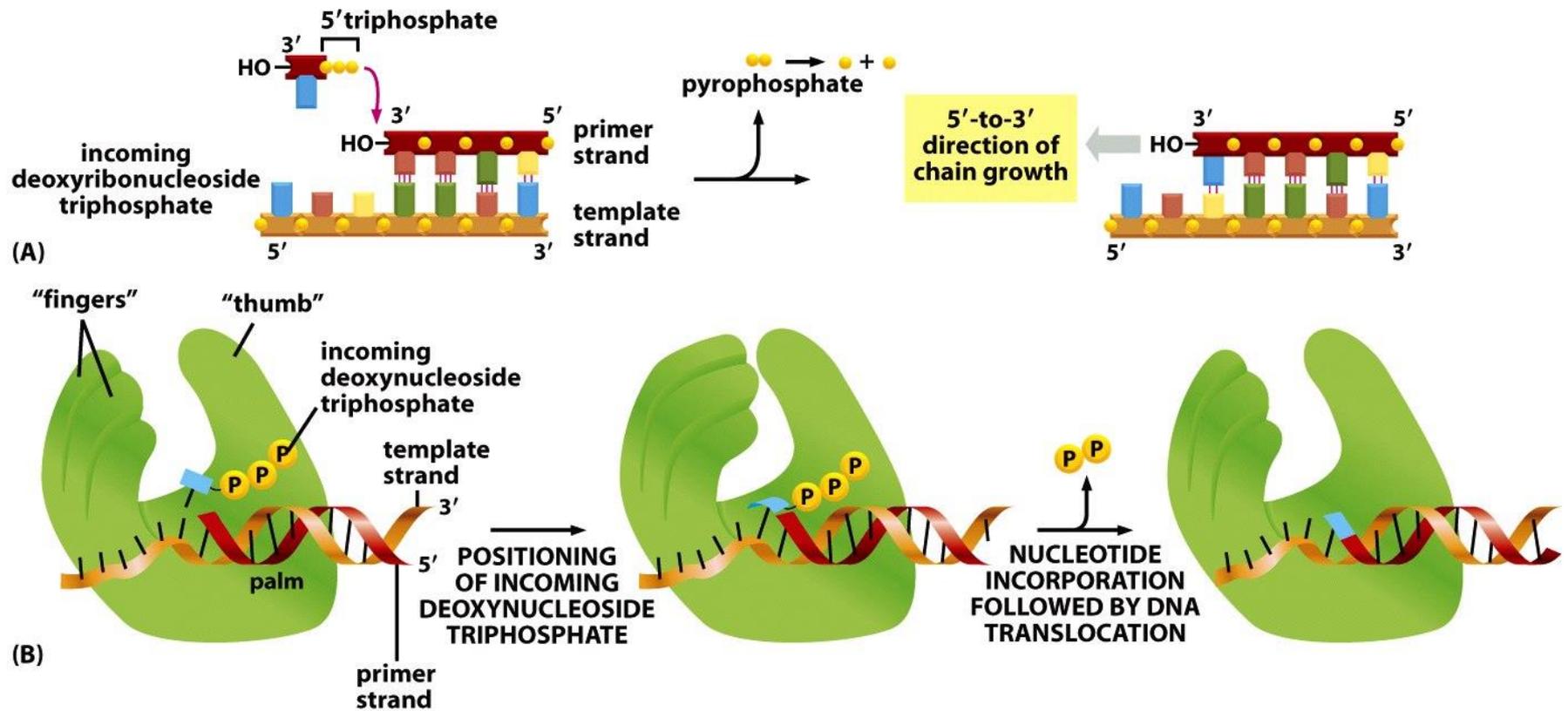


Figure 5-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

# DNS és RNS egyaránt sokszorosítható

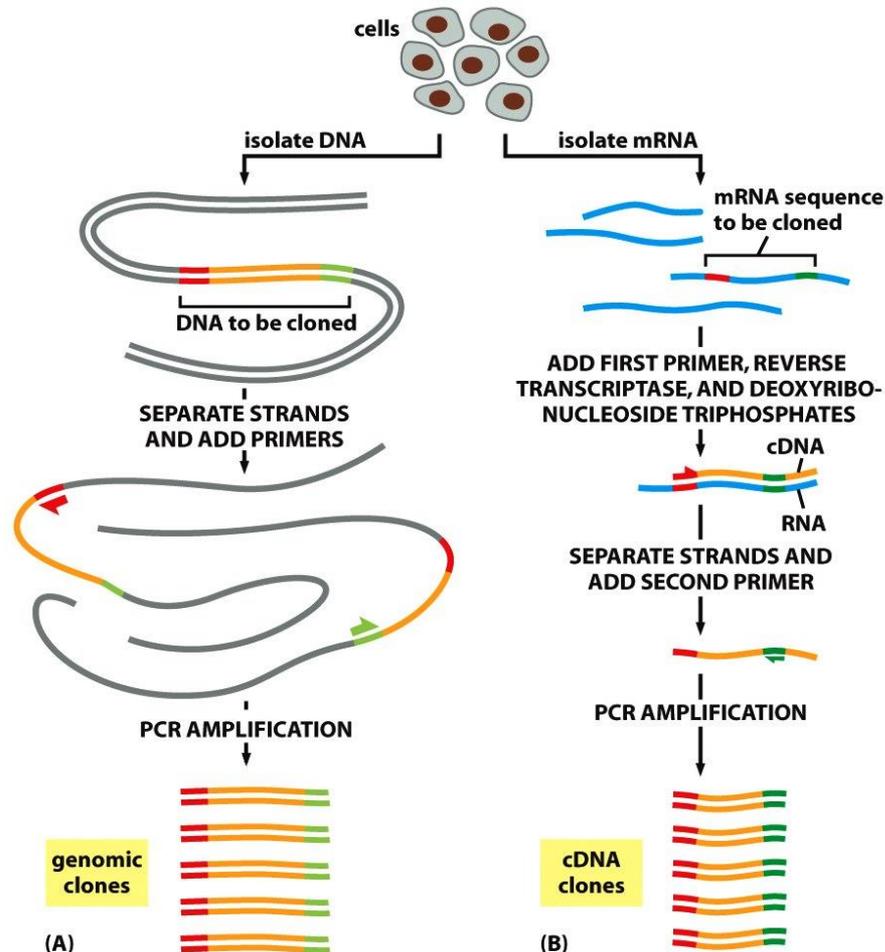
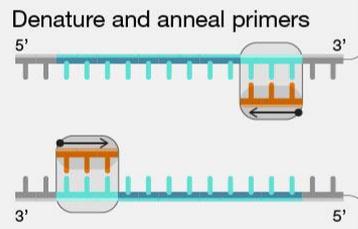
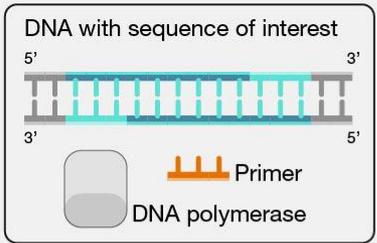


Figure 8-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

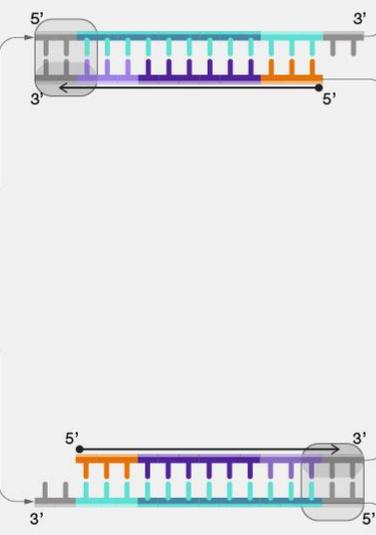
# Polymerase Chain Reaction (PCR)



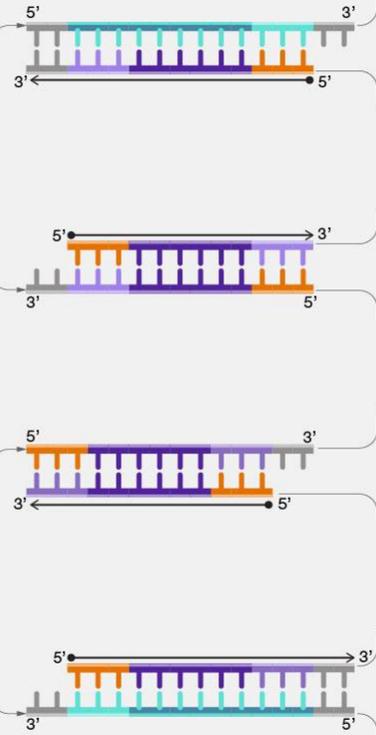
[www.dnalc.org](http://www.dnalc.org)



