

ΔΙΔΑΚΤΕΑ ΥΛΗ

Gene Cloning (T.A. Brown)

Βλέπε αντίστοιχες εικόνες

Ανασυνδυασμένο DNA (D. Watson et al.)

Κεφάλαιο 4 1 [σελίδες 09-136]

Κεφάλαιο 6 [σελίδες: 175-177]

Κεφάλαιο 10 [σελίδες 337-356]

Κεφάλαιο 13 [σελίδες: 445-456]

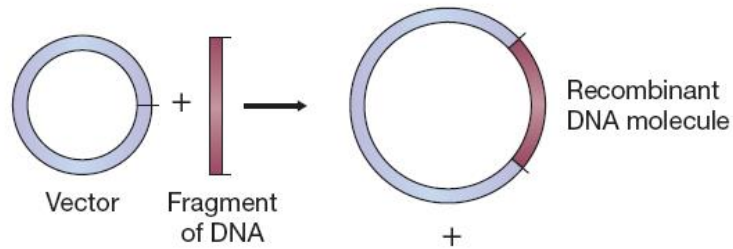
Ανασυνδυασμένο DNA

Έκφραση και ρύθμιση των γονιδίων

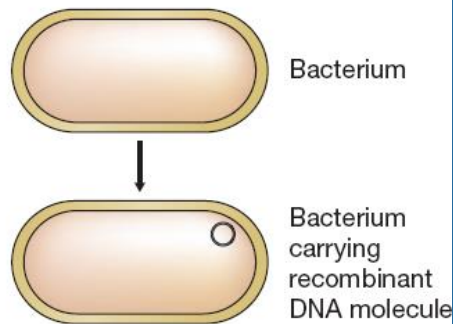
- Λειτουργία των γονιδίων
- Ιατρική διαγνωστική
- Φαρμακευτικά, γεωργικά και βιομηχανικά προϊόντα
- Γονιδιακή θεραπεία
(Knockdown-Knockout-gene replacement)

Κλωνοποίηση

1 Construction of a recombinant DNA molecule



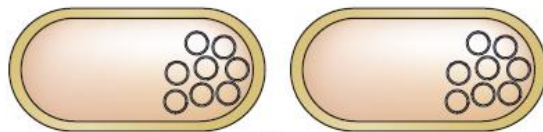
2 Transport into the host cell



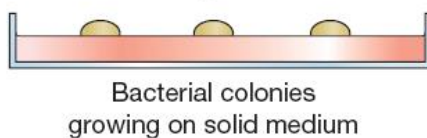
3 Multiplication of recombinant DNA molecule



