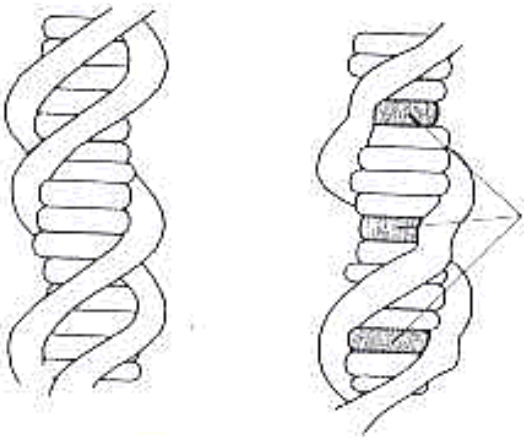
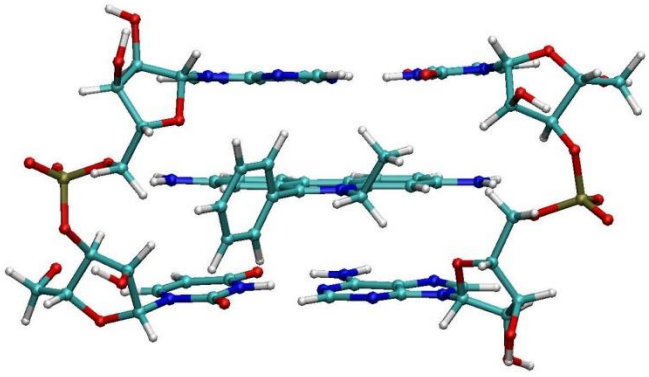
5. Előadás
Nukleinsavak kimutatása, szekvenálás

A nukleinsav kimutatás



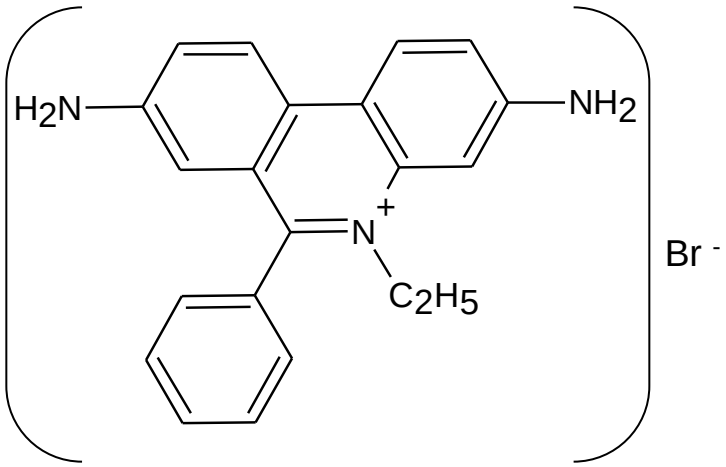


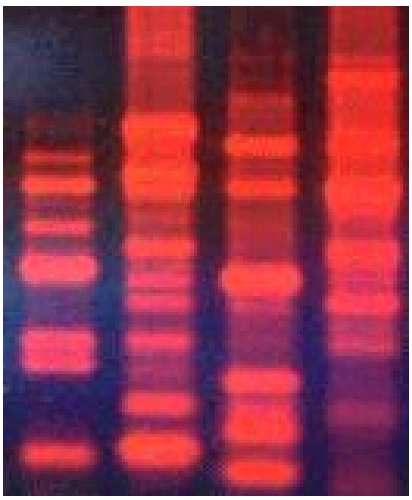
interkaláció

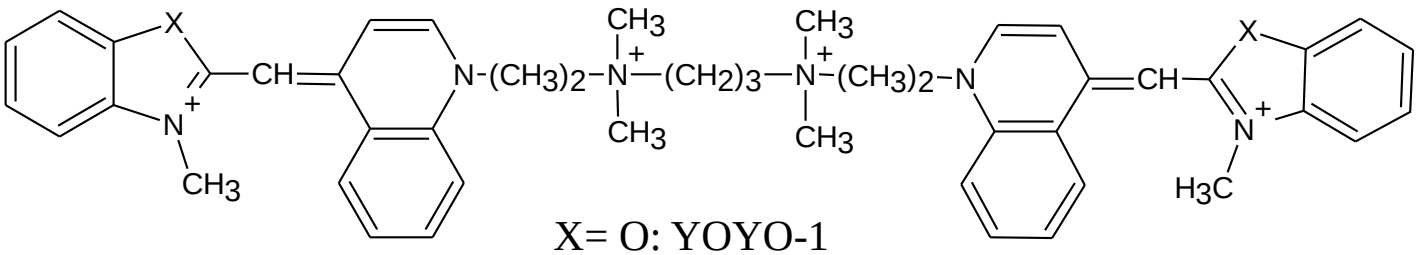
etidiumbromid

3,8-diamino-5-etil-6-fenil-fenantrédiumbromid

fluoreszcencia: λ_g=254-366 nm λ_e=590 nm



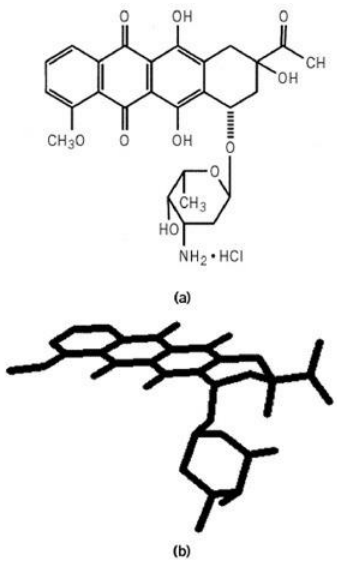


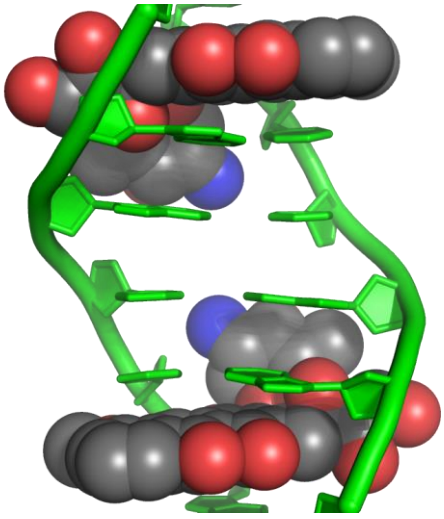


X= O: YOYO-1

X= S: TOTO-1

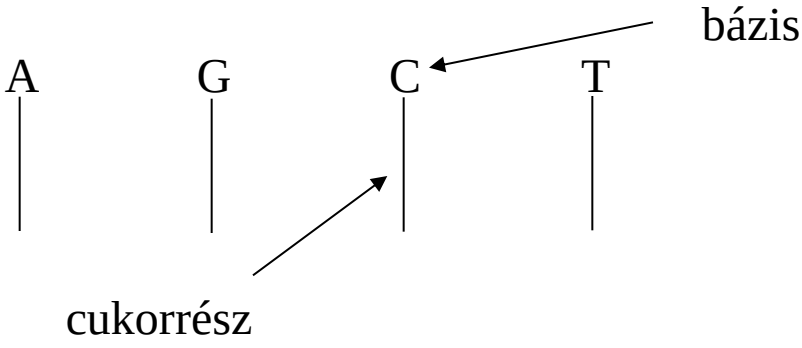
Anthraciklin antibiotikumok

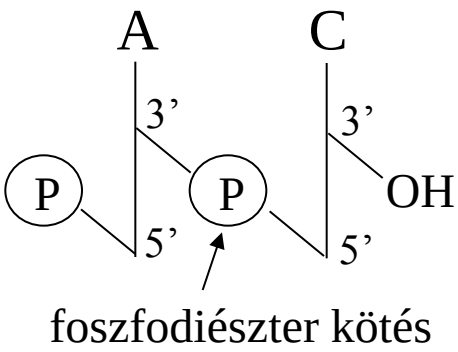




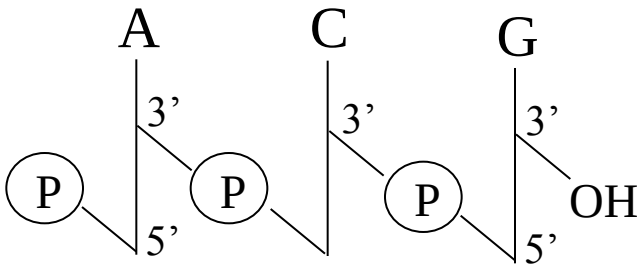
daunomycin és doxorubicin

Rövidítések





foszfodiészter kötés

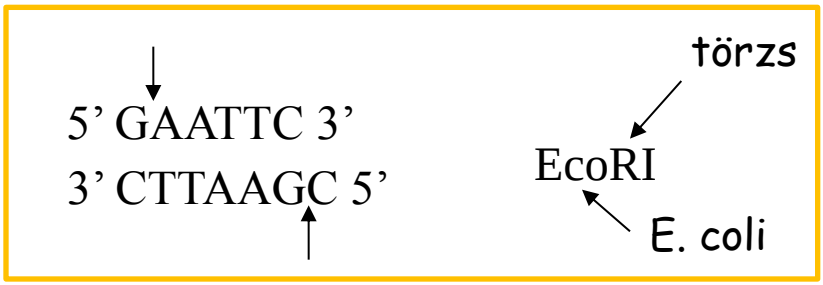


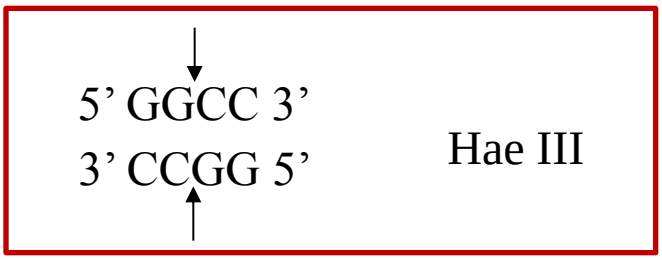
pApCpG [ACG]

http://what-when-how.com/molecular-biology/daunomycin-molecular-biology/

Szekvencia meghatározása

1. Fragmentálás restrikciós enzimekkel
(W. Arber, H. Smith, D. Nathans, 1978 Nobel-díj)





Cél: feldarabolás
5'-3' irányban haladva
a hasítás pontos helye:
a '/' jelnél

Néhány endonukleáz enzim (>2000)