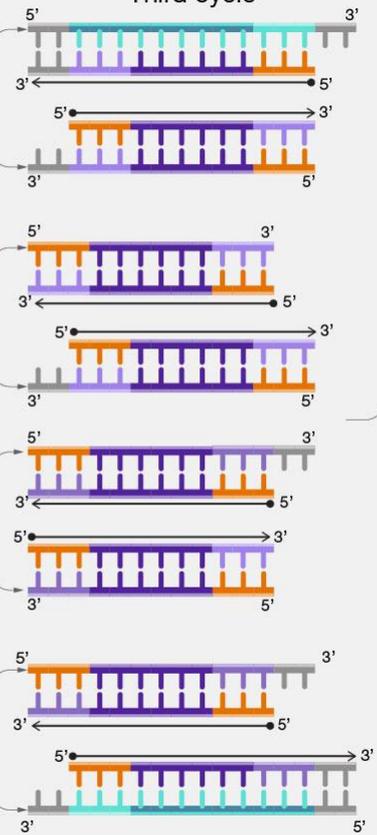
First cycle



Second cycle

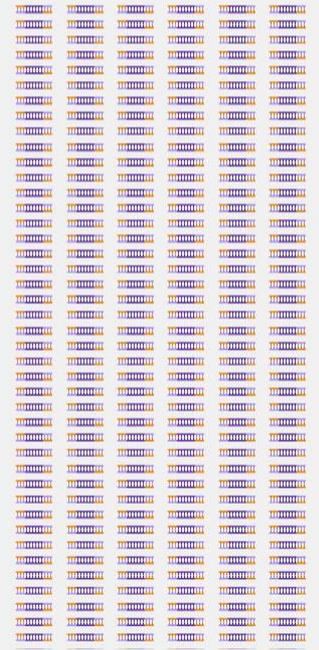


Third cycle



20 to 30 cycles

Millions of copies



# STR analysis

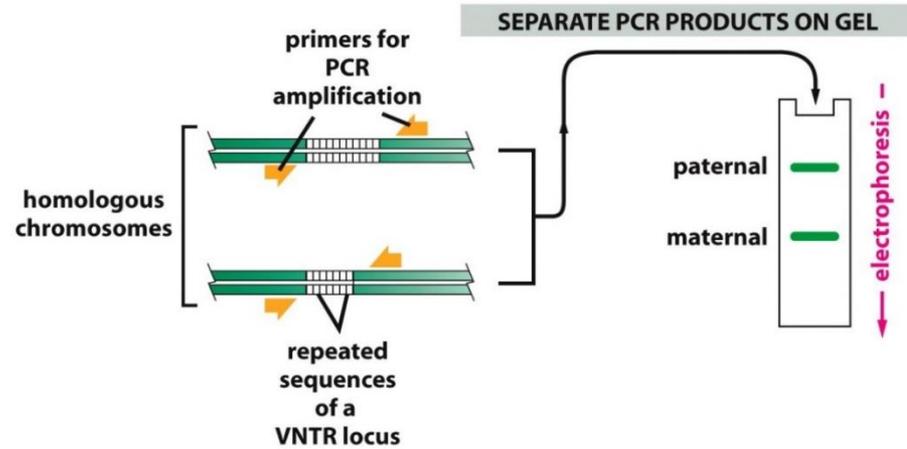


Figure 8-47a Molecular Biology of the Cell 5/e (© Garland Science 2008)

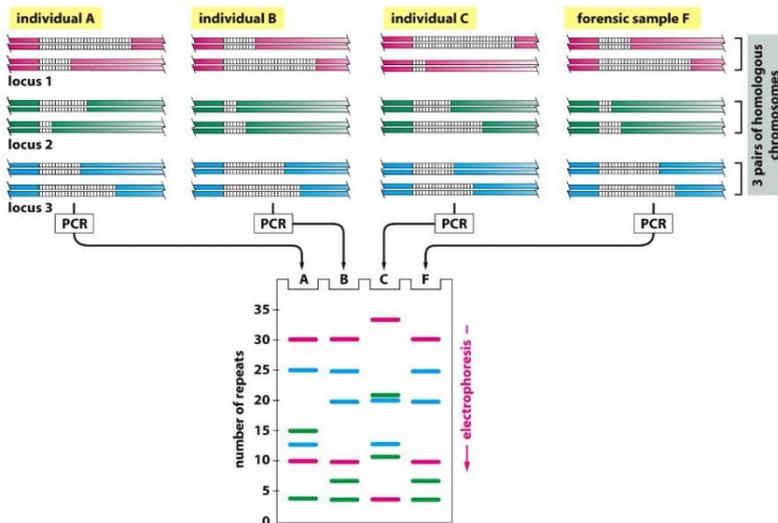
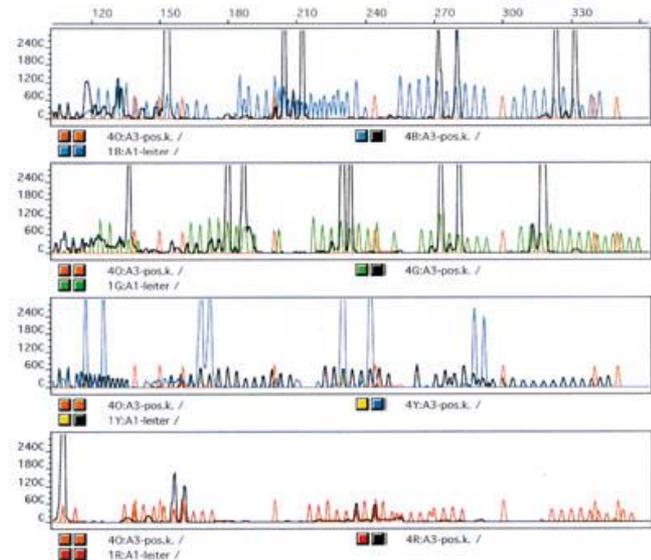


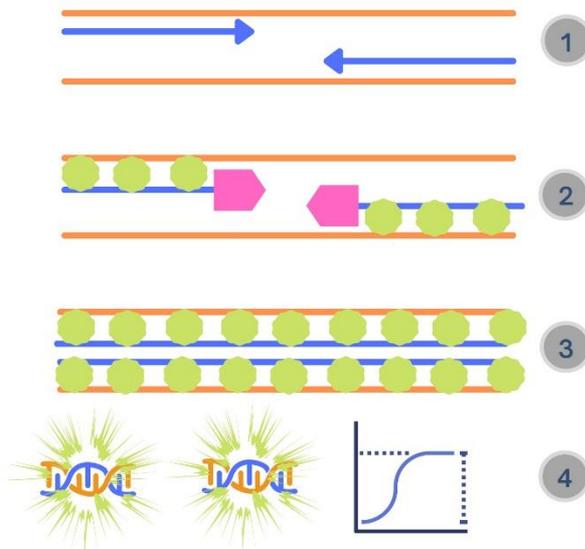
Figure 8-47b Molecular Biology of the Cell 5/e (© Garland Science 2008)



# Valós idejű PCR

## RT-qPCR chemistries

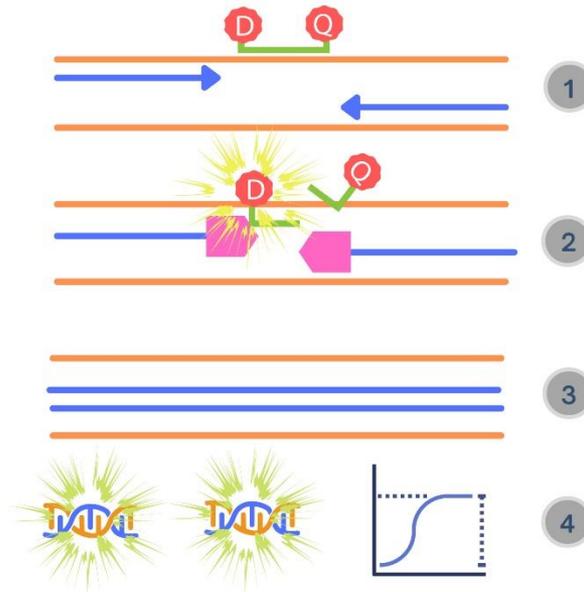
Dye-based RT-qPCR



- 1 cDNA template is denatured
- 2 Dye binds to dsDNA
- 3 Fluorescence signals are detected
- 4 Fluorescence increase with more synthesized DNA

Interkalálódó festékekkel

Probe-based RT-qPCR



- 1 cDNA template is denatured
- 2 Probe binds to target dsDNA
- 3 Fluorescence signals are detected
- 4 Fluorescence increase with more synthesized DNA

TaqMan próbával

# Sanger-szekvenálás

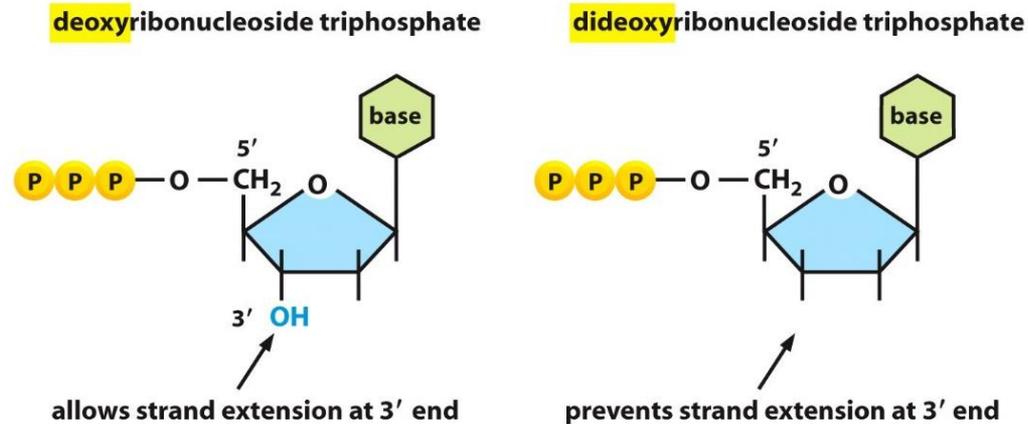


Figure 8-50a Molecular Biology of the Cell 5/e (© Garland Science 2008)