4 Division of host cell

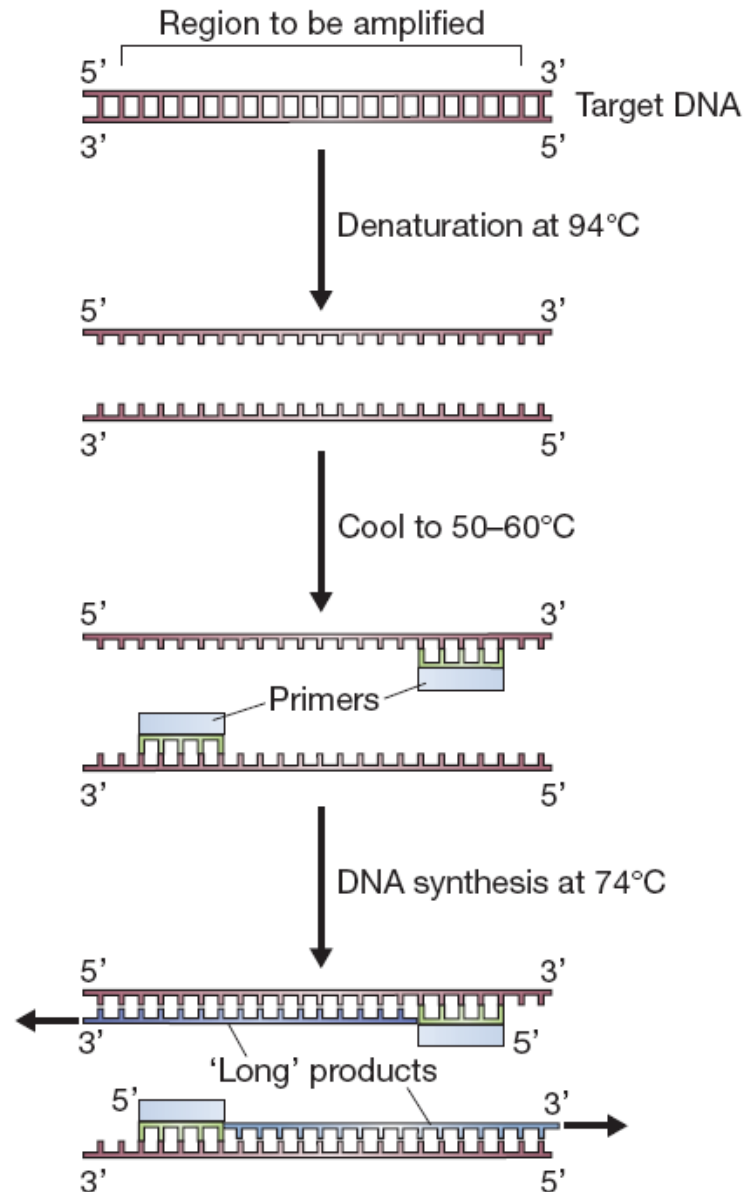


5 Numerous cell divisions resulting in a clone

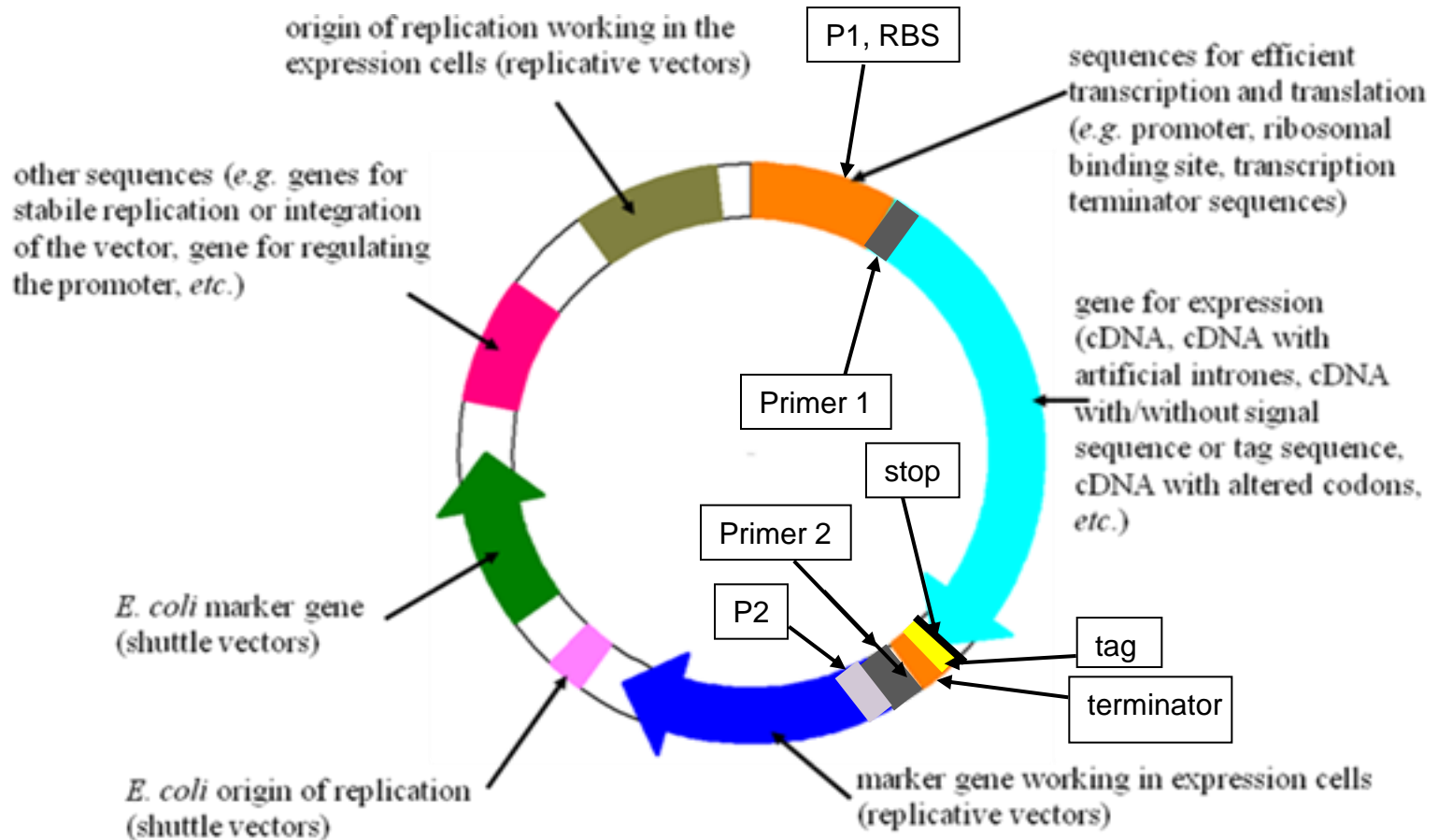


PCR

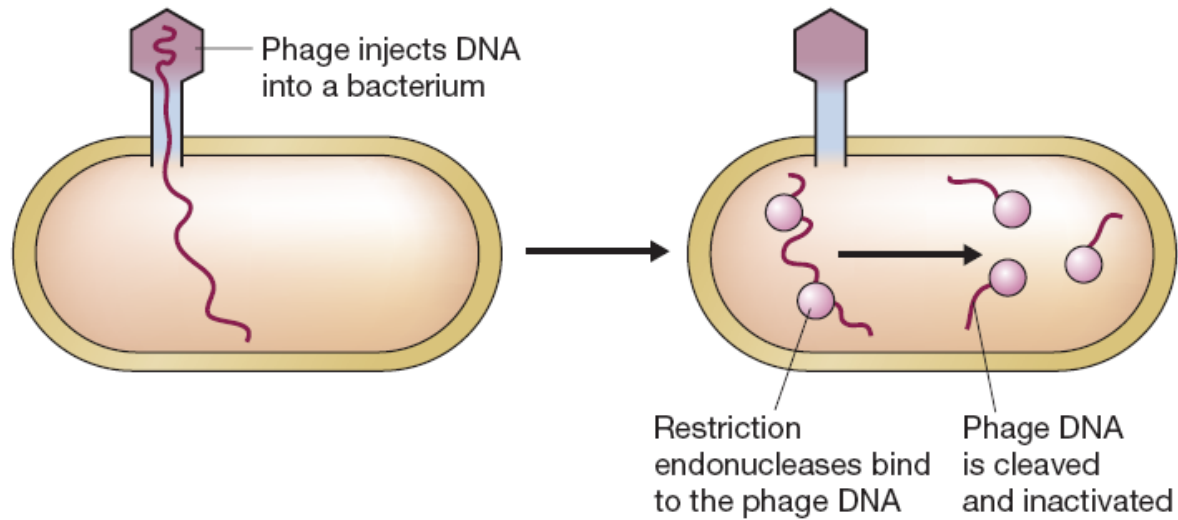
F- 1.1, 9.2



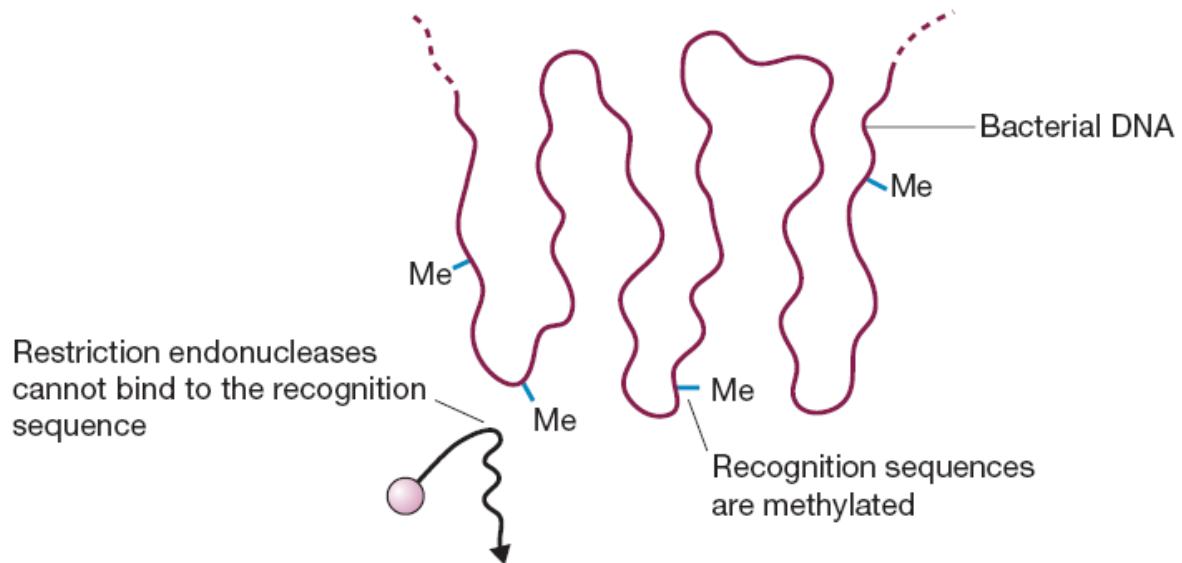
Ειδικά πλασμίδια



(a) Restriction of phage DNA



(b) Bacterial DNA is not cleaved



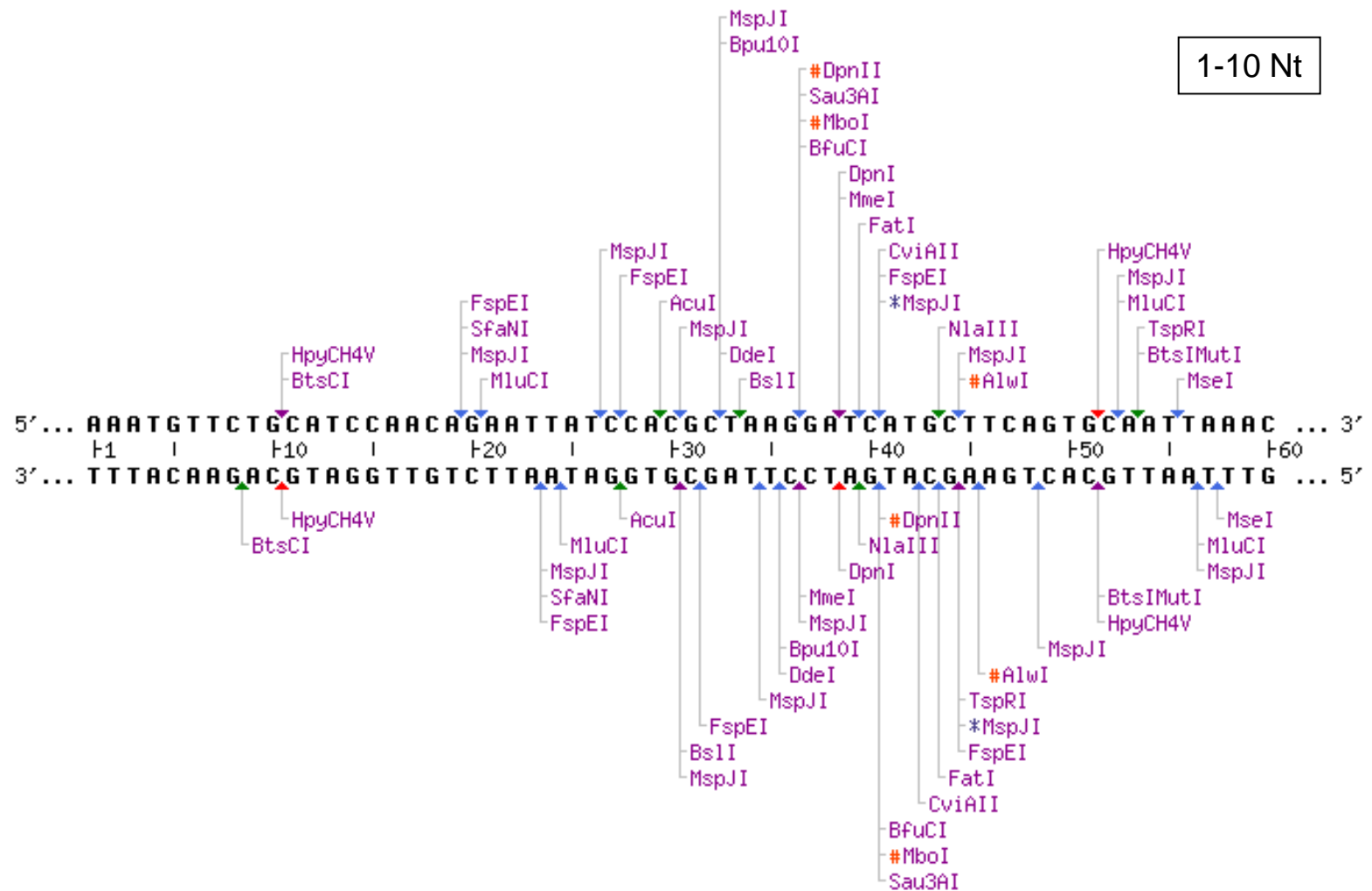
Ένζυμα περιορισμού

Πίνακας 4.1

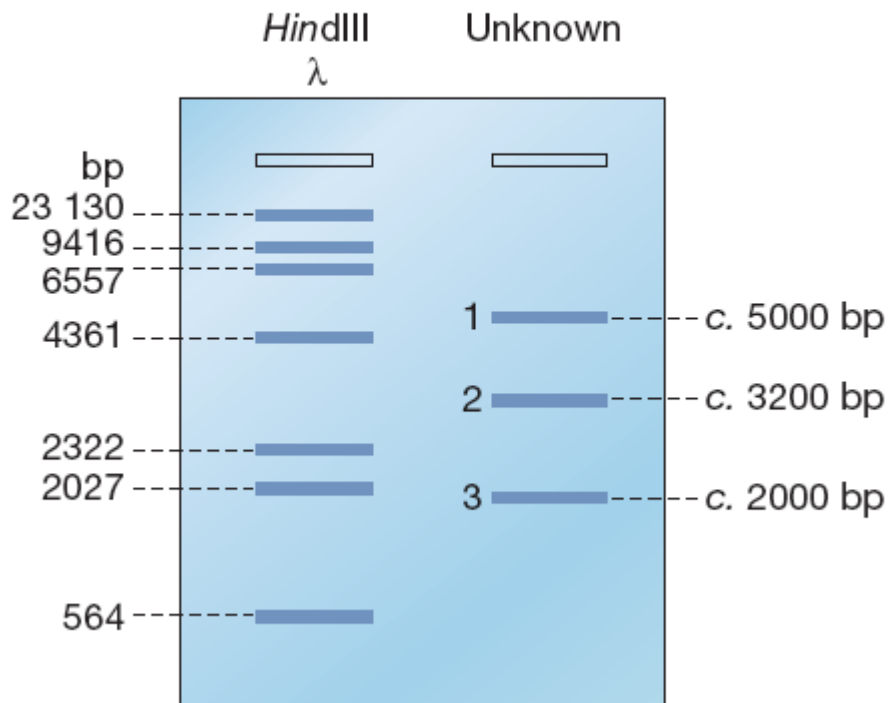
Μικροοργανισμός	Ένζυμο	Αλληλουχία αναγνώρισης	Σημειώσεις
<i>Haemophilus aegyptius</i>	HaeIII	5' ... G G C C ... 3' 3' ... C C G G ... 5'	Λεία άκρα
<i>Thermus aquaticus</i>	TaqI	5' ... T C G A ... 3' 3' ... A G C T ... 5'	5' μονόκλιωνα άκρα
<i>Haemophilus haemolyticus</i>	HhaI	5' ... G C G C ... 3' 3' ... C G C G ... 5'	3' μονόκλιωνα άκρα
<i>Desulfovibrio desulfuricans</i>	DdeI	5' ... C T N A G ... 3' 3' ... G A N T C ... 5'	
<i>Moraxella bovis</i>	MboII	5' ... G A A G A (N) ₈ ... 3' 3' ... C T T C T (N) ₇ ... 5'	
<i>Escherichia coli</i>	EcoRV	5' ... G A T A T C ... 3' 3' ... C T A T A G ... 5'	
	EcoRI	5' ... G A A T T C ... 3' 3' ... C T T A A G ... 5'	4 bp → 4 ⁴ = 1/250 bp 6 bp → 4 ⁶ = 1/4000 bp 8 bp → 4 ⁸ = 1/65000 bp
<i>Providencia stuarti</i>	PstI	5' ... C T G C A G ... 3' 3' ... G A C G T C ... 5'	
<i>Microcoleus</i>	MstII	5' ... C C T N A G G ... 3' 3' ... G G A N T C C ... 5'	
<i>Nocardia otitidiscaviarum</i>	NotI	5' ... G C G G C C G C ... 3' 3' ... C G C C G G C G ... 5'	

Τα περισσότερα ένζυμα είναι ομοδιμερή αναγνωρίζουν παλίνδρομες αλληλουχίες

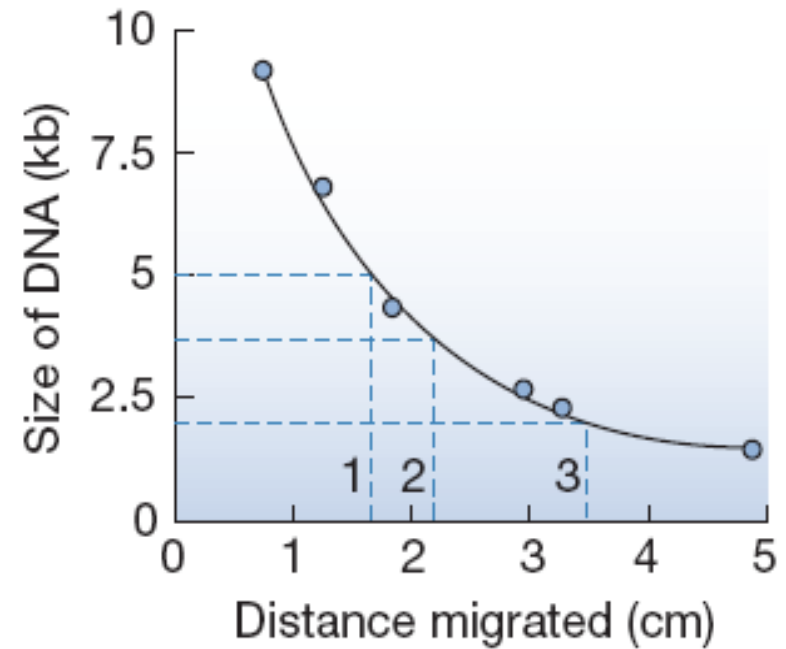
Ένζυμα περιορισμού



(a) Rough estimation by eye

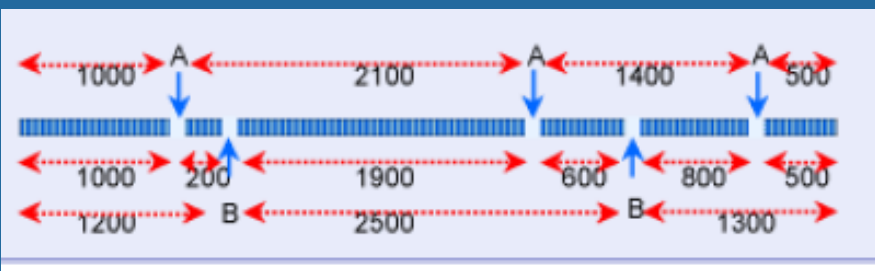
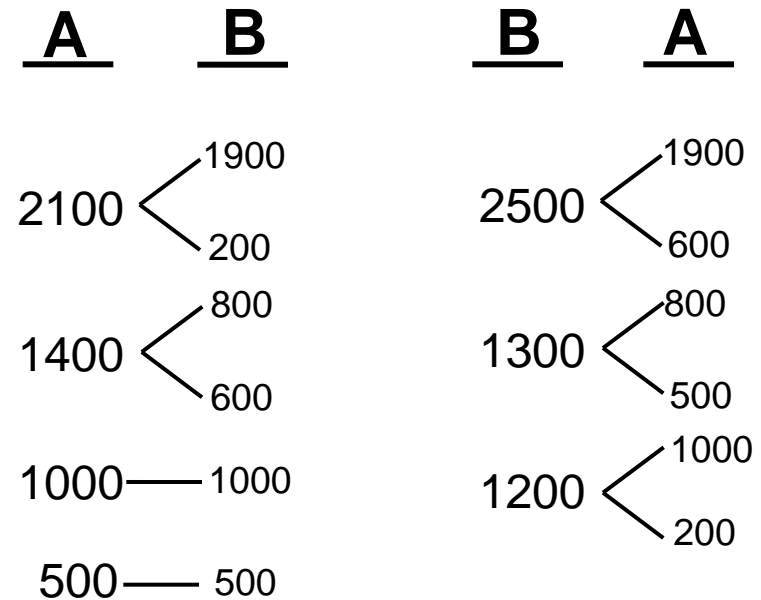
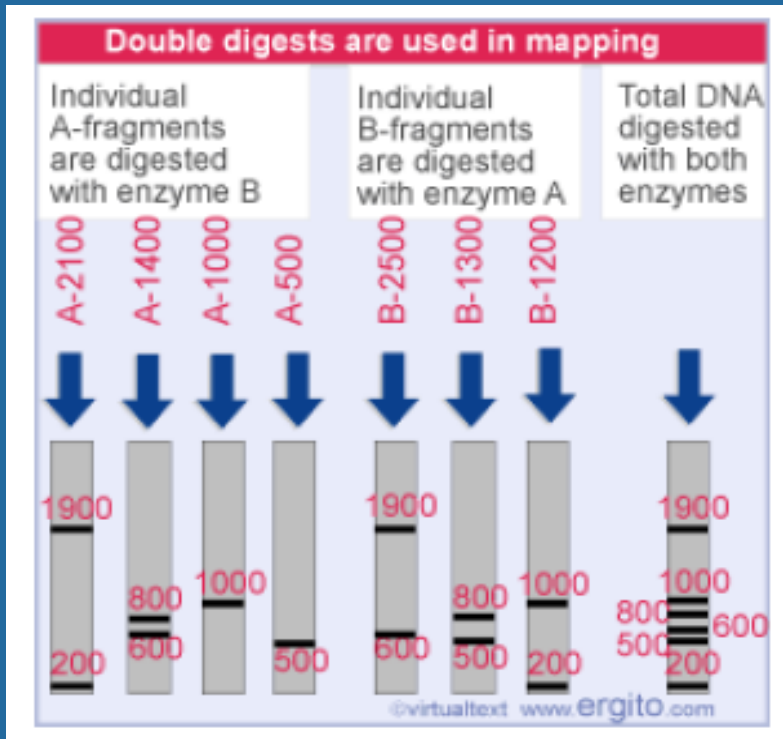


(b) Accurate graphical estimation



Συναρμολόγηση μικρών τμημάτων DNA

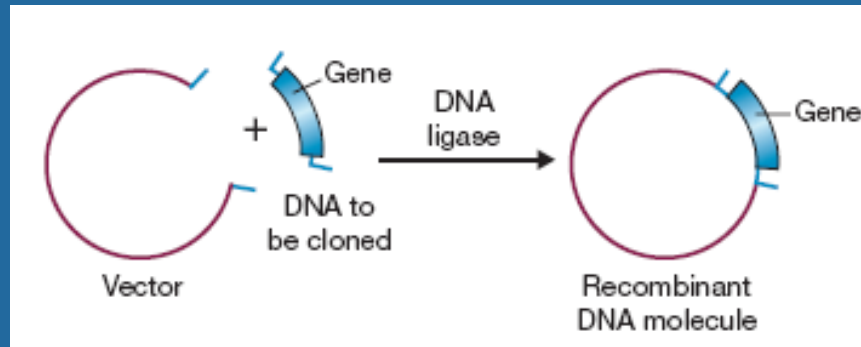
Τμήμα 5 Kb



Αλληλούχηση κάθε τμήματος και συναρμολόγηση

Σύνδεση τμημάτων DNA

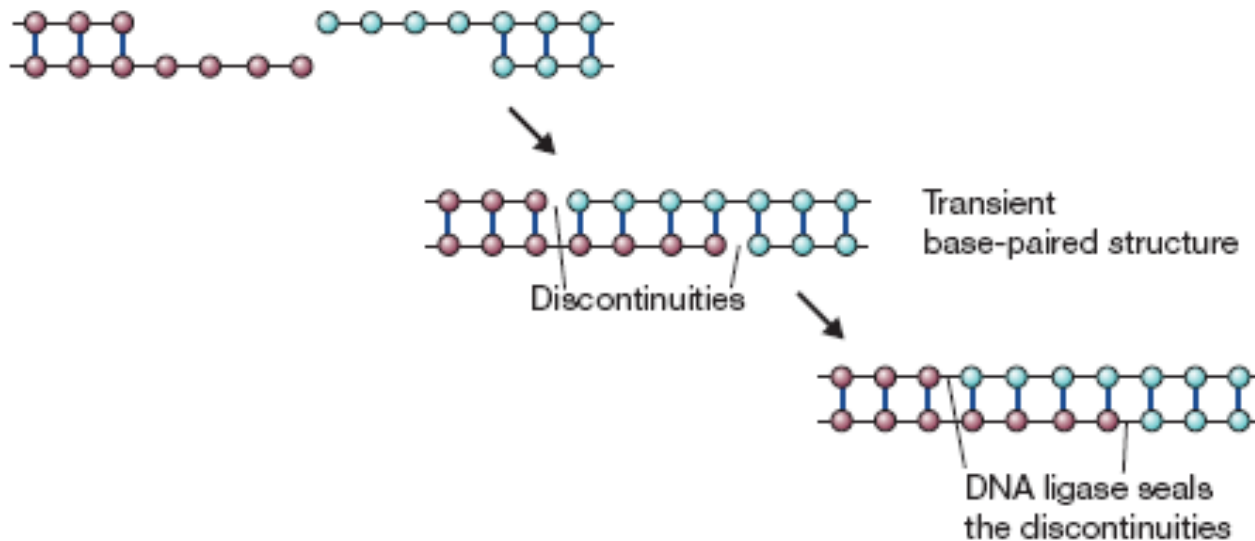
F- 4.19, 4.20



(a) Ligating blunt ends



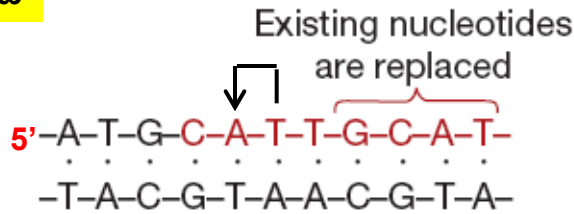
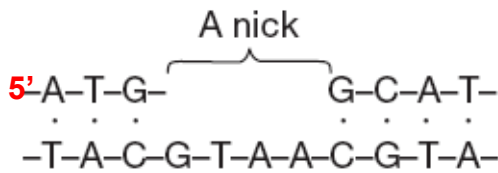
(b) Ligating sticky ends



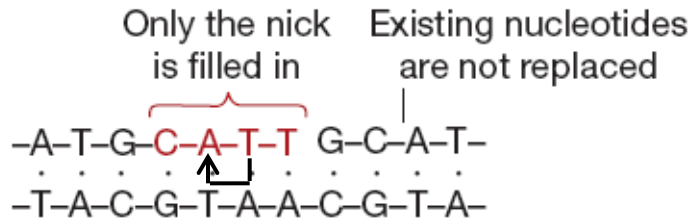
Ένζυμα με συμβατά άκρα

Ένζυμο	Αλληλουχία	Προϊόν	Μονόκλιωνα άκρα
BamHI	GGATCC	G GATCC CCTAG G	5'-GATC
BglII	AGATCT	A GATCT TCTAG A	5'-GATC
BclI	TGATCA	T GATCA ACTAG T	5'-GATC
Sau3AI	NGATCN	N GATCN NCTAG N	5'-GATC

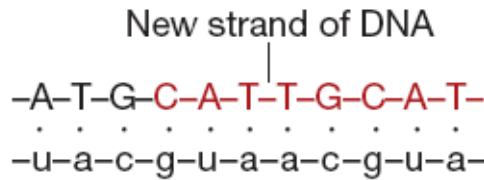
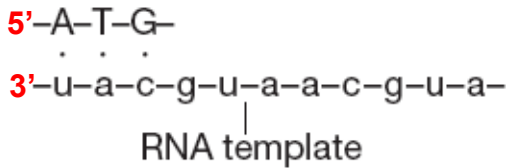
(b) DNA polymerase I **5'→3' & 3'→5' έξω**



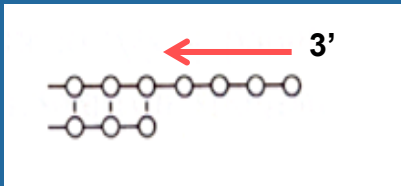
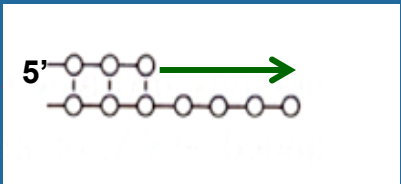
(c) The Klenow fragment (T4 DNA πολυμεράση) **3'→5' έξω**



