Betekintés a bionalitikába

Mészáros Tamás
SE-OVI

Biológiailag eredetű anyagok azonosítása

Vírusok

Mikrobák

- morfológia, fenotípus
- fertőző képesség
- nukleinsav, fehérje

Nukleinsavak

- direkt módon

Fehérjék

- direkt módon
- enzim aktivitáson keresztül

Anyagcseretermékek

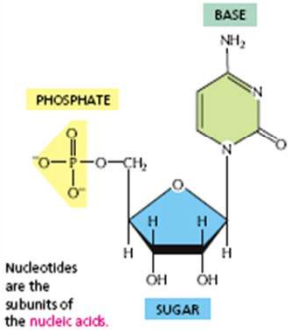
- direkt módon
- enzim aktivitáson keresztül

Nukleinsavak azonosítása

A nukleinsavak kémiailag azonosak

NUCLEOTIDES

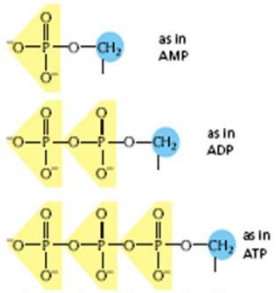
A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.



Nucleotides are the subunits of the nucleic acids.

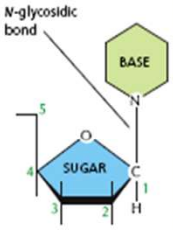
PHOSPHATES

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



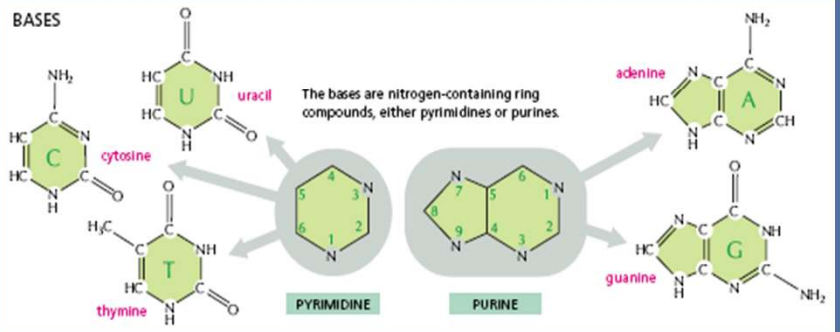
The phosphate makes a nucleotide negatively charged.

BASIC SUGAR LINKAGE



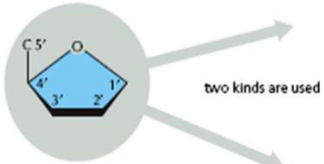
The base is linked to the same carbon (C1) used in sugar–sugar bonds.

BASES

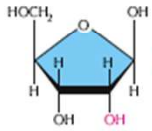


The bases are nitrogen-containing ring compounds, either pyrimidines or purines.

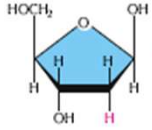
SUGARS



two kinds are used



β-D-ribose
used in ribonucleic acid



β-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.

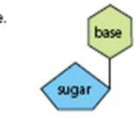
NOMENCLATURE

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

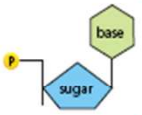
BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

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



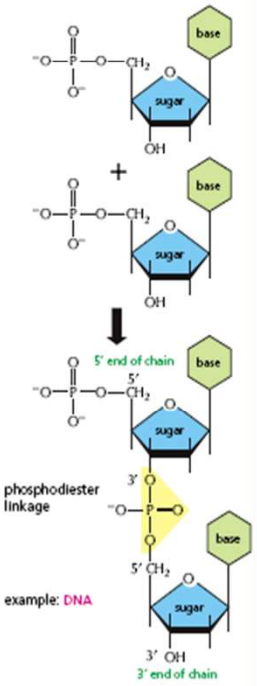
BASE + SUGAR = NUCLEOSIDE



BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

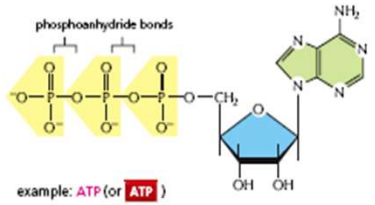
NUCLEIC ACIDS

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.



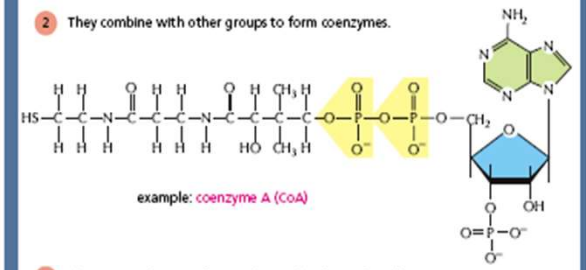
NUCLEOTIDES HAVE MANY OTHER FUNCTIONS

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



example: ATP (or ATP)

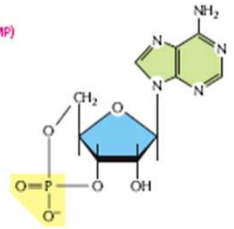
2. They combine with other groups to form coenzymes.



example: coenzyme A (CoA)

3. They are used as specific signaling molecules in the cell.

example: cyclic AMP (cAMP)



A komplementer bázisok párokat képeznek

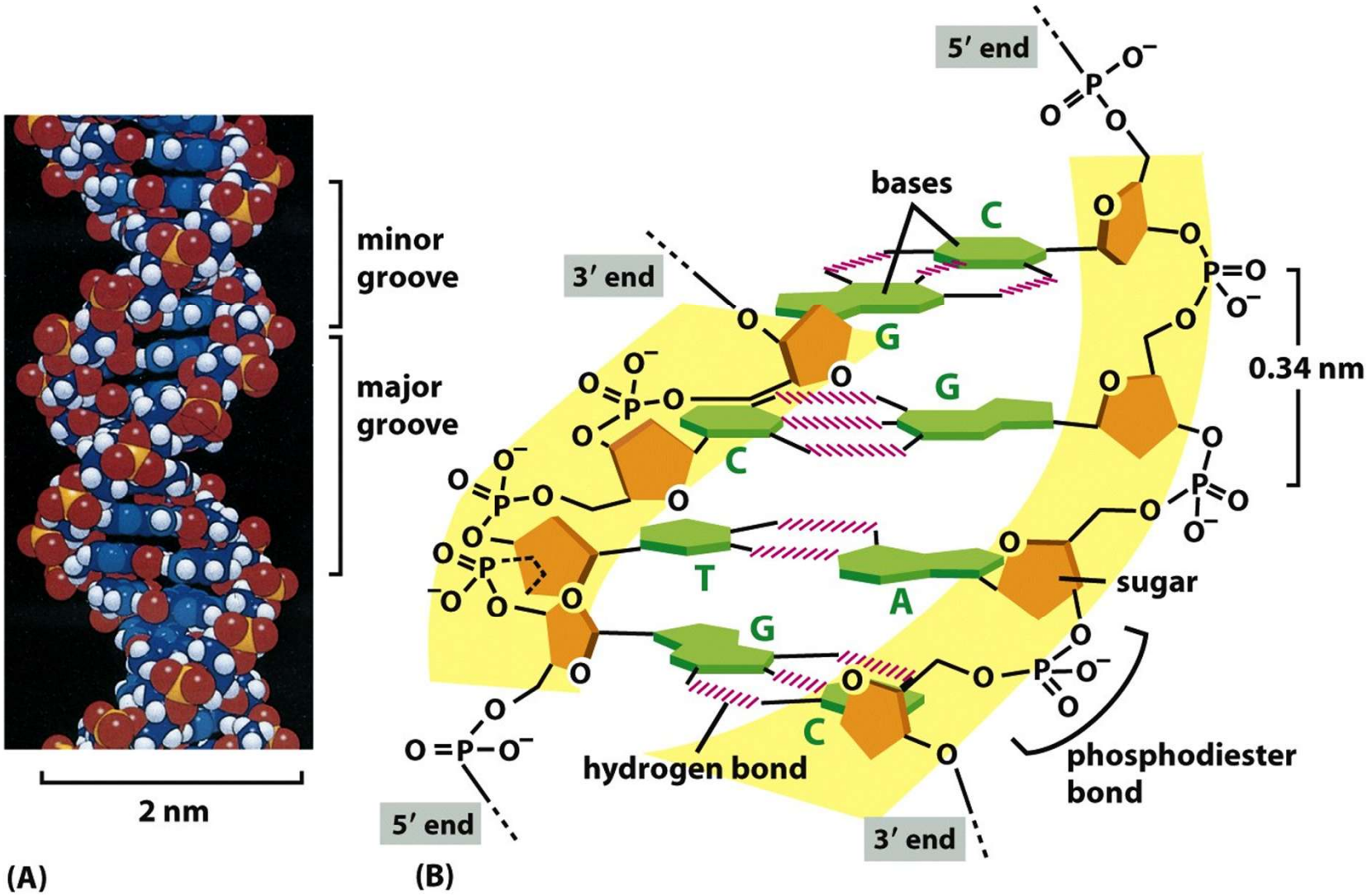


Figure 4-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

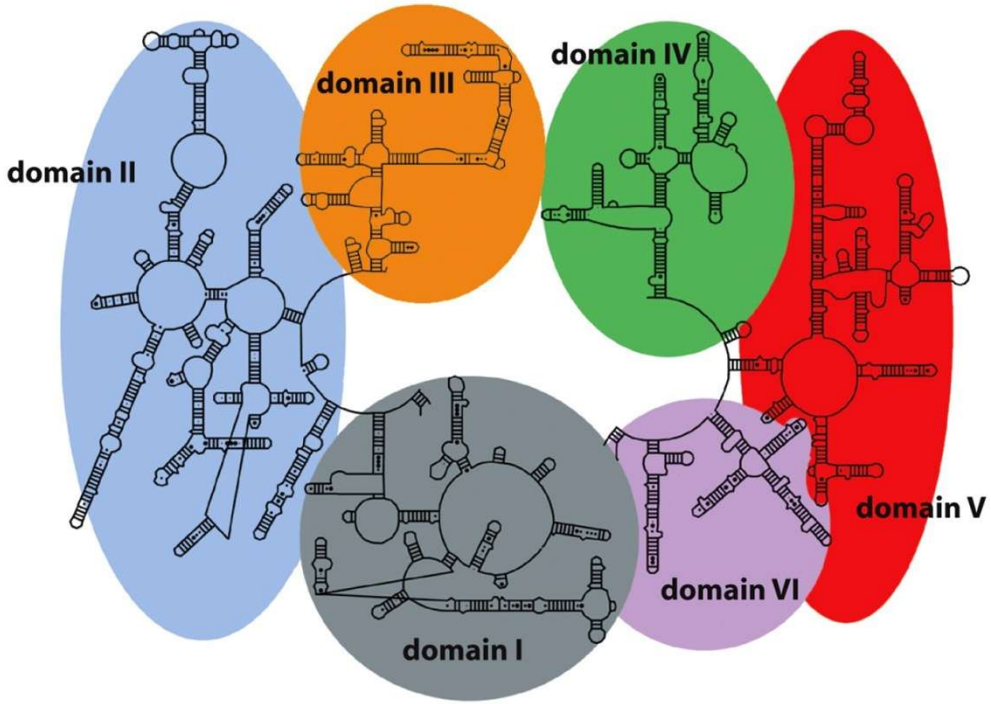


Figure 6-69b Molecular Biology of the Cell 5/e (© Garland Science 2008)

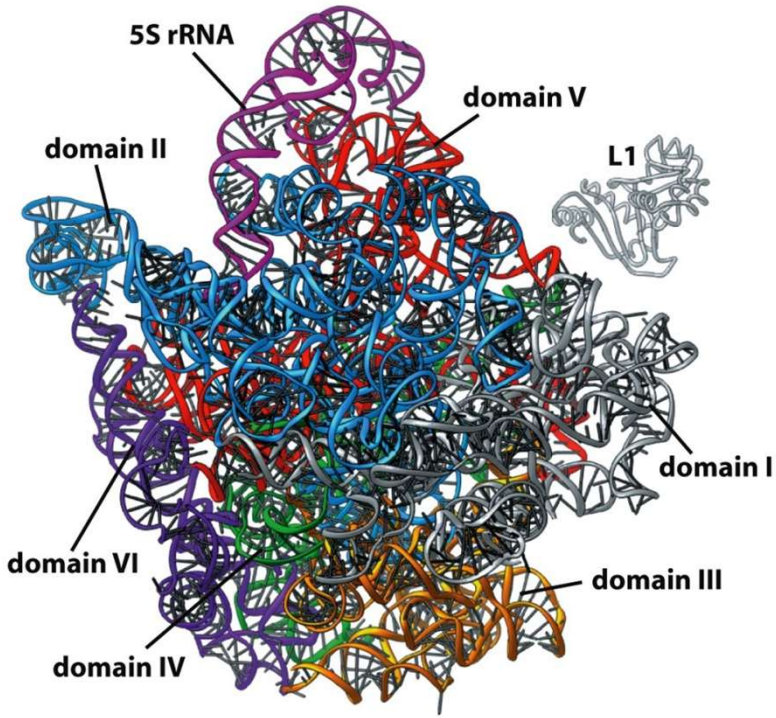


Figure 6-69a Molecular Biology of the Cell 5/e (© Garland Science 2008)

A nukleinsav azonosítás alapvető metodikája a hibridizáció

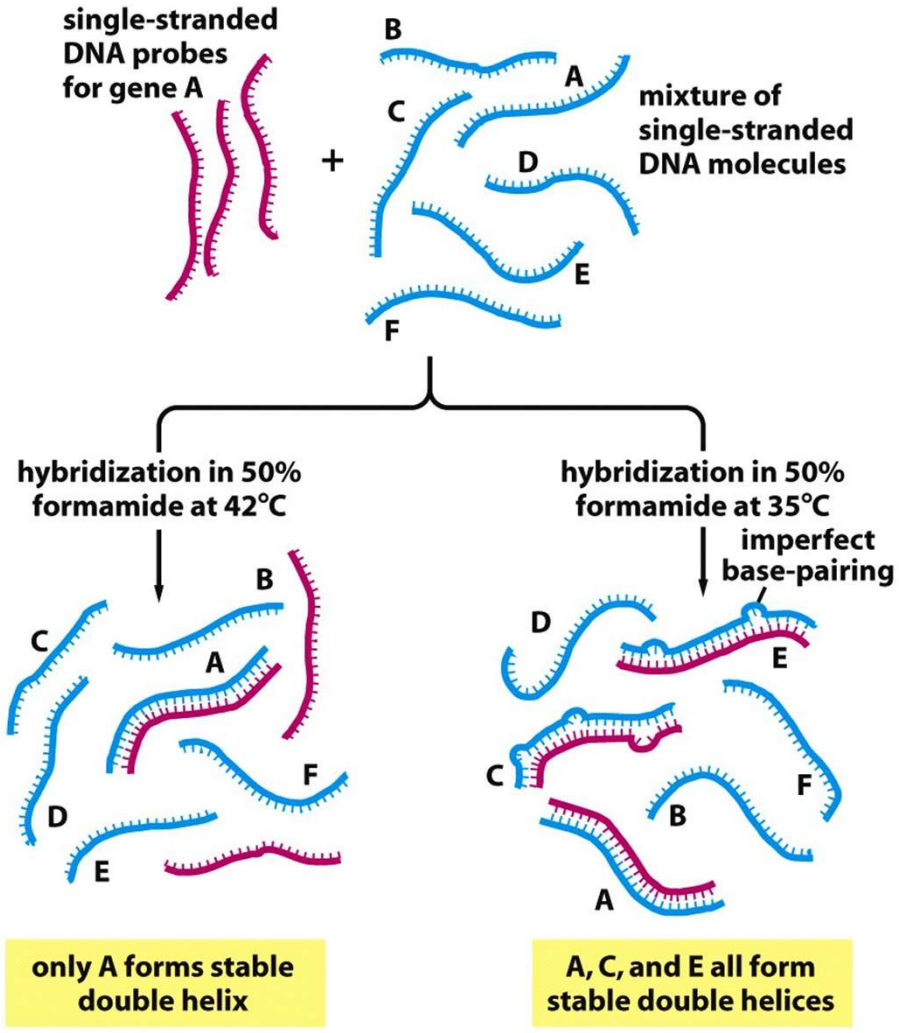


Figure 8-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Southern és Northern blot analízis