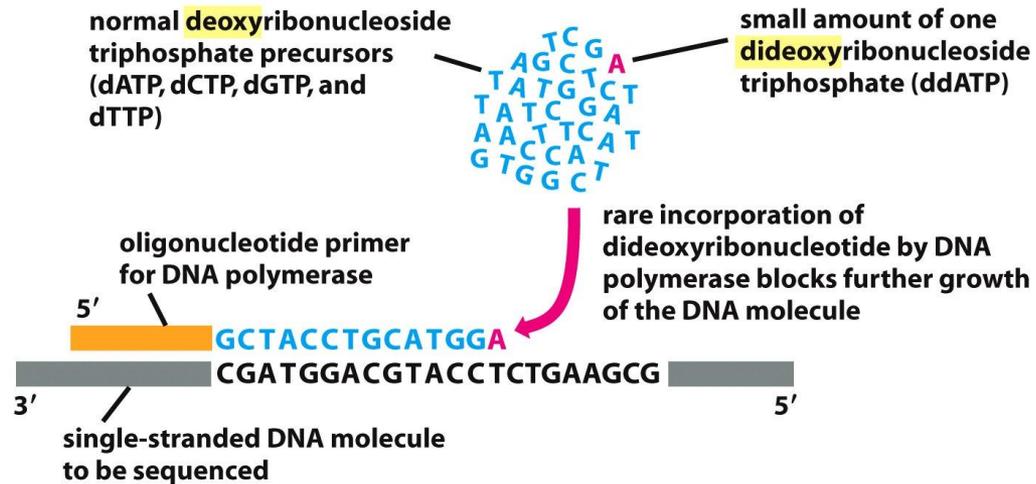
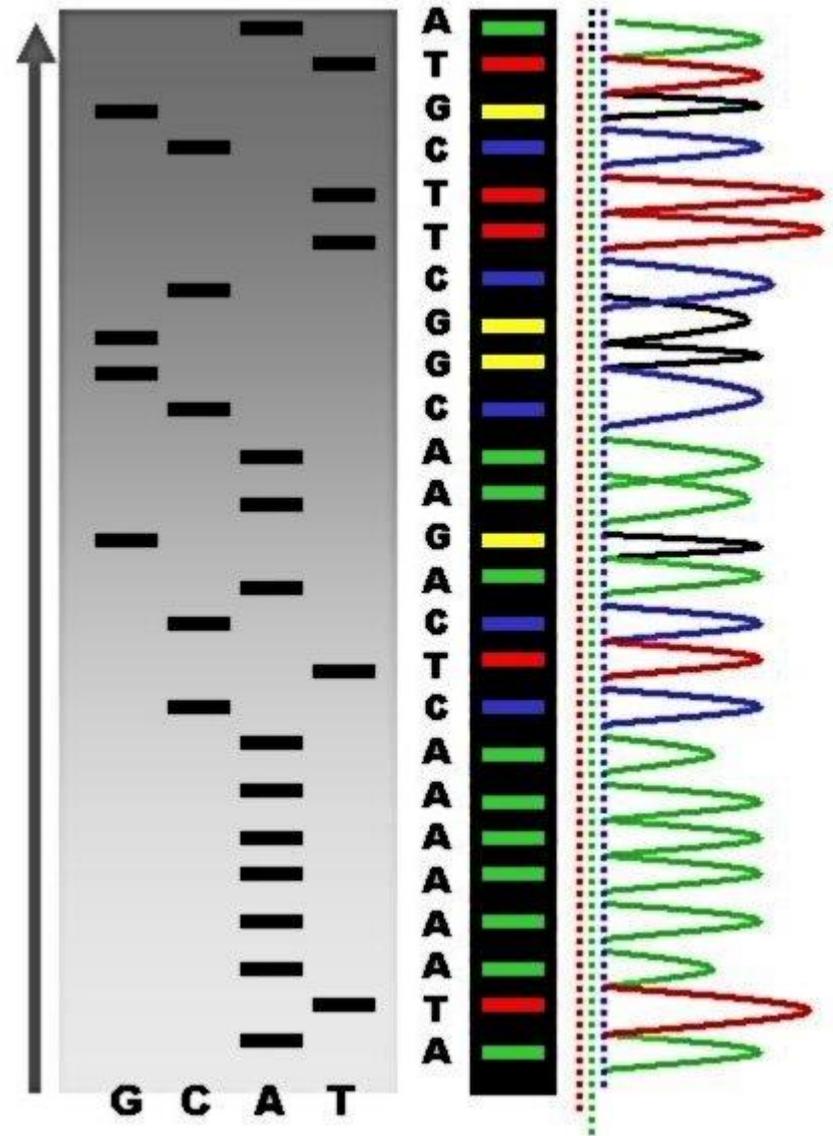
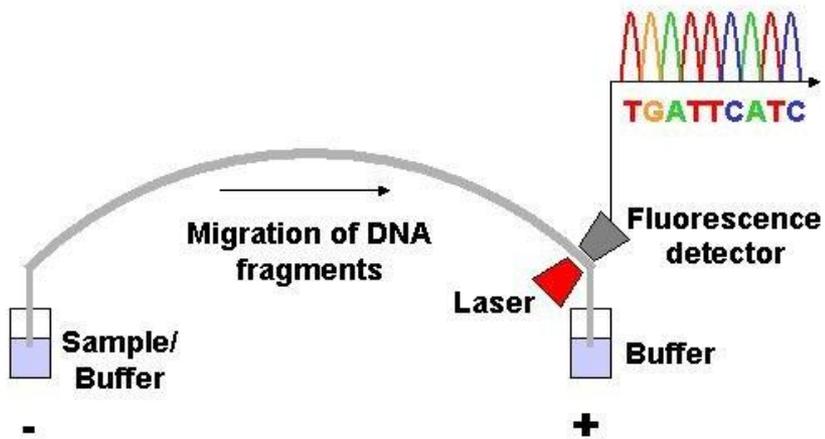
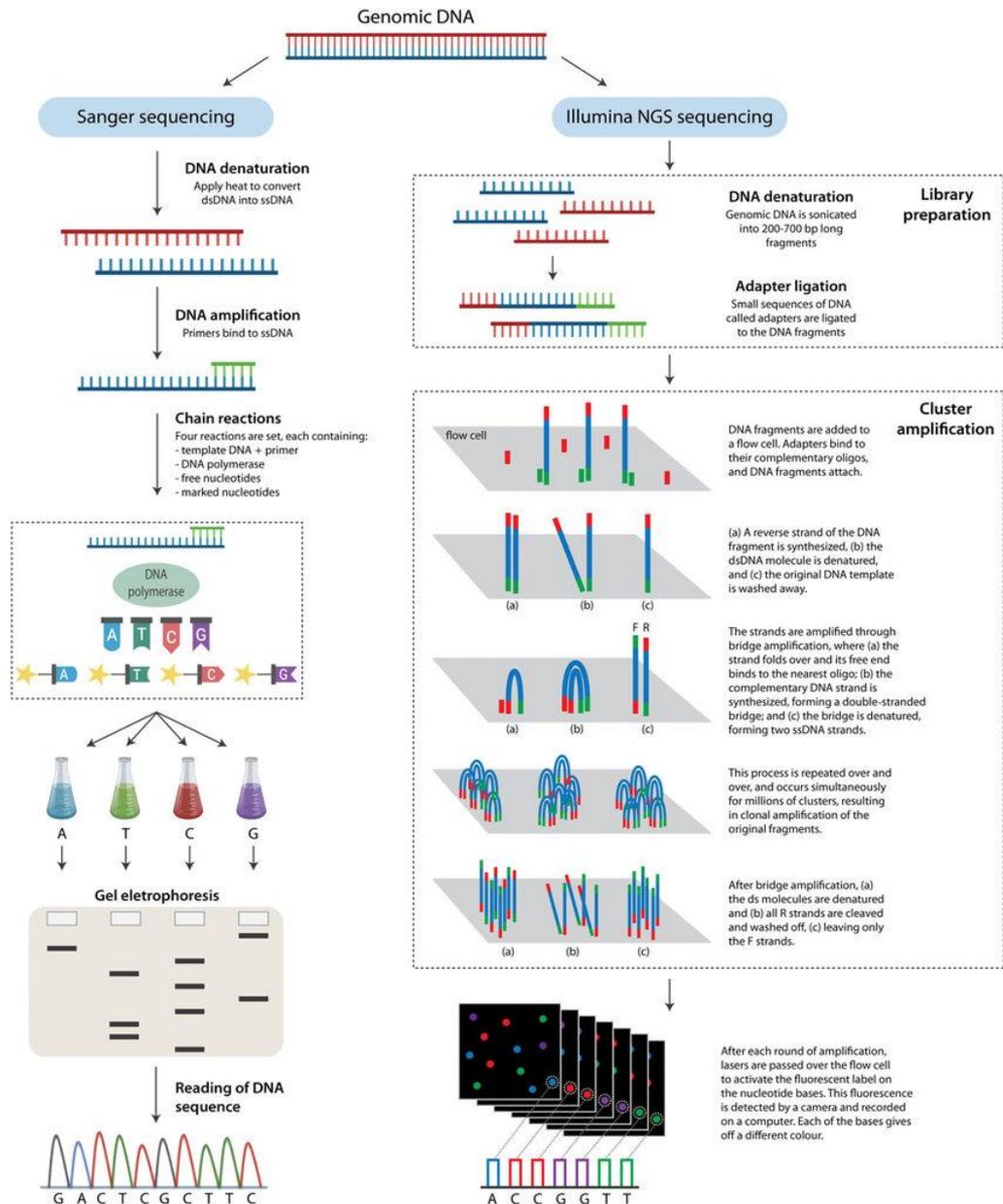


Figure 8-50b Molecular Biology of the Cell 5/e (© Garland Science 2008)



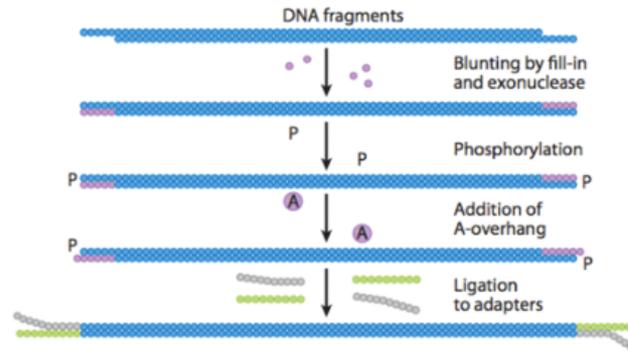


# Következő generációs szekvenálás

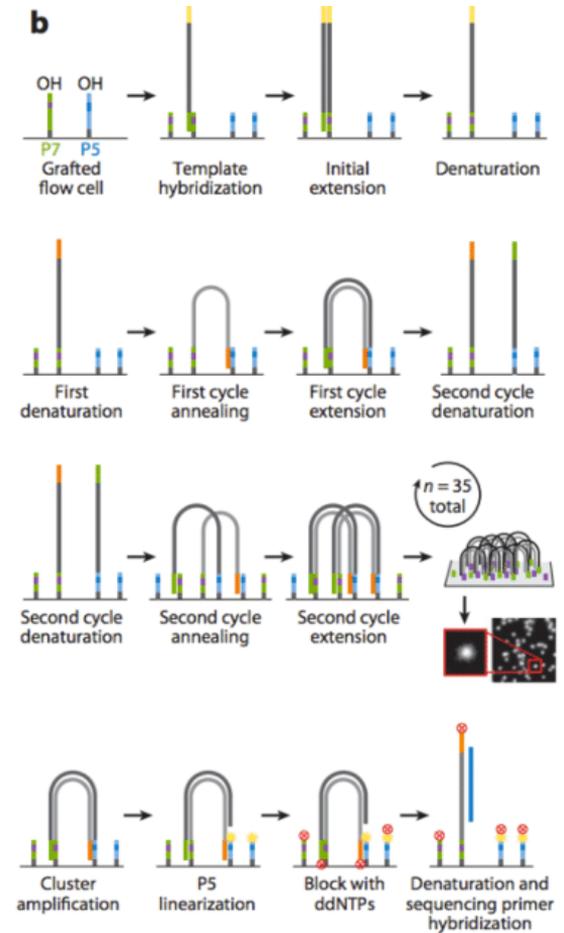


# Szekvenálás szintézissel

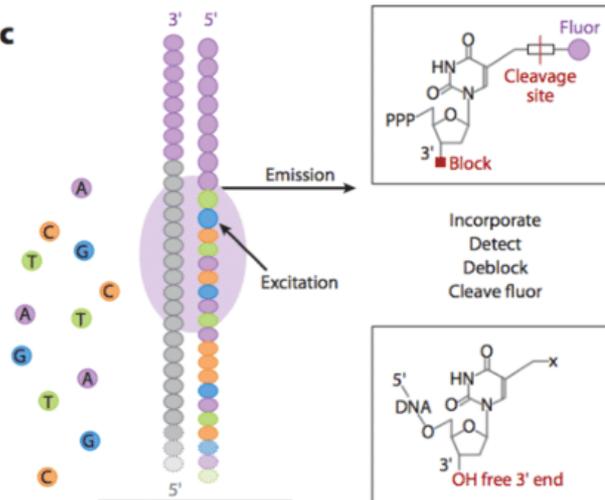
**a** Illumina's library-preparation work flow