(d) Reverse transcriptase



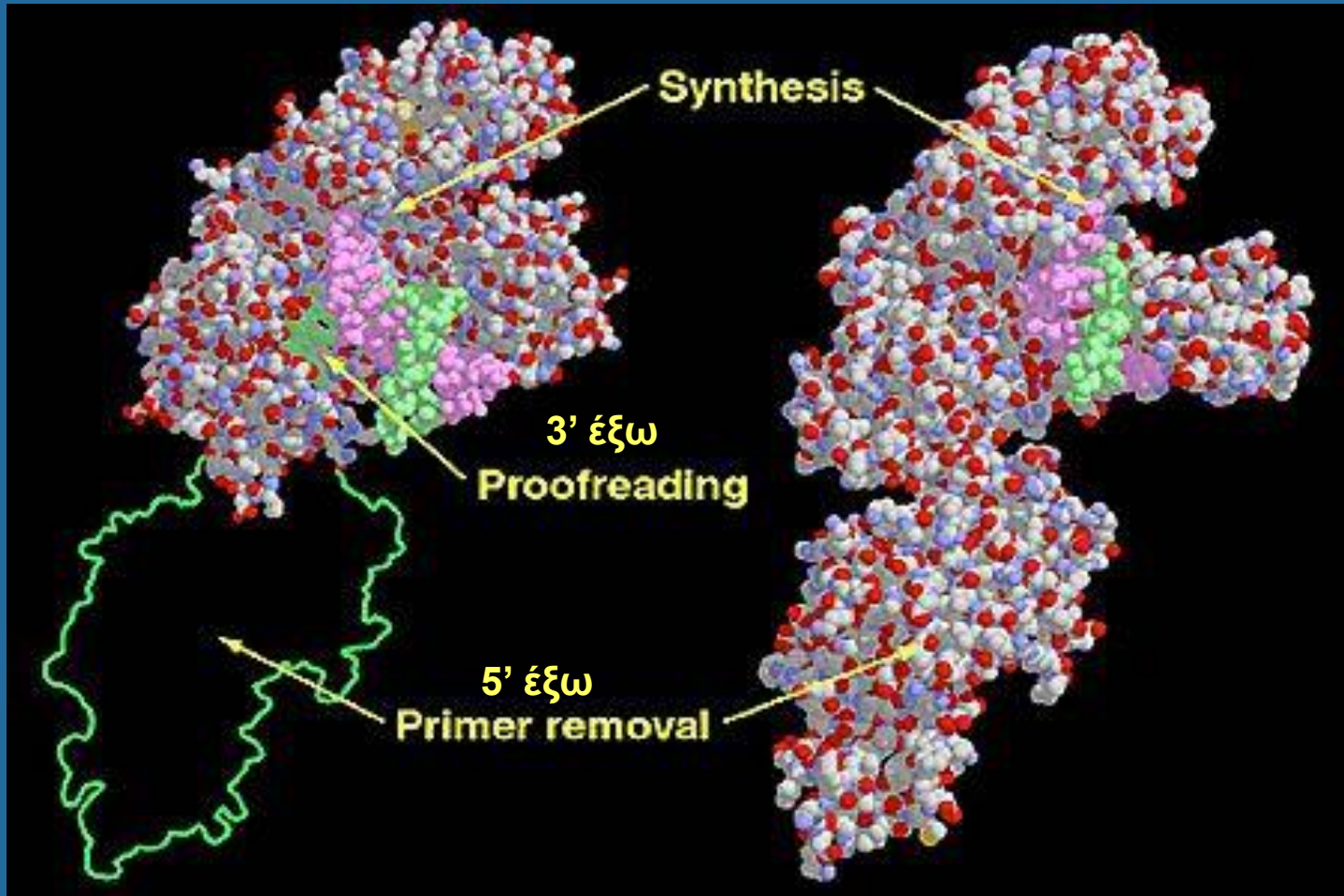
+ dNTPs-Γέμισμα



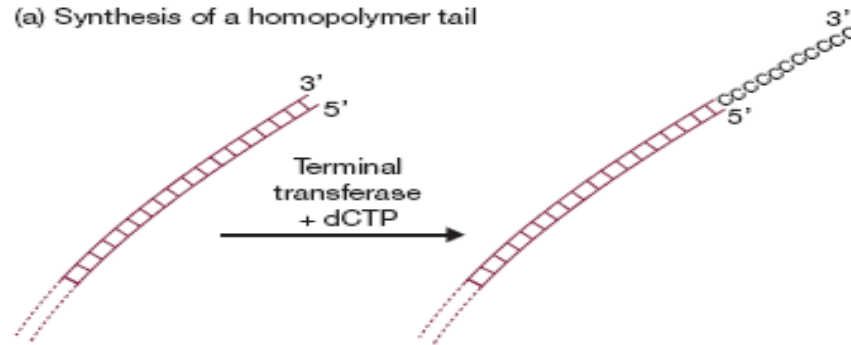
- dNTPs-Άδειασμα

Klenow

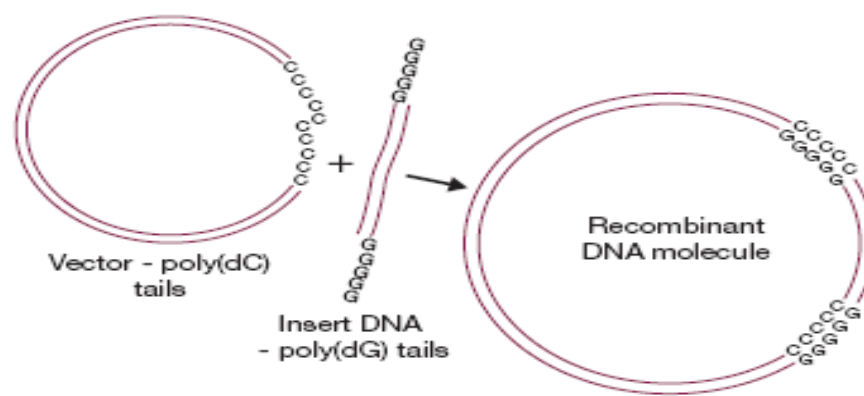
DNA πολυμεράση I



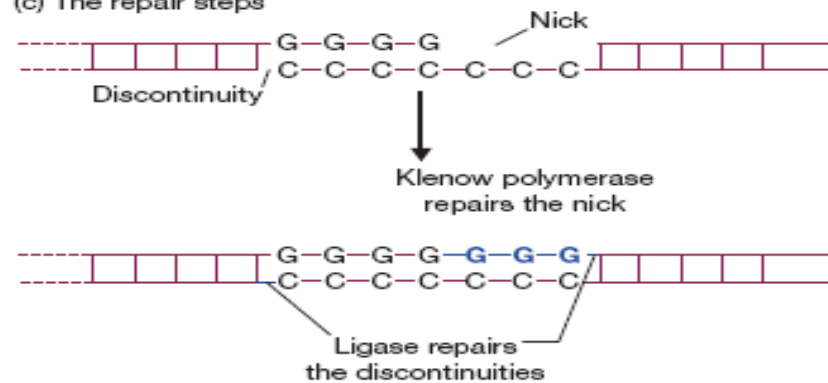
(a) Synthesis of a homopolymer tail



(b) Ligation of homopolymer tails



(c) The repair steps



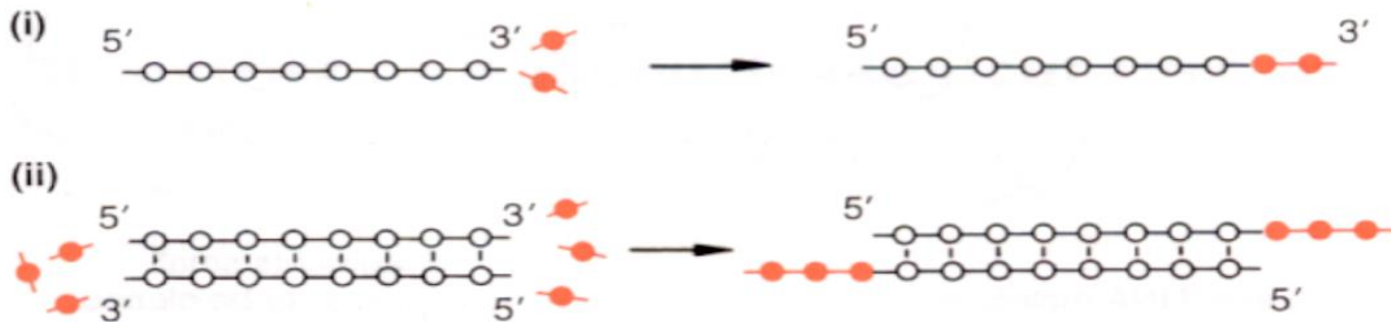
(a) Alkaline phosphatase



(b) Polynucleotide kinase



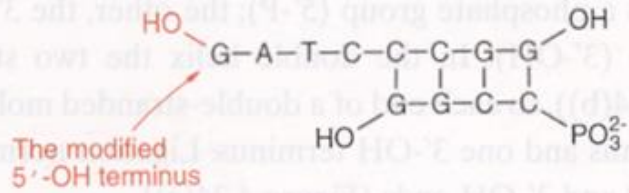
(c) Terminal deoxynucleotidyl transferase



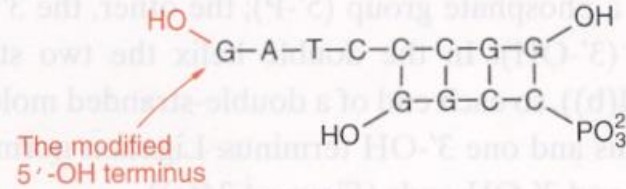
Adaptors (Προσαρμοστές)

F-4.23, 4.25

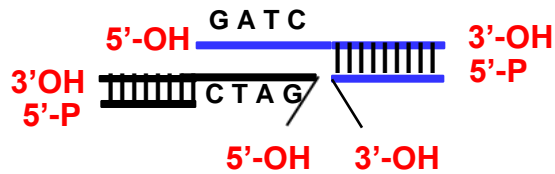
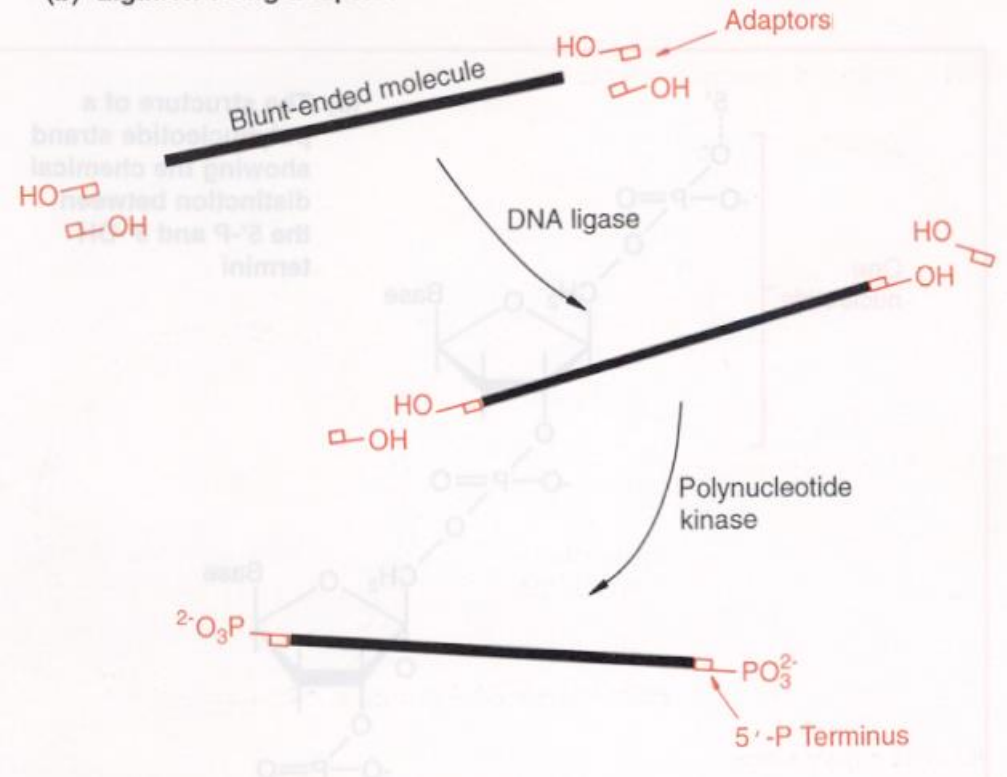
(a) The precise structure of an adaptor



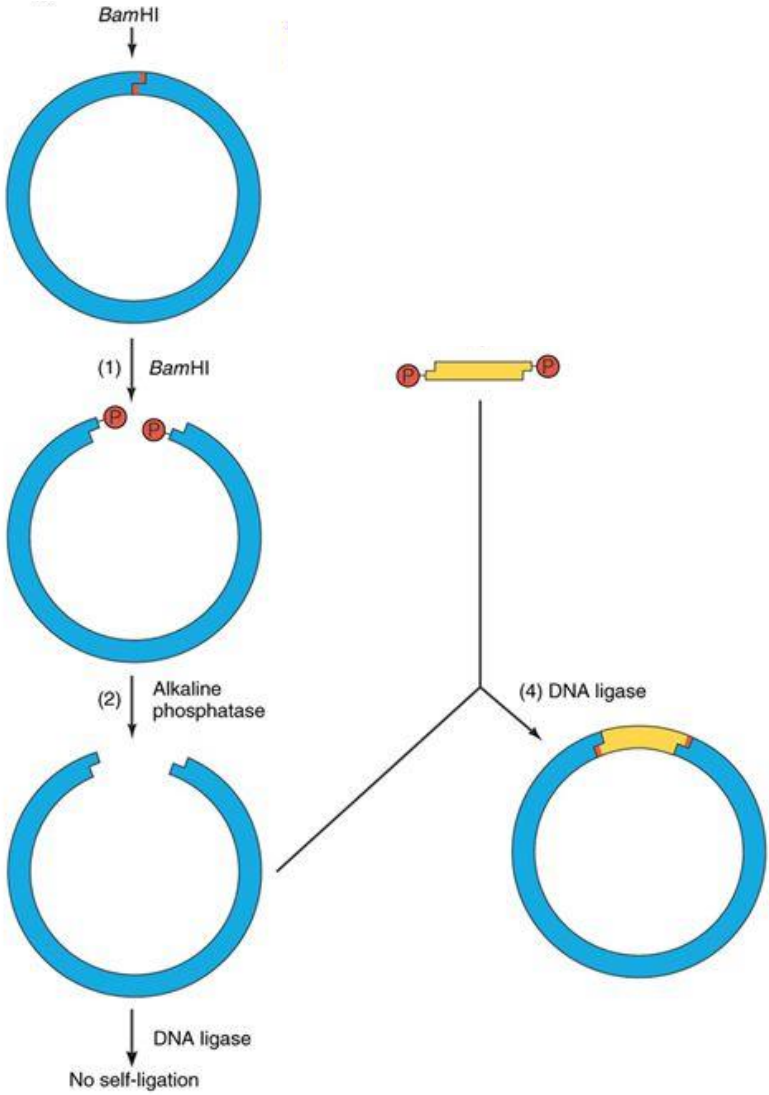
(a) The precise structure of an adaptor



(b) Ligation using adaptors



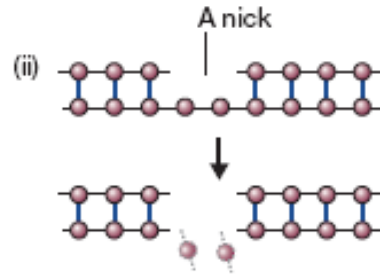
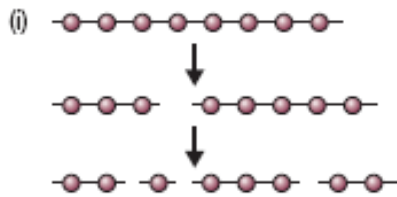
Cloning with adaptors



Νουκλεάσες

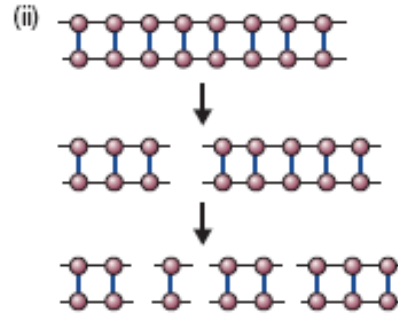
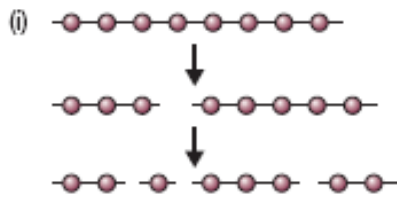
F- 4.2, 4.3

(a) S1 nuclease



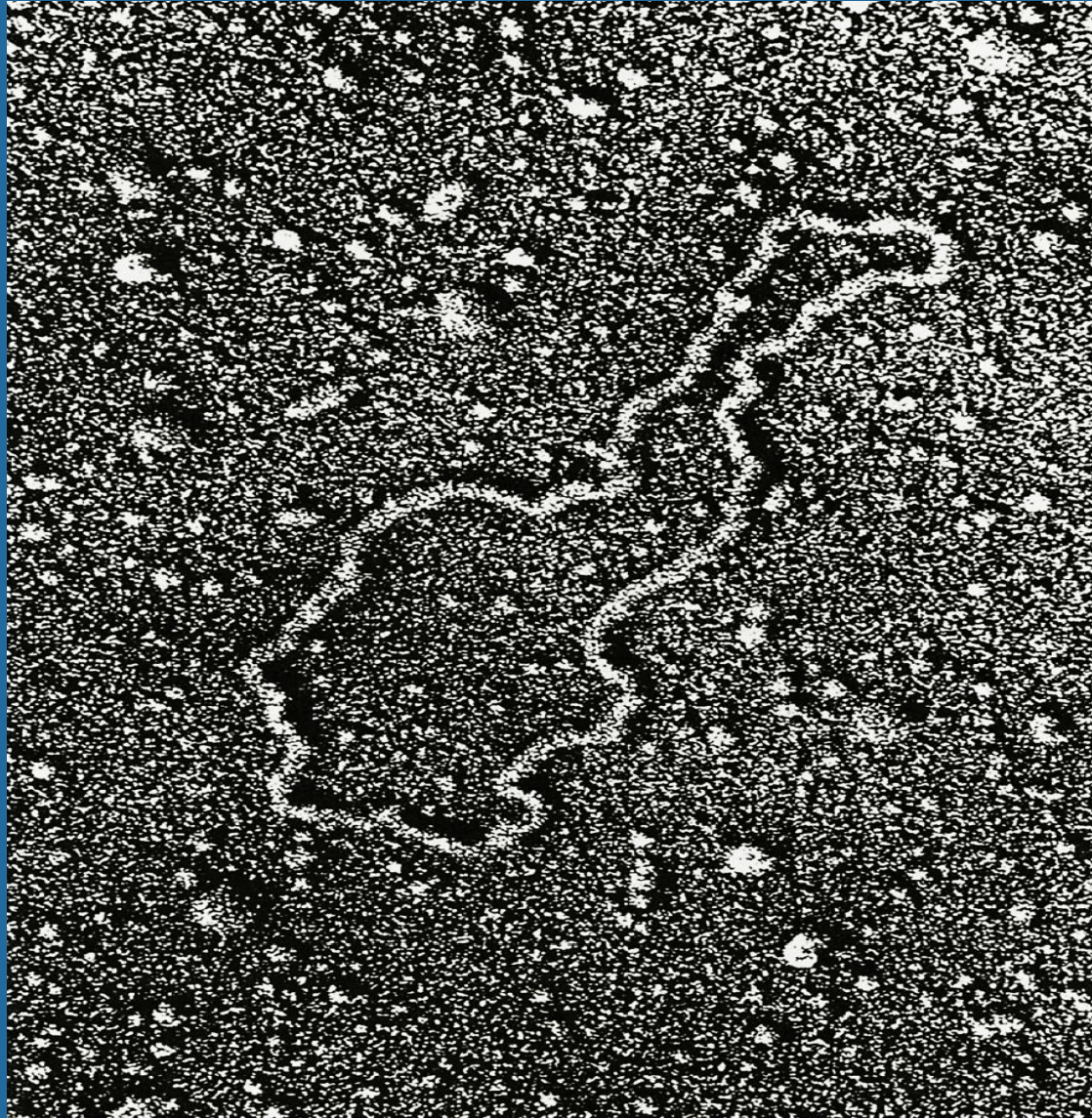
S1 νουκλεάση: S (DNA & RNA)

(b) DNase I



DNάση I: D,S (DNA & DNA-RNA)