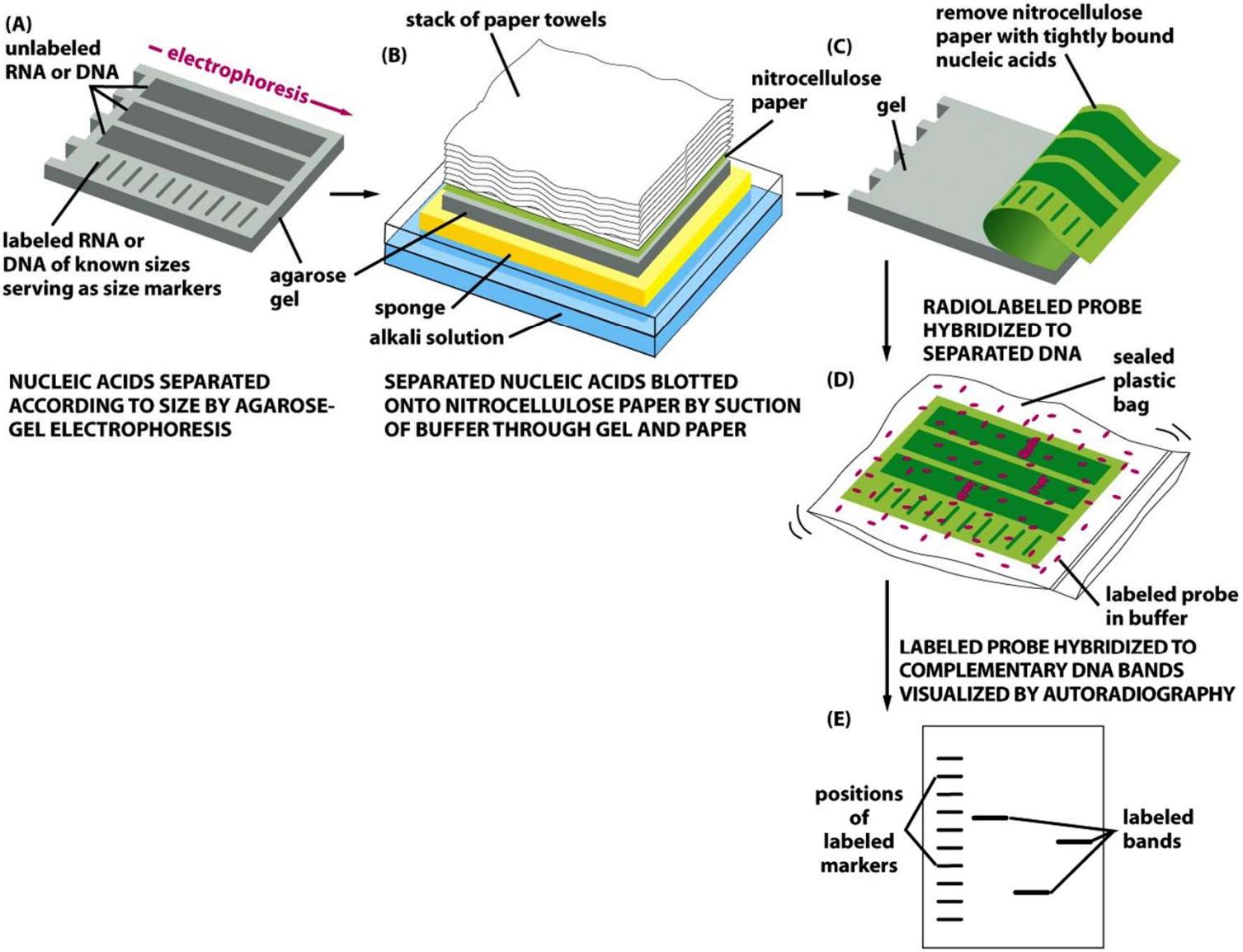
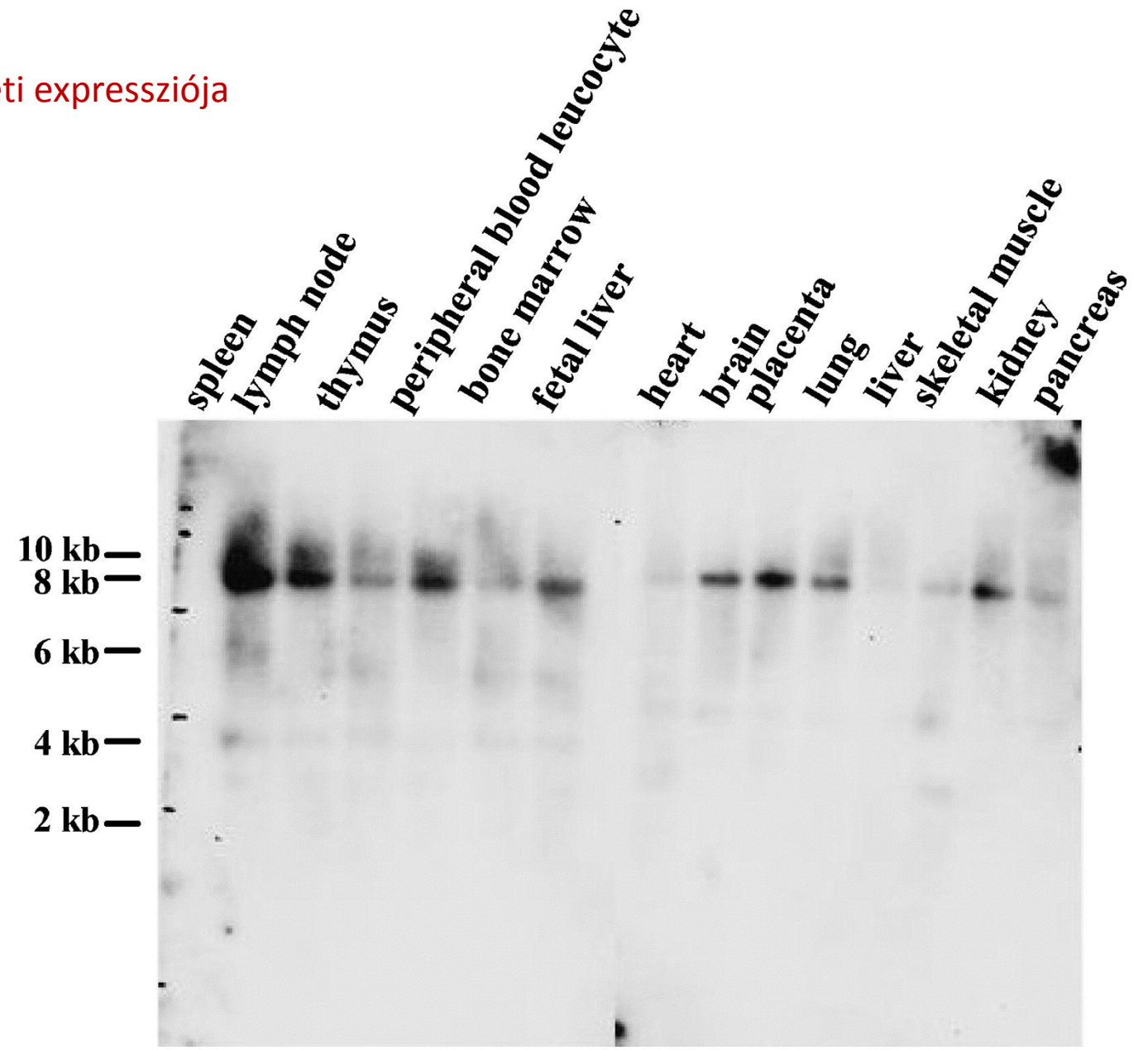


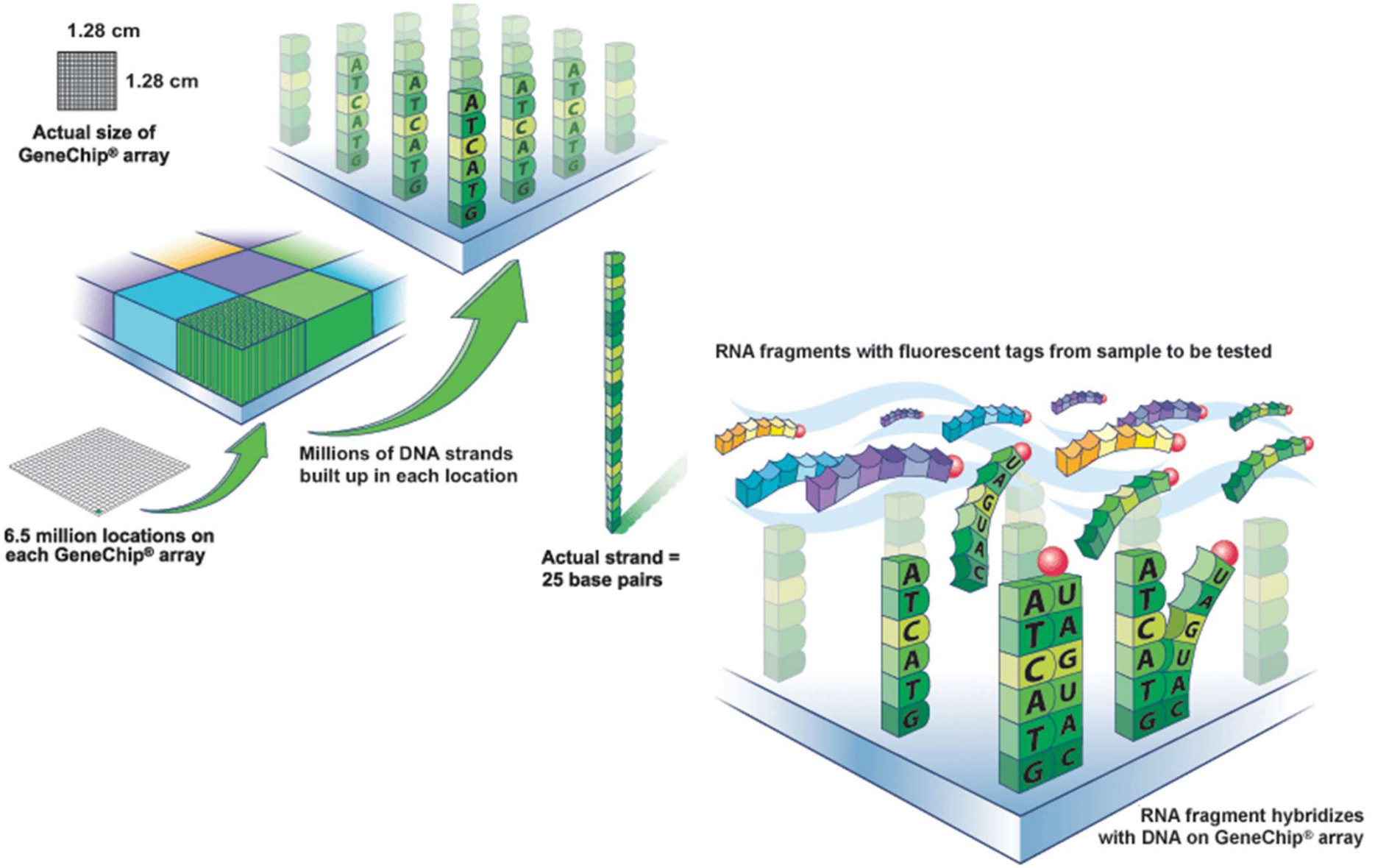
Figure 8-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

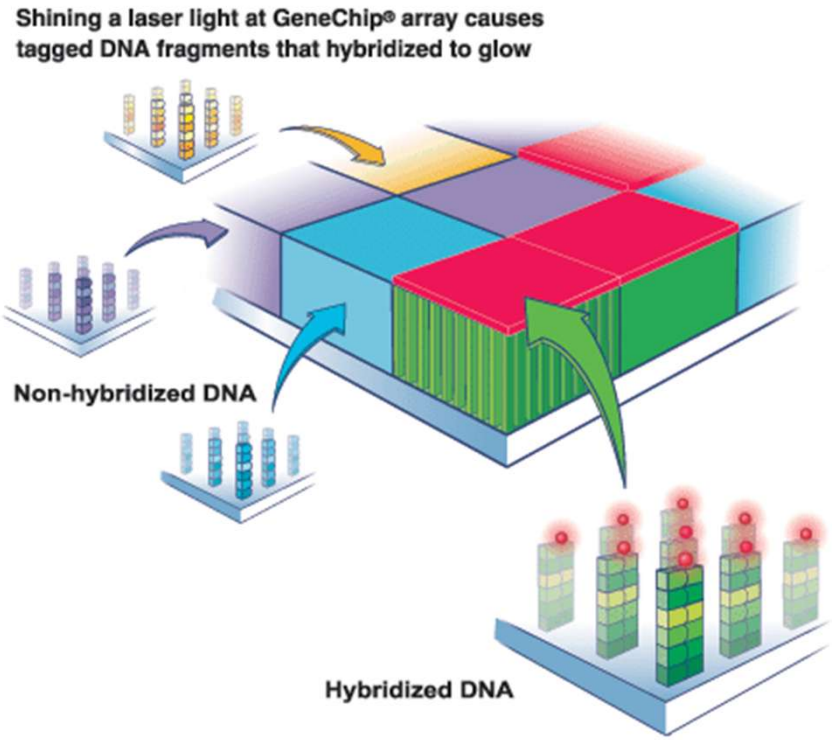
hMIN kináz szöveti expressziója

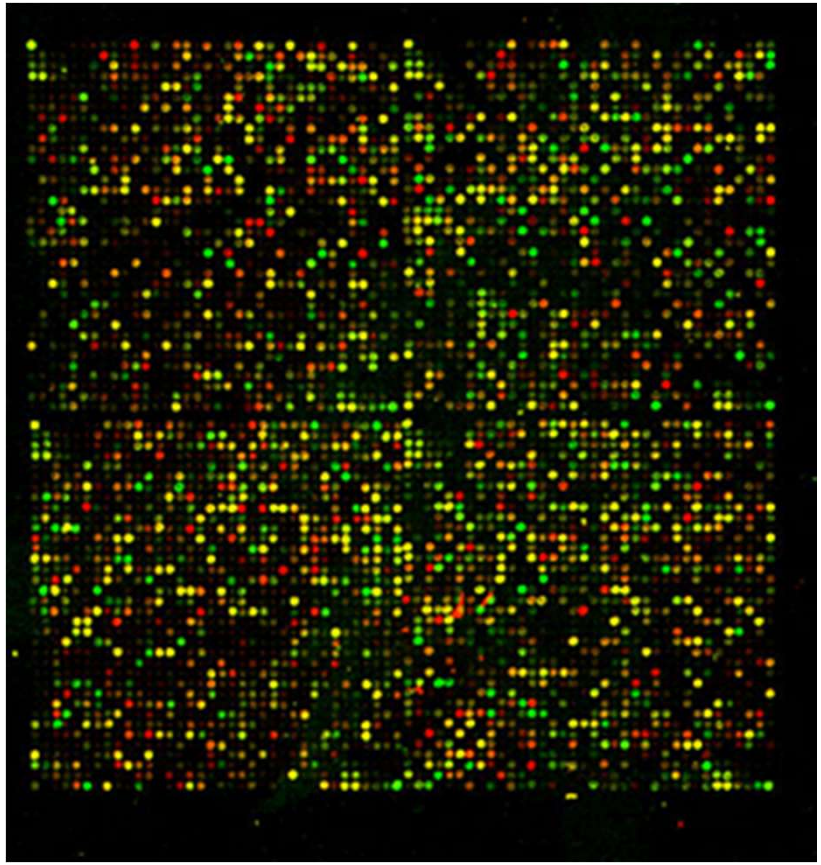


Biochemical and Biophysical Research Communications
Volume 300, Issue 3, 17 January 2003, Pages 694–698

Hibridizáció nagyléptékben, microarray




Shining a laser light at GeneChip® array causes tagged DNA fragments that hybridized to glow
Non-hybridized DNA
Hybridized DNA



Nukleinsav detektálás sokszorosítással

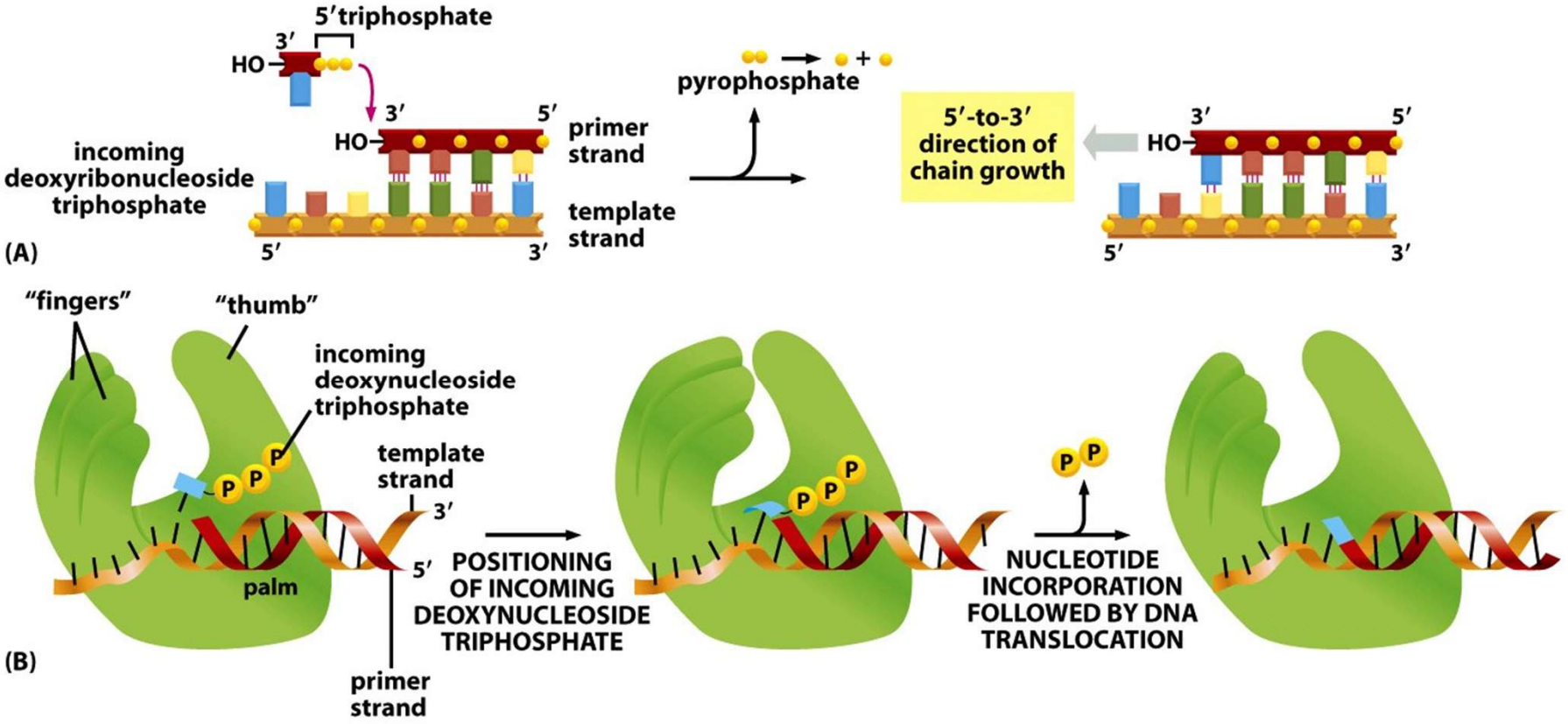


Figure 5-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Polymerase Chain Reaction