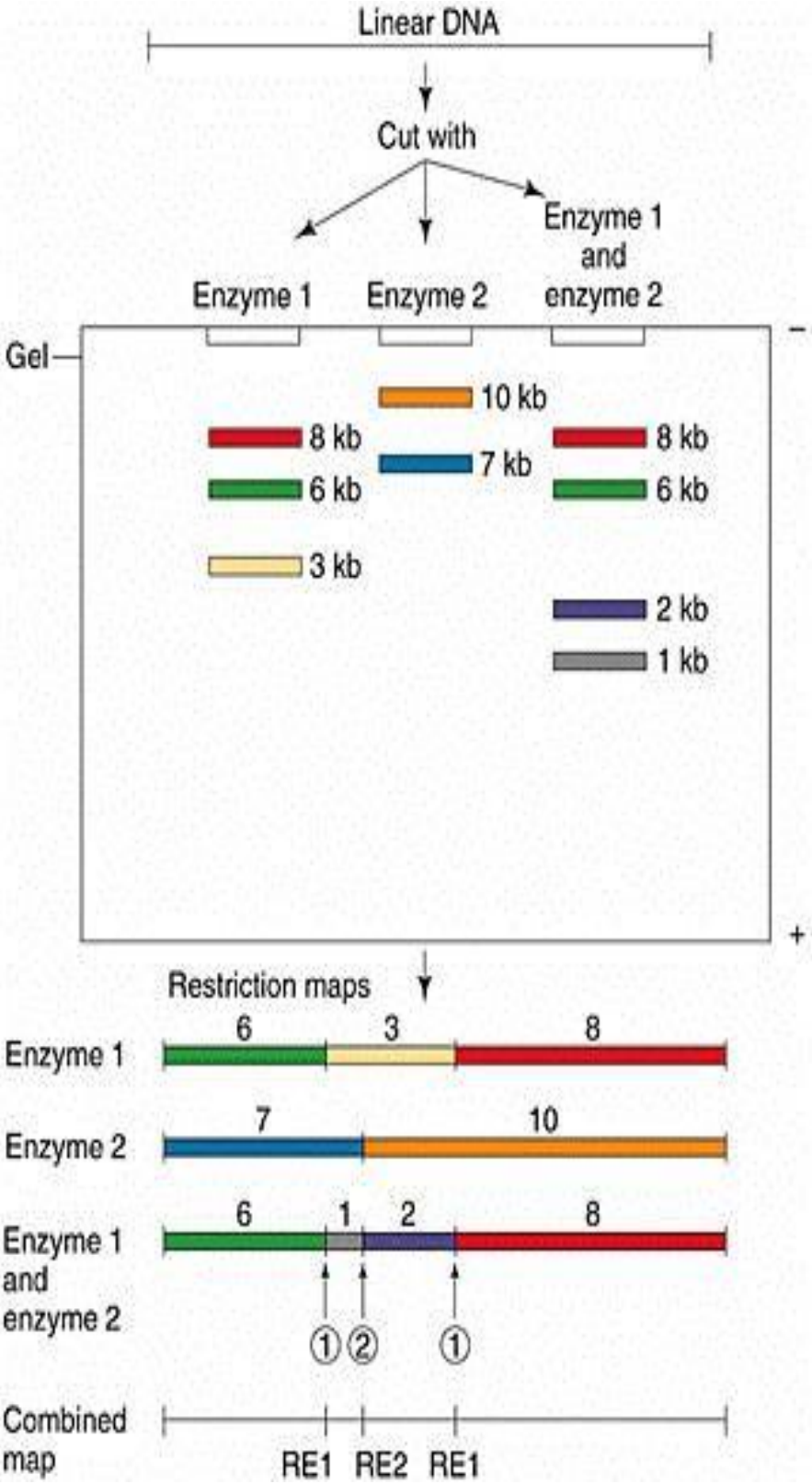
Bam HI	G/GATCC	*Bacillus amyloliquefaciens*H
Bst I	G/GATCC	*Bacillus stearothermophilus*1503-4R
Eco RI	G/AATTC	*Escherichia coli* RY 13
Fok I	$GGATGN_9$/ $CCTACN_{13}$/	*Flavobacterium okeanokoites*
Hind II	GTPy/PuAC	*Haemophilus influenzae* R_d
Hind I	A/AGCTT	*Haemophilus influenzae* R_d
Hpa II	C/CGG	*Haemophilus parainfluenzae*

2. Sorrend meghatározás

2a. Kémiai módosítás/hasítás (A. Maxam, W.Gilbert, 1977)

2b. Didezoxi beépítés-enzimes módszer (F. Sanger et al., 1977)

1. Fragmentálás restrikciós enzimekkel



2a. Kémiai módosítás/hasítás (A. Maxam, W. Gilbert, 1977)

1. lépés

Homogén „Single-stranded" DNS minta előállítása

$^{5'}$ATTGACTTAGCC$^{3'}$

2. lépés

Jelölés a szabad 5'-végen ^{32}P (*) (polinukleotid kináz, észter)

*ATTGACTTAGCC 12 mer

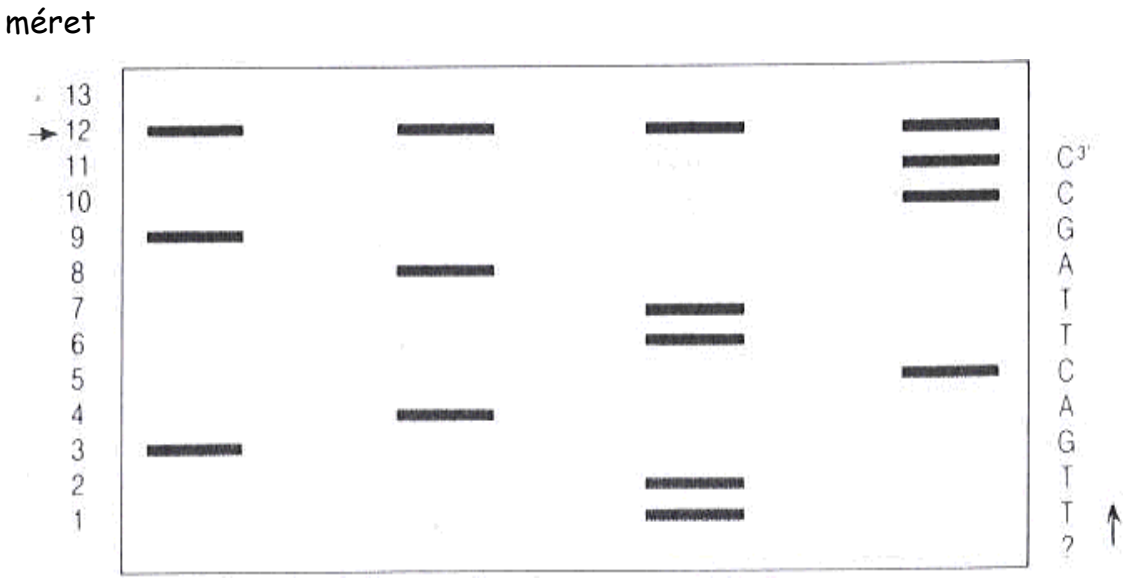
3. lépés

Kémiai hasítás

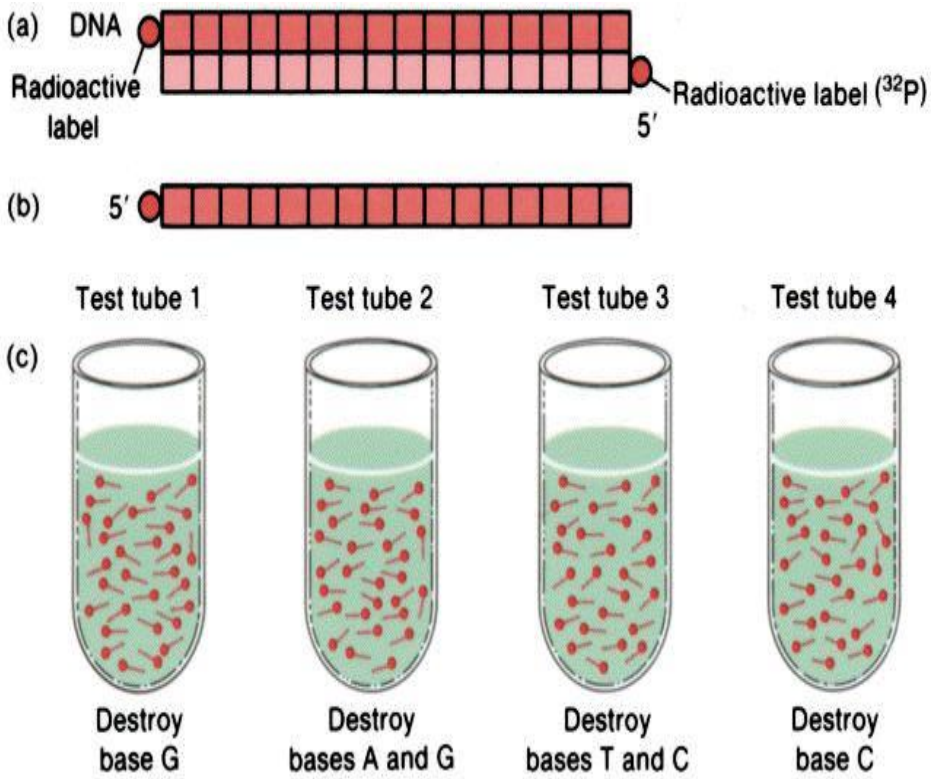
G reakció	A reakció + G reakció	T reakció + C reakció	C reakció
*ATTGACTTAGCC	*ATTGACTTAGCC	*ATTGACTTAGCC	*ATTGACTTAGCC
*ATTGACTTA	*ATTGACTTA	*ATTGACTTAGC	*ATTGACTTAGC
*ATT	*ATTGACTT	*ATTGACTTAG	*ATTGACTTAG
	*ATTG	*ATTGACT	*ATTGA
	*ATT	*ATTGAC	
		*ATTGA	
		*AT	
		*A	