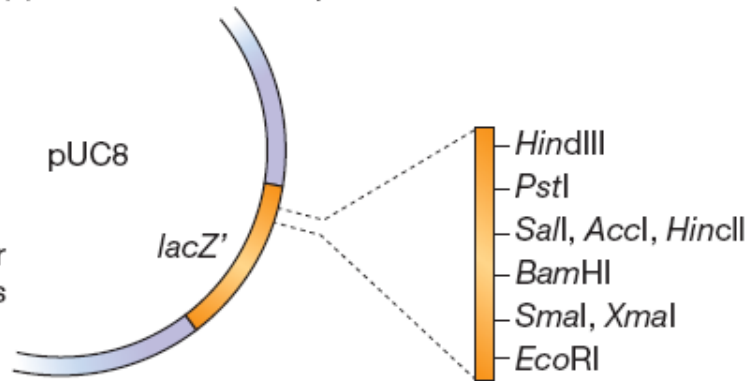
Κλωνοποίηση σε πλασμίδια



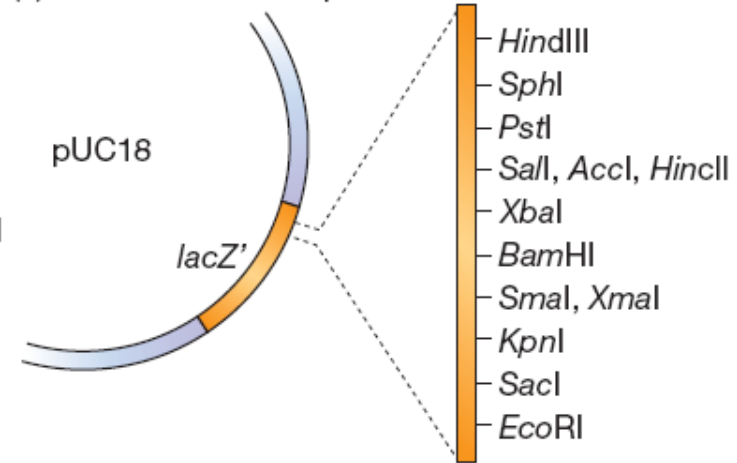
(a) pUC8



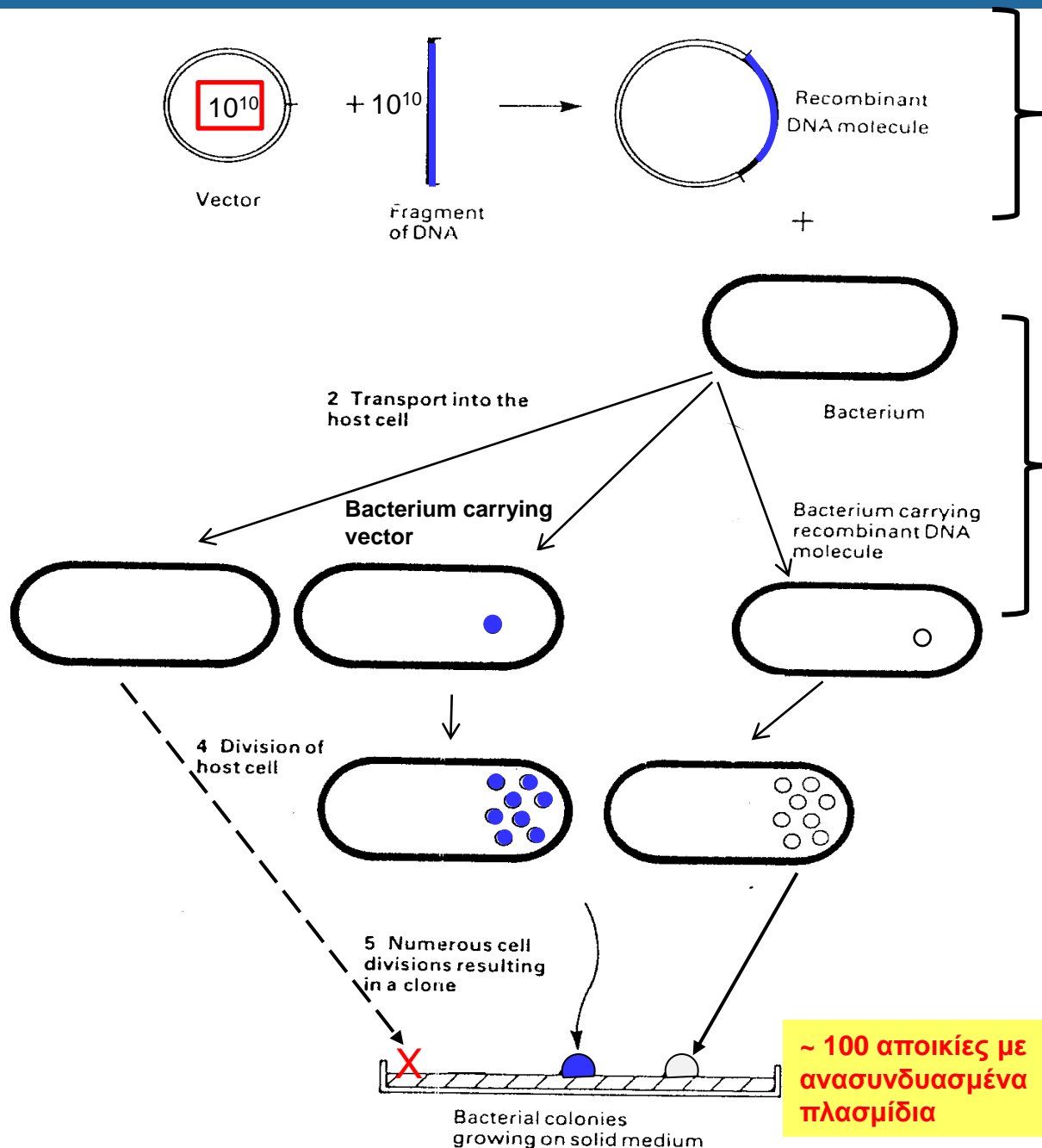
(b) Restriction sites in pUC8



(c) Restriction sites in pUC18



Κλωνοποίηση σε πλασμίδια



Συχνότητα ανασυνδυασμένων πλασμιδίων: $10^{-3} - 10^{-4}$

Συχνότητα μετασχηματισμού: $10^{-4} - 10^{-5}$

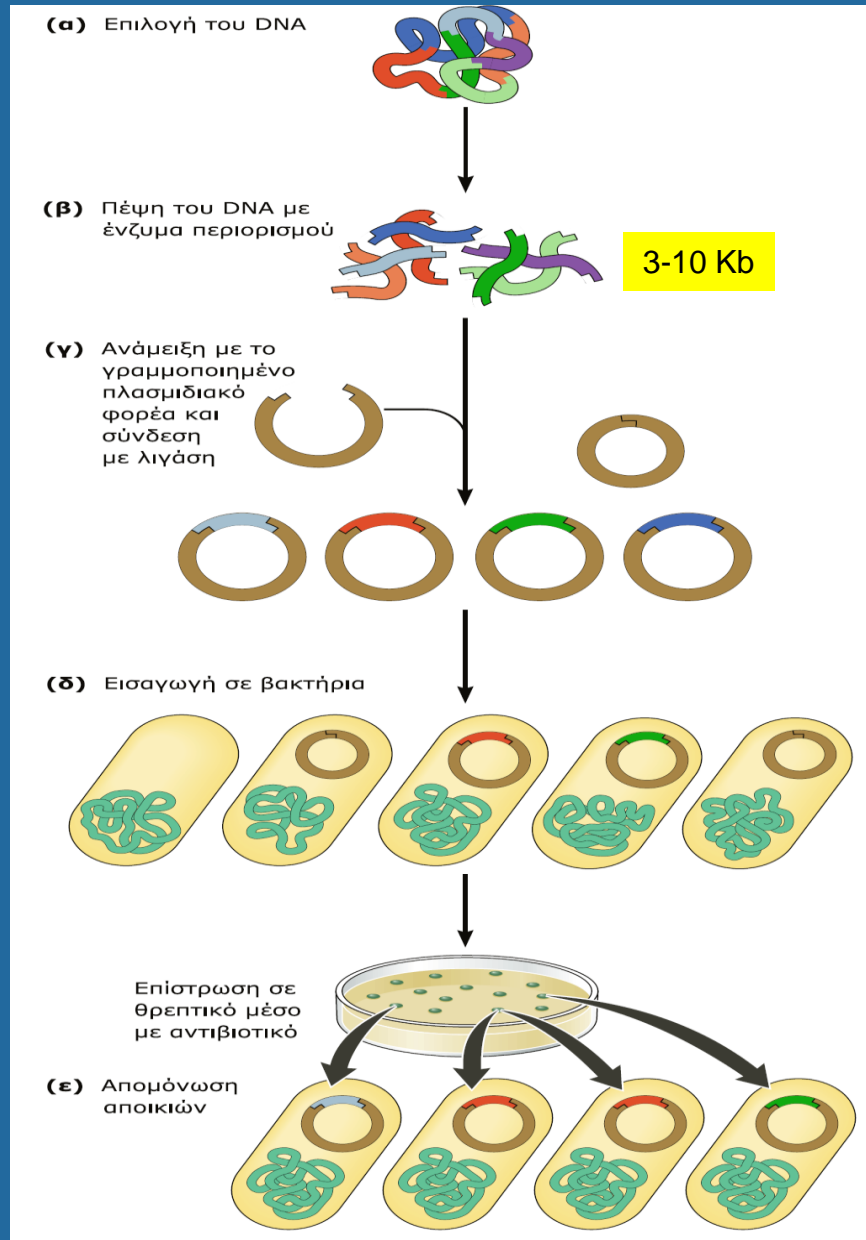


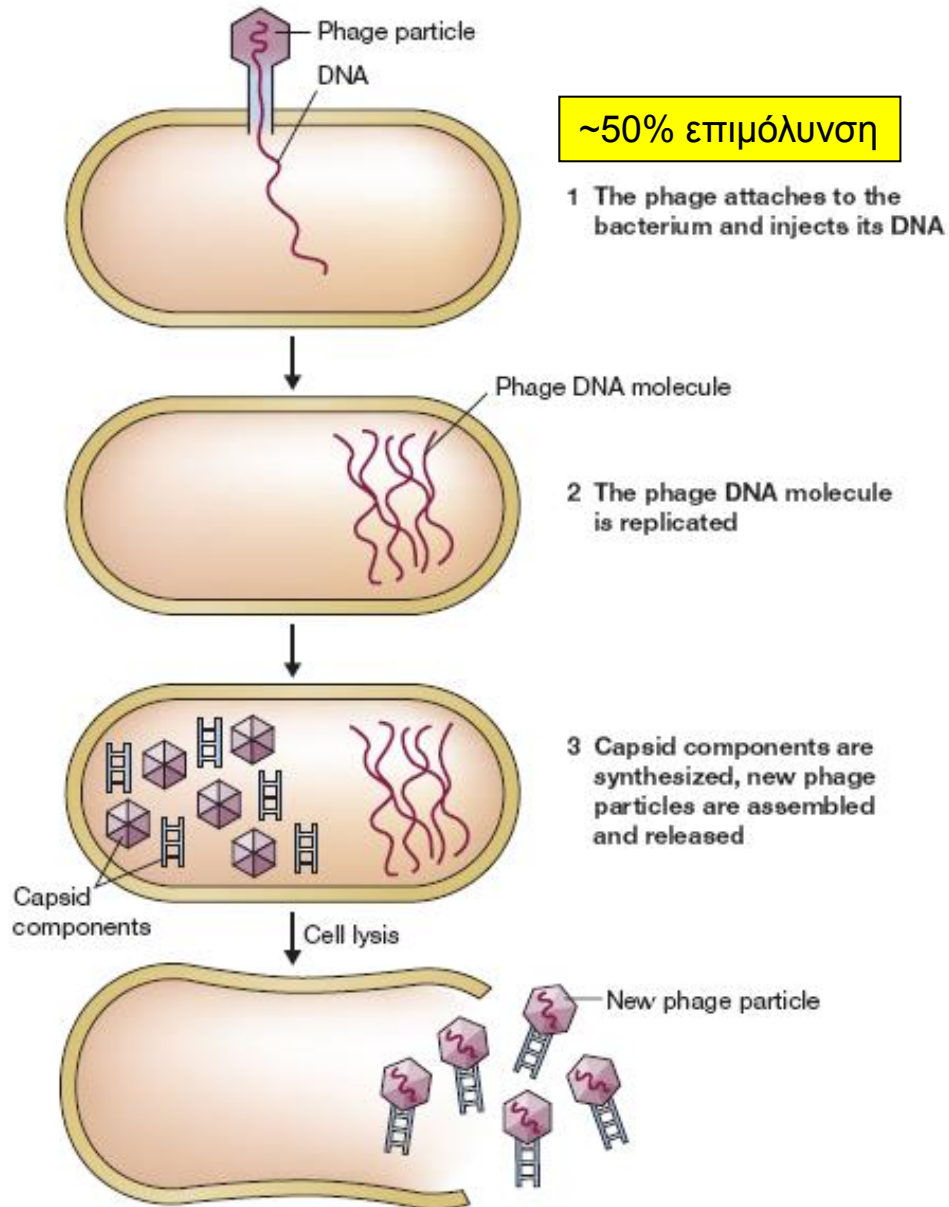
~ 100 αποικίες με ανασυνδυασμένα πλασμίδια

Μία αποικία *E. coli*
~ 10^6 βακτήρια

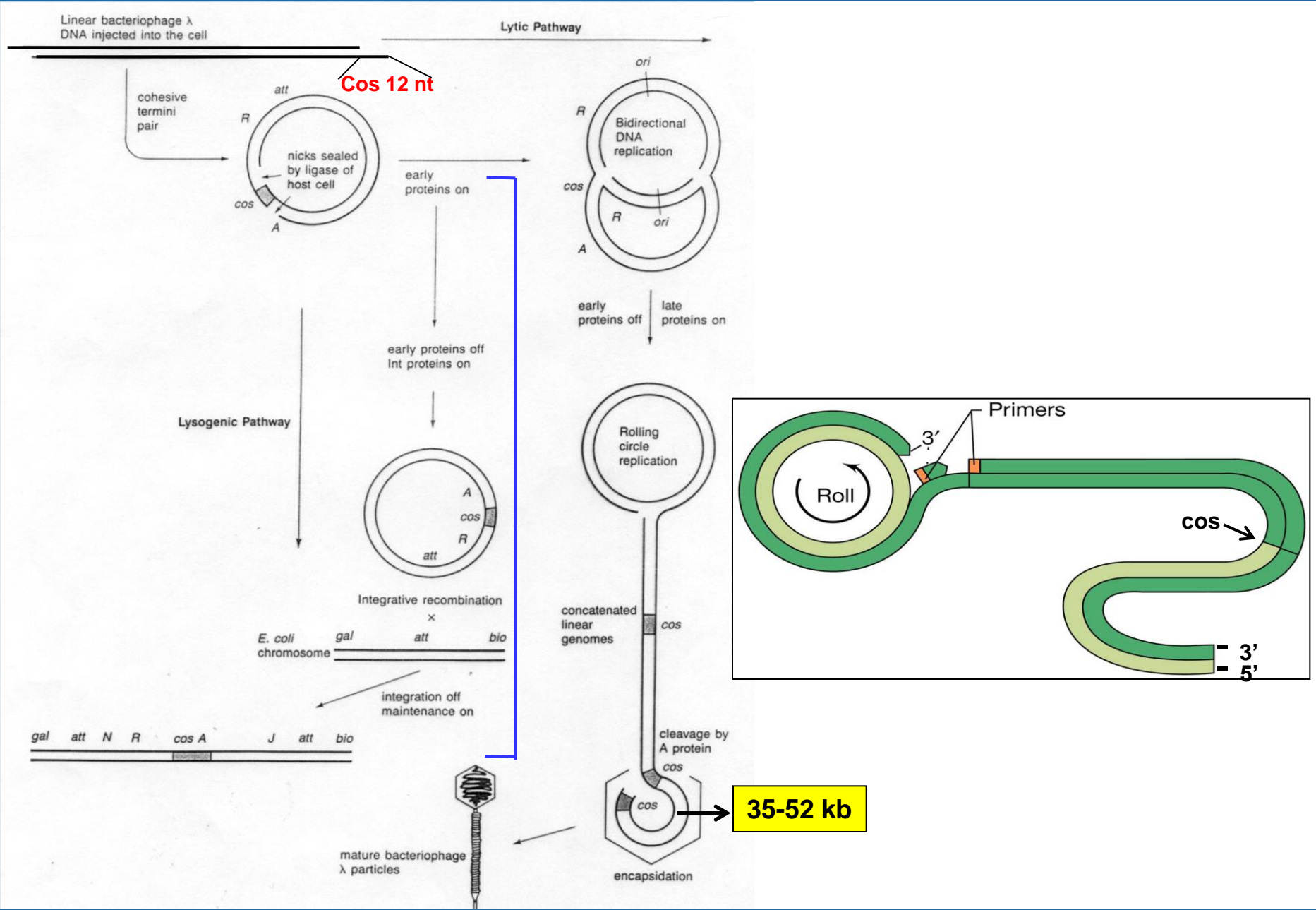
Βιβλιοθήκες σε πλασμίδια

ΕΙΚΟΝΑ 4.5



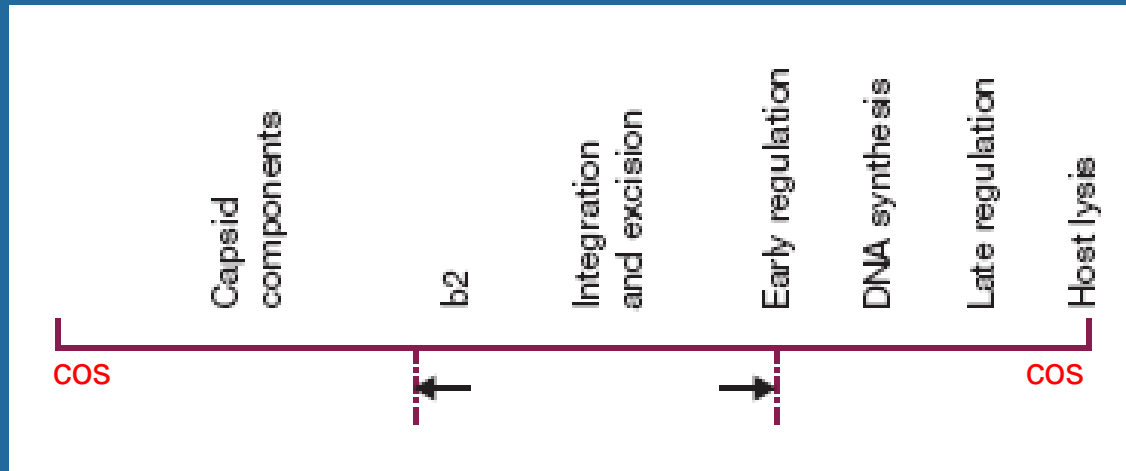


Κύκλος του λ



Τεχνητοί λ παρεμβολής (πολλαπλασιάζονται)