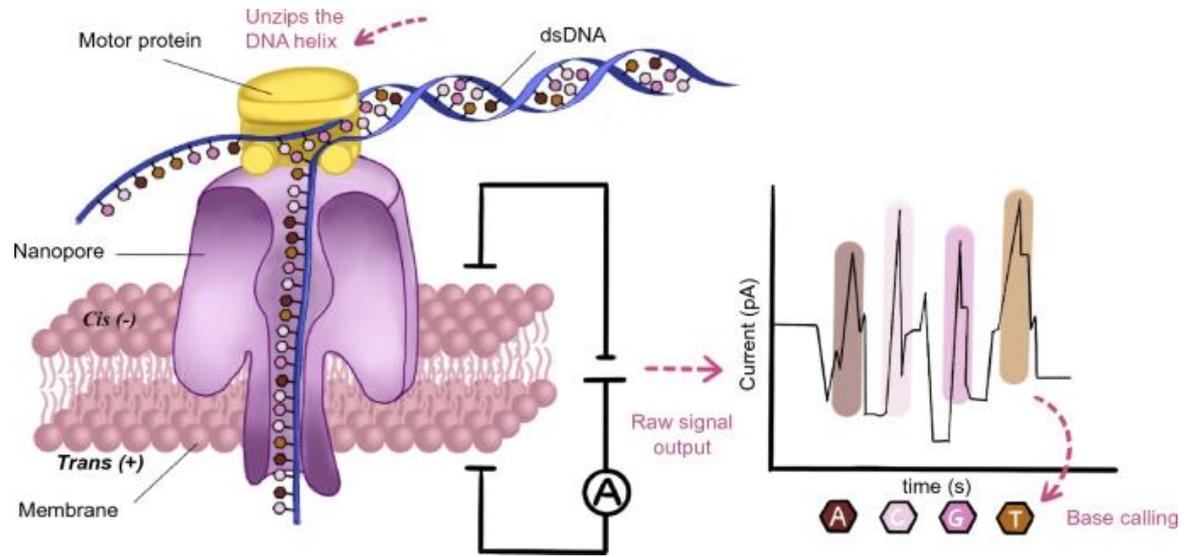
**b**



**c**



# Szekvenálás nanopórrussal



# A fehérjék változatos tulajdonságú aminosavakból épülnek fel

### THE AMINO ACID

The general formula of an amino acid is

R is commonly one of 20 different side chains. At pH 7 both the amino and carboxyl groups are ionized.

### OPTICAL ISOMERS

The  $\alpha$ -carbon atom is asymmetric, which allows for two mirror image (or stereo-) isomers, L and D.

Proteins consist exclusively of L-amino acids.

### FAMILIES OF AMINO ACIDS

The common amino acids are grouped according to whether their side chains are

- acidic
- basic
- uncharged polar
- nonpolar

These 20 amino acids are given both three-letter and one-letter abbreviations.

Thus: alanine = Ala = A

### BASIC SIDE CHAINS

**lysine**  
(Lys, or K)

**arginine**  
(Arg, or R)

**histidine**  
(His, or H)

This group is very basic because its positive charge is stabilized by resonance.

These nitrogens have a relatively weak affinity for an  $H^+$  and are only partly positive at neutral pH.

### PEPTIDE BONDS

Amino acids are commonly joined together by an amide linkage, called a peptide bond.

**Peptide bond:** The four atoms in each gray box form a rigid planar unit. There is no rotation around the C-N bond.

Proteins are long polymers of amino acids linked by peptide bonds, and they are always written with the N-terminus toward the left. The sequence of this tripeptide is histidine-cysteine-valine.

These two single bonds allow rotation, so that long chains of amino acids are very flexible.

### ACIDIC SIDE CHAINS

**aspartic acid**  
(Asp, or D)

**glutamic acid**  
(Glu, or E)

### UNCHARGED POLAR SIDE CHAINS

**asparagine**  
(Asn, or N)

**glutamine**  
(Gln, or Q)

Although the amide N is not charged at neutral pH, it is polar.

**serine**  
(Ser, or S)

**threonine**  
(Thr, or T)

**tyrosine**  
(Tyr, or Y)

The -OH group is polar.

### NONPOLAR SIDE CHAINS

**alanine**  
(Ala, or A)

**valine**  
(Val, or V)

**leucine**  
(Leu, or L)

**isoleucine**  
(Ile, or I)

**proline**  
(Pro, or P)  
(actually an imino acid)

**phenylalanine**  
(Phe, or F)

**methionine**  
(Met, or M)

**tryptophan**  
(Trp, or W)

**glycine**  
(Gly, or G)

**cysteine**  
(Cys, or C)

Disulfide bonds can form between two cysteine side chains in proteins.

-CH<sub>2</sub>-S-S-CH<sub>2</sub>-

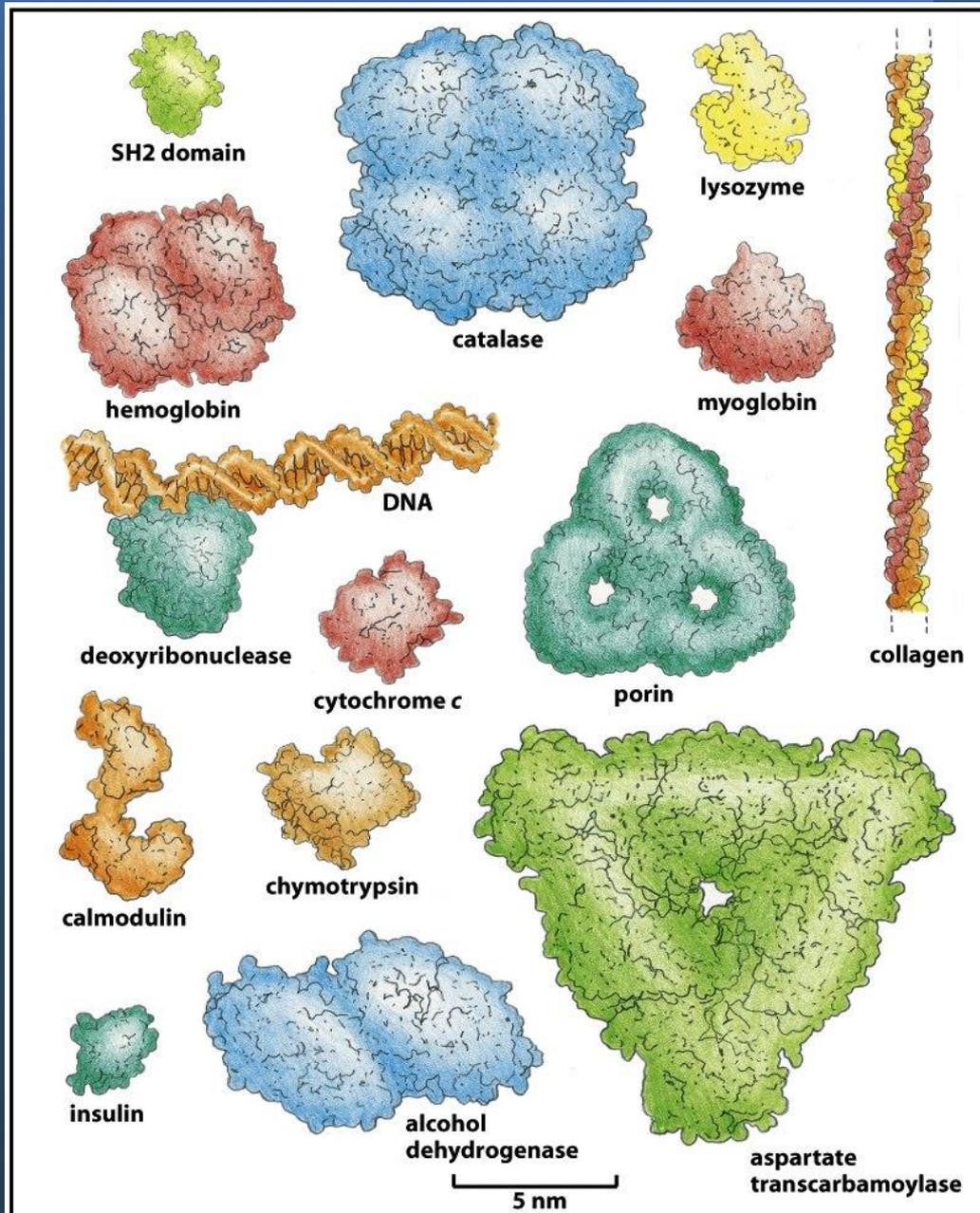
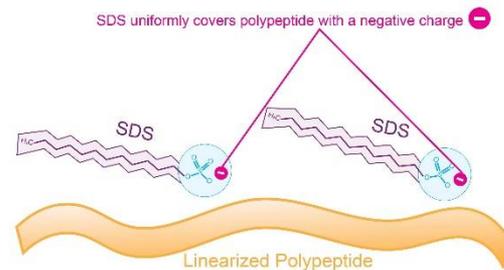
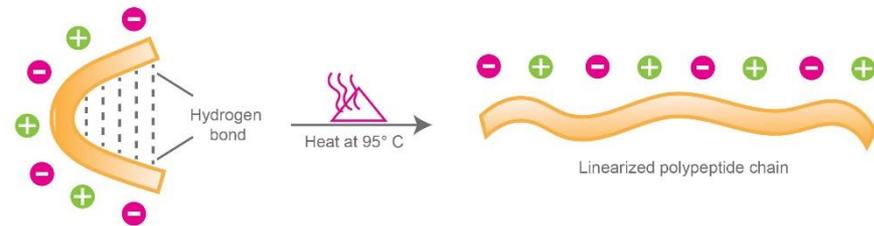
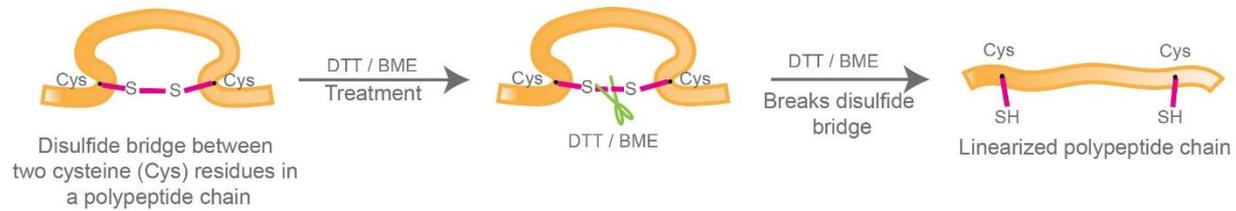
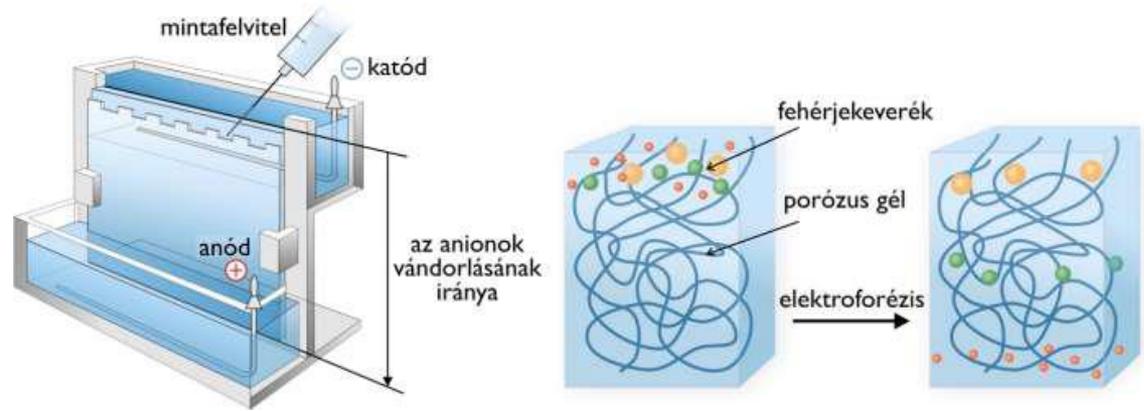
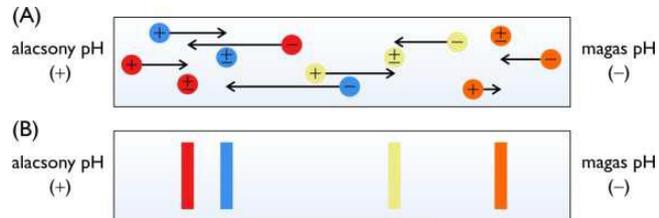