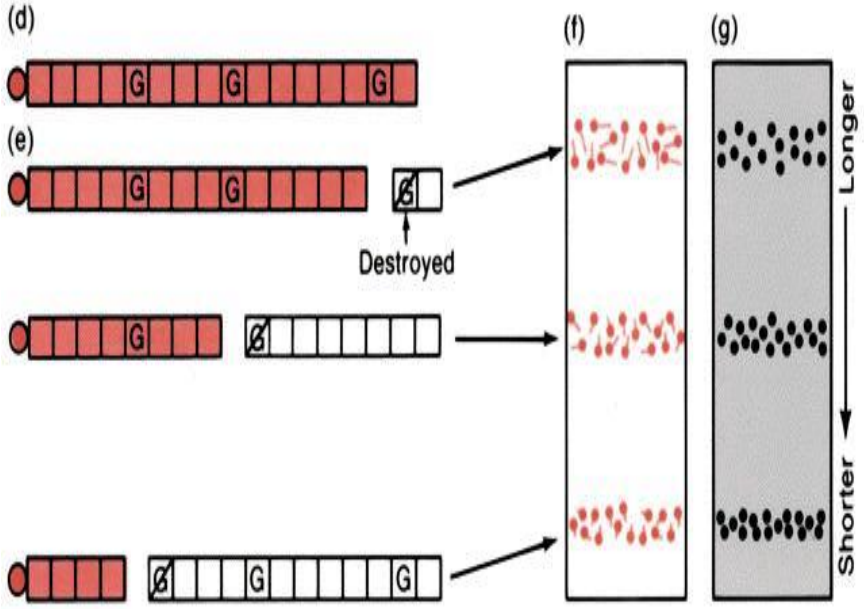
4. lépés: elektroforézis

5. lépés: autoradiográfia



6. lépés: szekvencia leolvasás

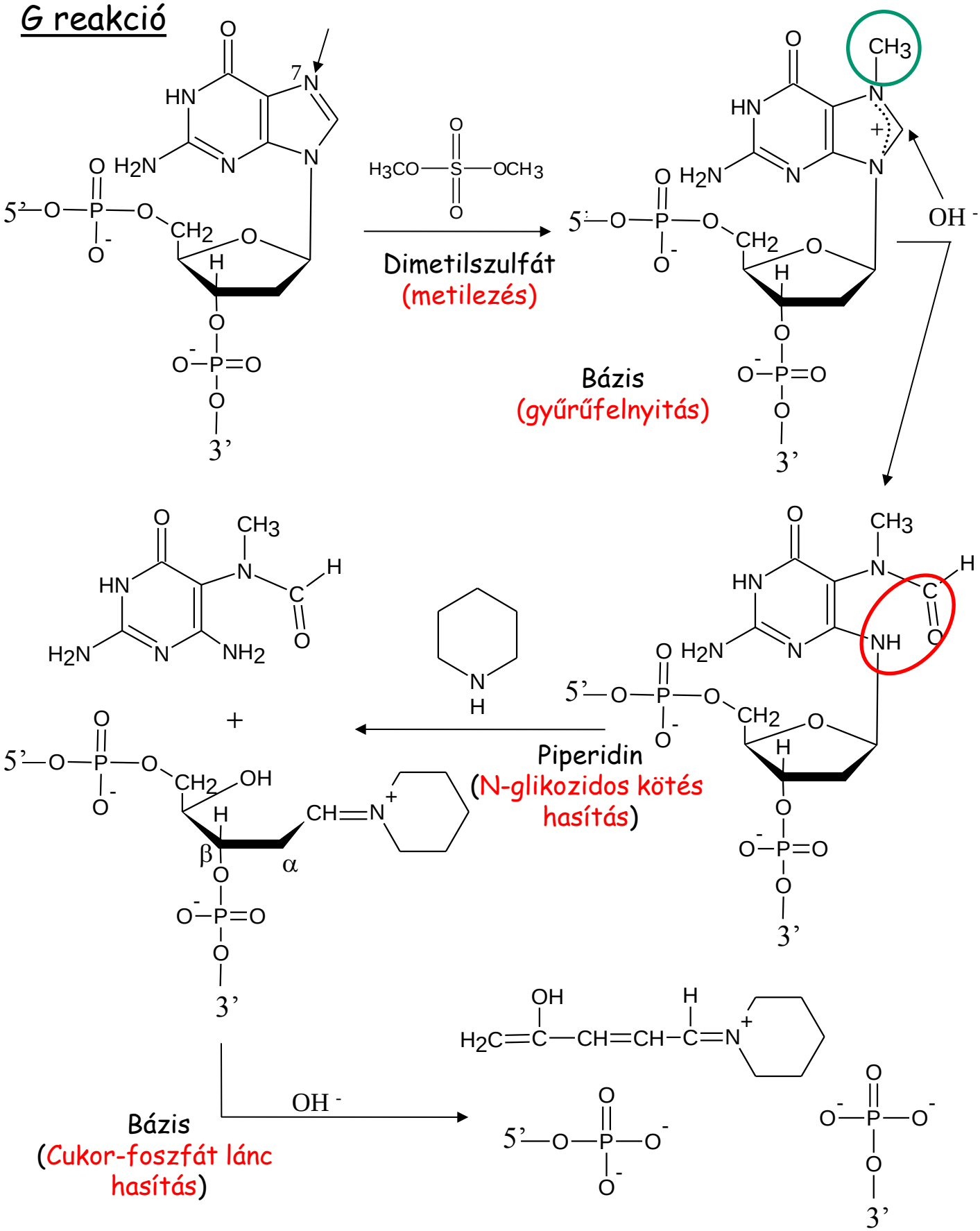




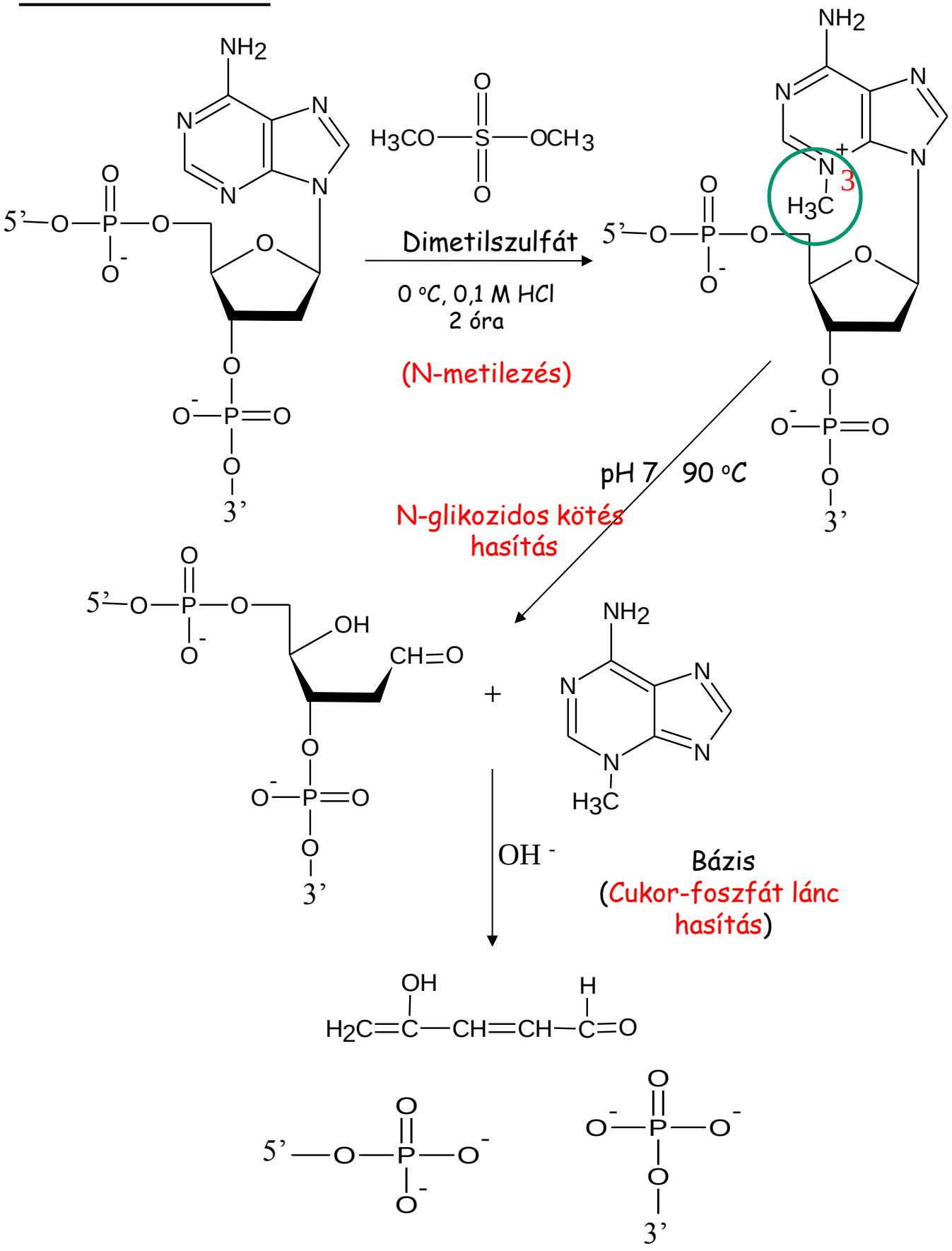
http://www.slideshare.net/DurgeshSirohi/different-methods-of-gene-sequencing

Kémiai hasítás: Purinok

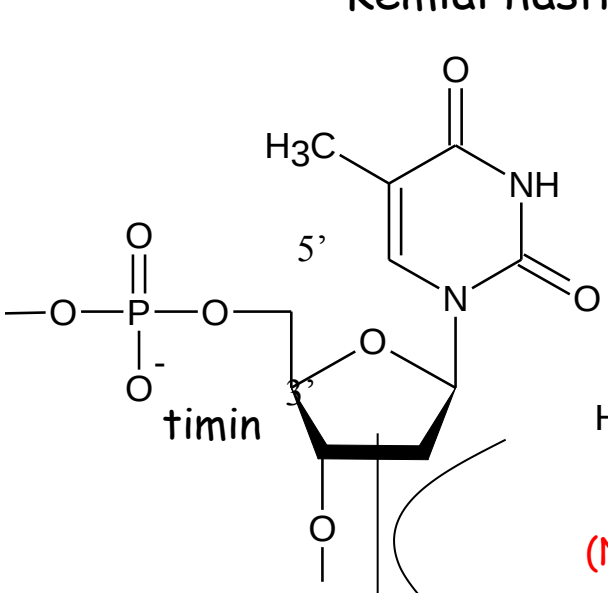
G reakció



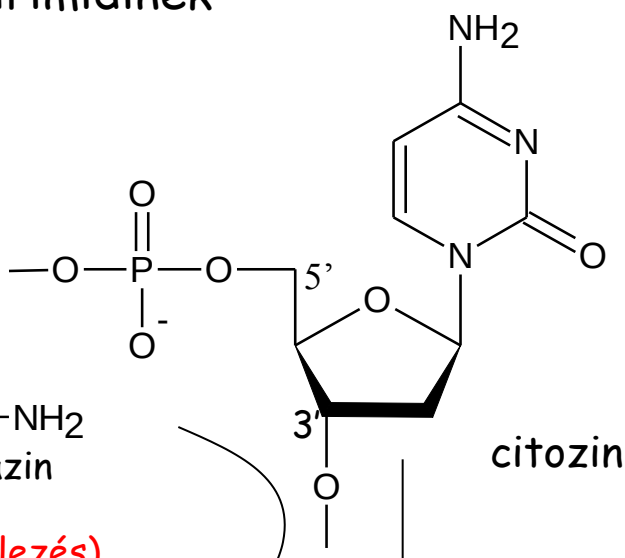
A > G: A reakció


Dimetilszulfát
0 °C, 0,1 M HCl
2 óra
(N-metilezés)
pH 7 90 °C
N-glikozidos kötés hasítás
OH -
Bázis
(Cukor-foszfát lánc hasítás)