F- 6.10, 6.12



(a) Construction of a λ insertion vector



Πλάκες

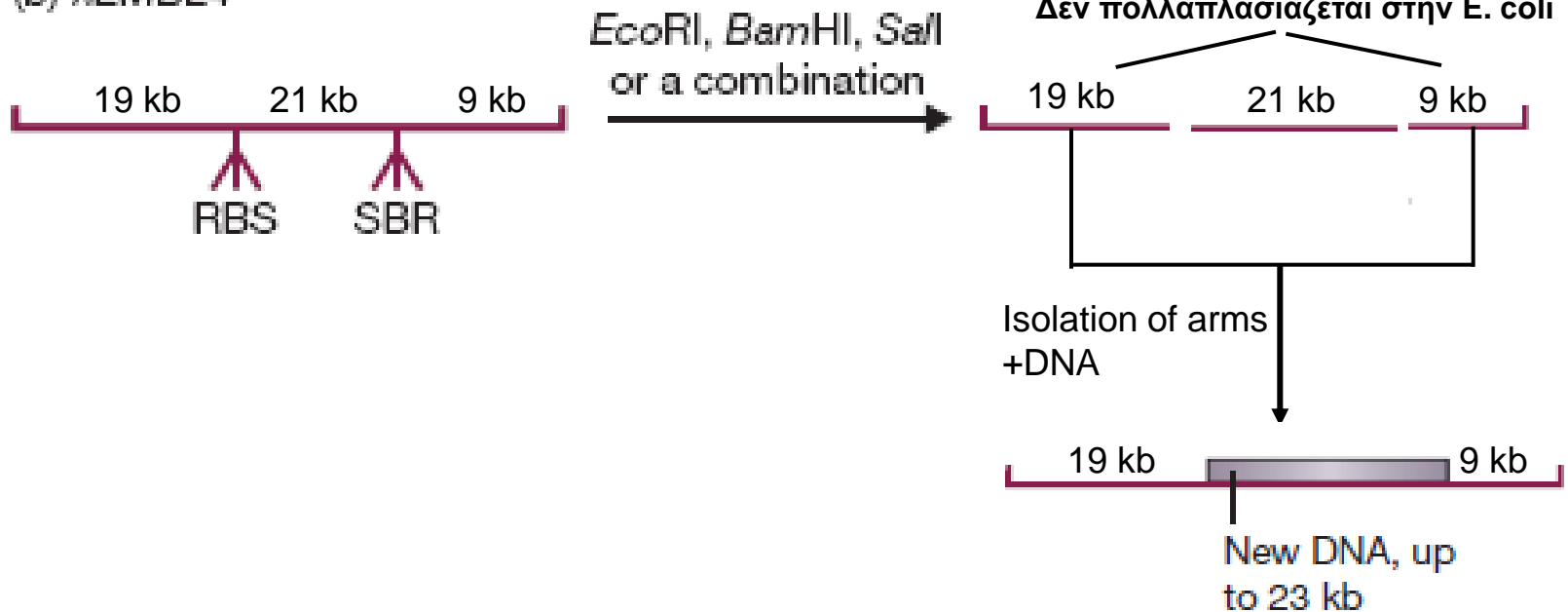
Επιμόλυνση

In vitro πακετάρισμα

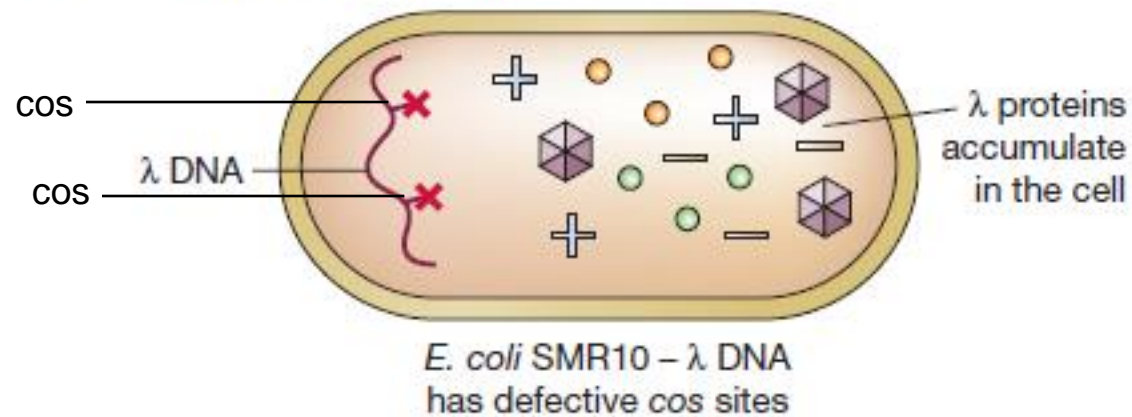
Τεχνητοί λ αντικατάστασης (Δεν πολλαπλασιάζονται)

F- 6.13

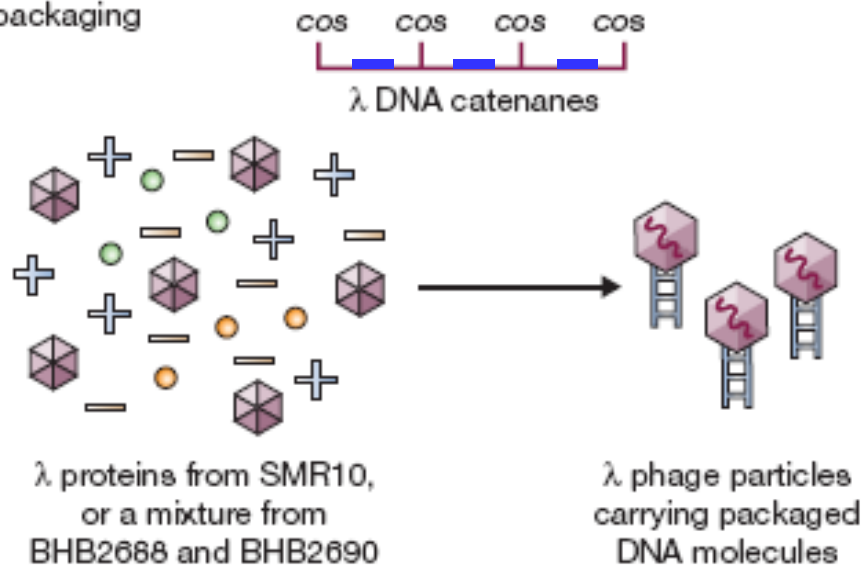
(b) λEMBL4



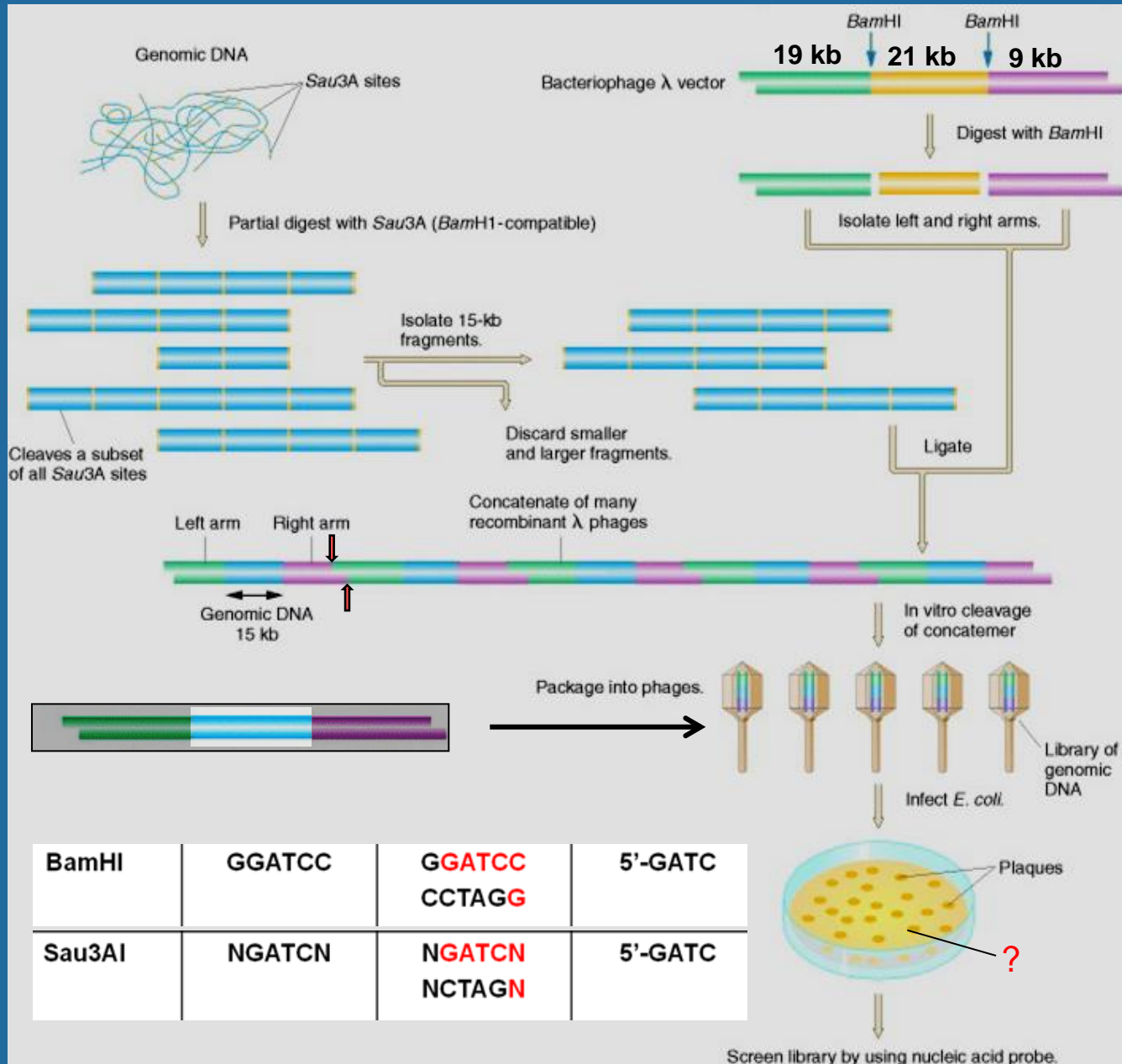
(a) A single-strain packaging system



(c) *In vitro* packaging



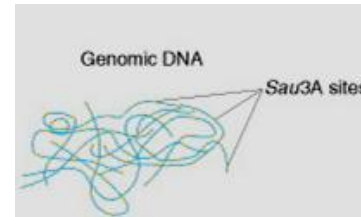
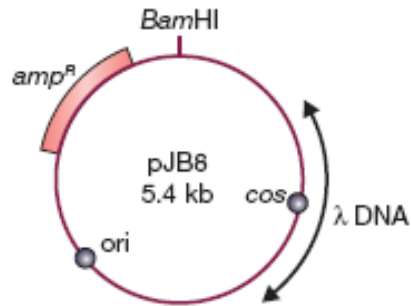
Δημιουργία βιβλιοθήκης



Κλωνοποίηση σε Κοσμίδια

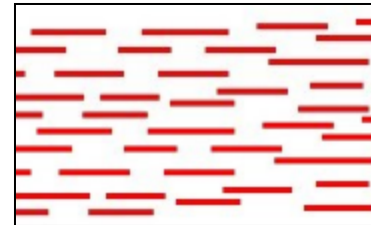
F- 6.15

(a) A typical cosmid

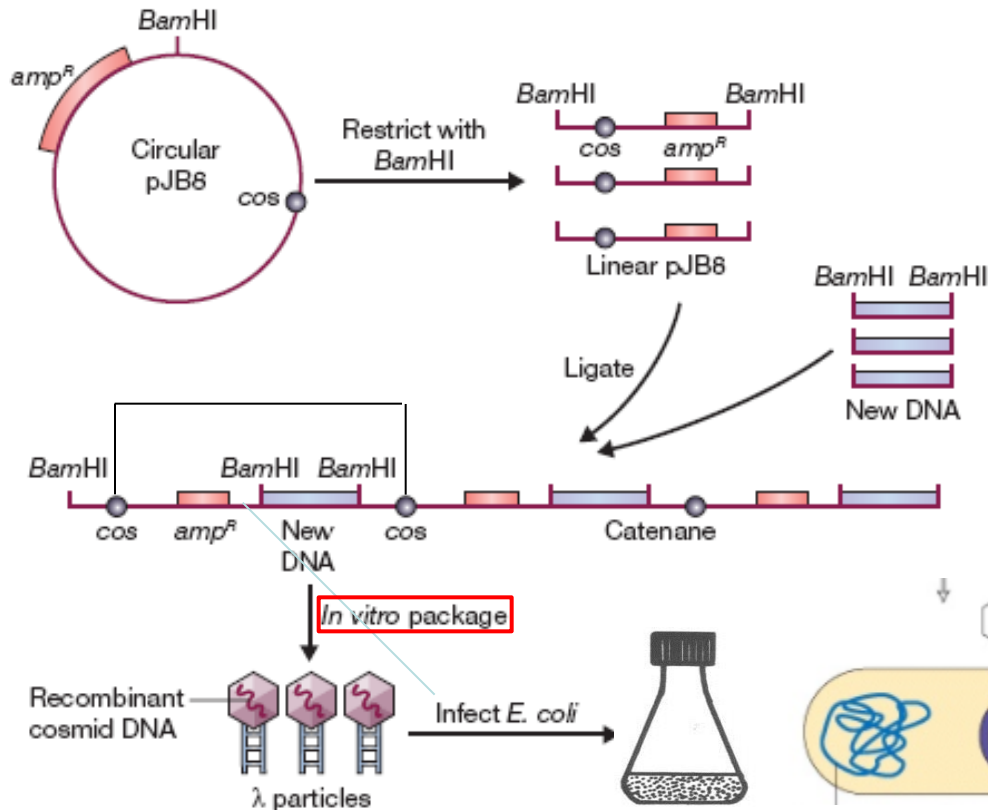


(40- 45 Kb)