Figure 3-23 Molecular Biology of the Cell 5/e (© Garland Science 2008)

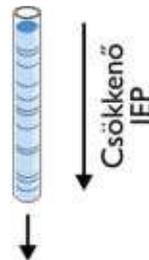
# Fehérjék méret szerinti elválasztása: SDS-PAGE



# 2D GE: IEF + SDS-PAGE



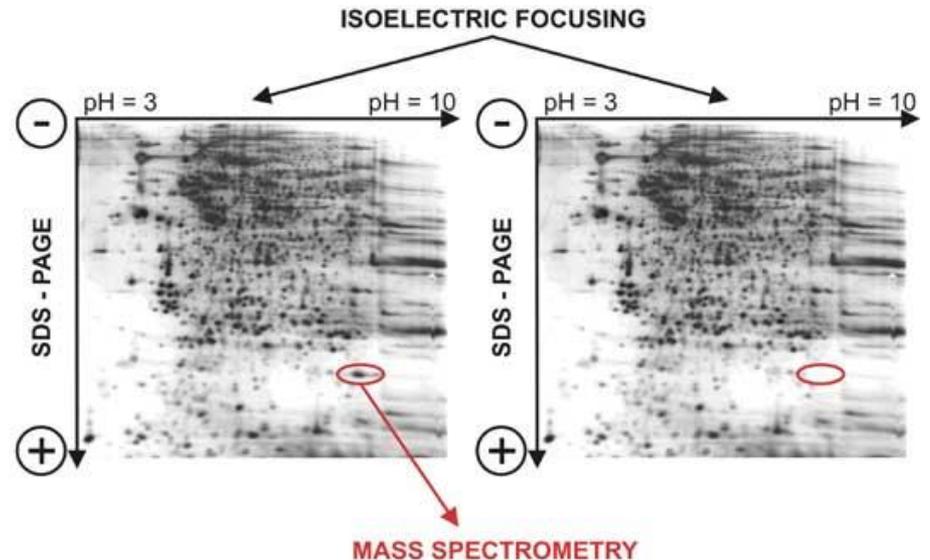
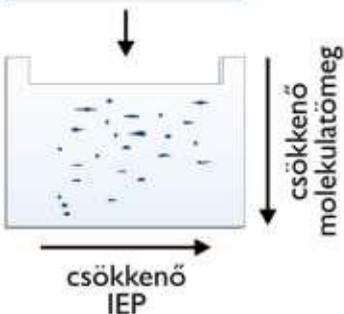
1. A fehérjék elválasztása izoelektromos pont alapján.



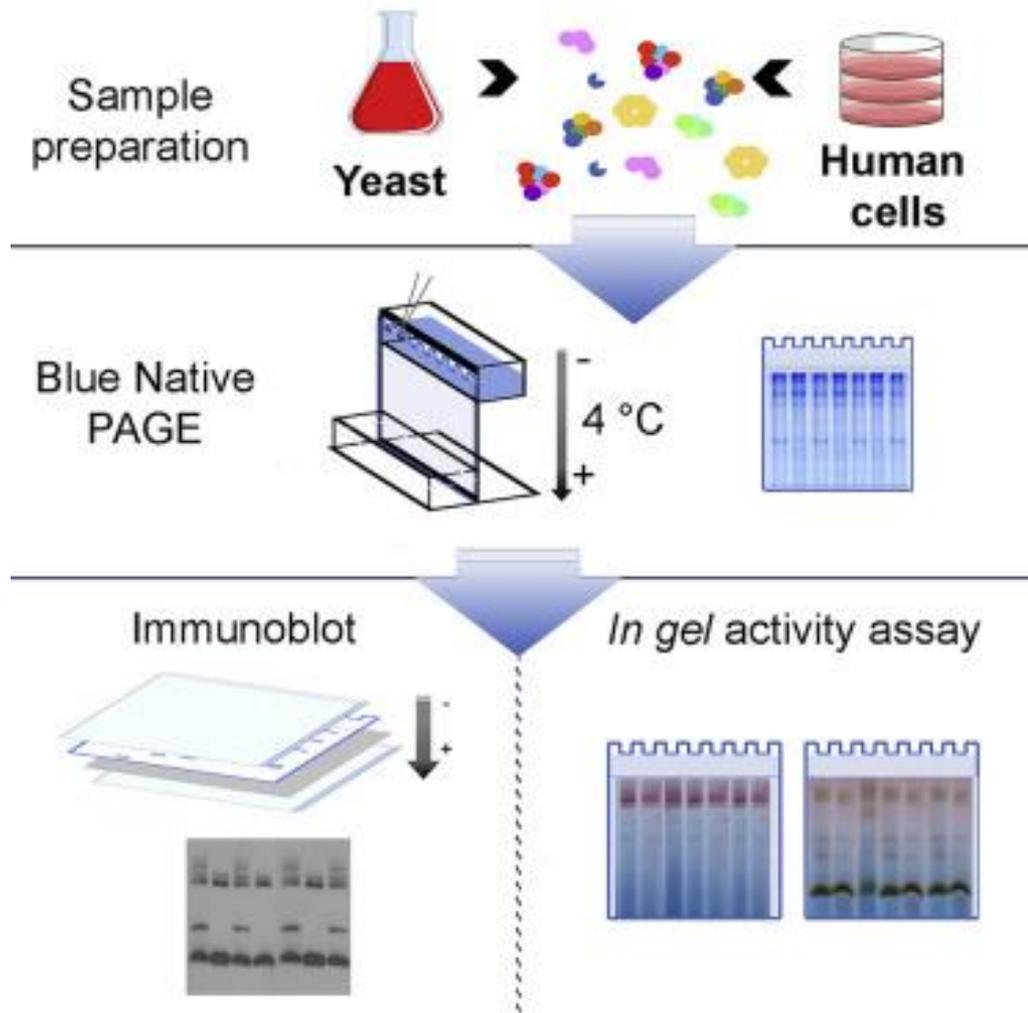
2. A minta beáztatása SDS oldatba és SDS-PA géltre helyezése.