Kémiai hasítás: Pirimidinek



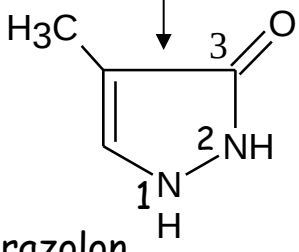
timin



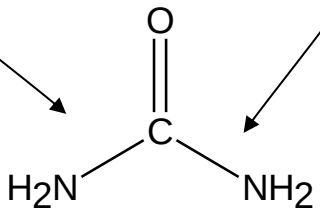
citozin

$H_2N—NH_2$
hidrazin

(N-metilezés)

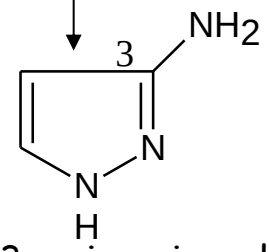


4-Me-3-pirazolon

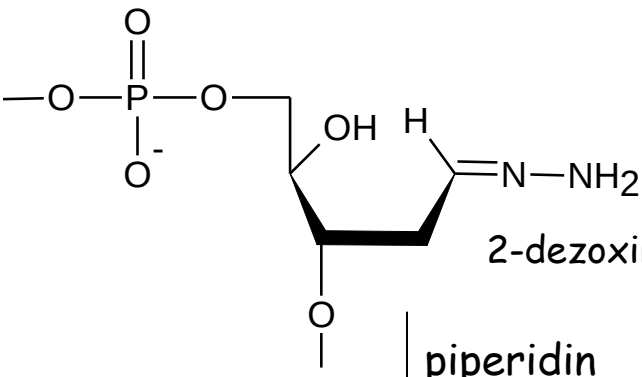


Urea

\+



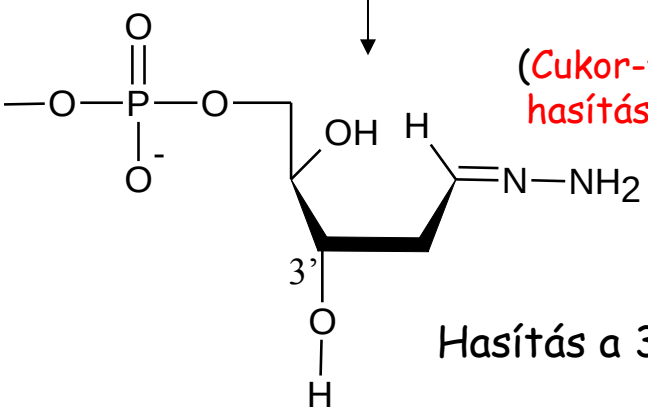
3-amino-pirazol



2-dezoxiribozil-hidrazon

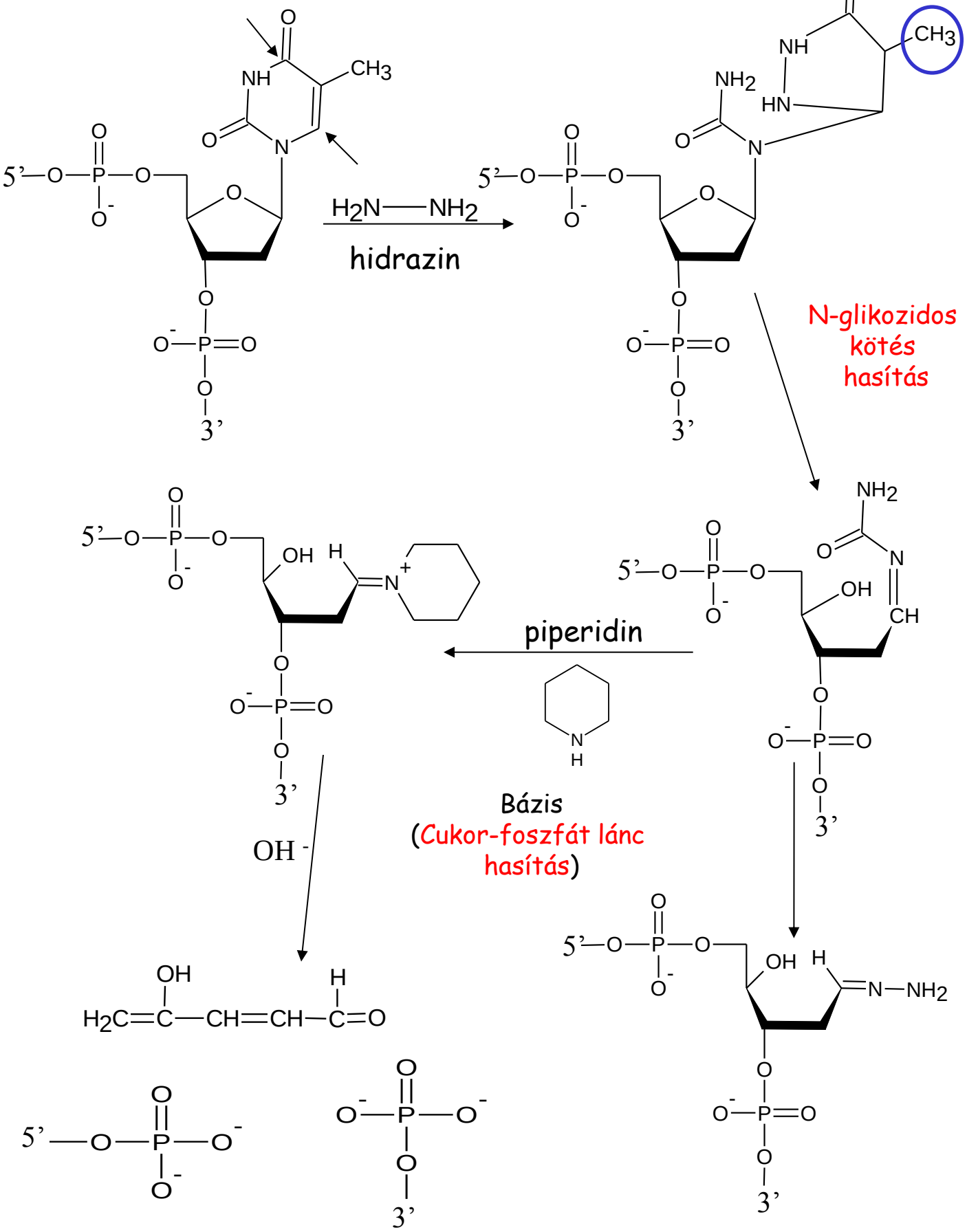
piperidin

Bázis
(Cukor-foszfát lánc hasítás a 3'- végen)