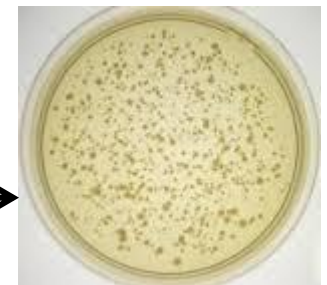
Sau3A



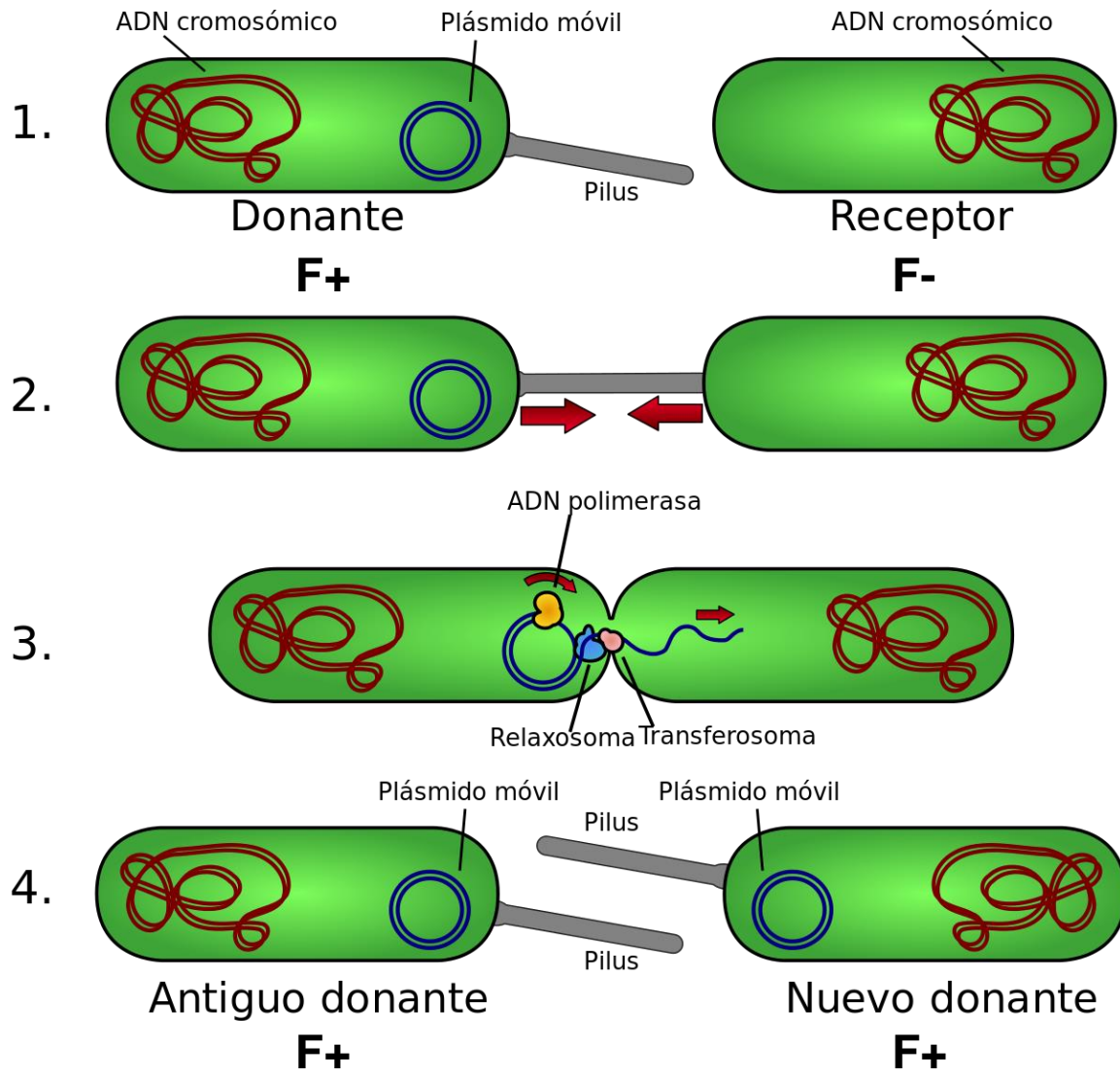
(b) Cloning with pJB6



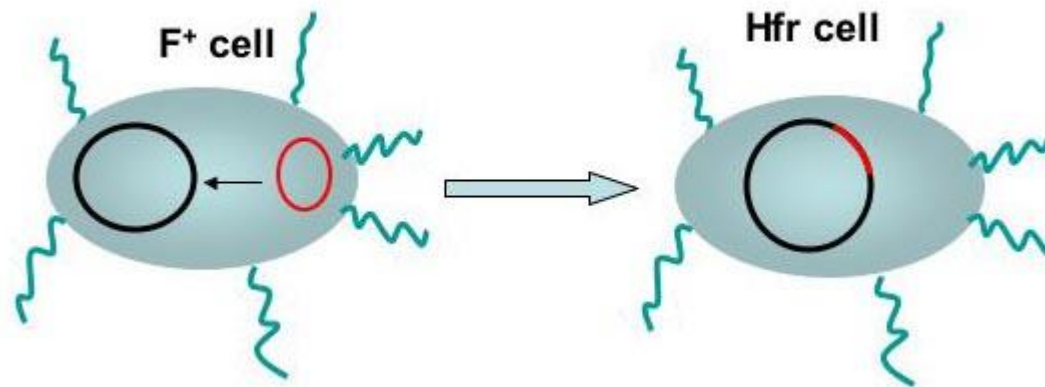
ΑΠΟΙΚΙΕΣ



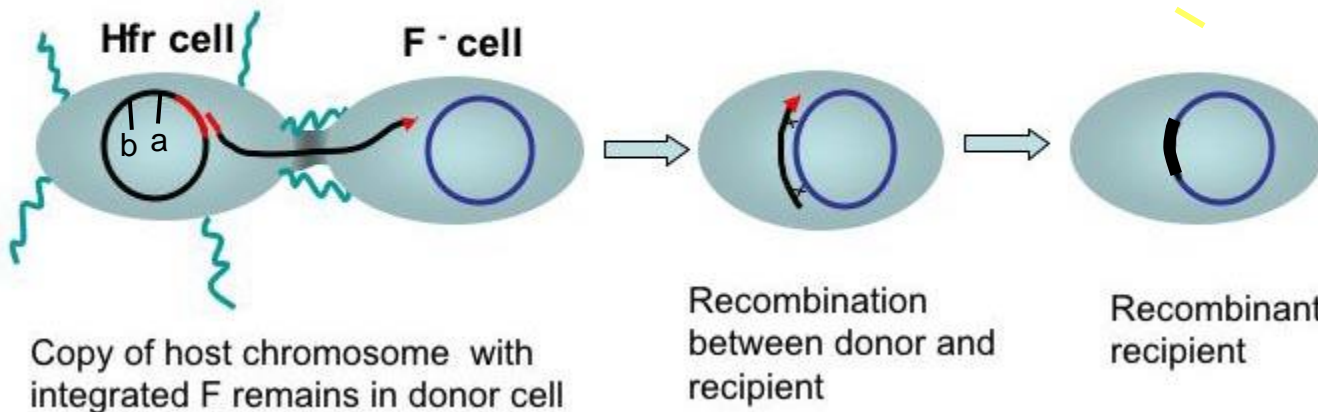
Fertility factor



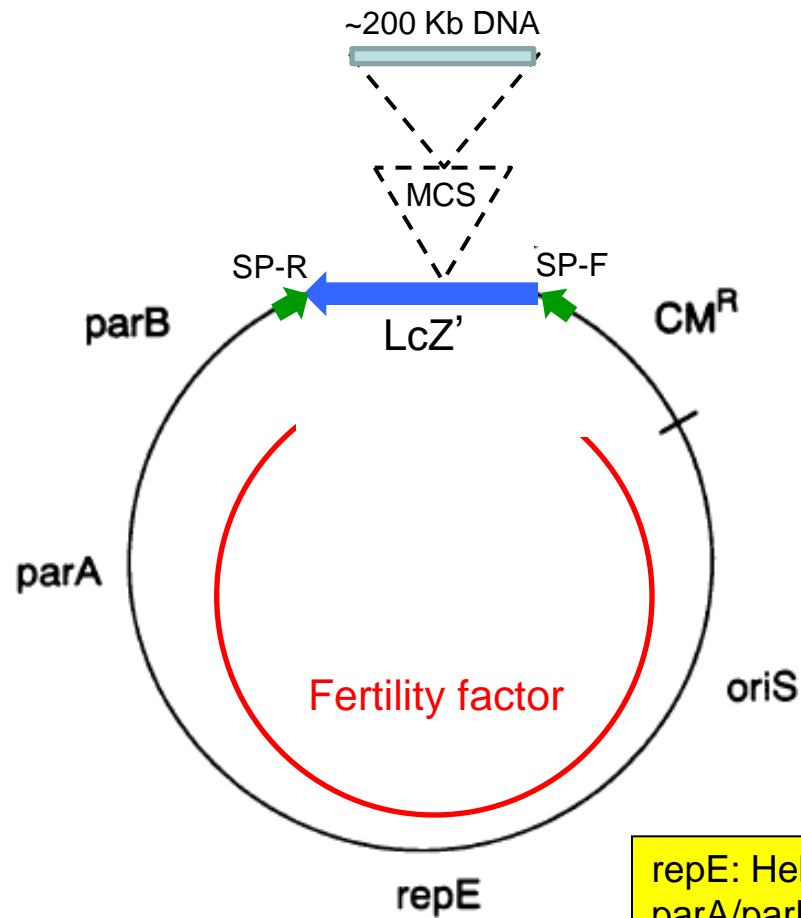
On rare occasions, F integrates into host chromosome



High Frequency Recombinant strains can donate chromosomal DNA of host to a recipient cell

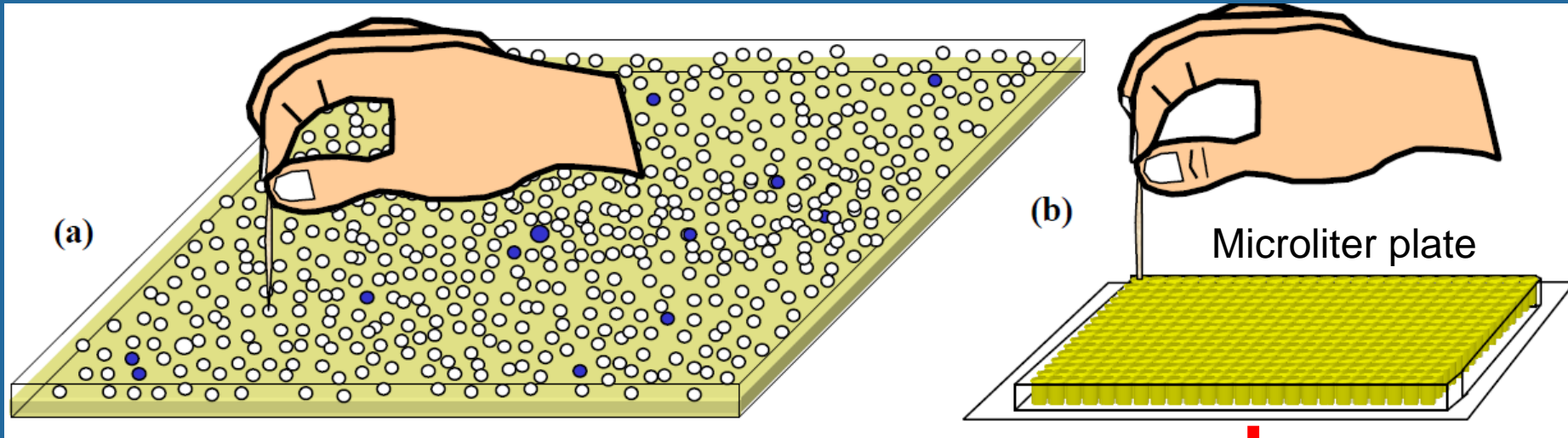


Κλωνοποίηση σε βακτηριακά τεχνητά χρωμοσώματα(Bacs)



repE: Helicase
parA/parB: Immunity, copy number (1-2)
and partitioning

Κλωνοποίηση σε βακτηριακά τεχνητά χρωσώματα(Bacs)



Ανθρώπινο γονιδίωμα= 3×10^9 bp →
 $1,5 \times 10^4$ τμήματα DNA των 2×10^5 bp
Μετασχηματισμός: $\sim 2 \times 10^{-3}$ αποικίες/τρυβλίο
→ ~ 100 τρυβλία με 10X κάλυψη του
γονιδιώματος

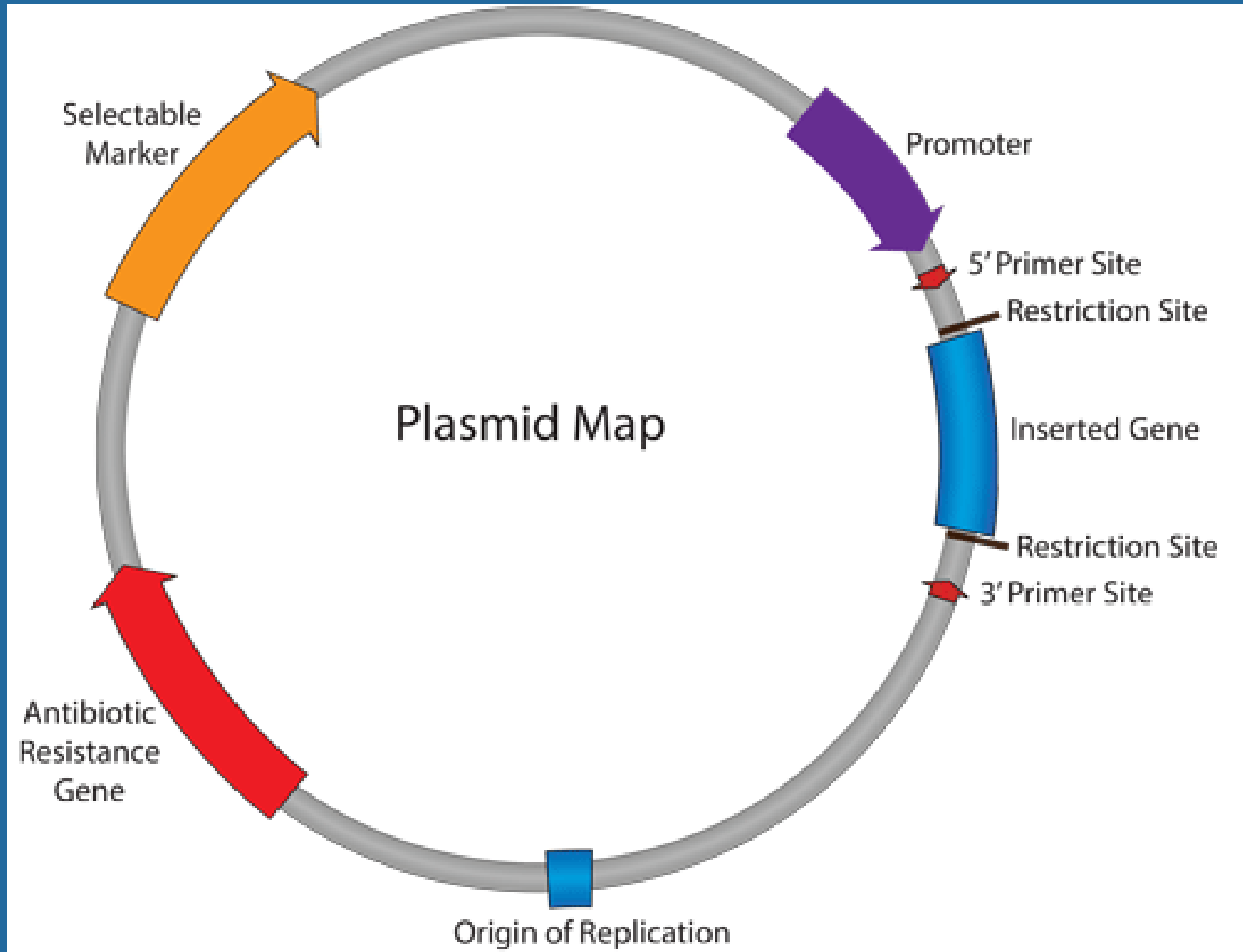
	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Table 6.1

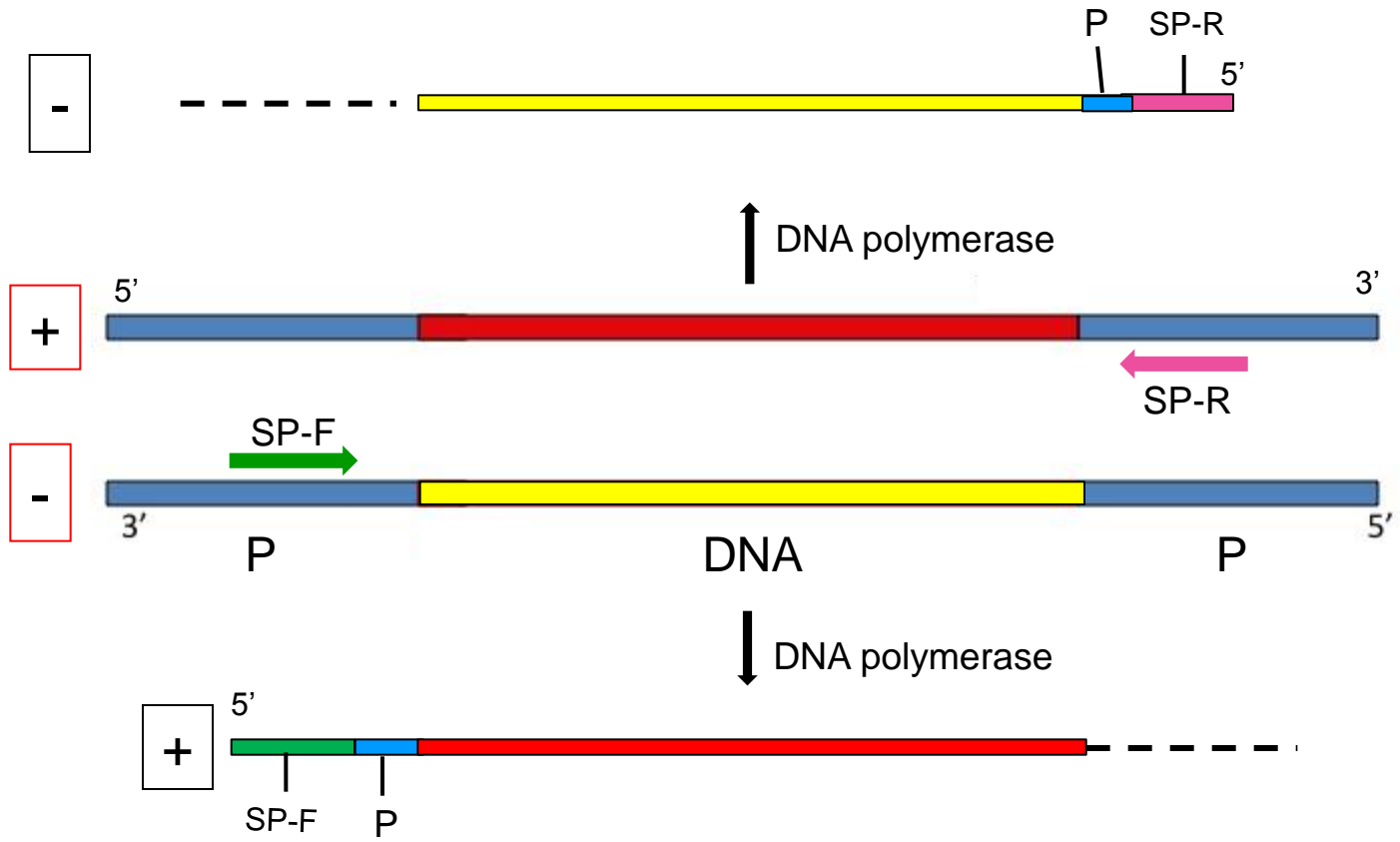
Number of clones needed for genomic libraries of a variety of organisms.