3. A minta elválasztása molekulatömeg szerint SDS-PAGE módszerrel.

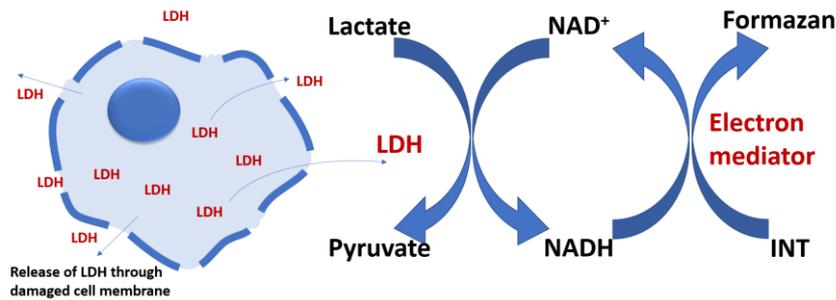


# (Blue) Native-PAGE



# Enzimaktivitás-mérés

LDH: életképesség-mérés



<https://www.creative-bioarray.com/ldh-cytotoxicity-assay.htm>

Májfunkció vizsgálata klinikumban: ALAT (sGPT)/ASAT (sGOT)

A central illustration of a human liver is surrounded by several conditions and their associated enzyme levels:

- Fatty liver disease**
- Hepatitis B**
- Hemochromatosis**
- Primary biliary cholangitis**
- Gilbert's syndrome**

Enzyme levels are indicated as follows:

- ↑ ALT **124 H**
- ↑ AST **111 H**
- ↑ Alk phos **204 H**

<https://www.straighthealthcare.com/liver-testing-and-diseases.html>

# Fehérjék szelektív azonosítása ellenanyagokkal

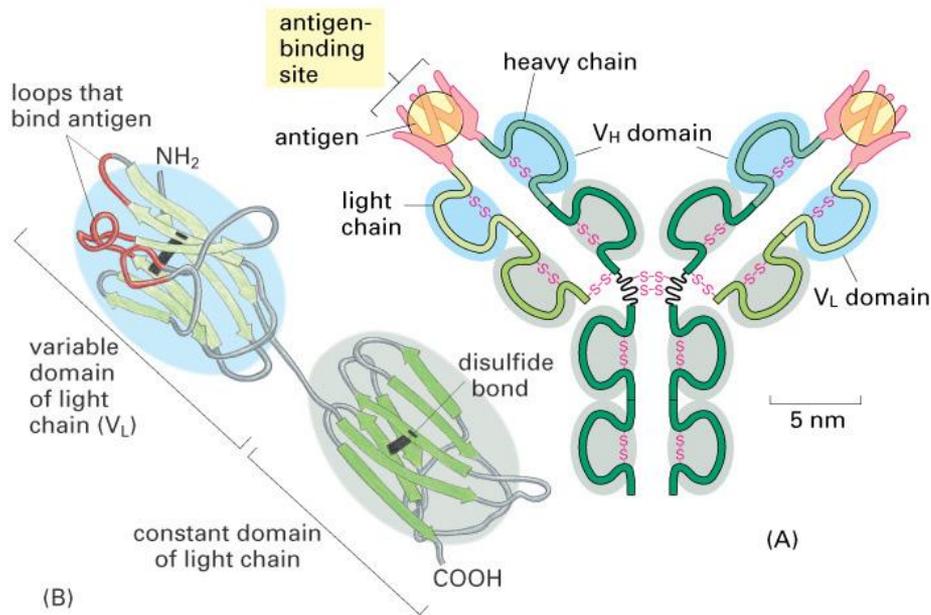
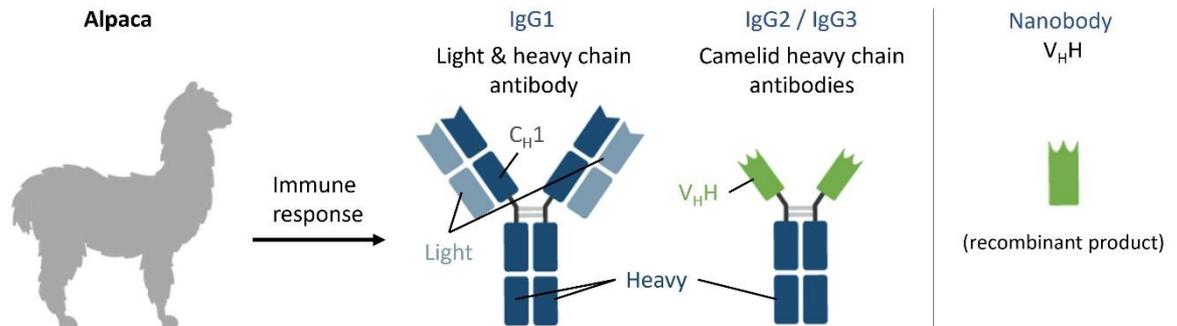
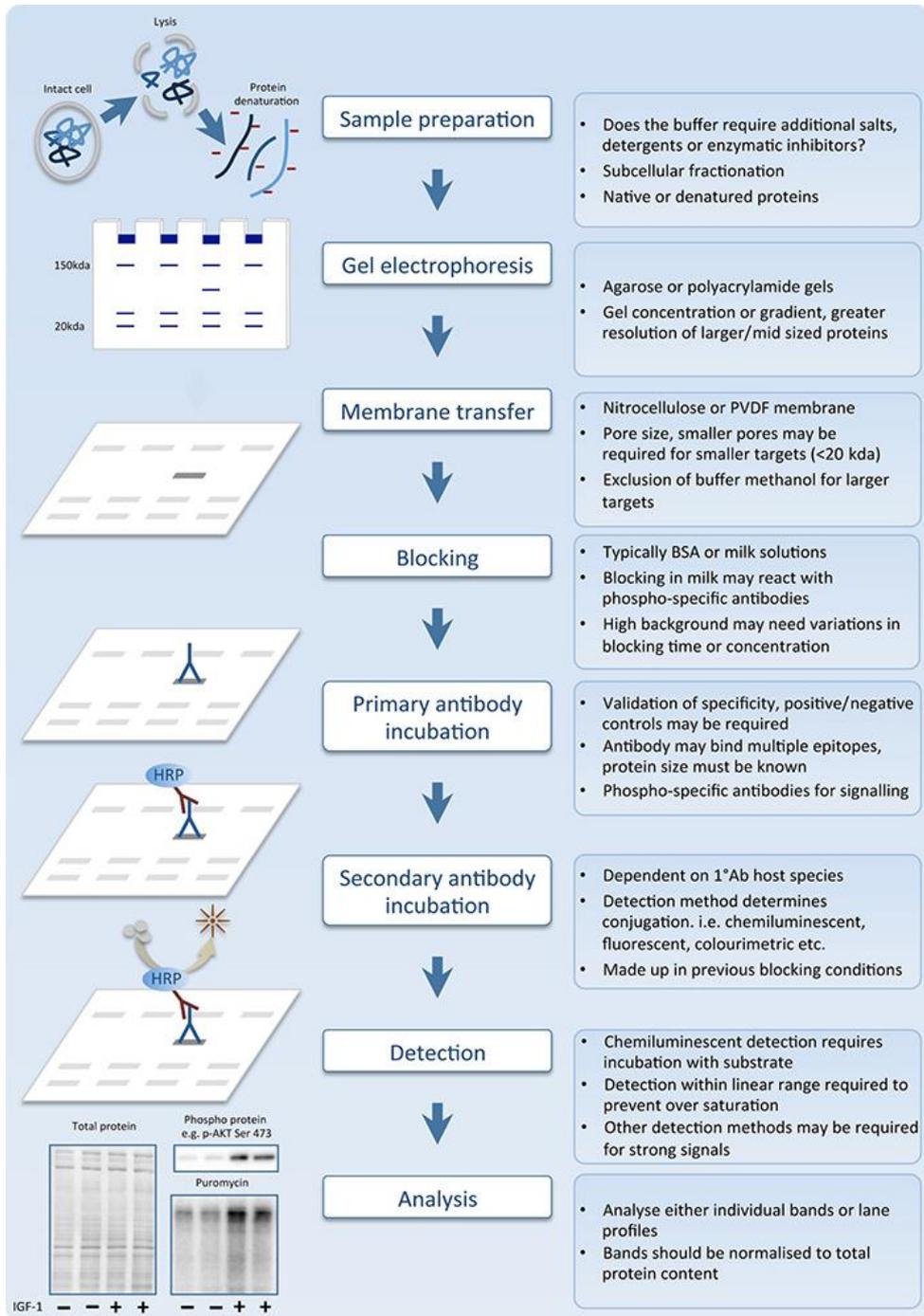


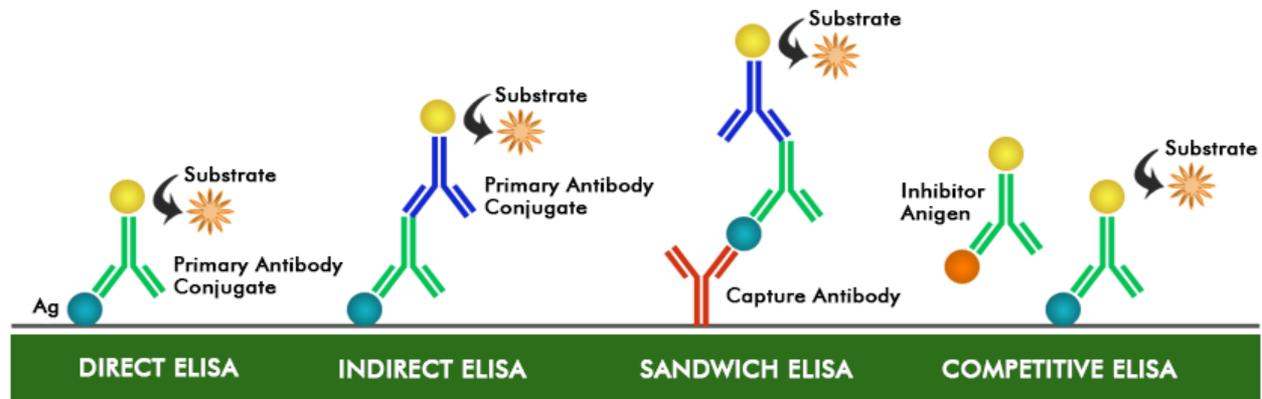
Figure 4-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)