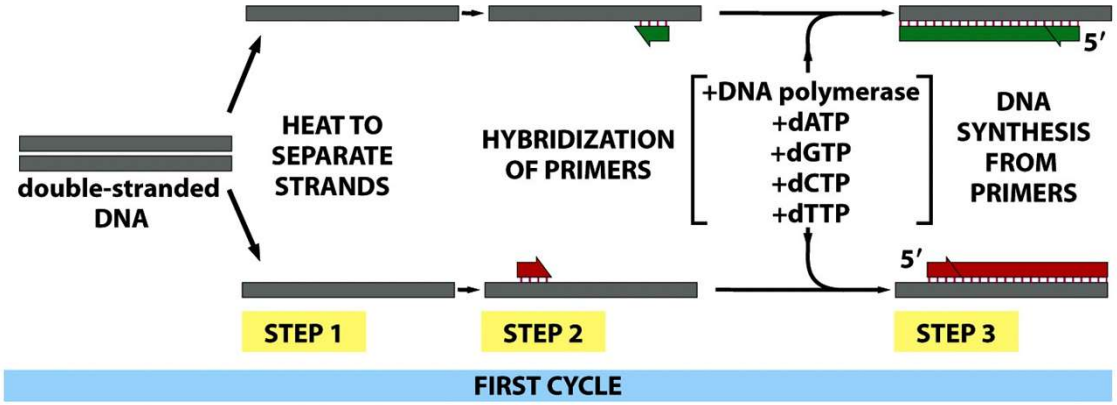


Figure 8-45a Molecular Biology of the Cell 5/e (© Garland Science 2008)

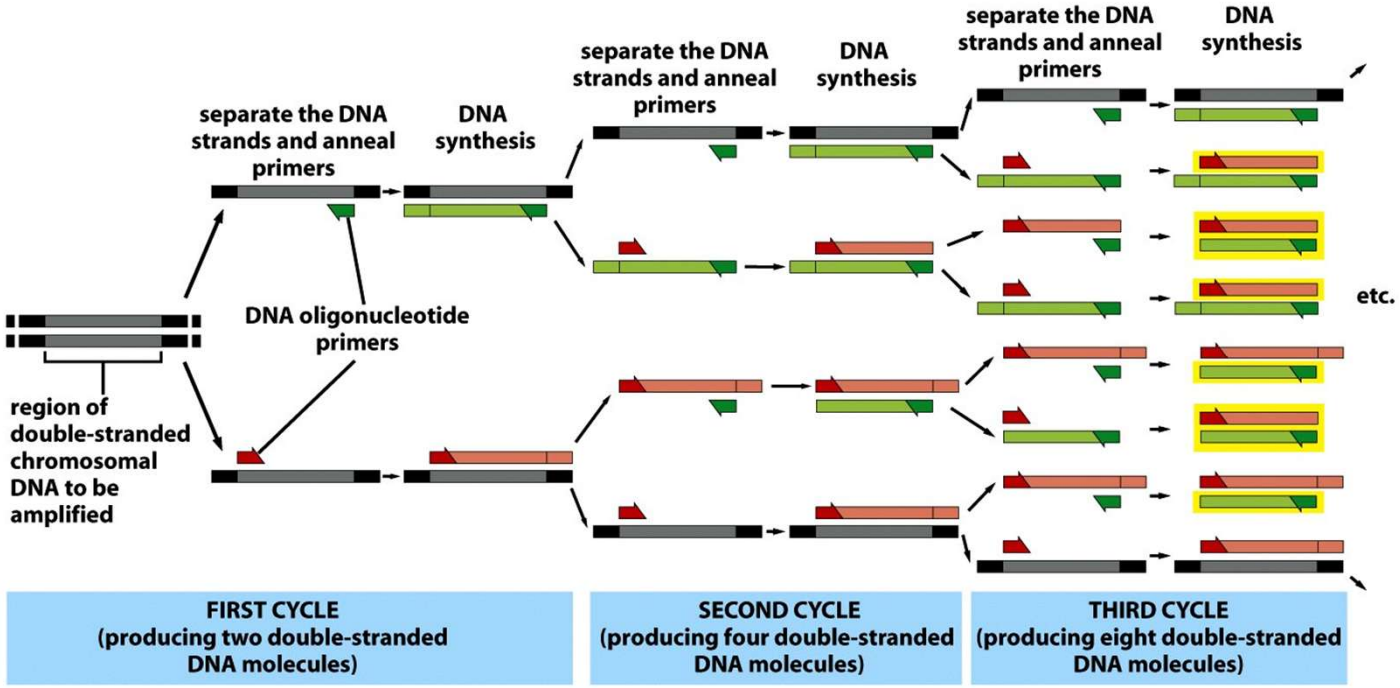


Figure 8-45b Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNS és RNS egyaránt sokszorosítható

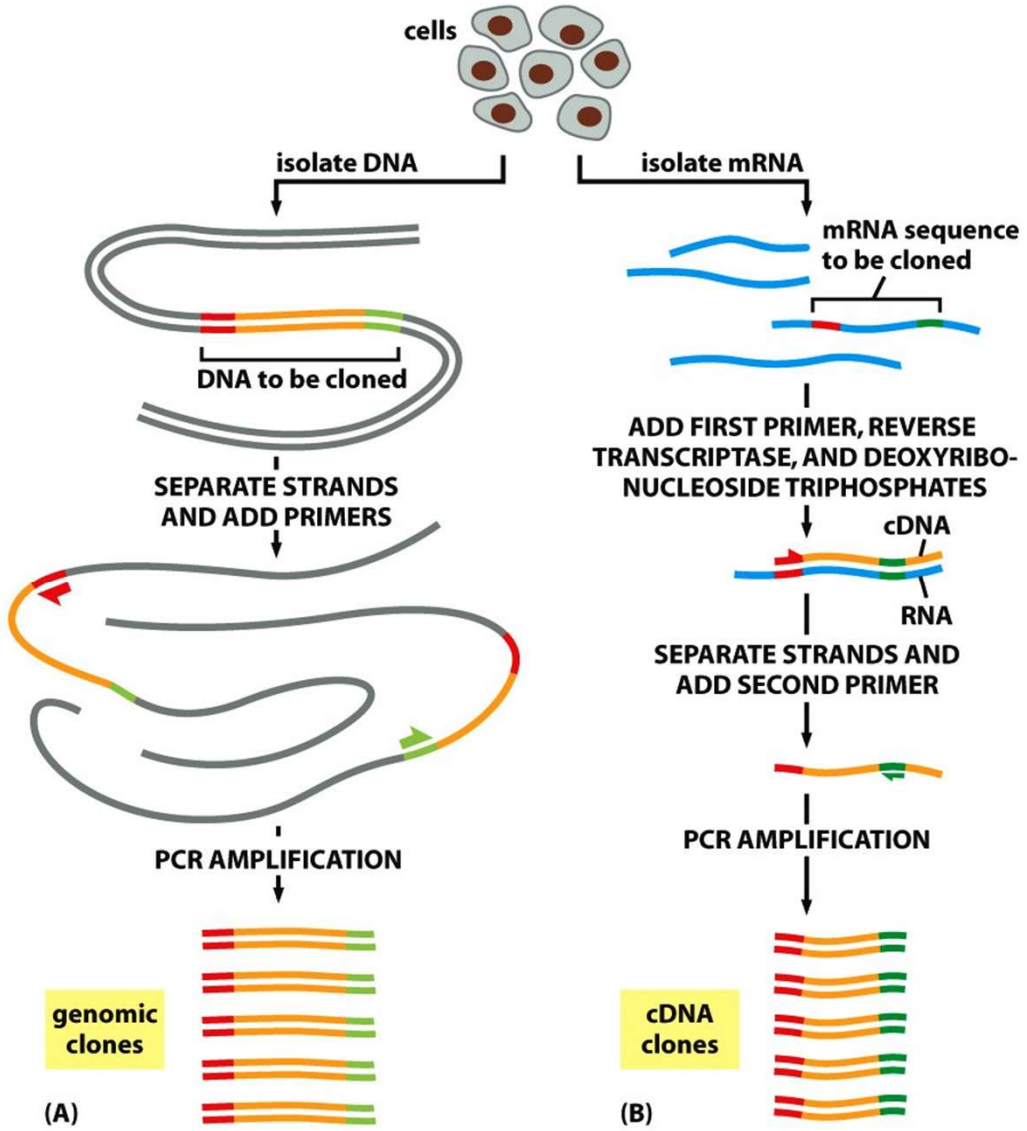


Figure 8-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

STR analízis

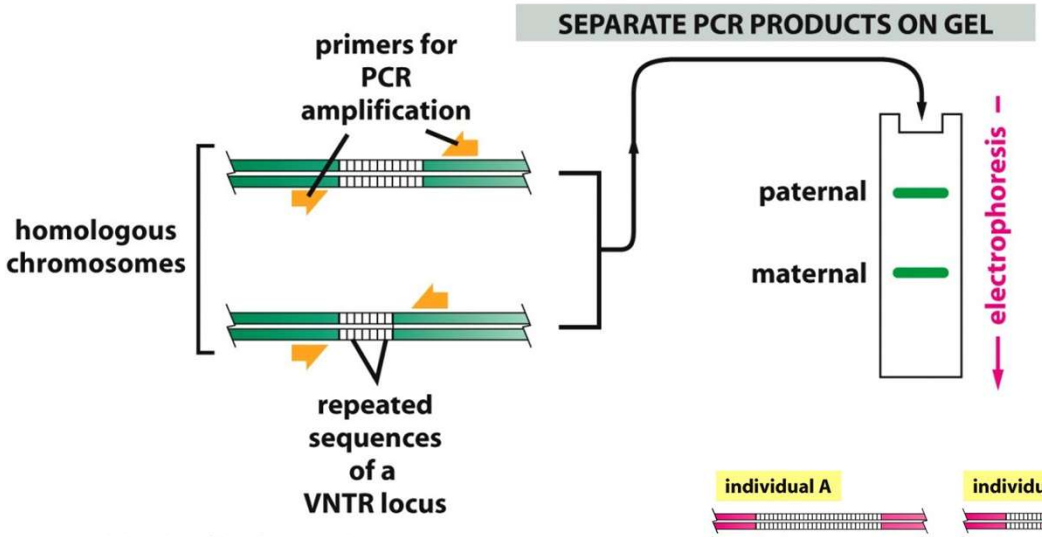
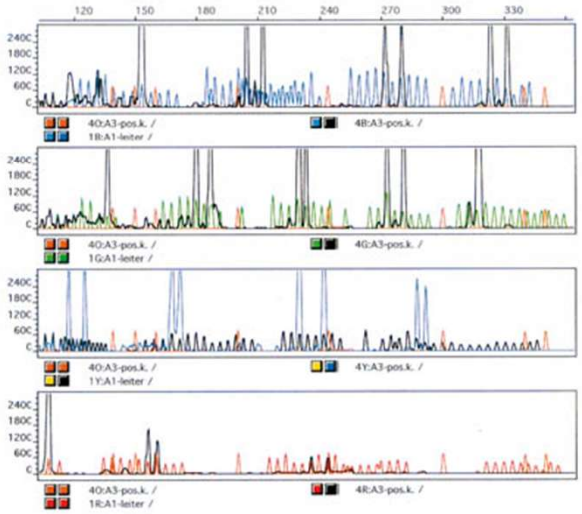


Figure 8-47a Molecular Biology of the Cell 5/e (© Garland Science 2008)



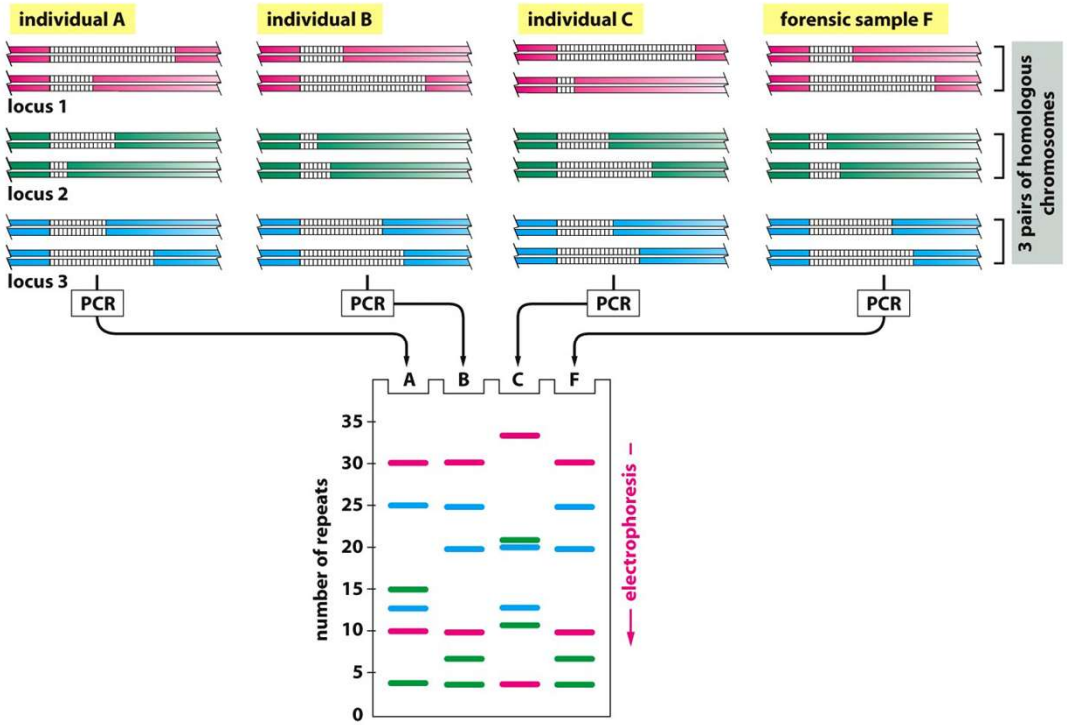
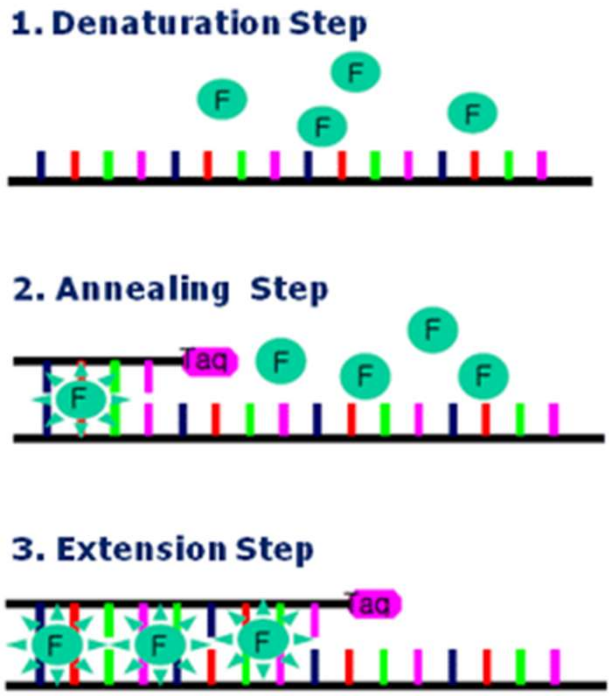
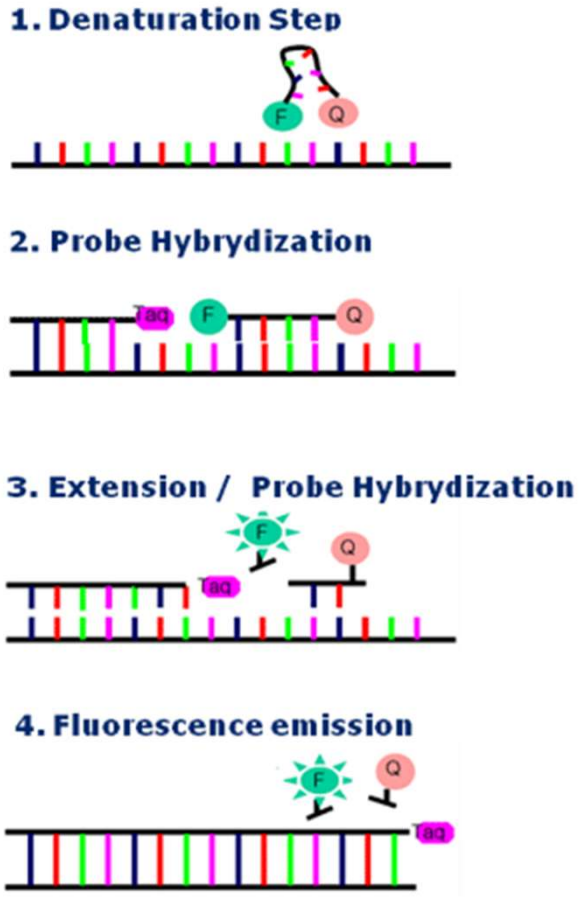