SPECIES	GENOME SIZE (bp)	NUMBER OF CLONES*		
		17 kb FRAGMENTS† Lamda phage	35 kb FRAGMENTS‡ Cosmids	200 Kb FRAGMENTS BAC plasmids
<i>E. coli</i>	4.6×10^8	(235)	(114)	(20)
<i>Saccharomyces cerevisiae</i>	1.8×10^7			
<i>Drosophila melanogaster</i>	1.2×10^8			
Rice	5.7×10^8			
Human	3.2×10^9	(~100.000 X3)	(60.000 X5)	(15.000 X10)
Frog	2.3×10^{10}			

Αλληλούχιση DNA



Αλληλούχιση DNA



Αλληλούχιση DNA Μέθοδος Sanger

EΙΚΟΝΑ 4.12

(a)

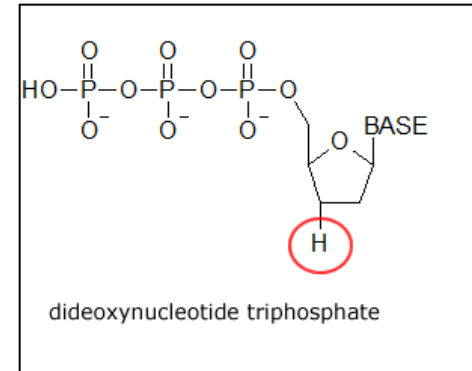
Δίκλωνο
DNA



Δημιουργία μονόκλωνης
μήτρας



Πραγματοποίηση τεσσάρων
αντιδράσεων



Νουκλεοτίδιο

A

T

G

C

Μείγμα νου-
κλεοτιδίων

dATP
dTTP
ddTTP
dGTP
dCTP
dATP★

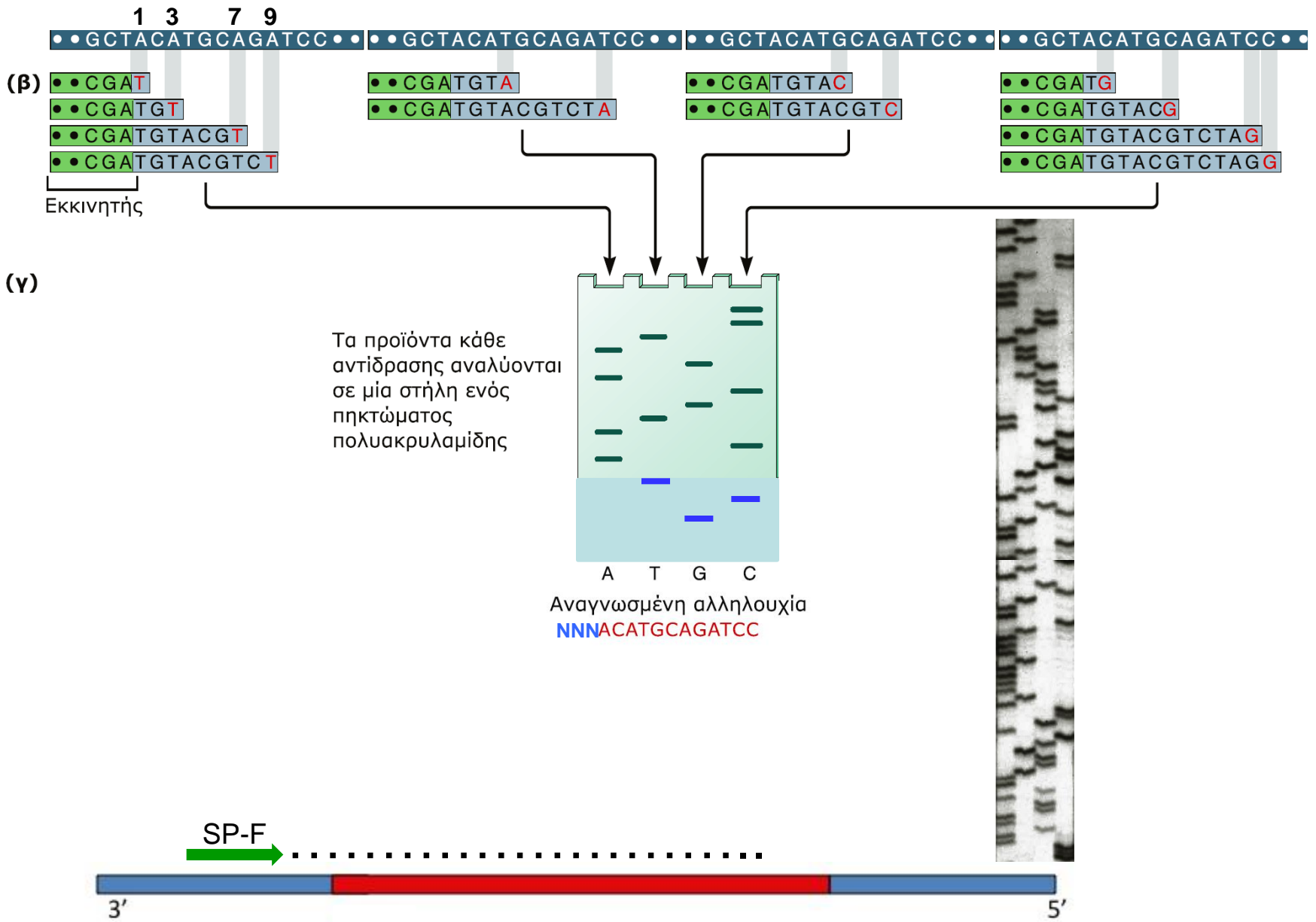
dATP
ddATP
dTTP
dGTP
dCTP
dATP★

dATP
dTTP
dGTP
dCTP
ddCTP
dATP★

dATP
dTTP
dGTP
ddGTP
dCTP
dATP★

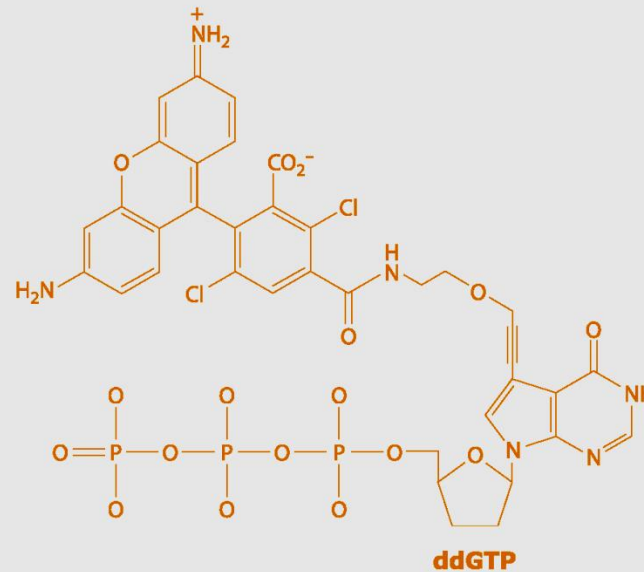
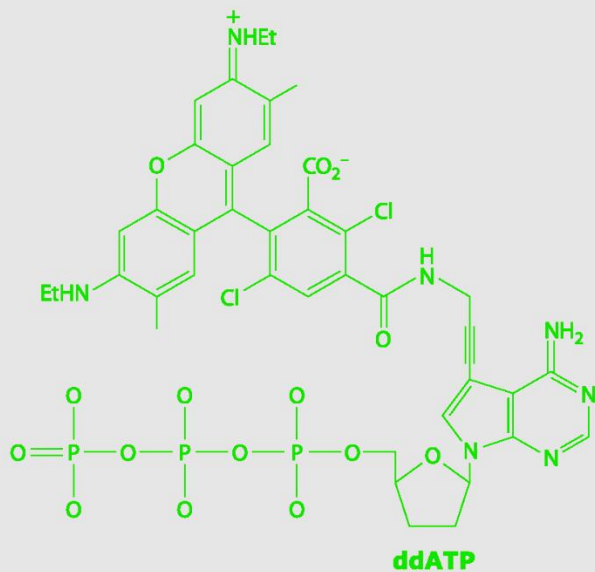
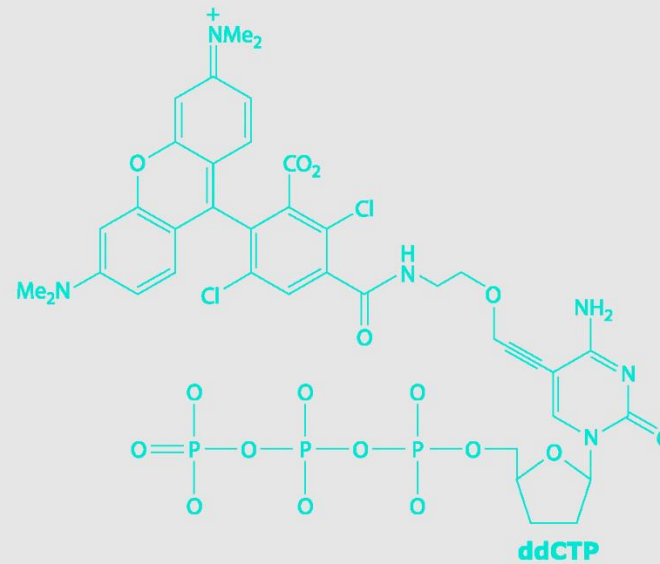
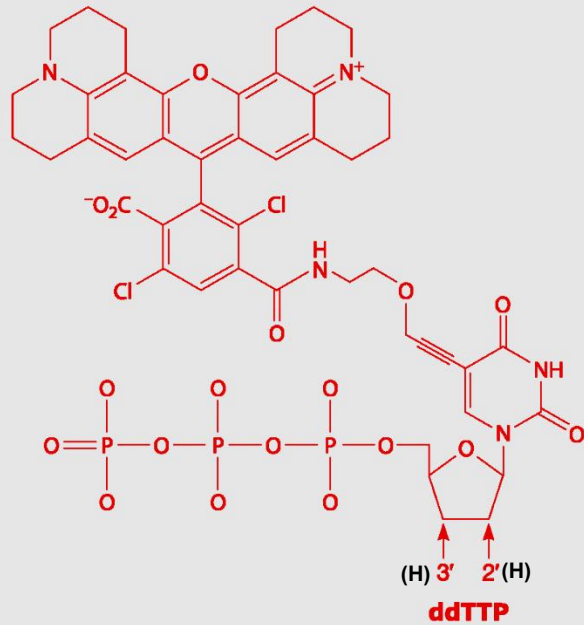
Προσθήκη DNA
πολυμεράσης

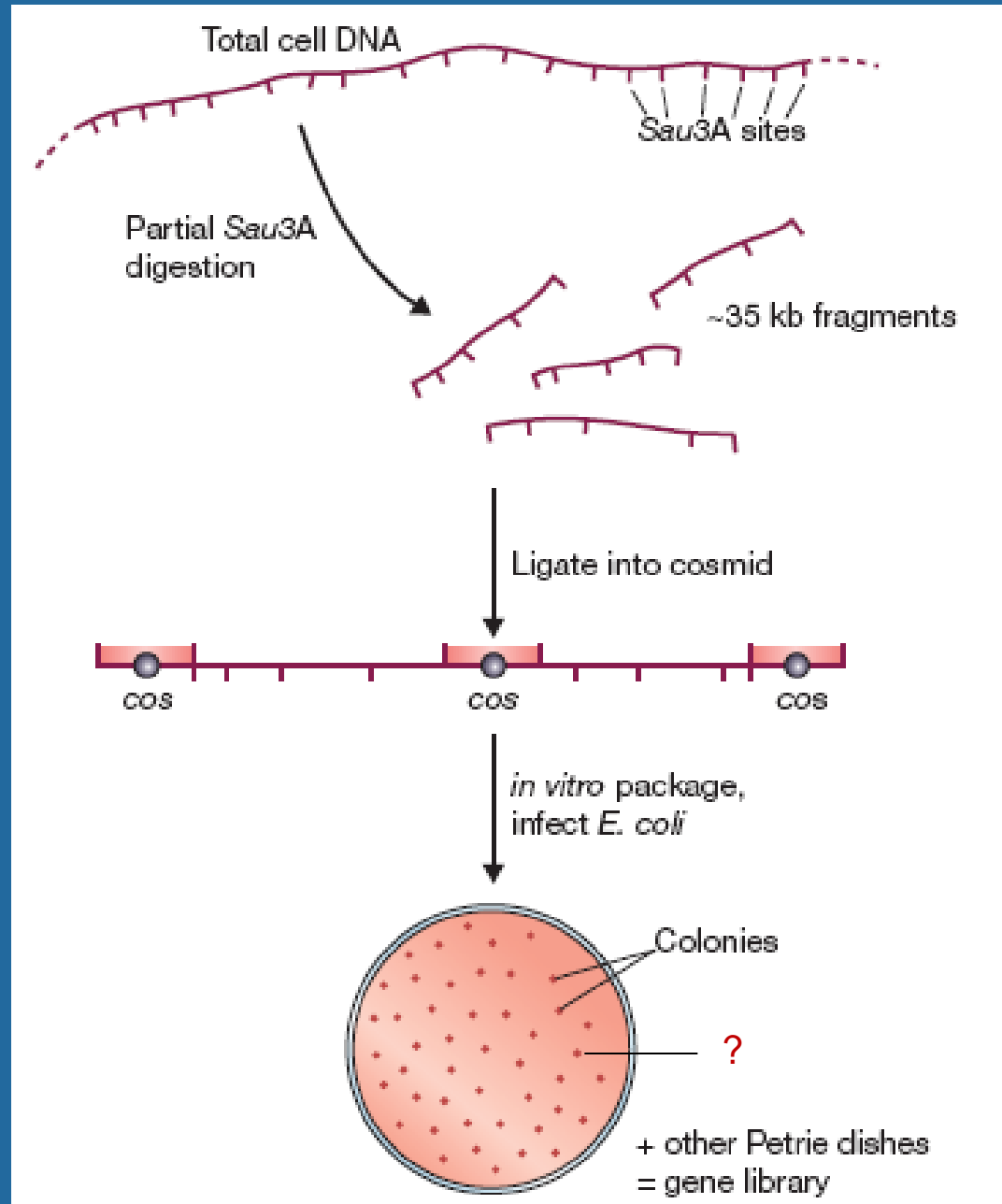




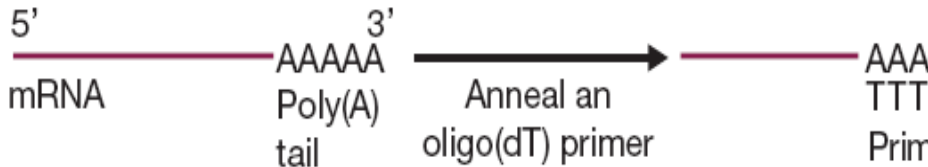
Δομές διδεοξυνουκλεοτιδίων (ddNTP) σημασμένων με φθορίζουσες χημικές ομάδες.

ΕΙΚΟΝΑ 10.10





(a) First strand synthesis



Reverse transcriptase

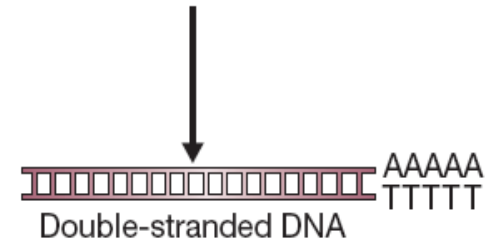
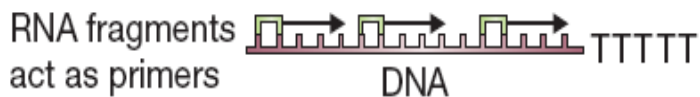
(b) RNA degradation



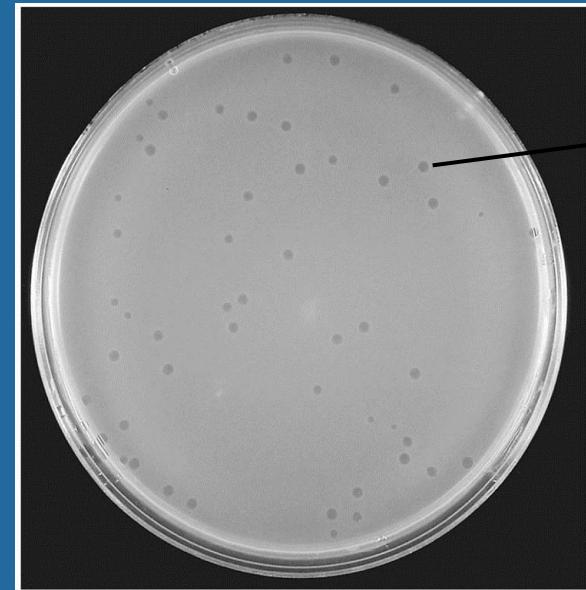
RNA fragments

DNA pol I

(c) Second strand synthesis



Κλωνοποίηση σε λ

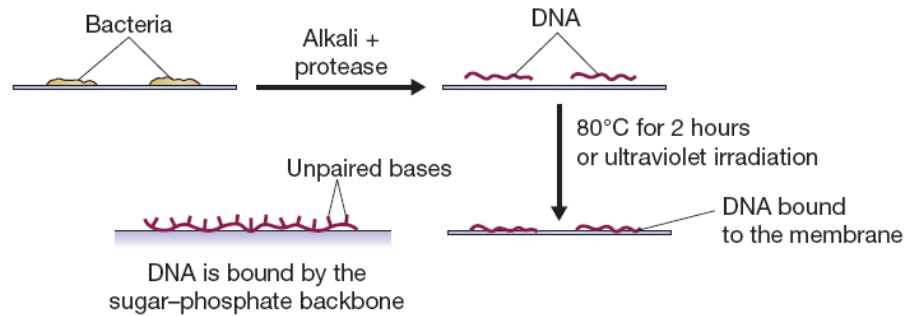


Πλάκα

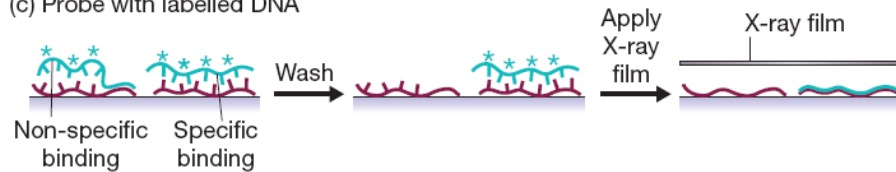
(a) Transfer colonies to nitrocellulose or nylon