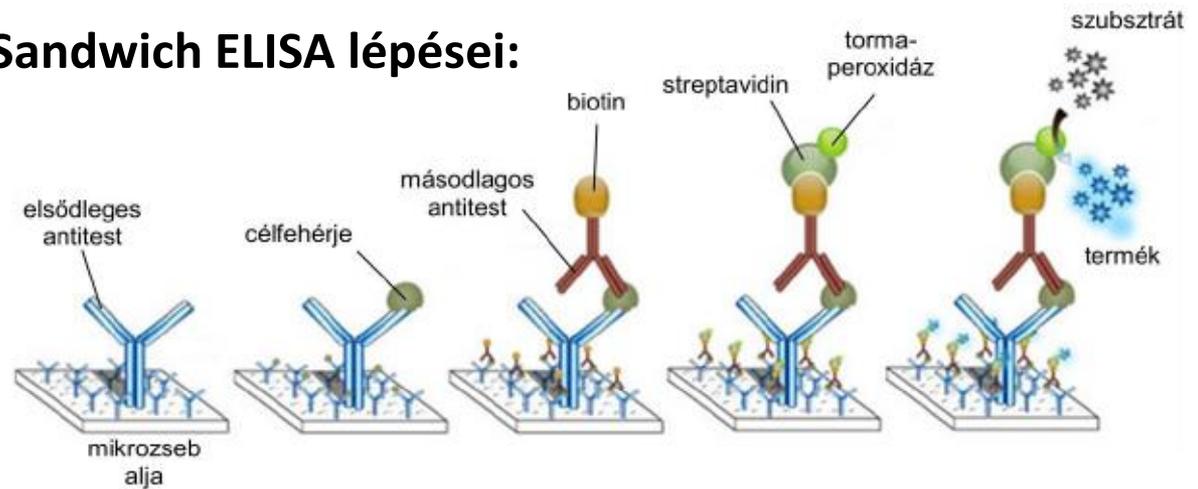
# Western-blot



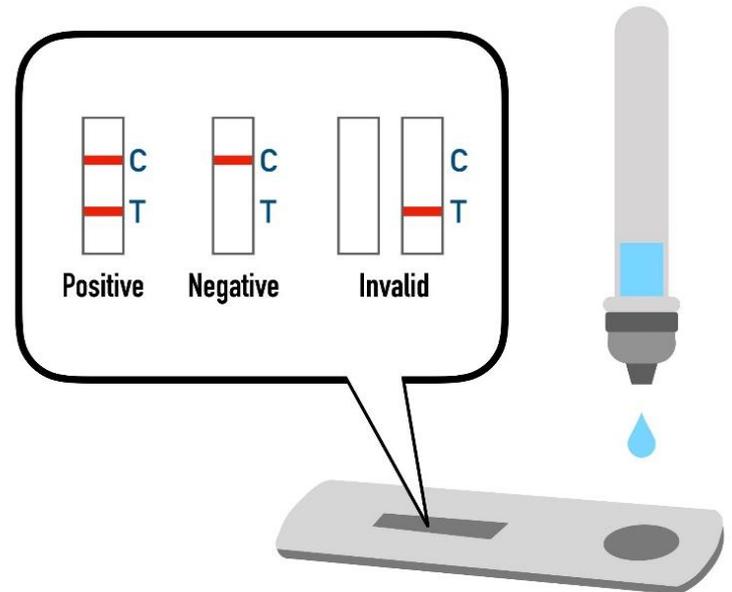
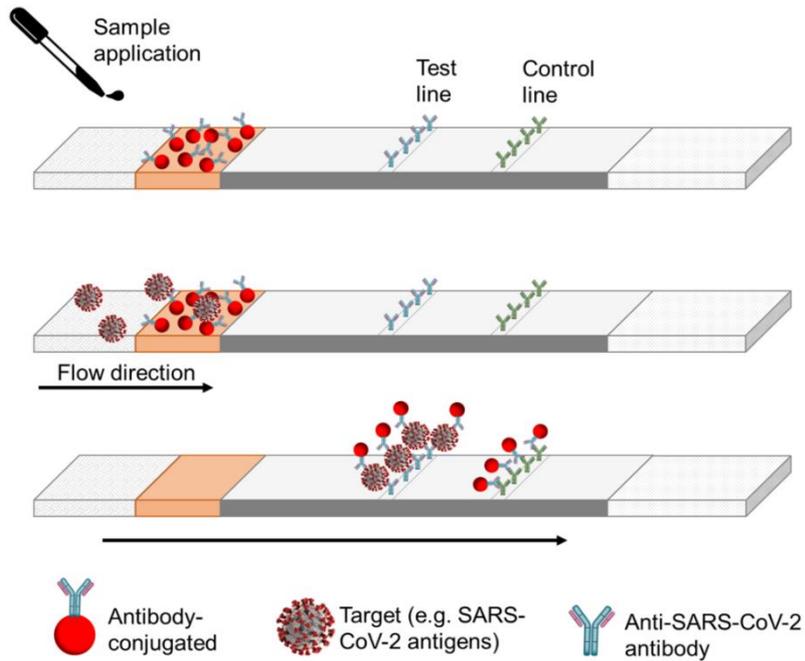
# Enzyme-Linked Immunosorbent Assay



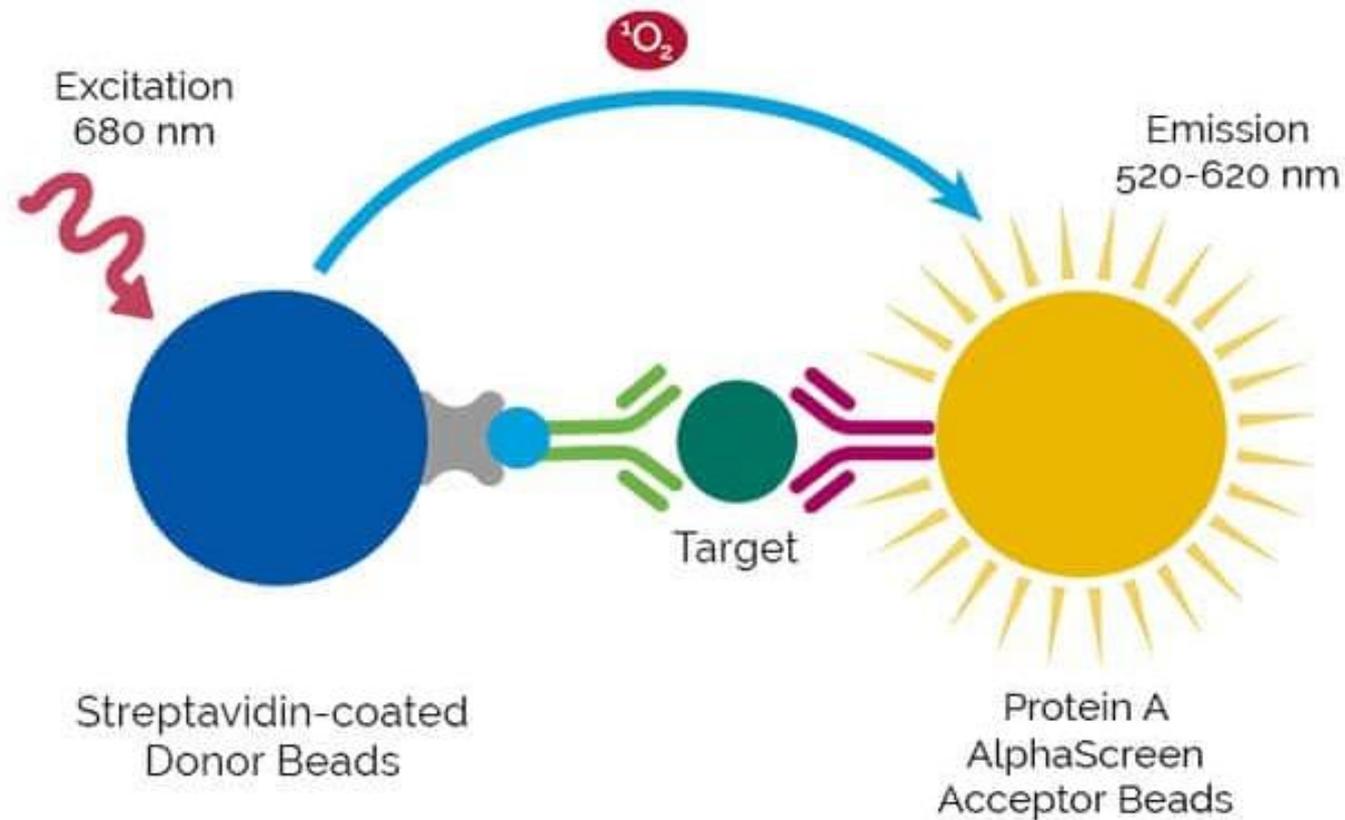
## Sandwich ELISA lépései:



# Lateral Flow Assay

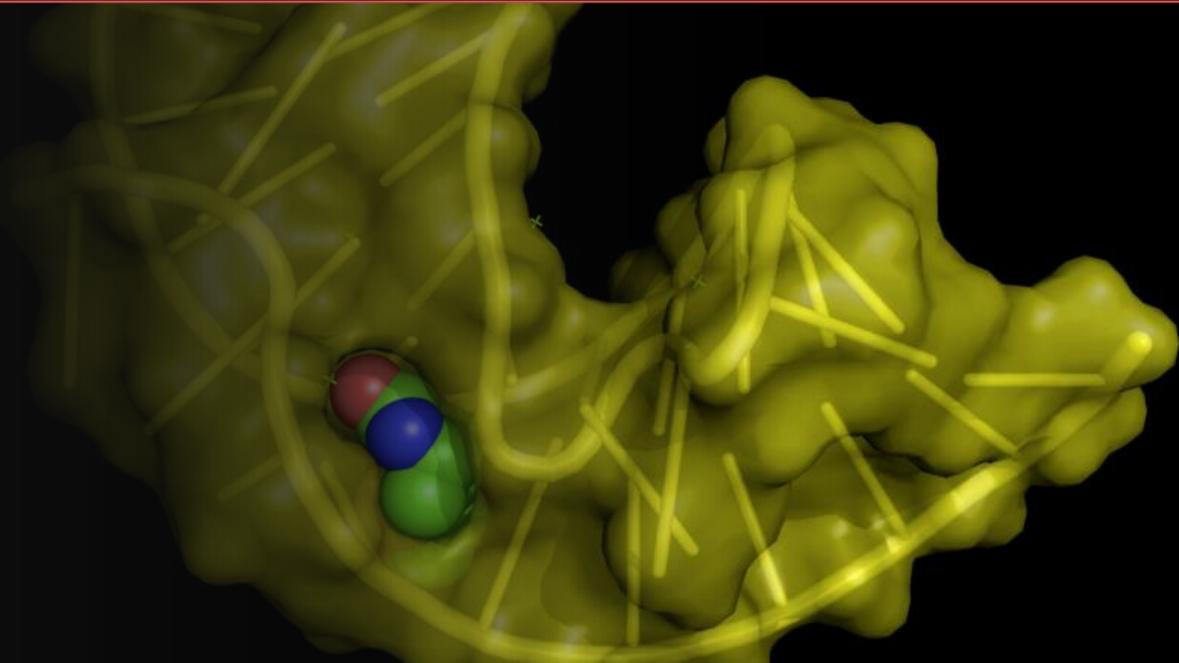


# Amplified Luminescent Proximity Homogeneous Assay (LISA)

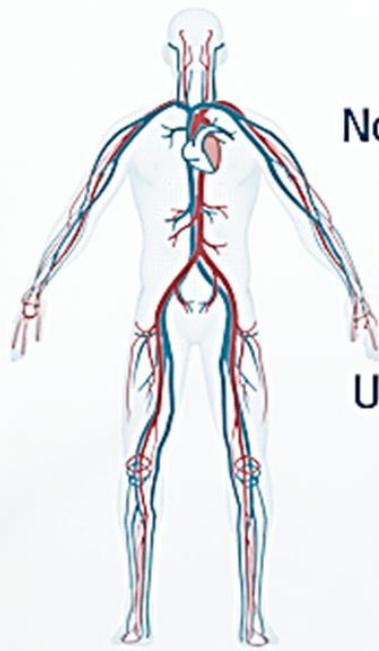


# Aptamerek, a nukleinsav alapú receptorok

---



# Az aptamerek előnyei



Advantages in  
Clinical Applicability

Non-Immunogenic



High Penetration



Unlimited Targets



Thermally Stable



Less Batch Variation



Cost Effective



Short Production Time

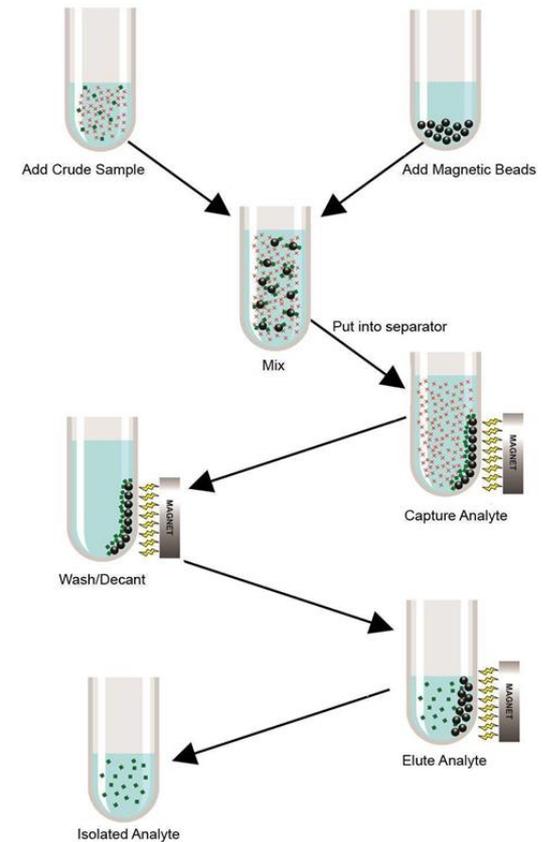
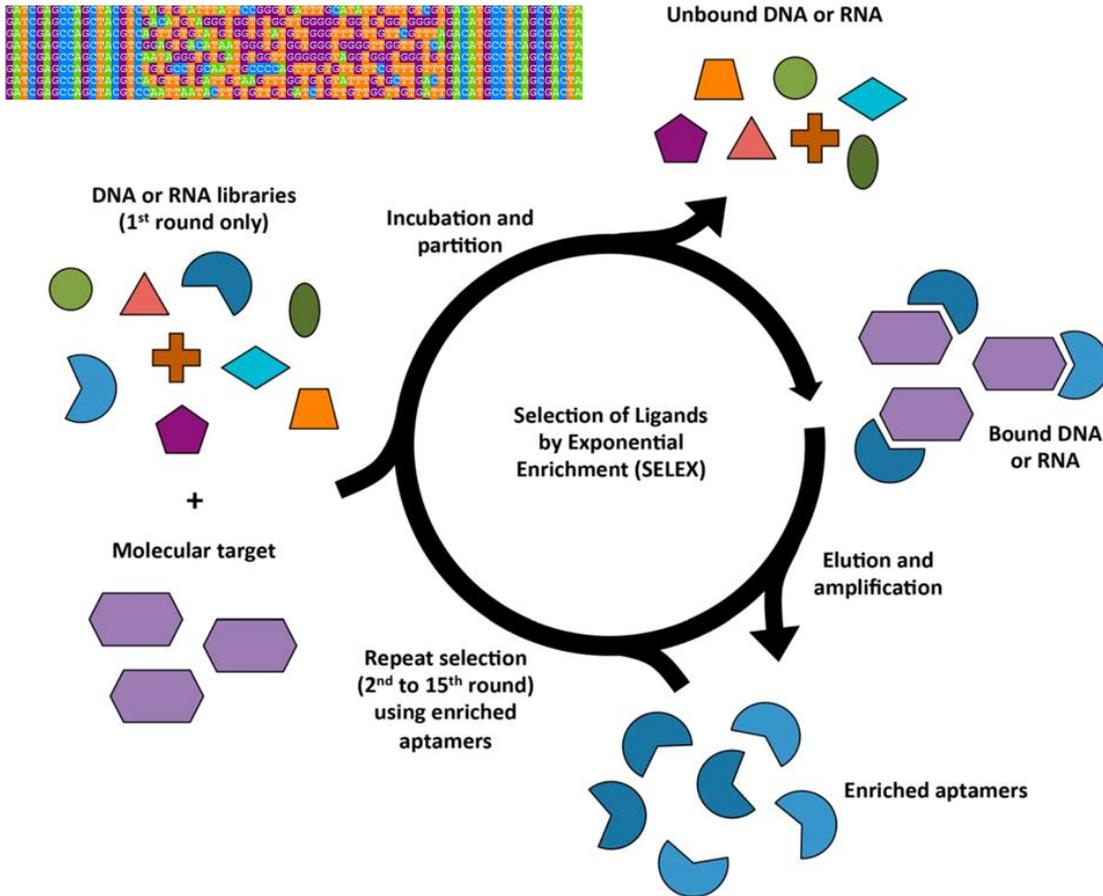


**Aptamer**



Advantages in  
Industrialization

# Aptamerek előállítása (SELEX)

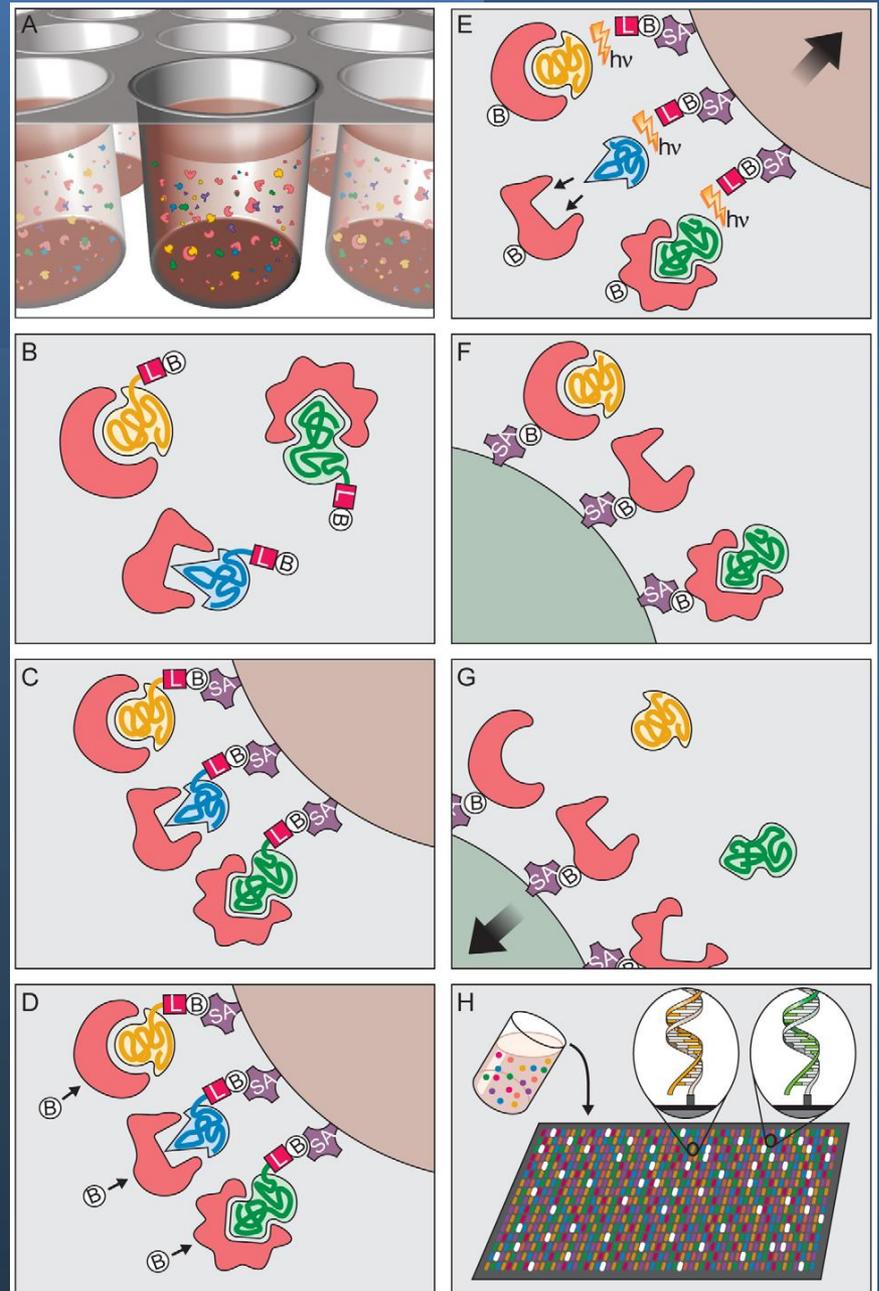




# Multiplex SOMAmer kölcsonhatás- vizsgálat

## Choose Disease-Specific Panels

Inflammation and immune response	938 analytes	<a href="#">View analytes</a>
Oncology	863 analytes	<a href="#">View analytes</a>
Neuroscience	1,316 analytes	<a href="#">View analytes</a>
Cardiovascular disease	953 analytes	<a href="#">View analytes</a>
Metabolic diseases	890 analytes	<a href="#">View analytes</a>
Respiratory	627 analytes	<a href="#">View analytes</a>
Cytokines	168 analytes	<a href="#">View analytes</a>





Köszönöm a figyelmet!