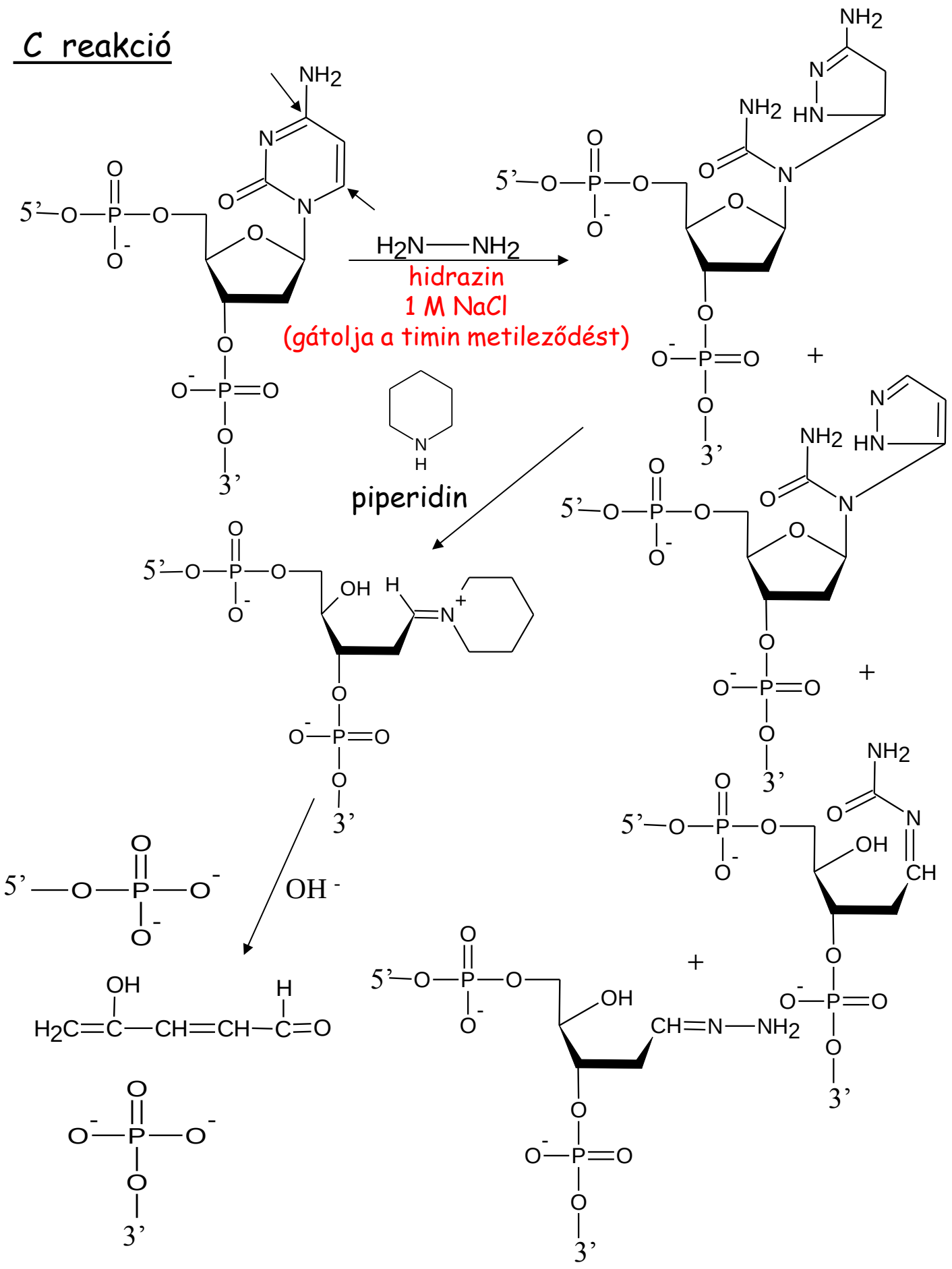


Hasítás a 3' végen

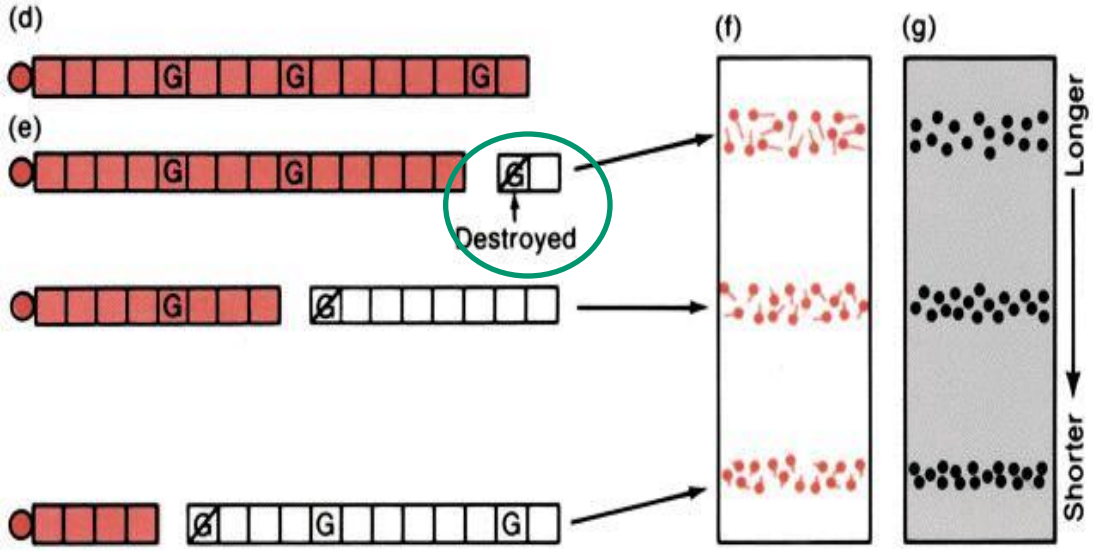
C + T: T reakció

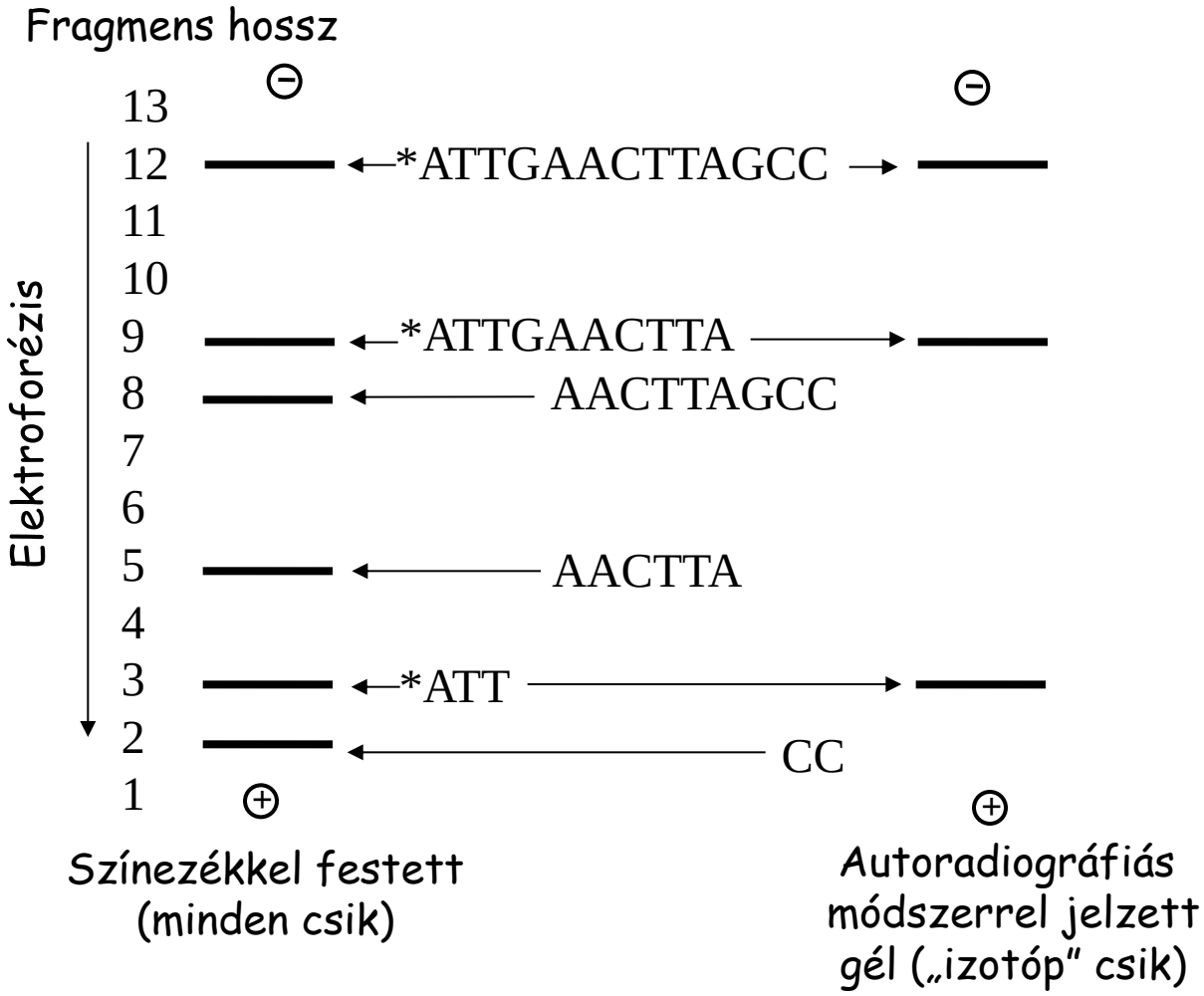

H2N—NH2
hidrazin
N-glikozidos kötés hasítás
piperidin
Bázis
(Cukor-foszfát lánc hasítás)
OH -