Figure 8-47b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Valós idejű PCR



Interkaláló festékkel



http://eng.bioneer.com

TaqMan próbával

Nukleinsav detektálás szekvenálással

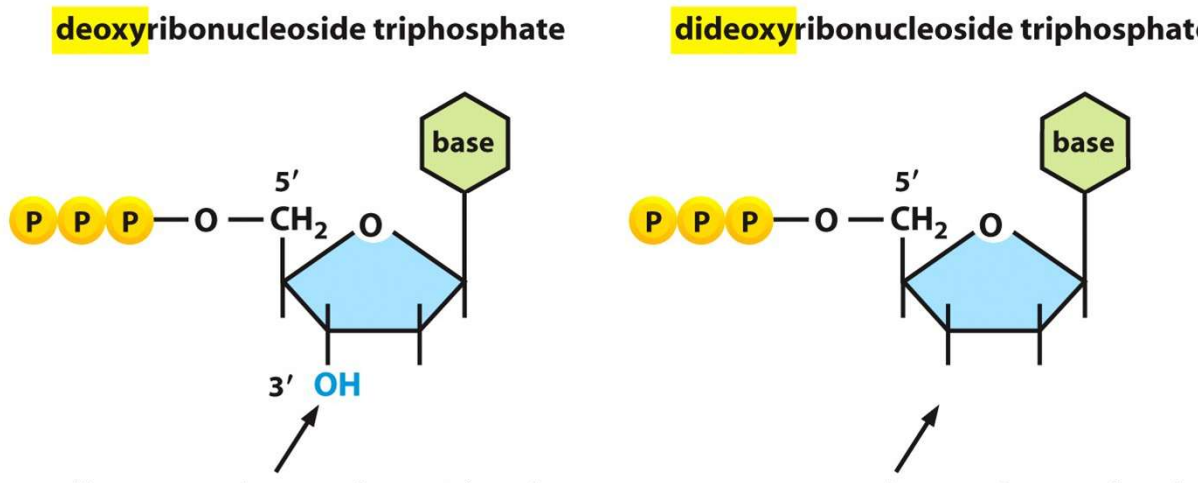


Figure 8-50a Molecular Biology of the Cell 5/e (© Garland Science 2008)

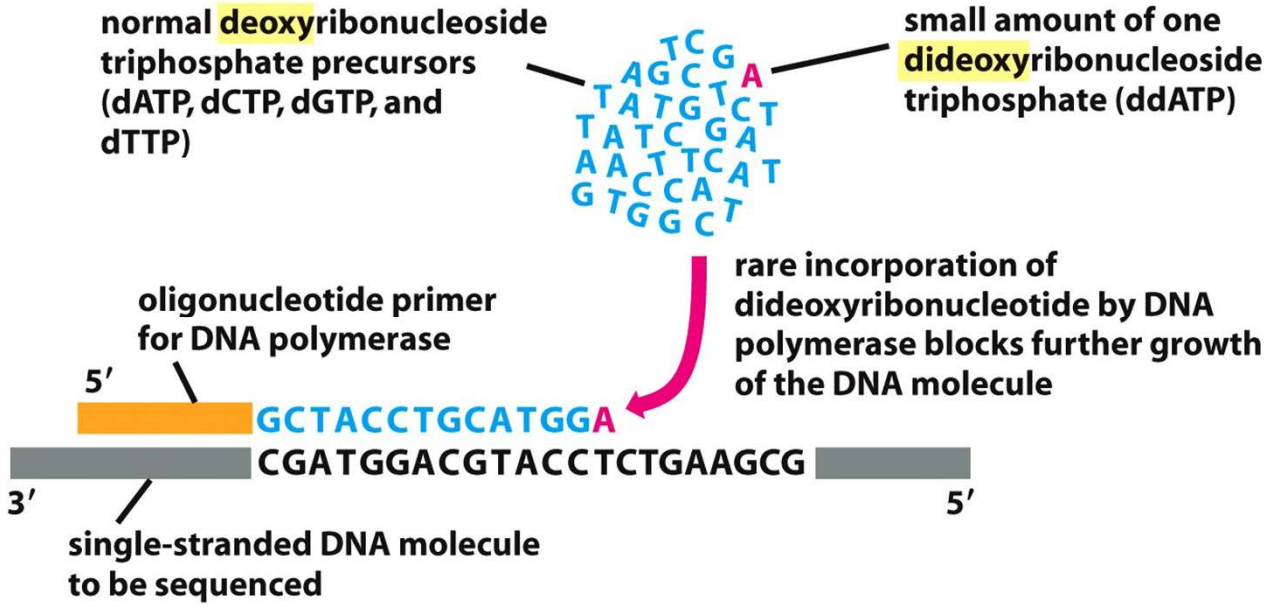
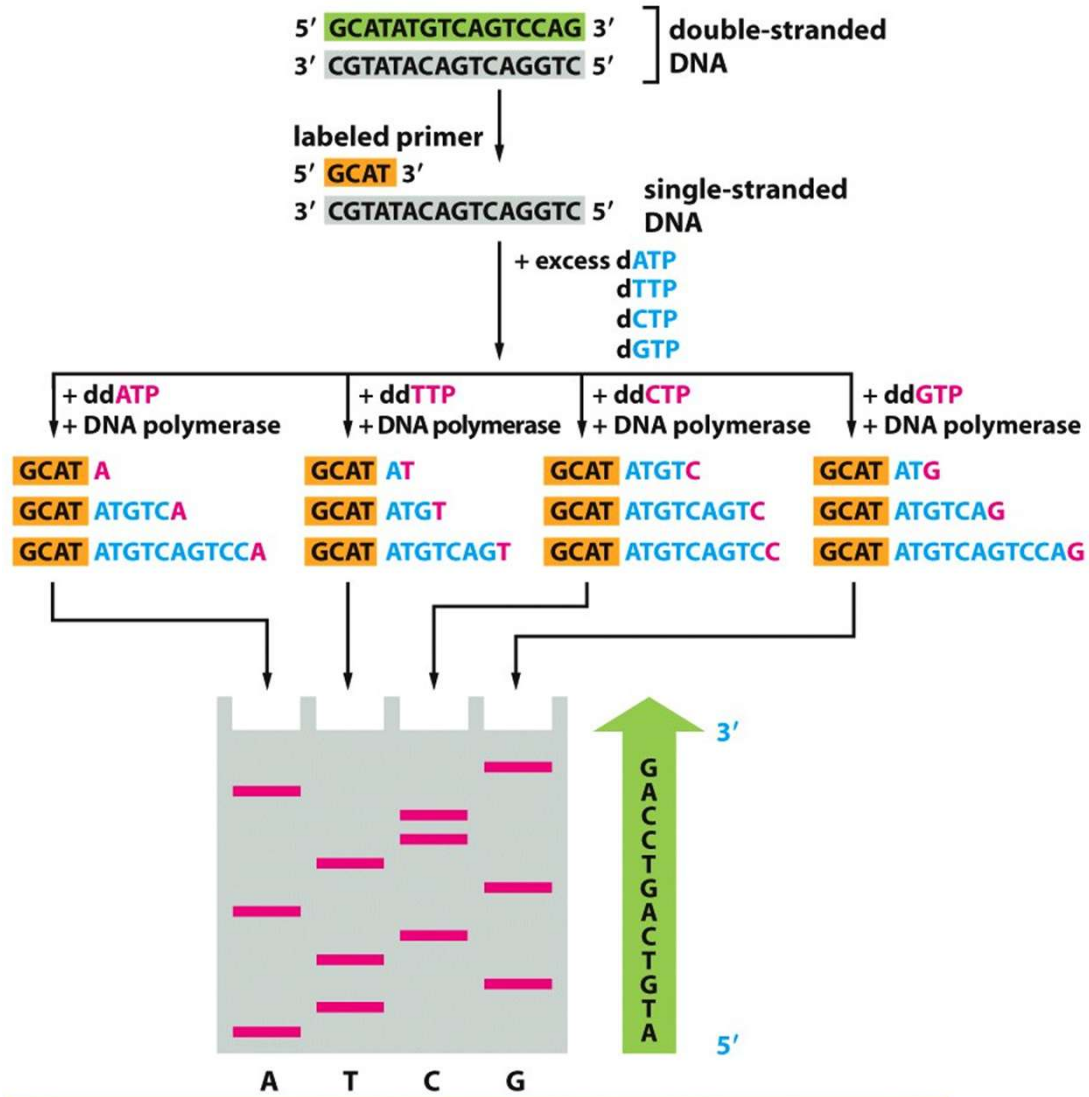


Figure 8-50b Molecular Biology of the Cell 5/e (© Garland Science 2008)



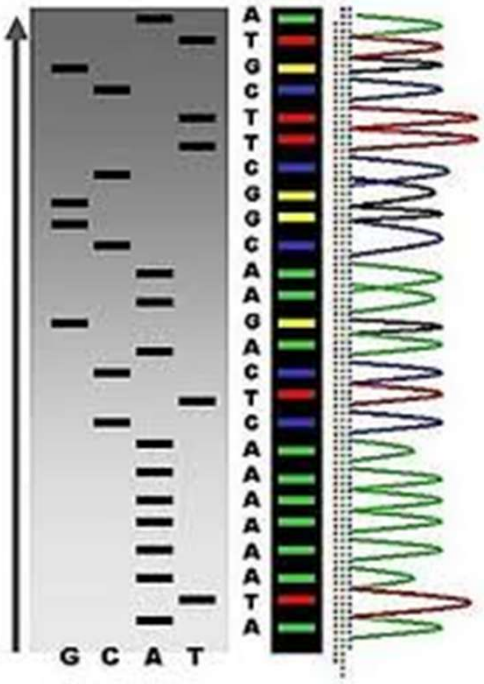
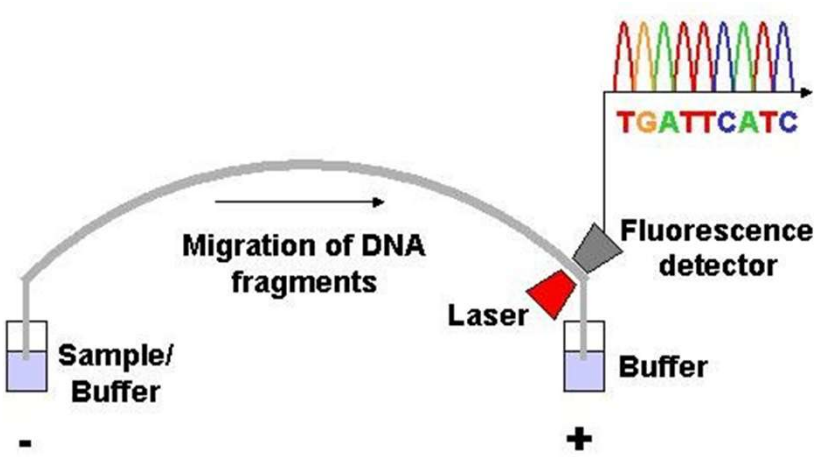
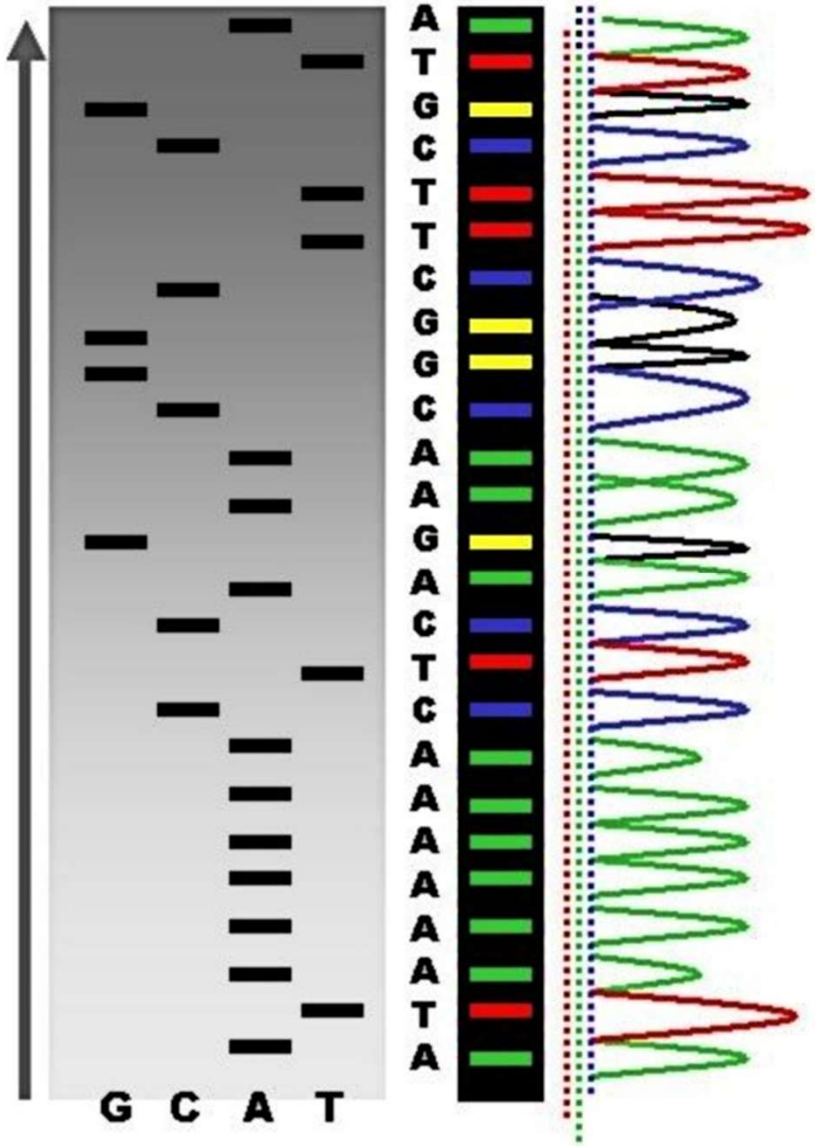


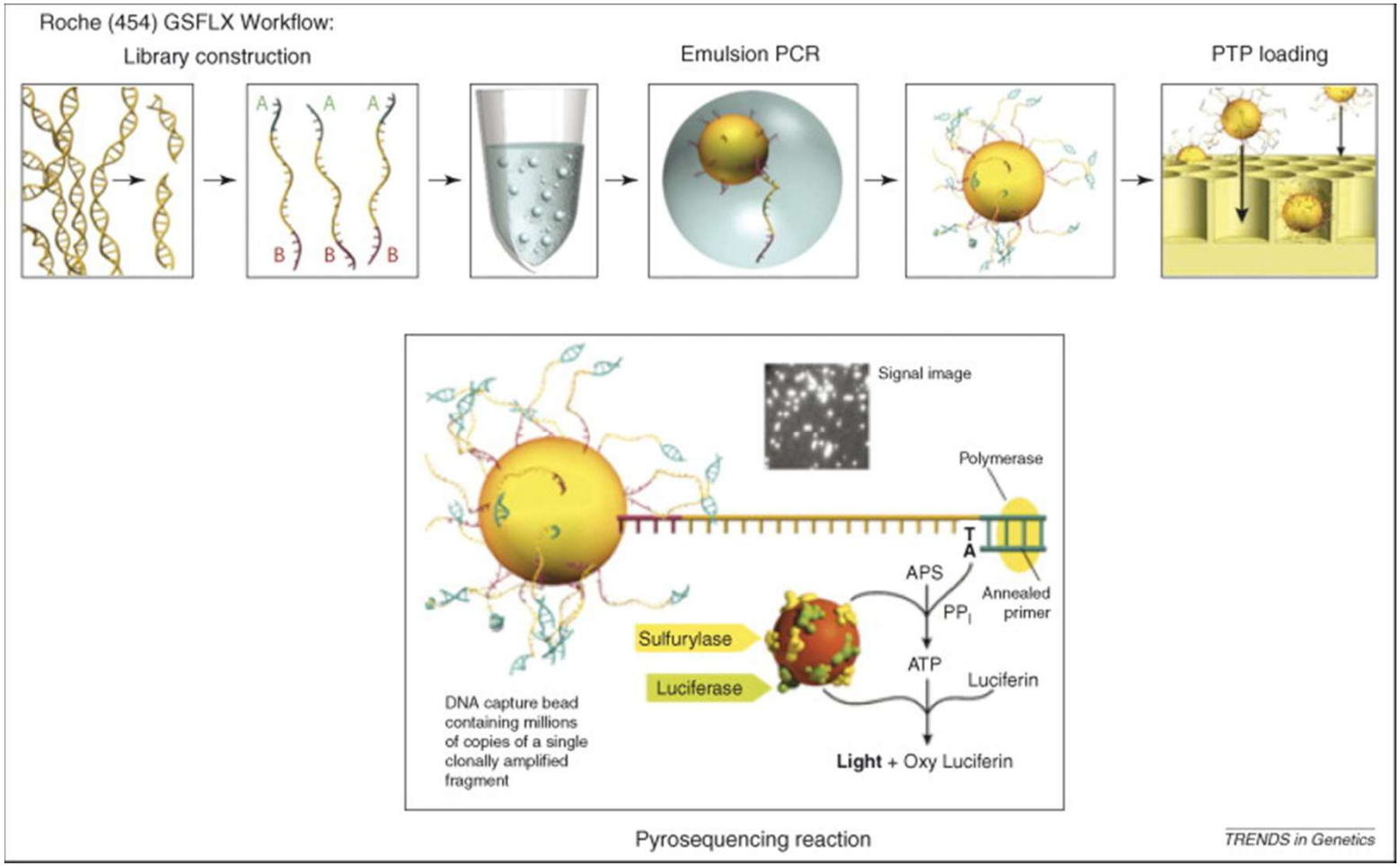
Figure 8-50c Molecular Biology of the Cell 5/e (© Garland Science 2008)