C reakció

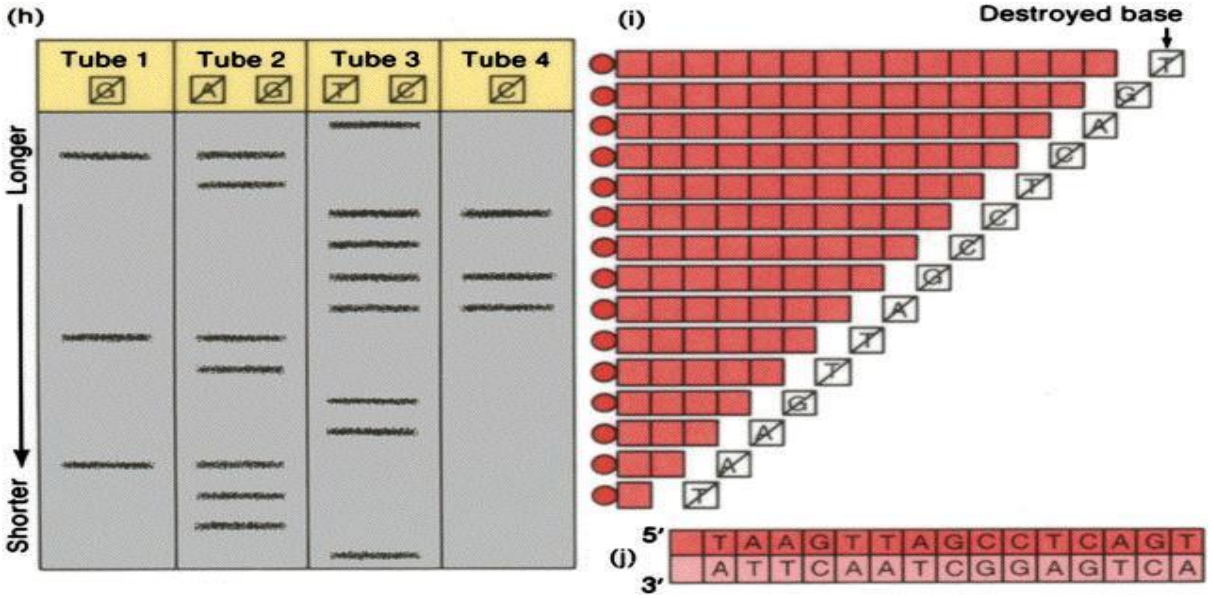

H2N—NH2
hidrazin
1 M NaCl
(gátolja a timin metileződést)
piperidin
OH -

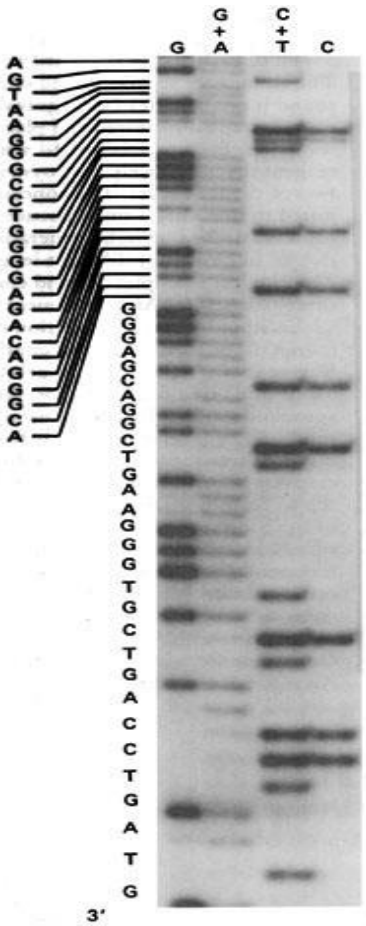
Példa: G hasítás után

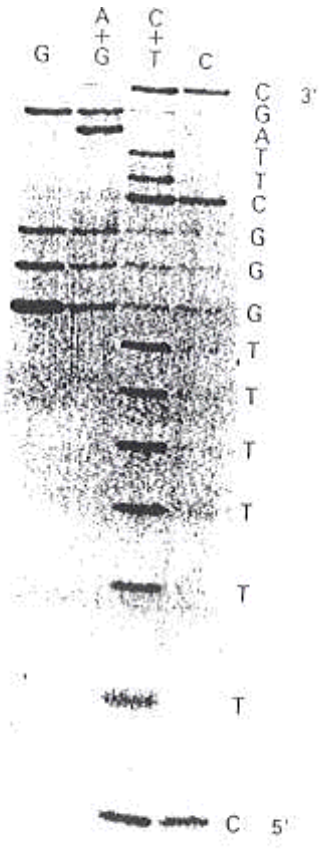




Maxam-Gilbert módszer - kiértékelés

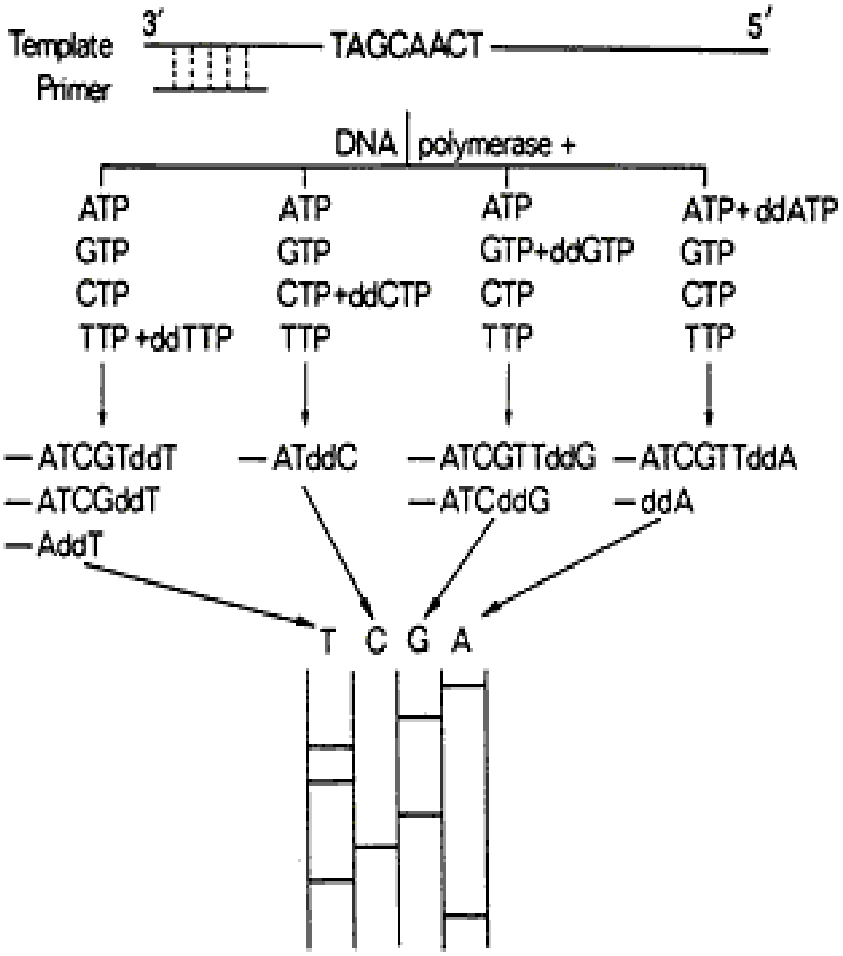


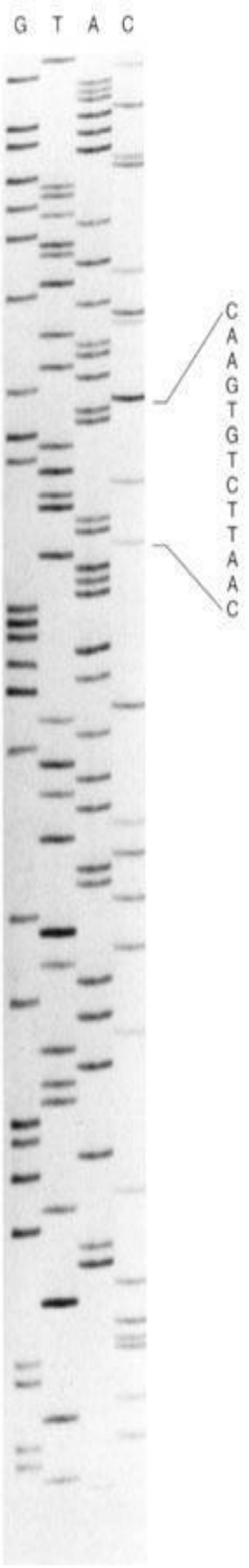




CTTTTTTGGGCTTAGC

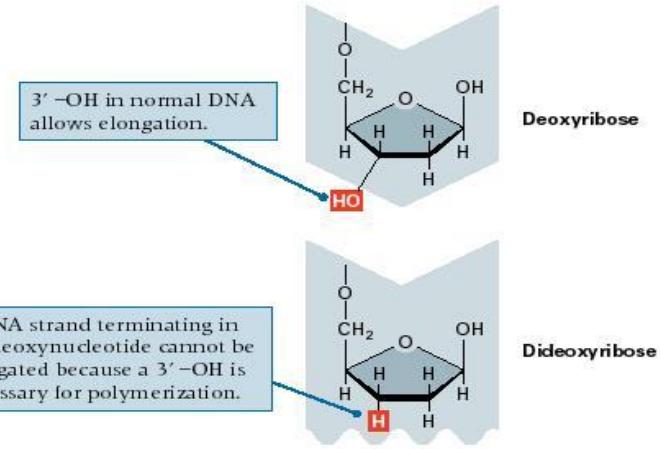
2b. Didezoxi nuleotid beépítés – enzimes módszer (F. Sanger et al., 1977)

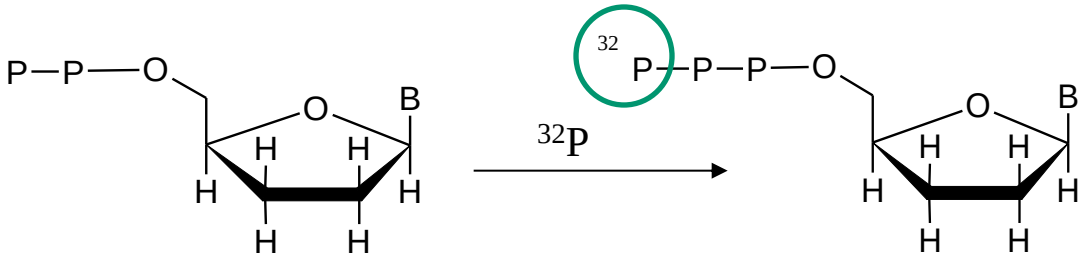




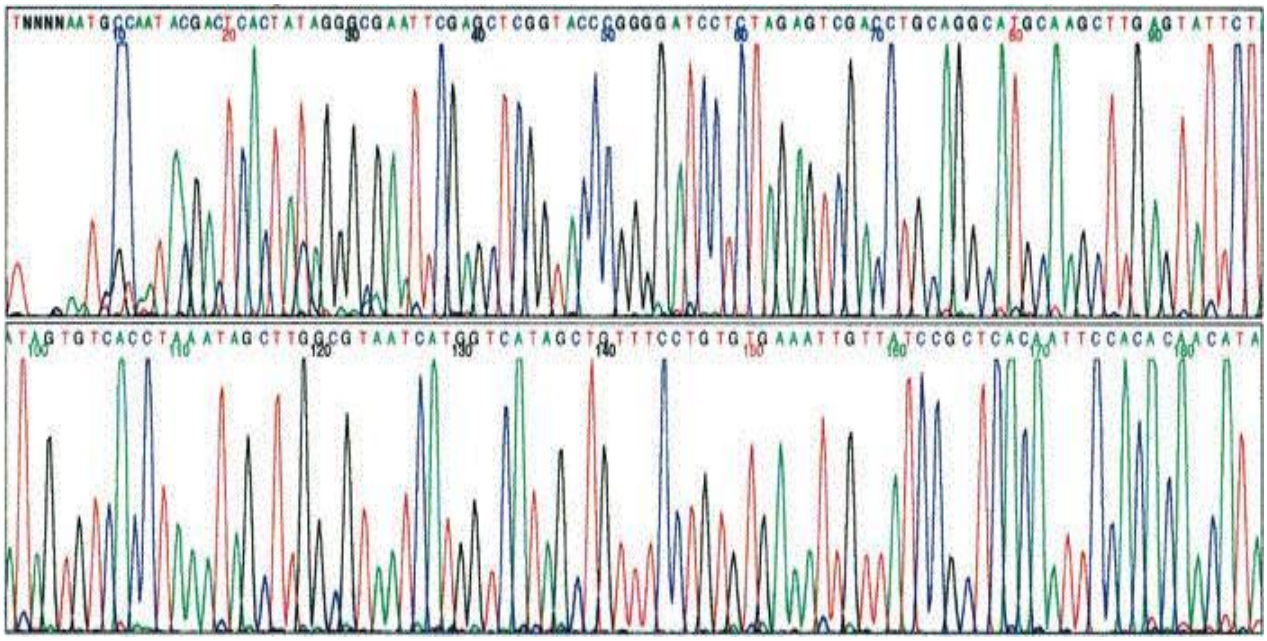
1. lépés: Didezoxi nukleotid jelölése

a) Radioaktív izotóp (^{32}P)

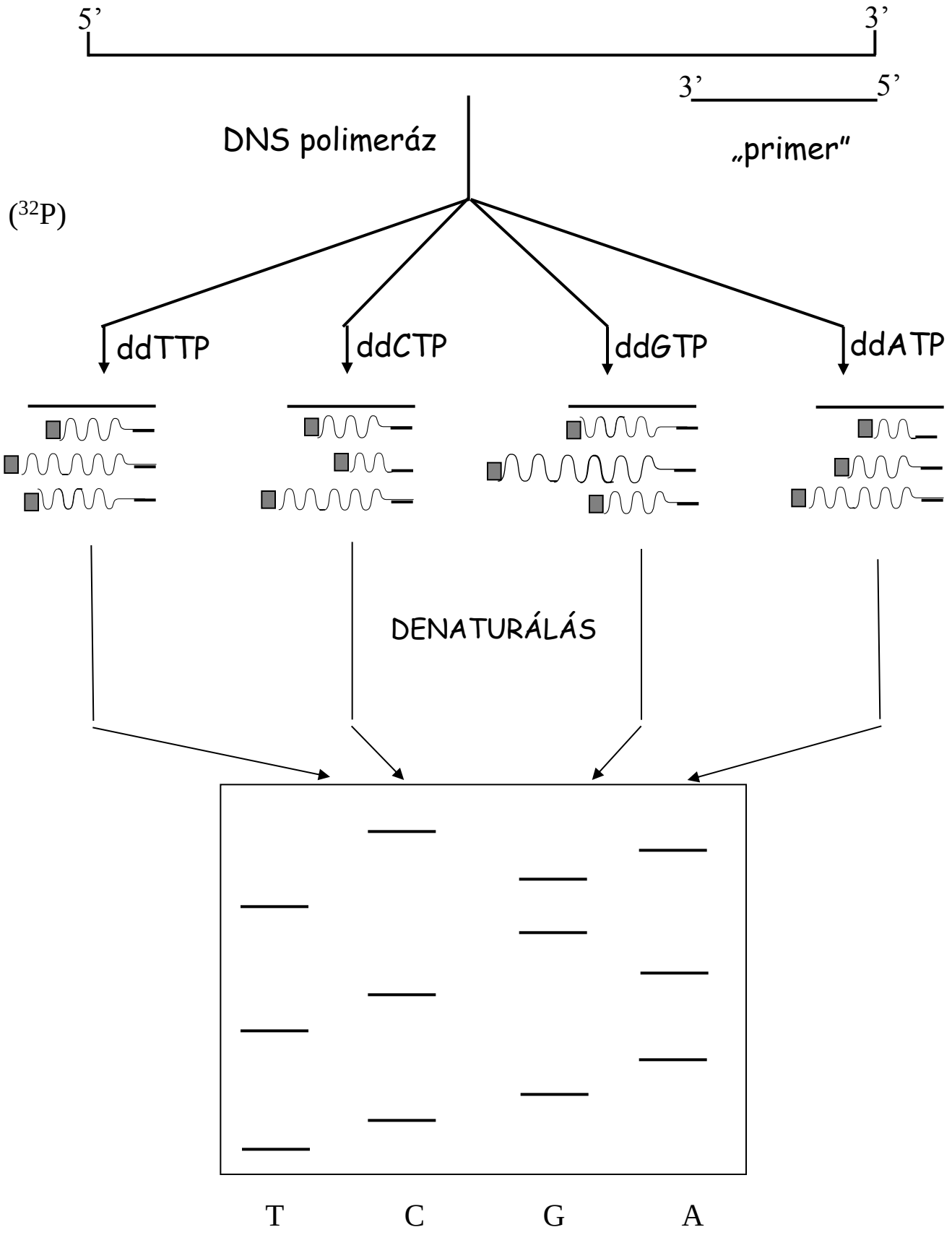




b) Fluorofór (fluoreszcens jelölés, L. E. Hood, 1986)



Áttekintés


5’
3’
3’
5’
DNS polimeráz
„primer”
(^{32}P)
ddTTP
ddCTP
ddGTP
ddATP
DENATURÁLÁS
T
C
G
A

2. lépés A DNS-polimeráz müködésének blokkolása

szekvenálandó DNS

3′ —— GAATTCGCTAATGC ——
5′ —— CTTAA

primer

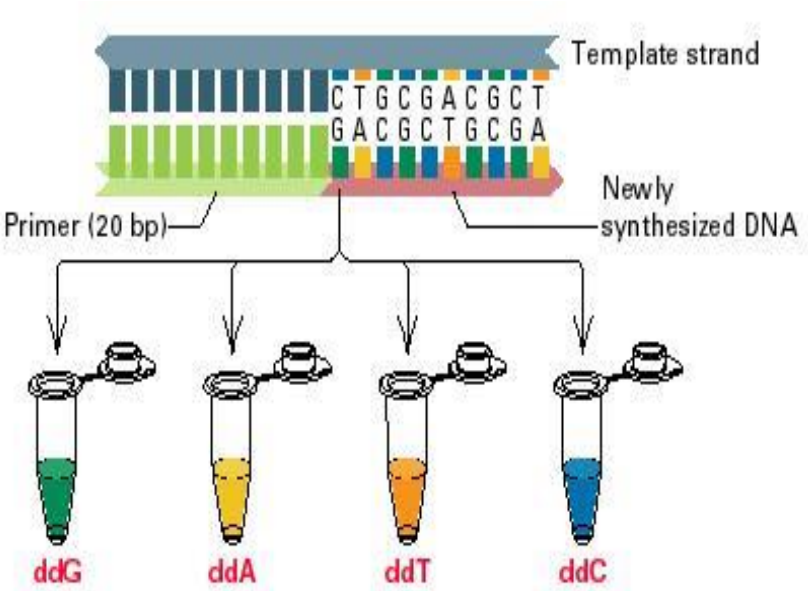
DNS polimeráz I
dATP, dTTP, dCTP, dGTP
+ didezoxi analóg (ddATP)