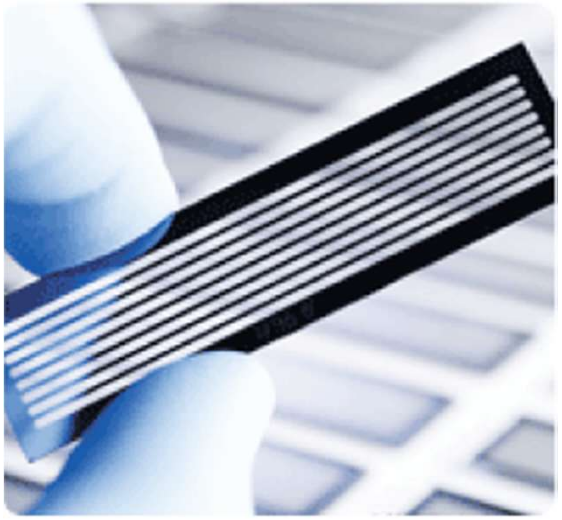

TGATTCATC
Migration of DNA fragments
Fluorescence detector
Laser
Sample/ Buffer
Buffer
-
+

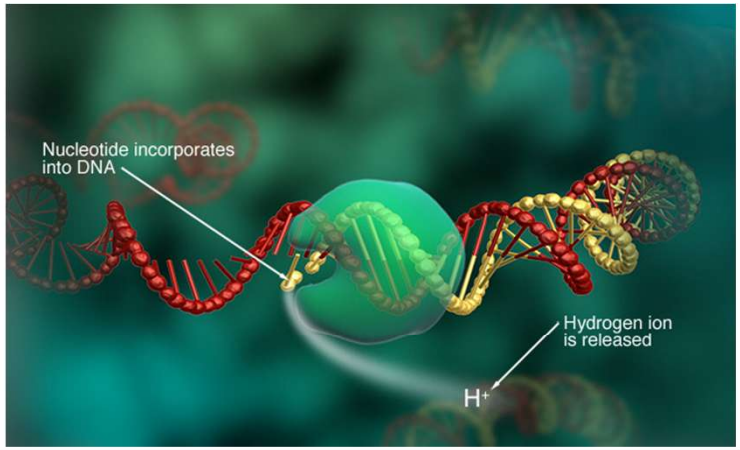

377-387

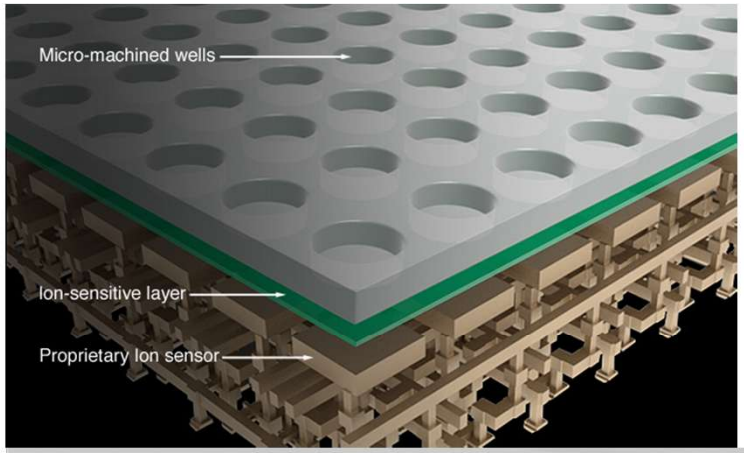

A T G C T T C G G C A A G A C T C A A A A A A A T A
G C A T

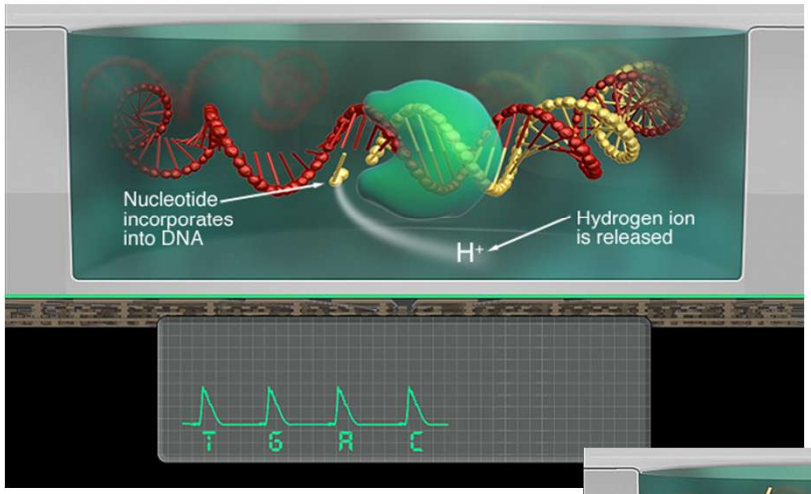
Következő generációs szekvenálások

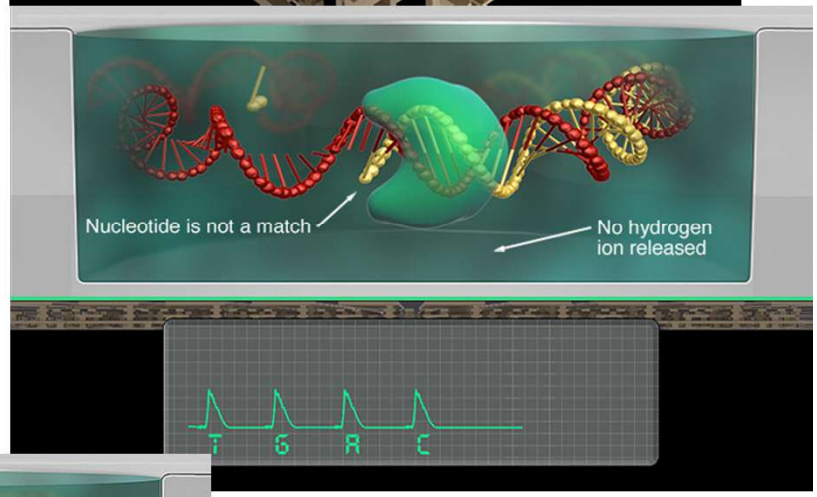


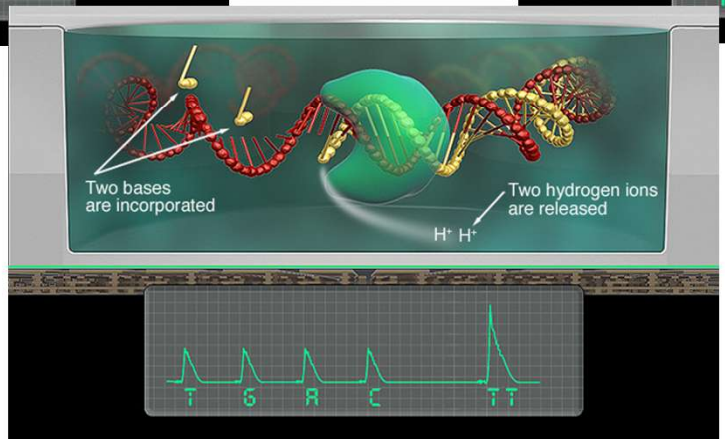



Nucleotide incorporates into DNA
Hydrogen ion is released
H+


Micro-machined wells
Ion-sensitive layer
Proprietary Ion sensor


Nucleotide incorporates into DNA
Hydrogen ion is released
H+
T G A C

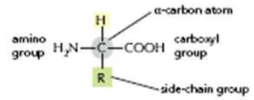

Nucleotide is not a match
No hydrogen ion released
T G A C


Two bases are incorporated
Two hydrogen ions are released
H+ H+
T G A C TT

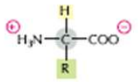
A fehérjék változatos tulajdonságú aminosavakból épülnek fel

THE AMINO ACID

The general formula of an amino acid is

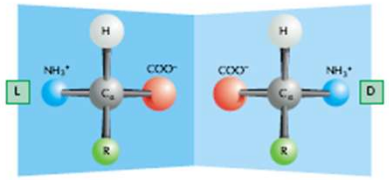


R is commonly one of 20 different side chains. At pH 7 both the amino and carboxyl groups are ionized.



OPTICAL ISOMERS

The α-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

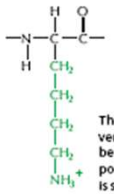
- acidic
- basic
- uncharged polar
- nonpolar

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

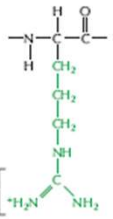
Thus: alanine = Ala = A

BASIC SIDE CHAINS

lysine (Lys, or K)

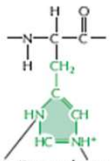


arginine (Arg, or R)



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

histidine (His, or H)

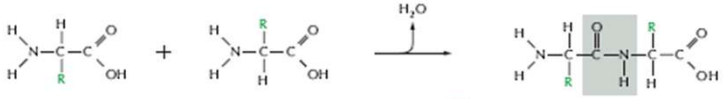


These nitrogens have a relatively weak affinity for an H^+ and are only partly positive at neutral pH.

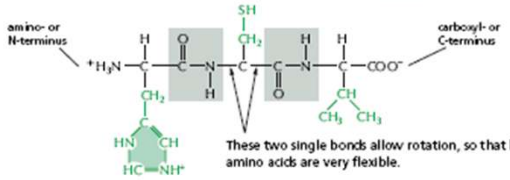
PEPTIDE BONDS

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.



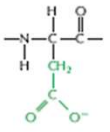
Proteins are long polymers of amino acids linked by peptide bonds, and they are always written with the N-terminus toward the left. The sequence of this tripeptide is histidine-cysteine-valine.



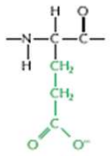
These two single bonds allow rotation, so that long chains of amino acids are very flexible.

ACIDIC SIDE CHAINS

aspartic acid (Asp, or D)

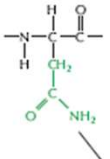


glutamic acid (Glu, or E)

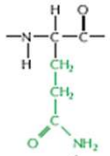


UNCHARGED POLAR SIDE CHAINS

asparagine (Asn, or N)

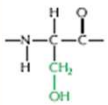


glutamine (Gln, or Q)

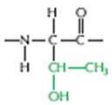


Although the amide N is not charged at neutral pH, it is polar.

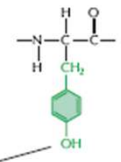
serine (Ser, or S)



threonine (Thr, or T)



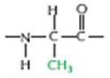
tyrosine (Tyr, or Y)



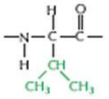
The –OH group is polar.

NONPOLAR SIDE CHAINS

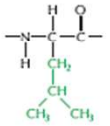
alanine (Ala, or A)



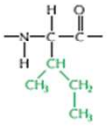
valine (Val, or V)



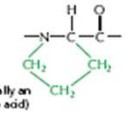
leucine (Leu, or L)



isoleucine (Ile, or I)

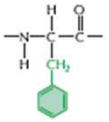


proline (Pro, or P)

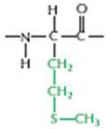


(actually an imino acid)

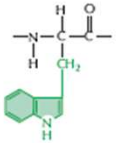
phenylalanine (Phe, or F)



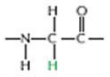
methionine (Met, or M)



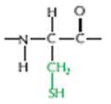
tryptophan (Trp, or W)



glycine (Gly, or G)



cysteine (Cys, or C)