3′ —— GAATTCGCTAATGC ——
5′ —— CTTAA**GCGATTA**

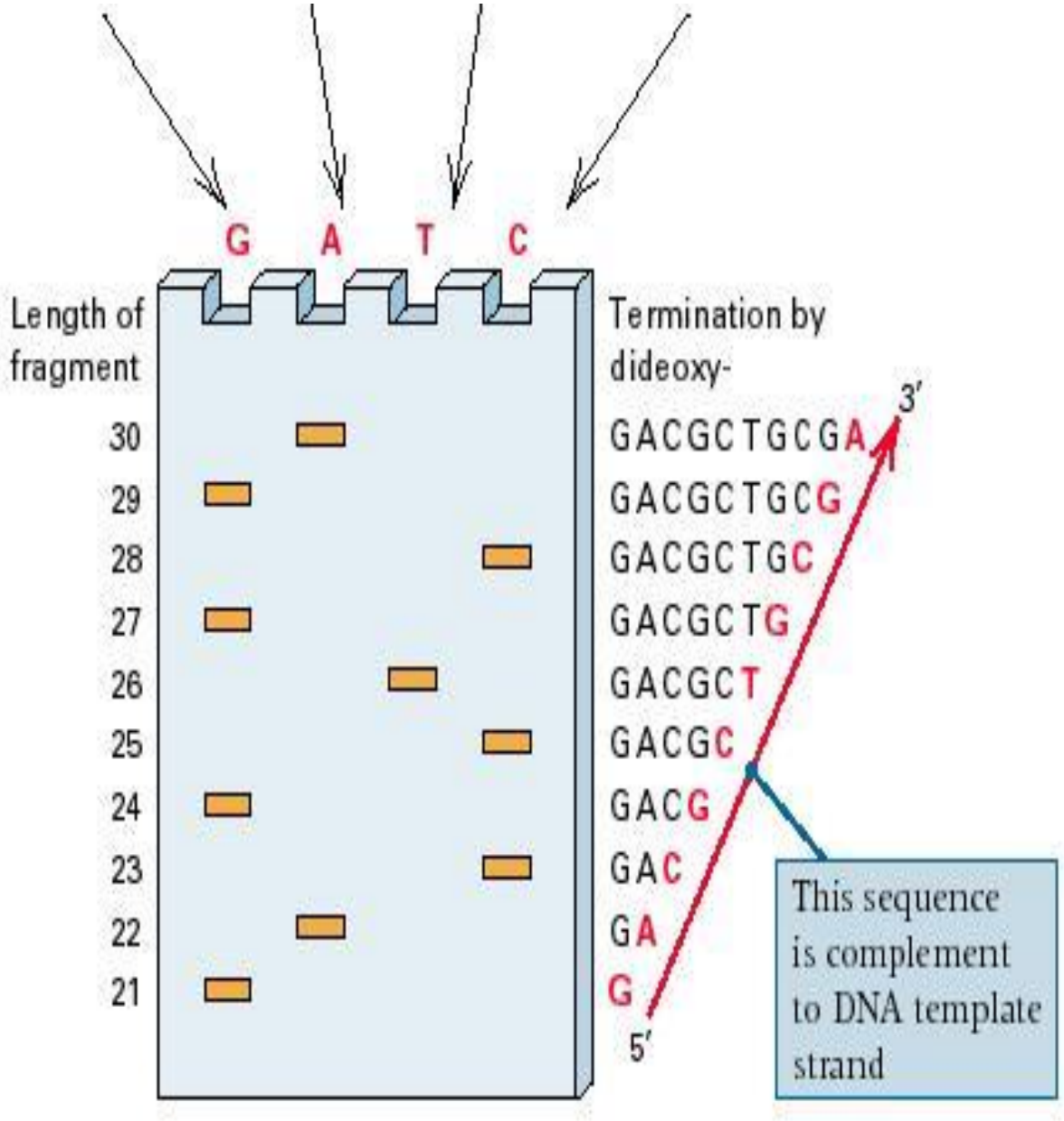
\+

3′ —— GAATTCGCTAATGC ——
5′ —— CTTAA**GCGA**

4 edény
4 analóg



3. lépés Gélelektroforézis



Leolvasás - komplement szál

Új-generációs szekvenálás

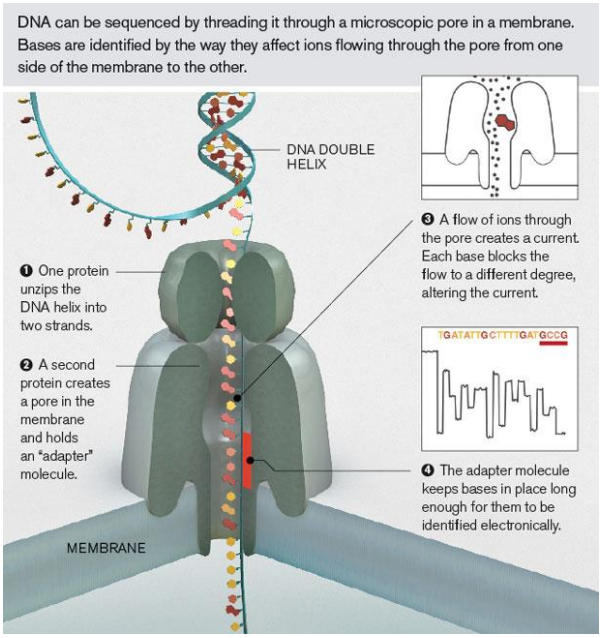
1. Piroszekvenálás

2. Illumina/Solexa szekvenálás

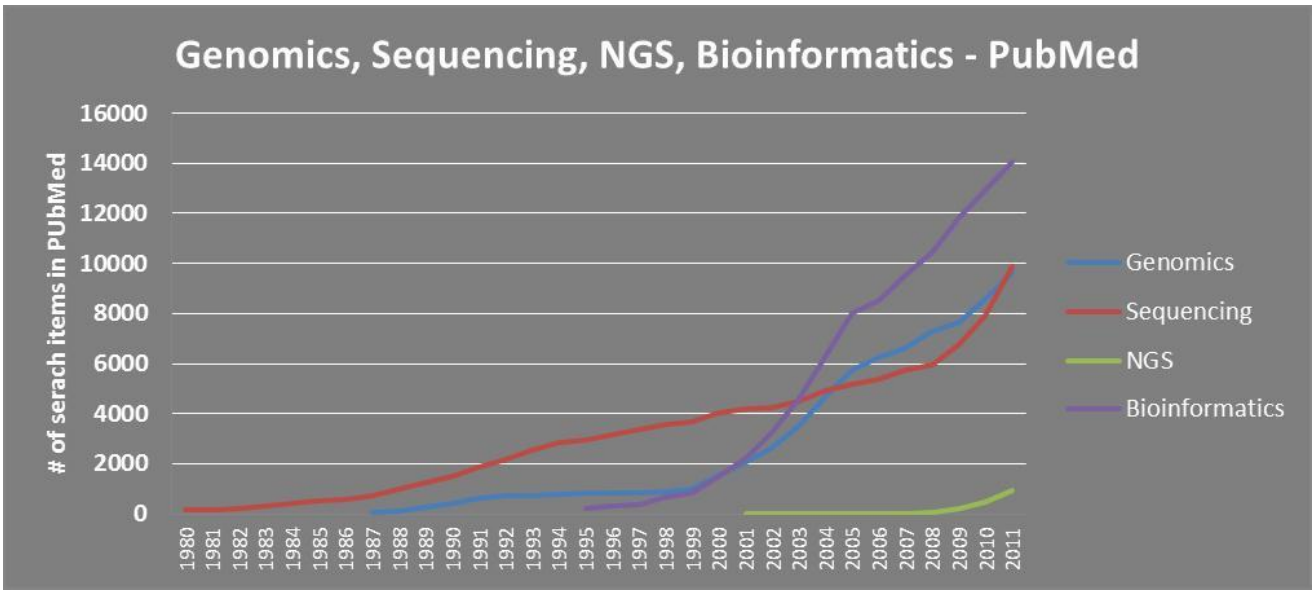
3. SOLiD (Sequencing by Oligonucleotid Ligation and Detection)

4. TSMS
   (True Single Molecule Sequencing)
   Valódi egymolekulás szekvenálás)

5. SMRT
   (Single Molecule Real-time)
   Egymolekulás valósidejű szekvenálás

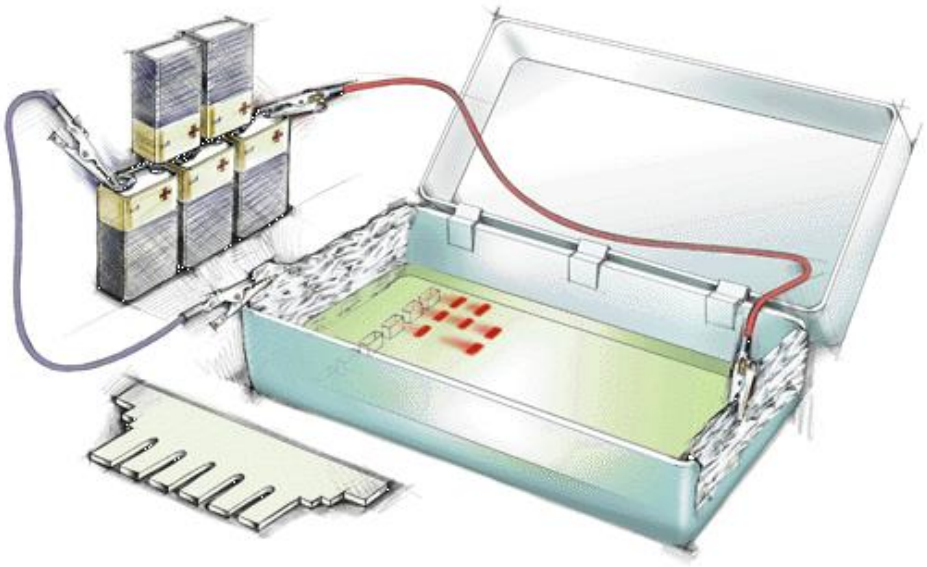


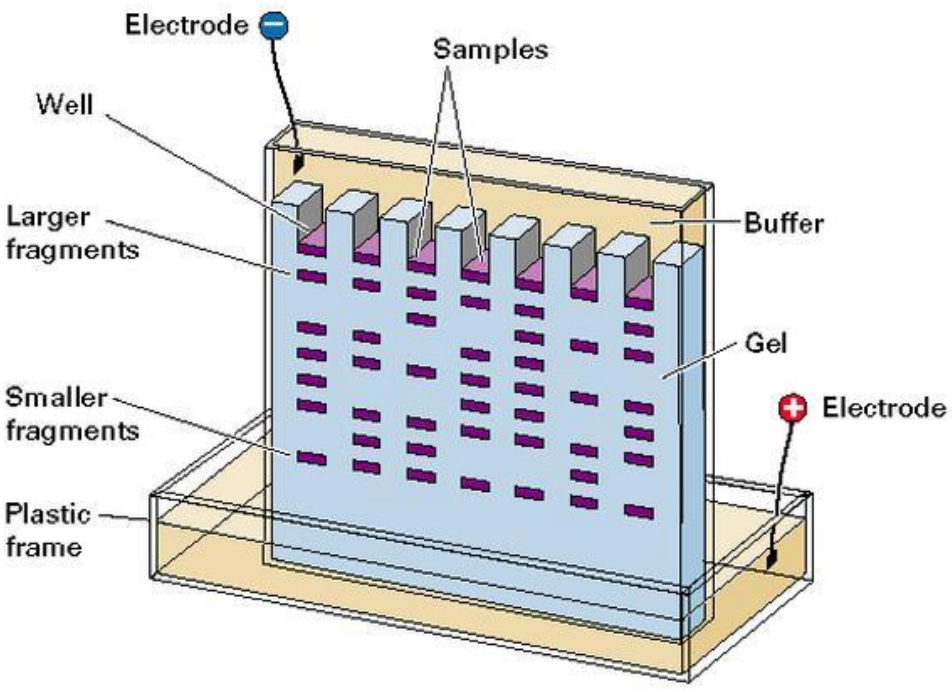
http://www2.technologyreview.com/news/427677/nanopore-sequencing/



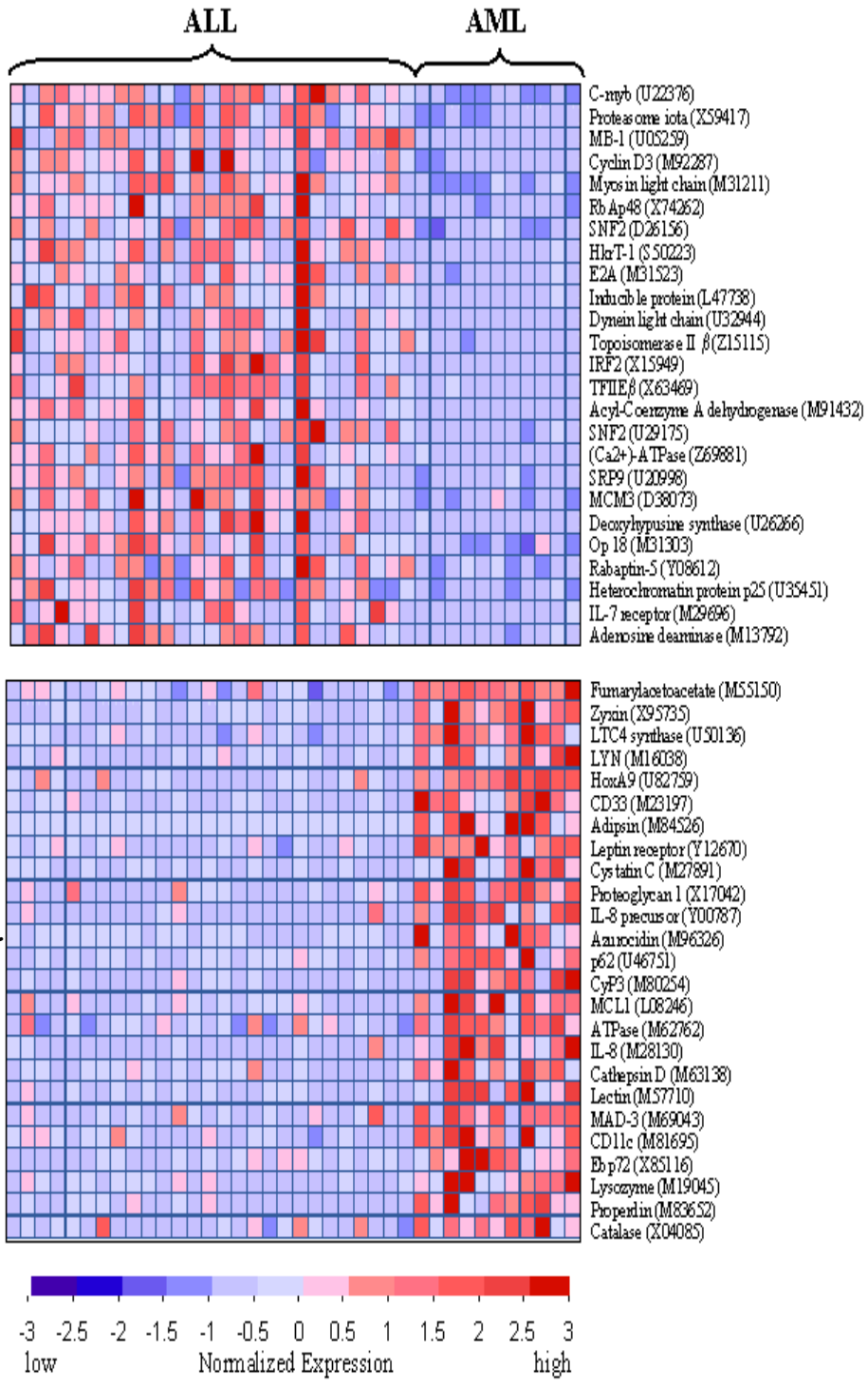
http://elte.prompt.hu/sites/default/files/tananyagok/Gentechnologia/ch05.html

DNS/oligonukleotid analitika (elektroforézis)





Expression levels of 50 genes most highly correlated with the acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).



Expressed in ALL

Expressed in AML

Expression levels greater than the mean: red, below the mean: blue.

TR Golub et al Science 286: 531 (1999)