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

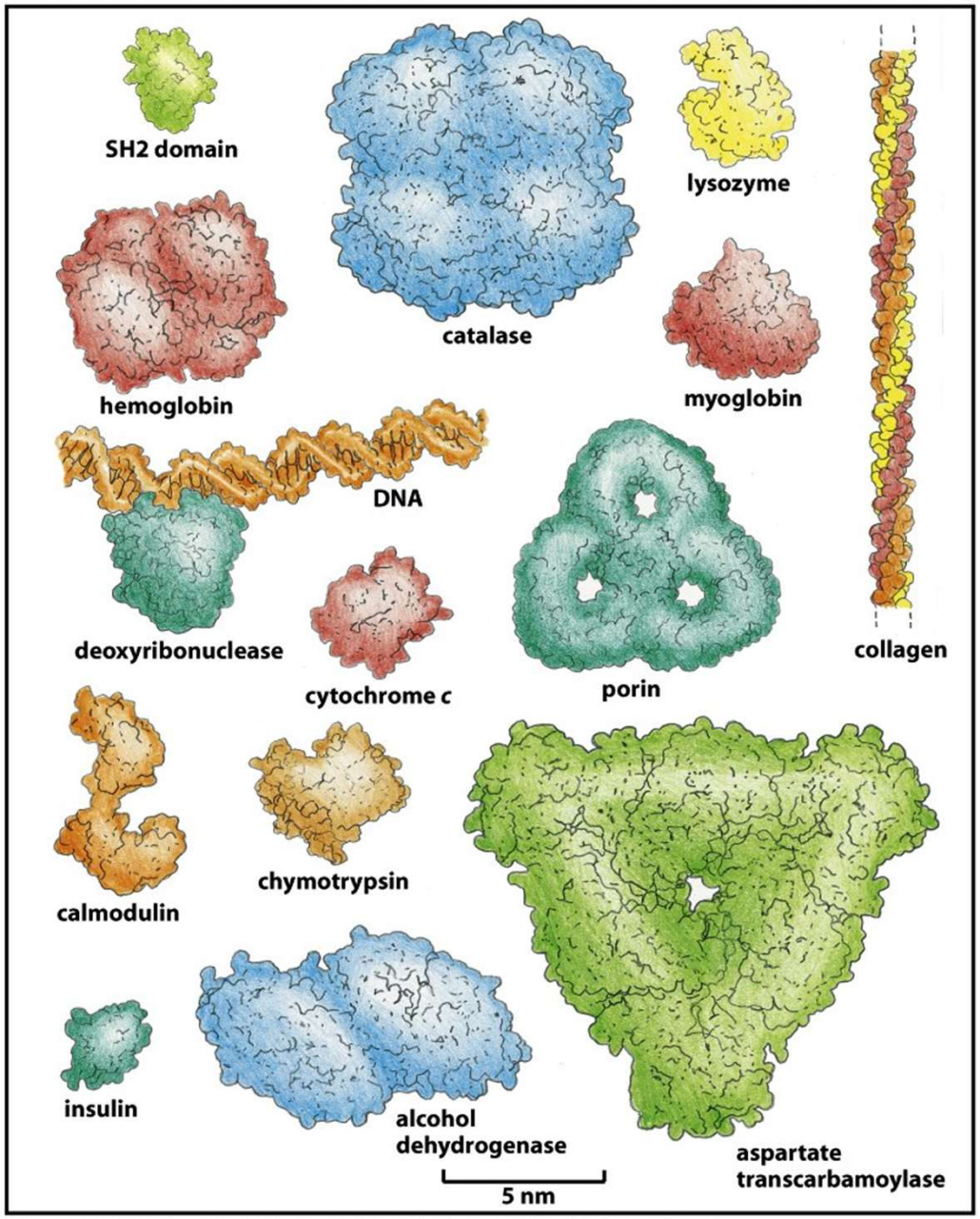
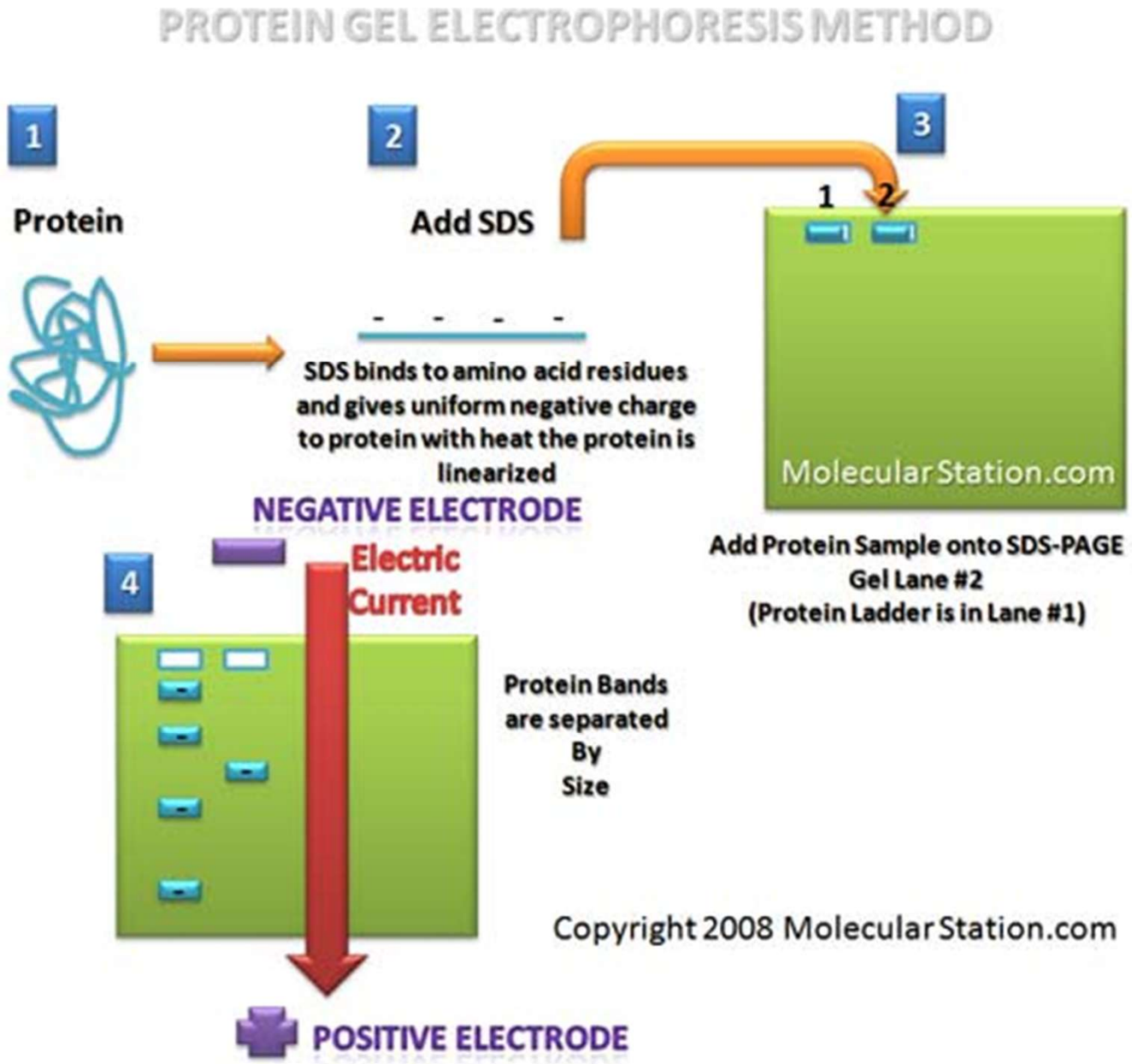
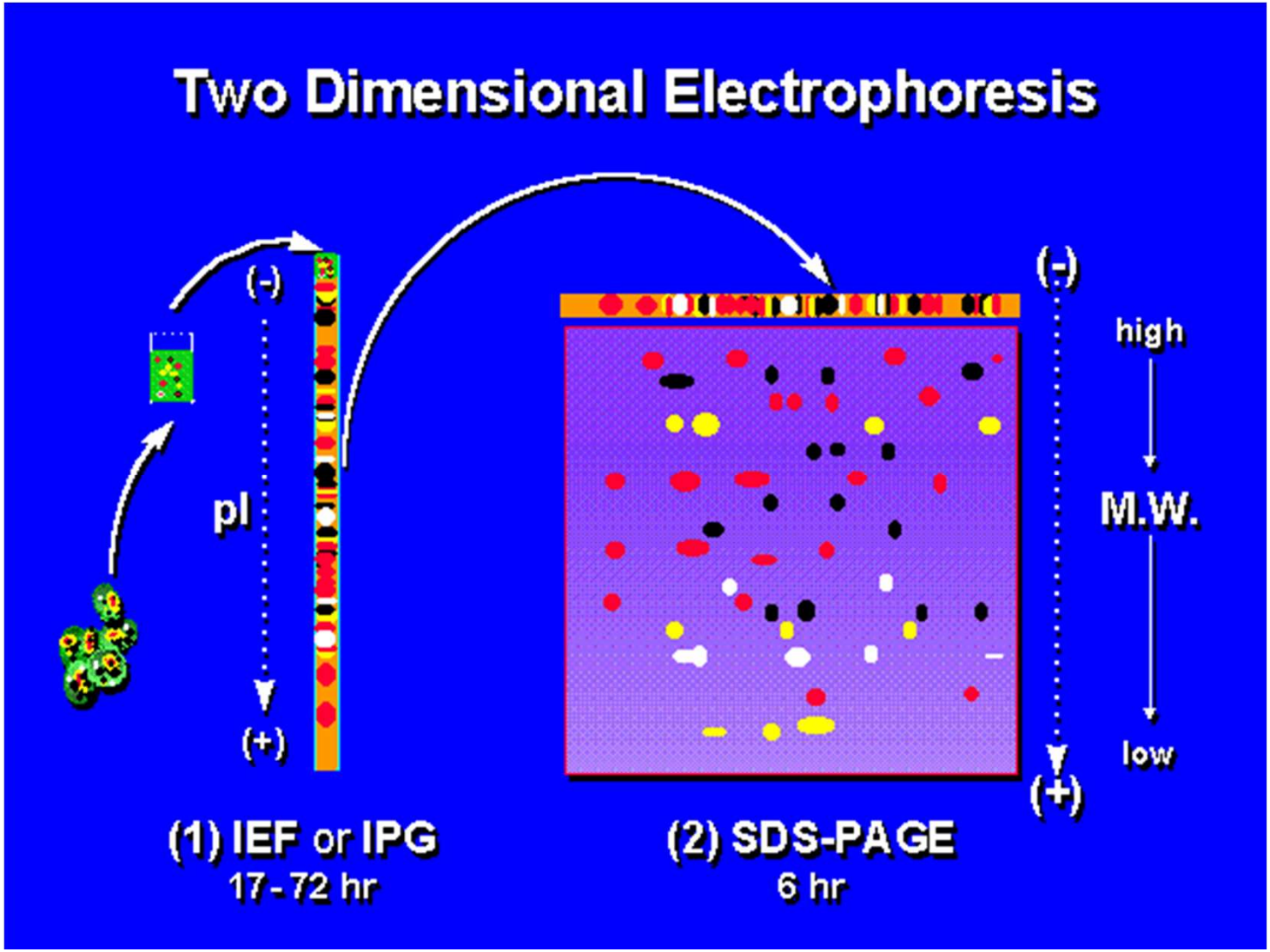
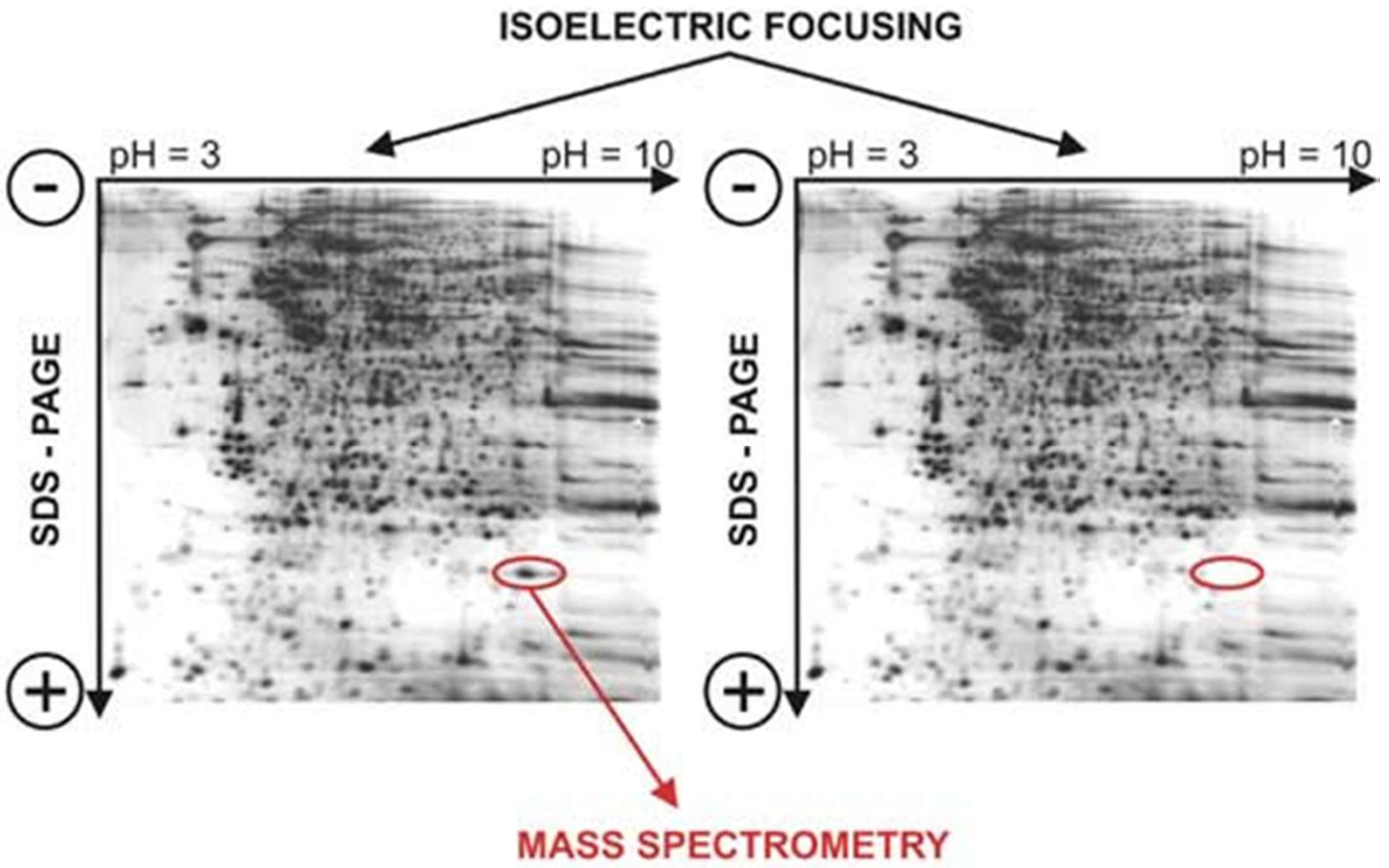


Figure 3-23 Molecular Biology of the Cell 5/e (© Garland Science 2008)


PROTEIN GEL ELECTROPHORESIS METHOD
1
Protein
2
Add SDS
3
1
2
SDS binds to amino acid residues and gives uniform negative charge to protein with heat the protein is linearized
MolecularStation.com
Add Protein Sample onto SDS-PAGE Gel Lane #2 (Protein Ladder is in Lane #1)
NEGATIVE ELECTRODE
4
Electric Current
Protein Bands are separated By Size
Copyright 2008 MolecularStation.com
POSITIVE ELECTRODE

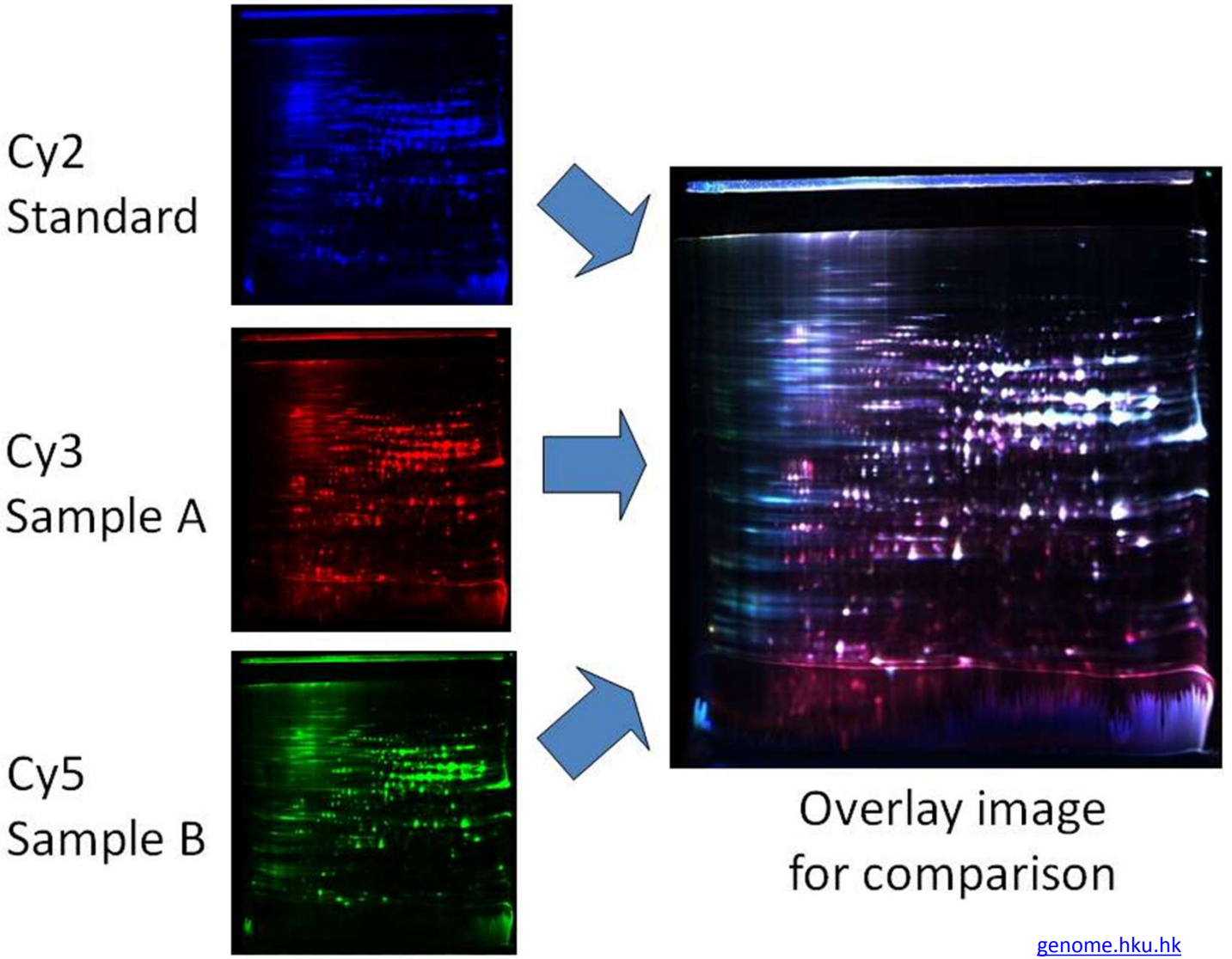


http://en.wikibooks.org/



http://biol.lf1.cuni.cz/ucebnice/en/proteomics.htm

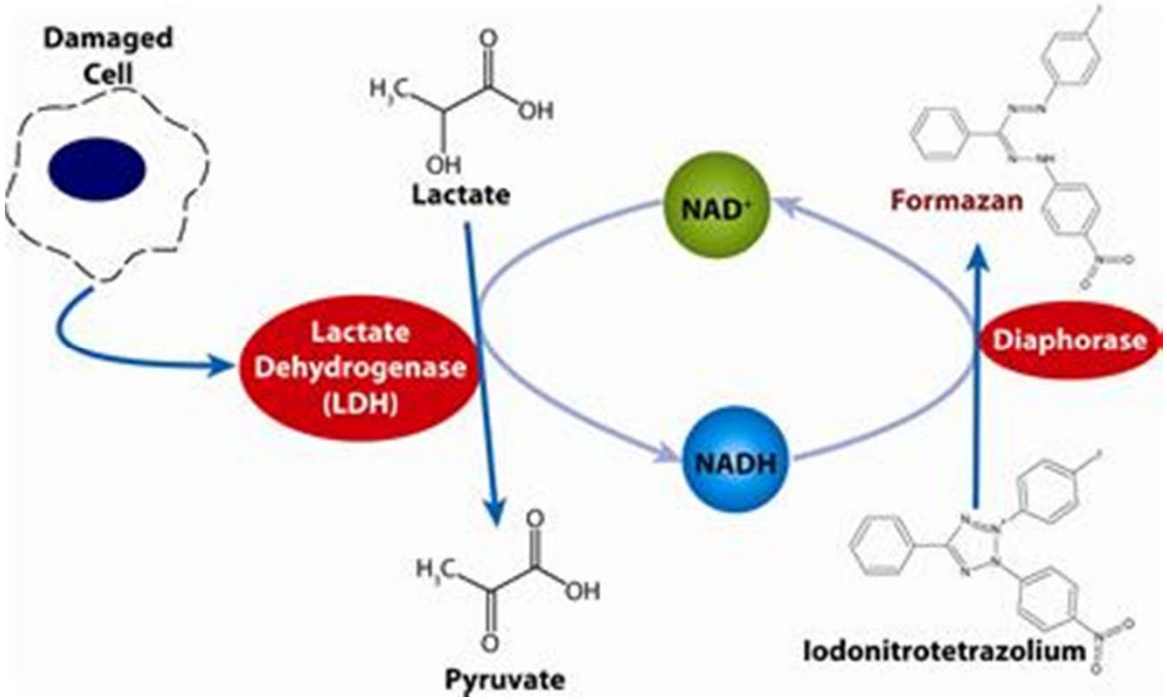
Difference Gel Electrophoresis



genome.hku.hk

Enzimaktivitások meghatározása

ALAT (sGPT)
ASAT (sGOT)
LDH



http://test.gbiosciences.com

Fehérjék szelektív azonosítása ellenanyagokkal

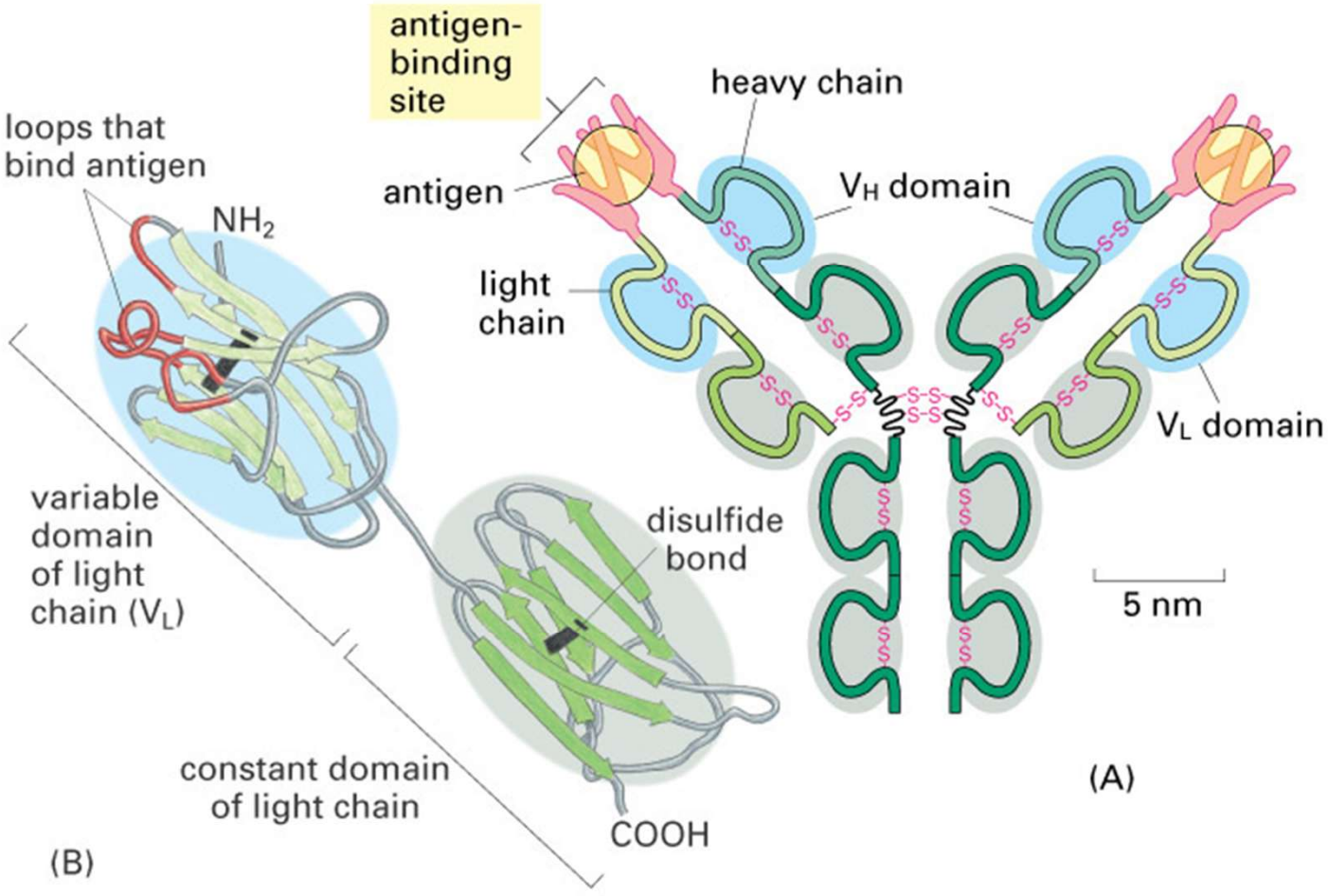


Figure 4-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Western blot

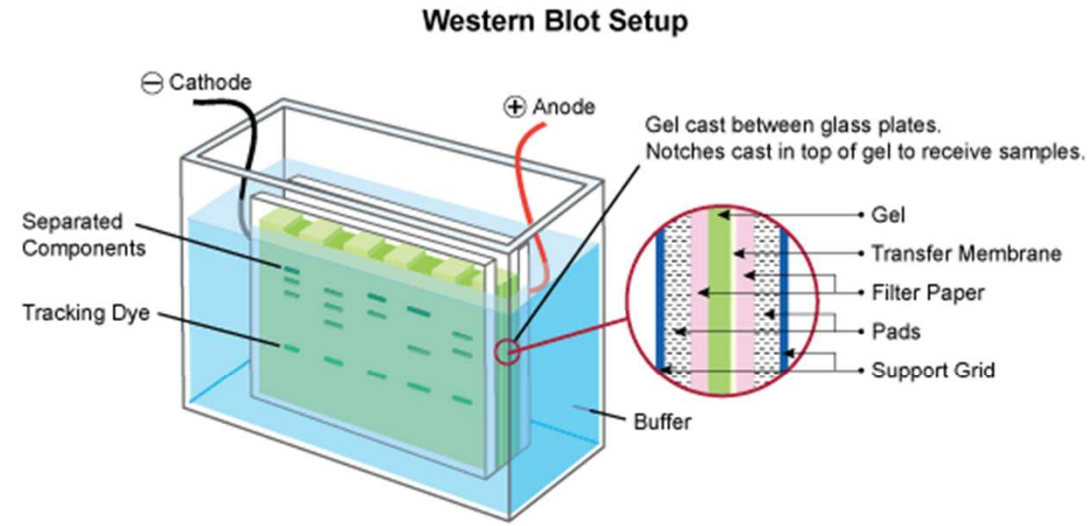


Diagram 1: *Illustration of Western Blot Setup.*

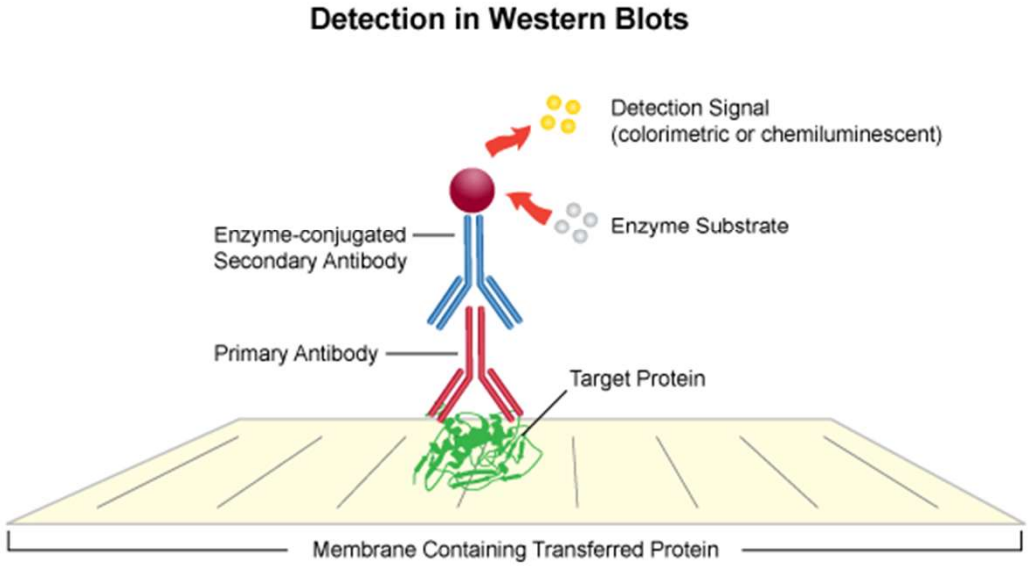
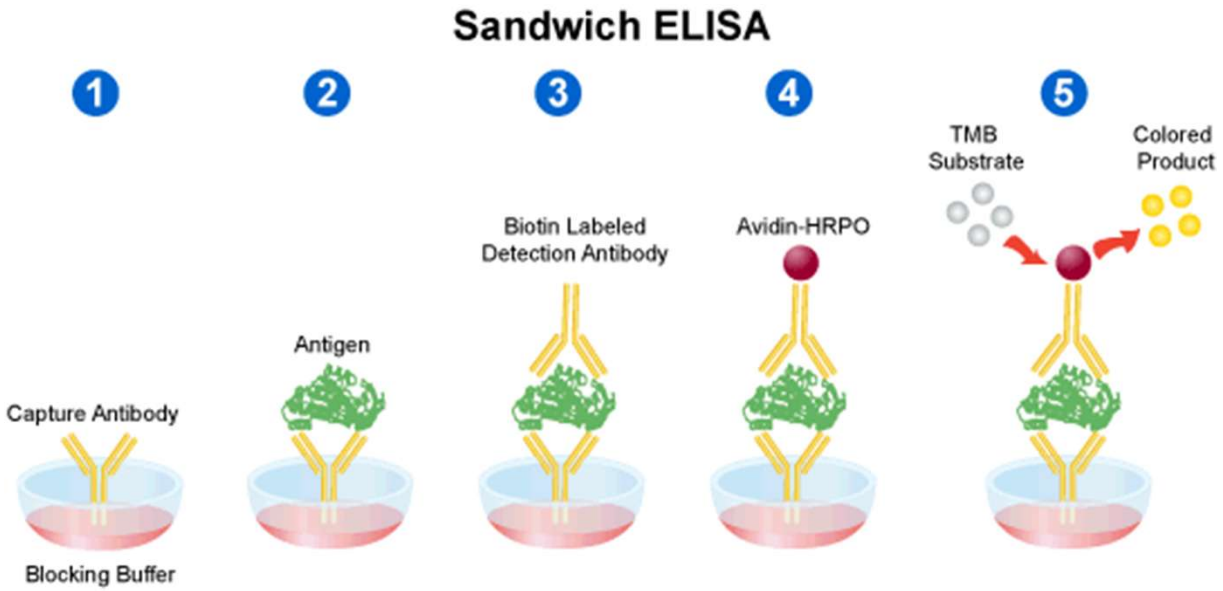


Diagram 2: *Illustration of detection in Western Blots.*

http://www.leinco.com

Enzyme-Linked Immunosorben Assay



1. **a.)** Plate is coated with a suitable capture antibody. **b.)** Blocking buffer is added to block remaining protein-binding sites on plate.
2. Sample is added to plate and any antigen present is bound by the capture antibody.
3. A suitable biotin labeled detection antibody is added to the plate and also binds to any antigen present in well.
4. UltraAvidin™-HRPO *(Leinco Prod. No. A106)* is added and binds the biotin labeled detection antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and converted by HRPO to a detectable form.

Diagram 1: *Illustration of Sandwich ELISA method.*

Indirect ELISA

1. Antigen/sample is added to plate.
2. Blocking buffer is added to block remaining protein-binding sites.
3. Next a suitable ***primary antibody*** is added.
4. A suitable ***secondary antibody – HRPO conjugate*** is then added which recognizes and binds to the primary antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and is converted by HRPO to detectable form.

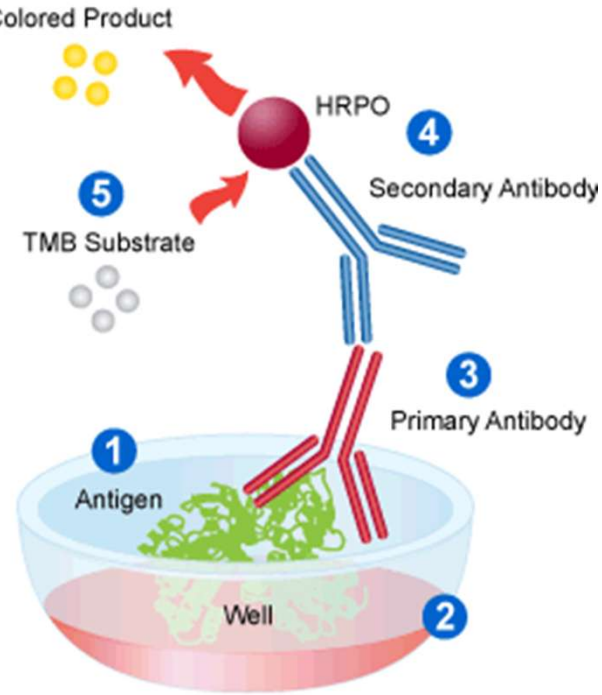
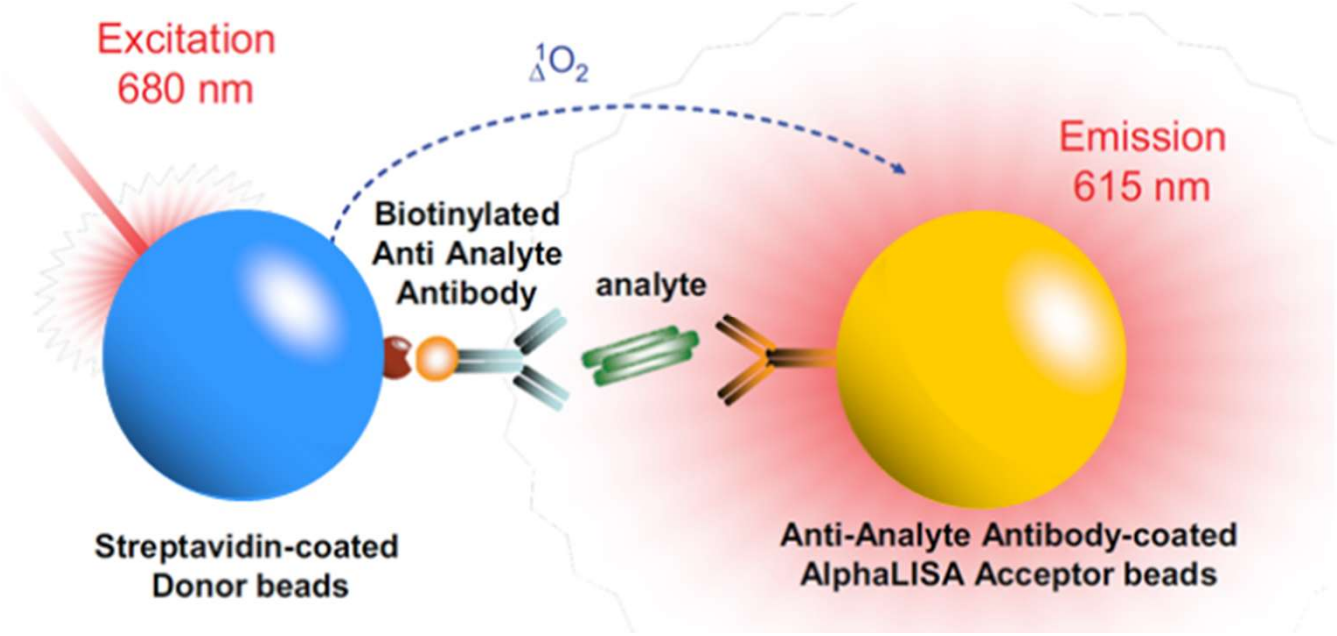
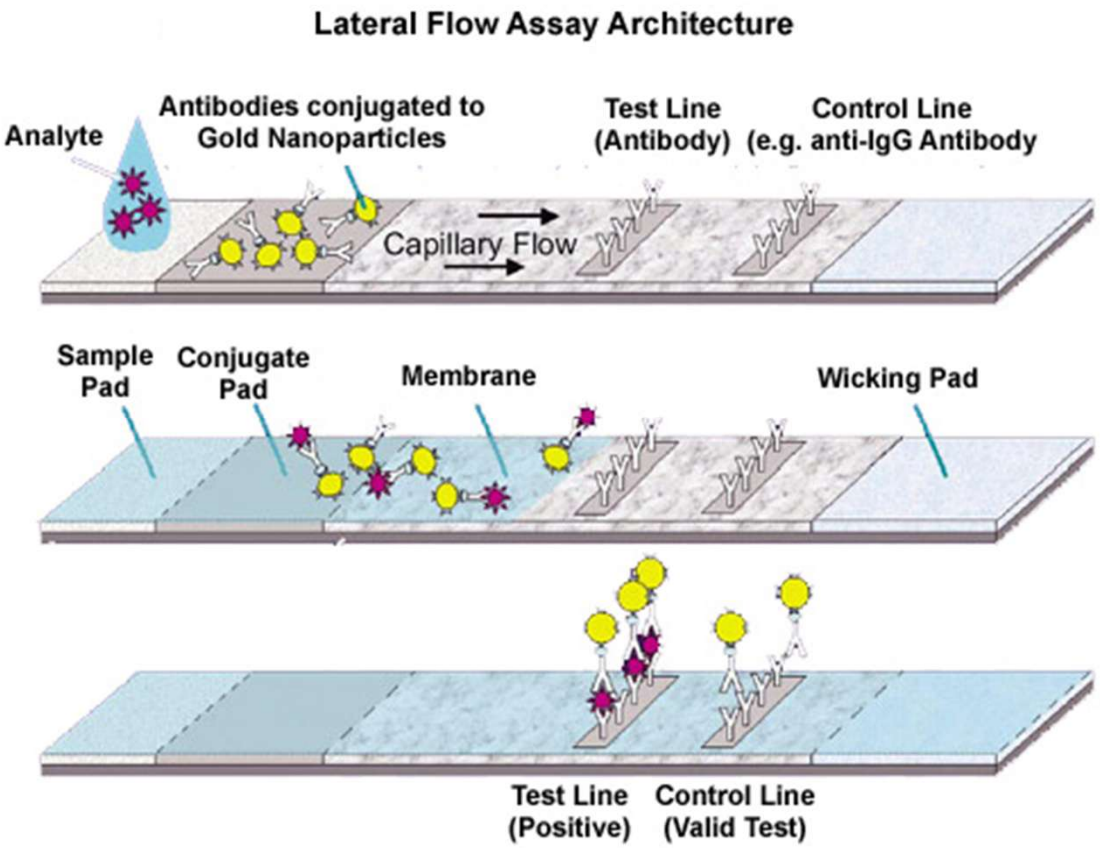


Diagram 1: *Illustration of Indirect ELISA method.*

Amplified Luminescent Proximity Homogeneous AssayLISA

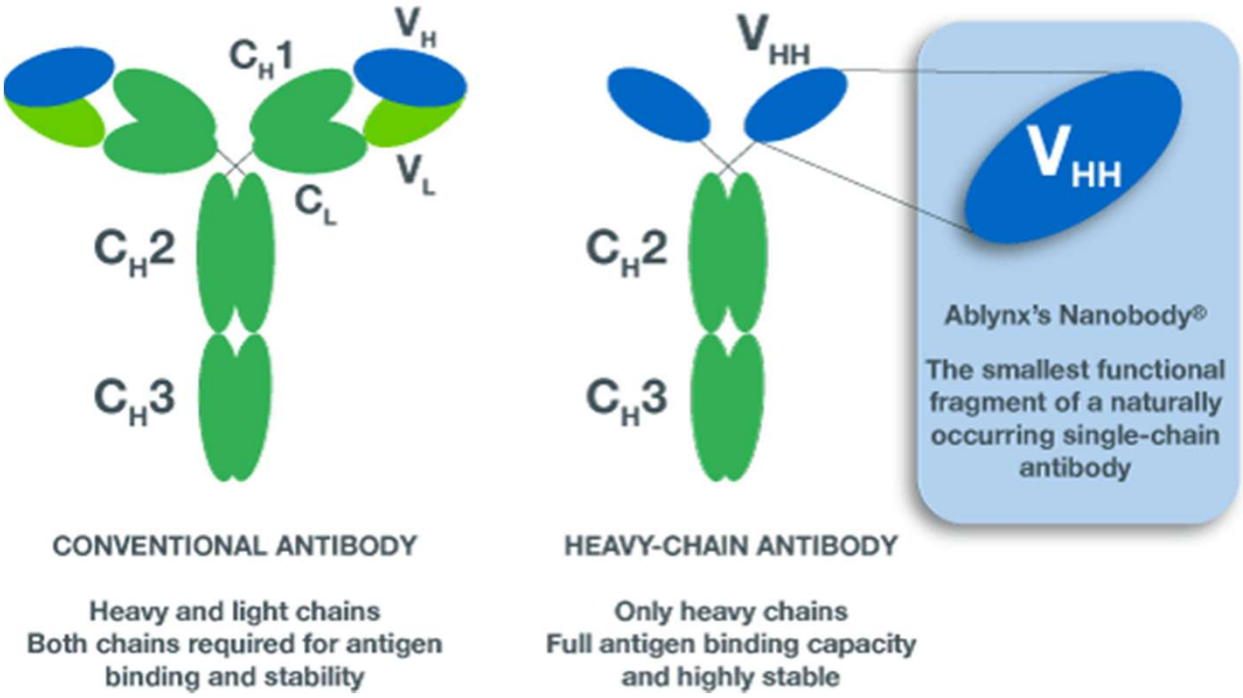


Lateral Flow Assay



http://www.cytodiagnostics.com

Nanobodies



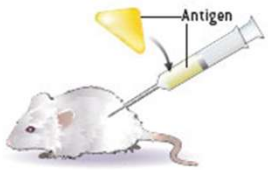
http://www.ablynx.com

CONSTRUCTING ANTIBODIES AND NANOBODIES

Creating an effective nanobody takes less time and money than a therapeutic antibody requires, according to scientists at Ablynx. In both cases, the immune system of a live animal performs the initial "design" of a protein that can latch onto the target molecule. Geneticists then tweak the DNA encoding that protein to add the properties desired in a medicine.

CONVENTIONAL MONOCLONAL ANTIBODIES

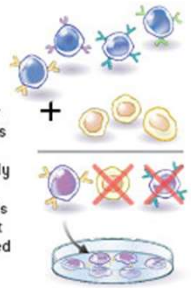
1 Immunization



Researchers inject a mouse with the target molecule. B cells of its immune system generate antibodies that recognize this antigen

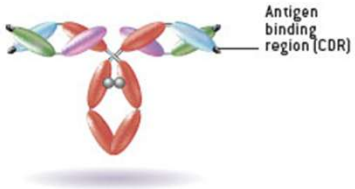
2 Fusion, Selection and Expansion

Mixing B cells (*blue*) with myeloma cancer cells (*orange*) creates hybridomas (*purple*) that divide indefinitely



Those hybridoma cells that make the correct antibody are identified and grown in culture

3 Harvesting Antibodies



Mouse antibody

The culture secretes copies of the antibody, which are then purified and tested

4 Humanization

Chimeric antibody | Humanized antibody | Human antibody

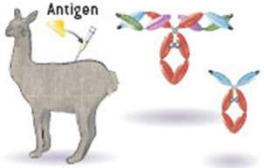
Antibody fragment (Fab)

Domain antibody

Genetic engineers can replace pieces of the mouse antibody with human segments (*orange*) and can also trim the antibody to create fragments of various sizes

ABLYNX NANOBODIES

1 Immunization

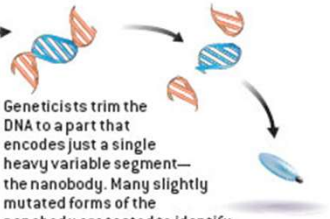


Allama or camel is immunized and produces both normal (*left*) and heavy-chain-only (*right*) antibodies against the target

2 Isolation and Cloning

From a blood sample, biologists identify cells that produce a heavy-chain-only antibody with high affinity for the target. They then obtain the DNA sequence for the genes that code for the antibody

3 Genetic Engineering



Geneticists trim the DNA to a part that encodes just a single heavy variable segment—the nanobody. Many slightly mutated forms of the nanobody are tested to identify the one that is most medically useful

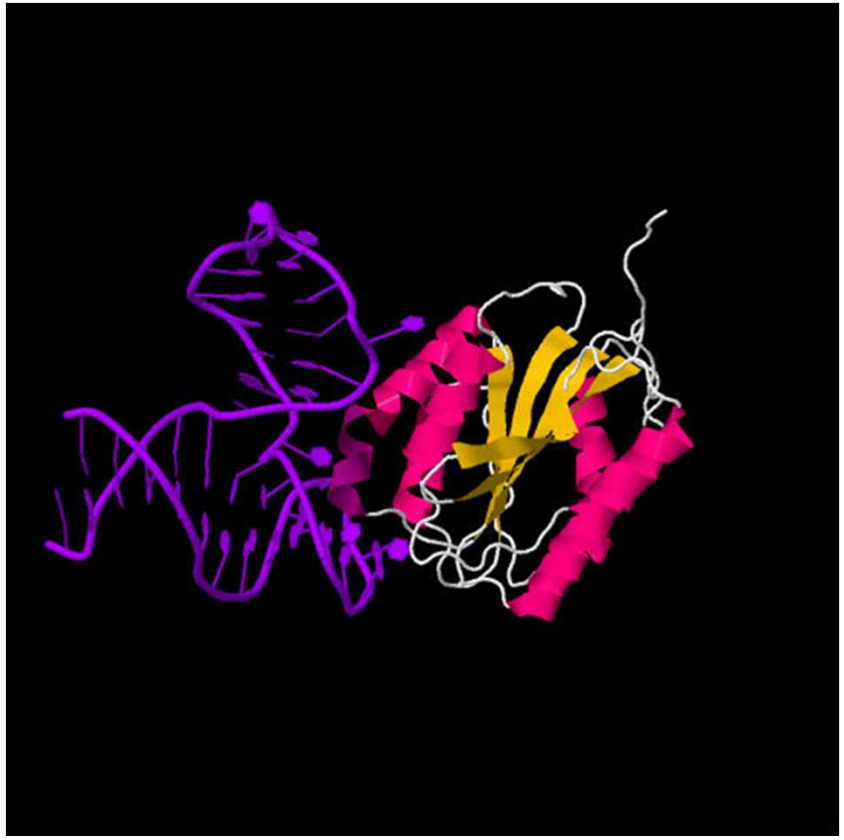
4 Construction of Nanobody Medicine

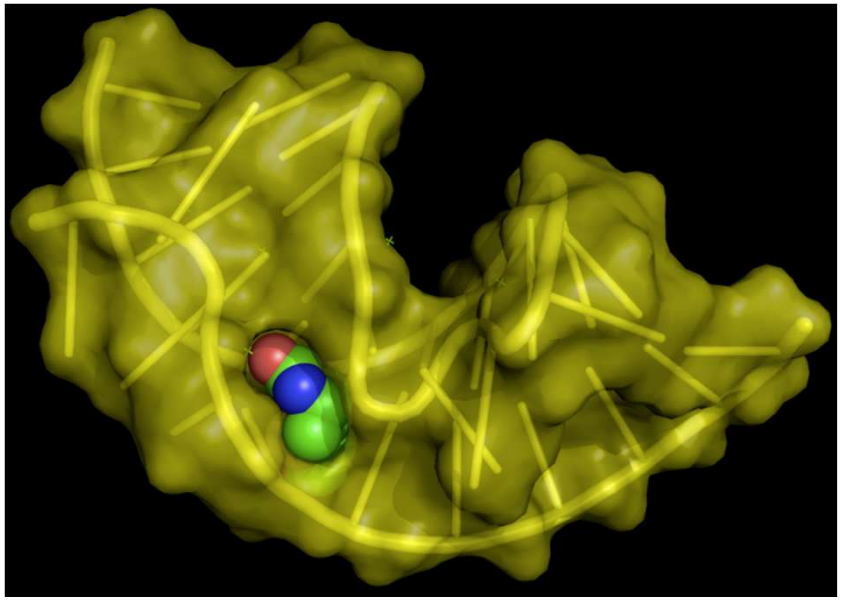
Single nanobody | Humanized nanobody | Multivalent nanobody | Bispecific nanobody | Bifunctional nanobody

Nanobody genes can be spliced with genes for other nanobodies or other biochemicals to create medicines that are then produced in bacteria, fungi or yeast cultures

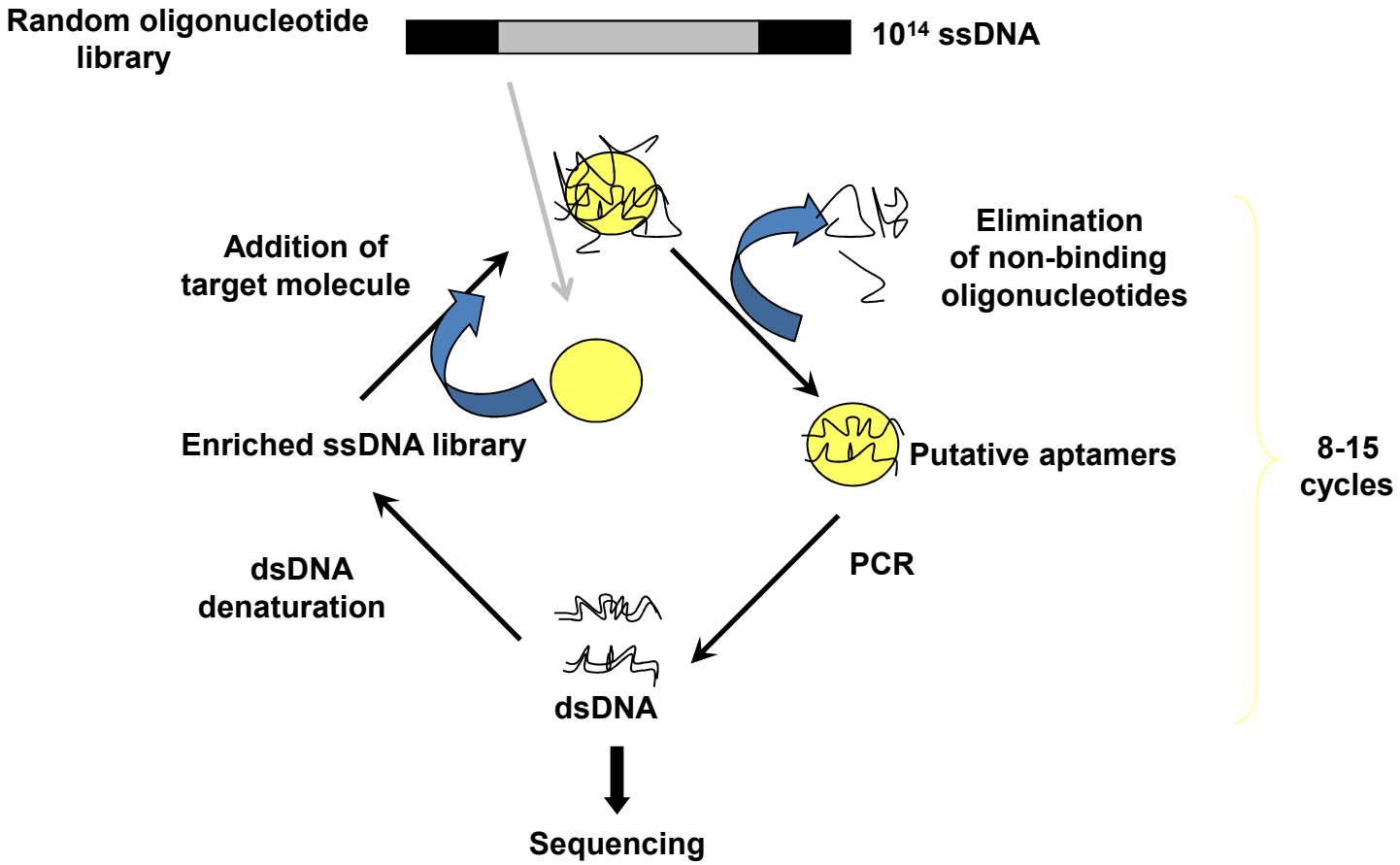
Scientific American **293**, 78 - 83 (2005)

Aptamerek, nukleinsav receptorok

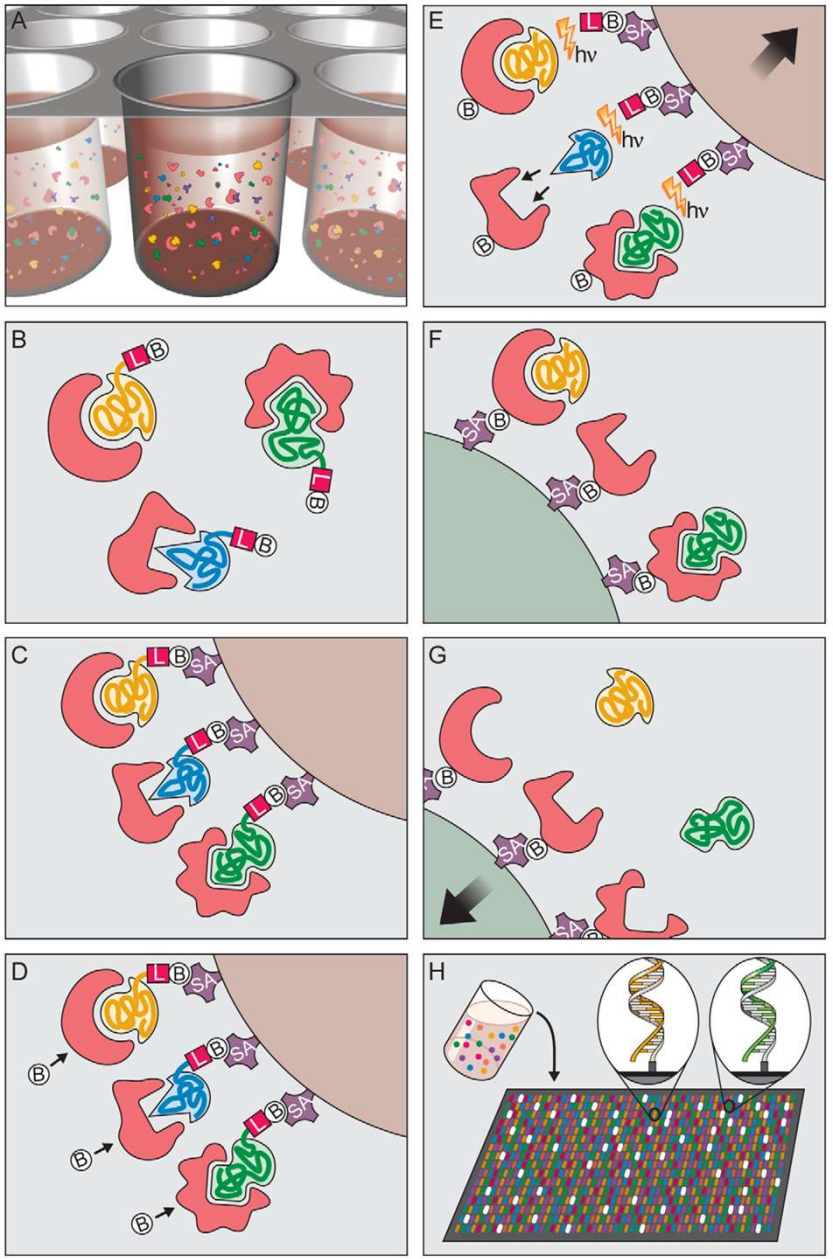




SELEX


Random oligonucleotide library
10^{14} ssDNA
Addition of target molecule
Elimination of non-binding oligonucleotides
Enriched ssDNA library
Putative aptamers
8-15 cycles
dsDNA denaturation
PCR
dsDNA
Sequencing

Principle of multiplex SOMAmer affinity assay



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