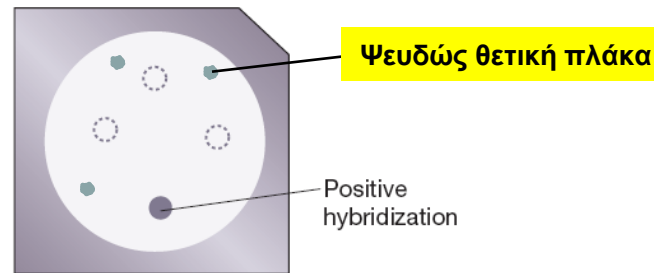
(b) Degrade cells, purify DNA

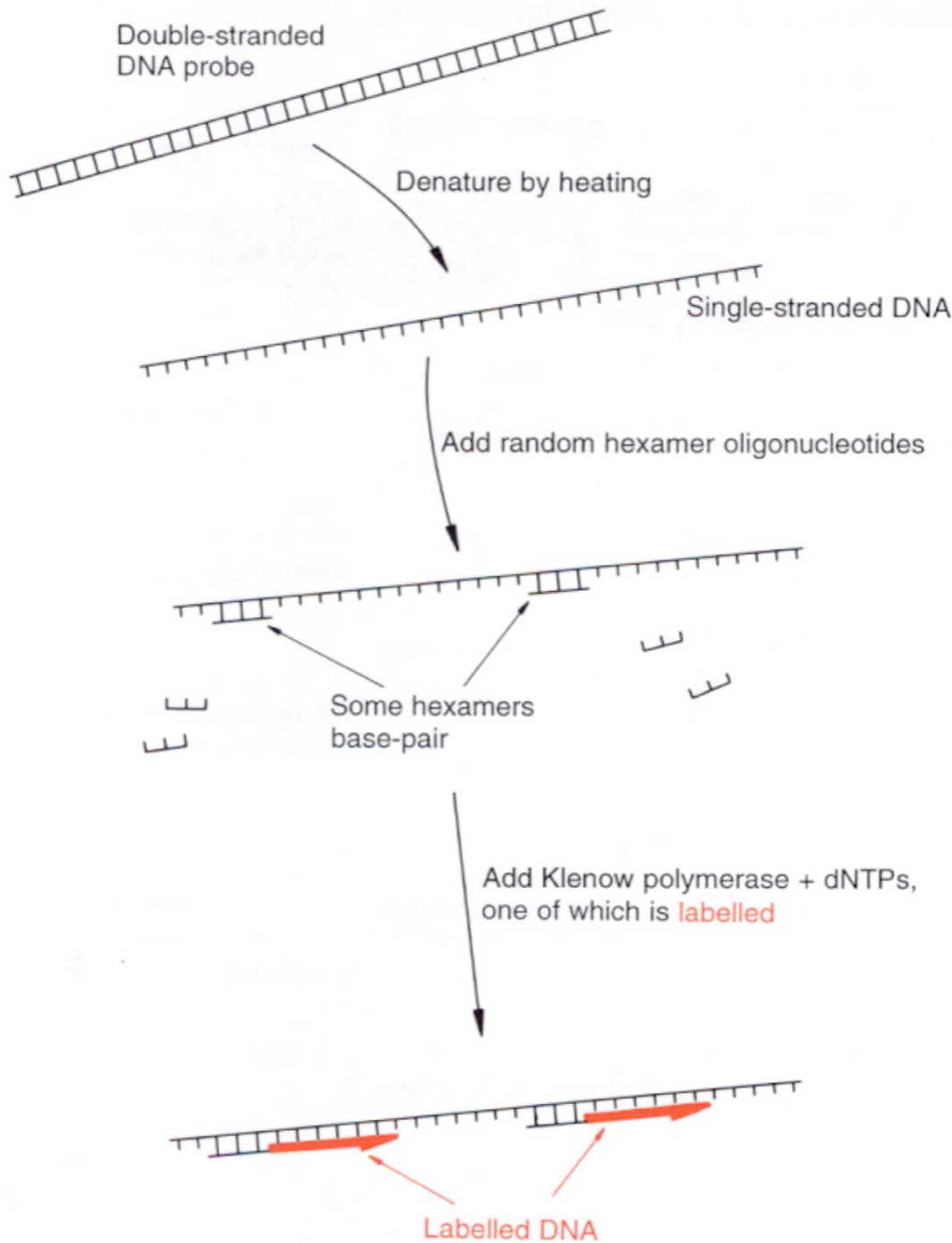


(c) Probe with labelled DNA



(d) The resulting autoradiograph





Σήμανση DNA - Ιχνηθέτης

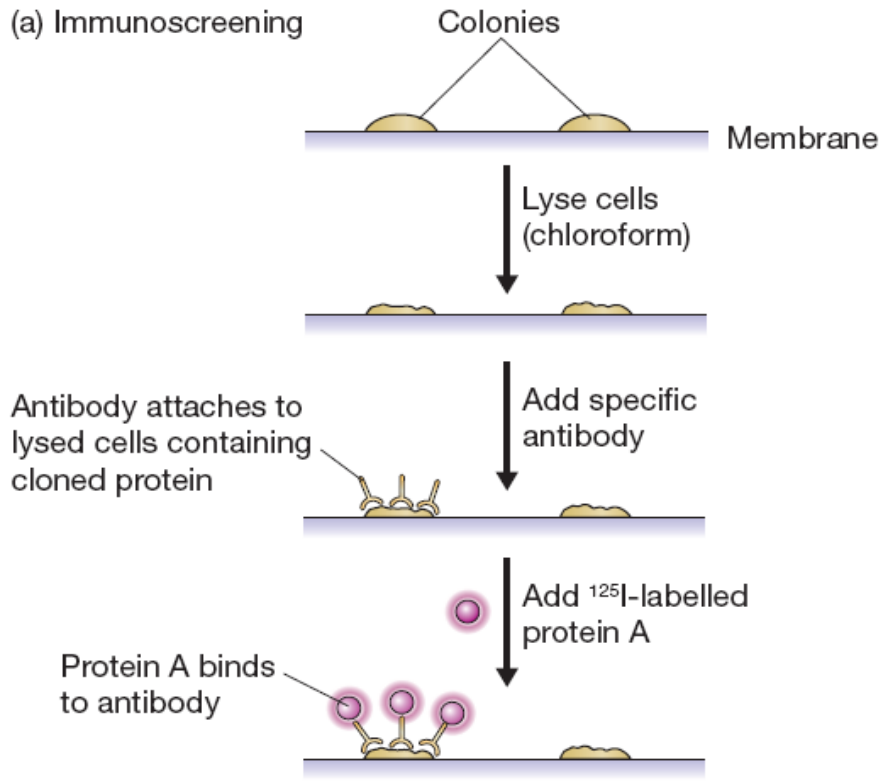
Εκφυλισμένα ολιγονουκλεοτίδια

F- 8.14

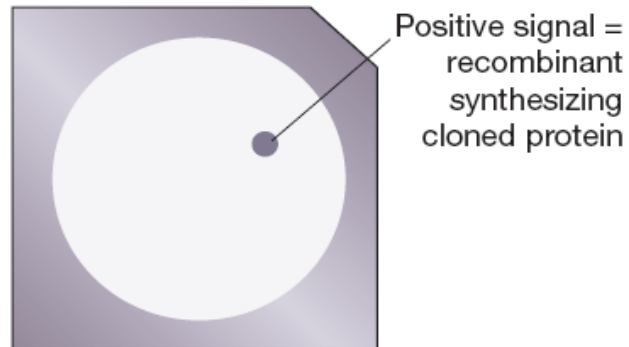
GLY-SER-ALA-LYS-LYS-GLY-ALA-THR-LEU-PHE-LYS-THR-ARG-CYS-GLU-
15
LEU-CYS-HIS-THR-VAL-GLU-LYS-GLY-GLY-PRO-HIS-LYS-VAL-GLY-PRO-
30
ASN-LEU-HIS-GLY-ILE-PHE-GLY-ARG-HIS-SER-GLY-GLN-ALA-GLN-GLY-
45
TYR-SER-TYR-THR-ASP-ALA-ASN-ILE-LYS-LYS-ASN-VAL-LEU-TRP-ASP-
60
GLU-ASN-ASN-MET-SER-GLU-TYR-LEU-THR-ASN-PRO-LYS-LYS-TYR-ILE-
75
PRO-GLY-THR-LYS-MET-ALA-PHE-GLY-GLY-LEU-LYS-LYS-GLU-LYS-ASP-
90
ARG-ASN-ASP-LEU-ILE-THR-TYR-LEU-LYS-LYS-ALA-CYS-GLU¹⁰³

Επιλογή κλώνων με αντισώματα Βιβλιοθήκες έκφρασης

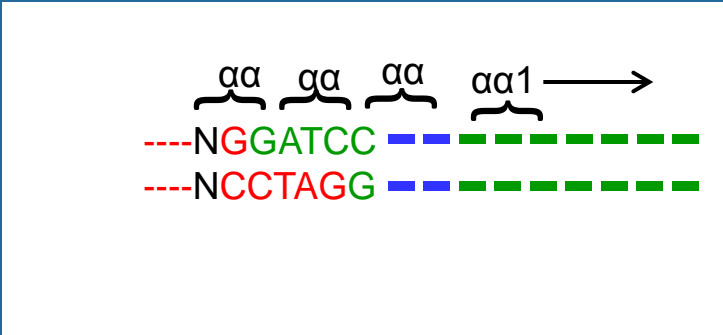
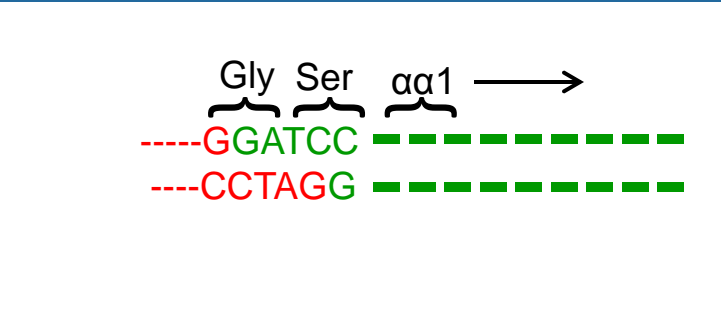
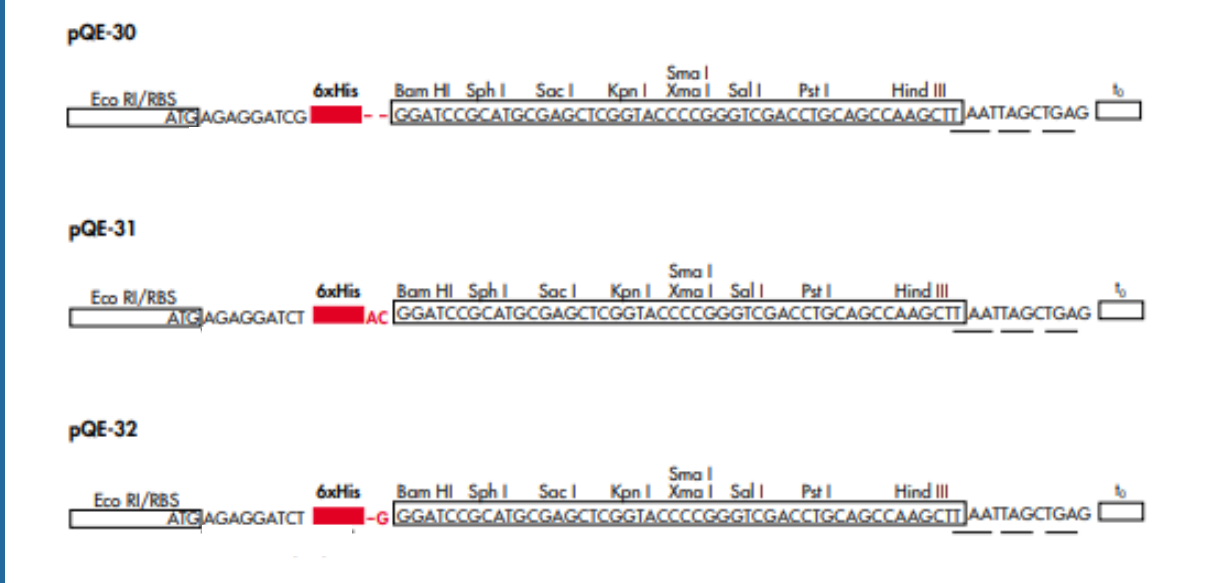
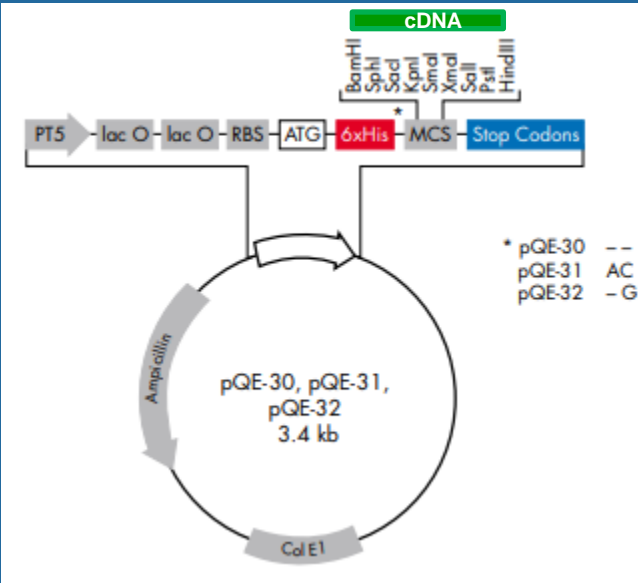
(a) Immunoscreening

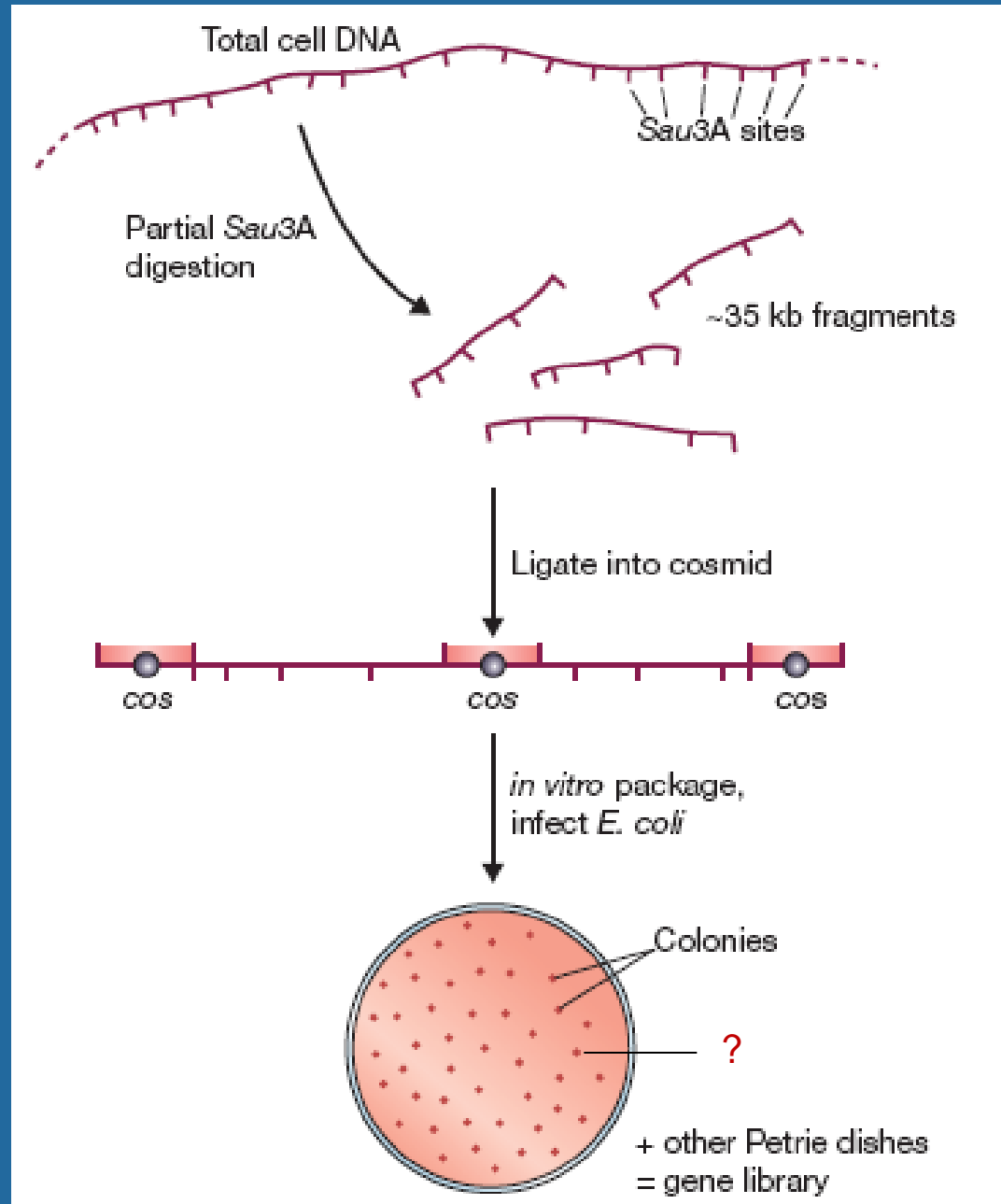


(b) The resulting autoradiograph

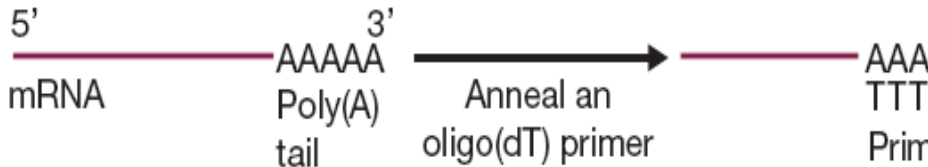


Πλασμίδια έκφρασης πρωτεϊνών





(a) First strand synthesis



Reverse transcriptase

(b) RNA degradation

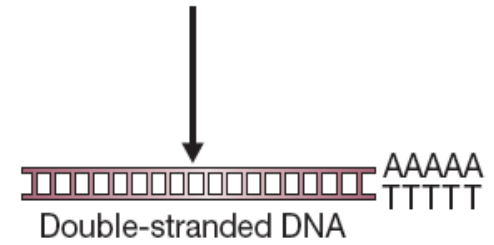
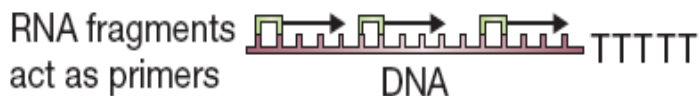


RNase HI

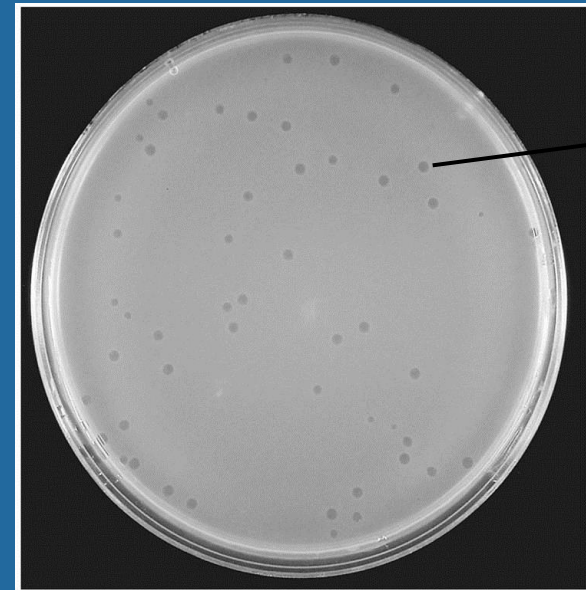


DNA pol I

(c) Second strand synthesis

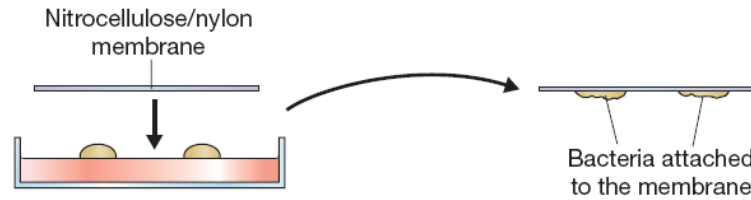


Κλωνοποίηση σε λ

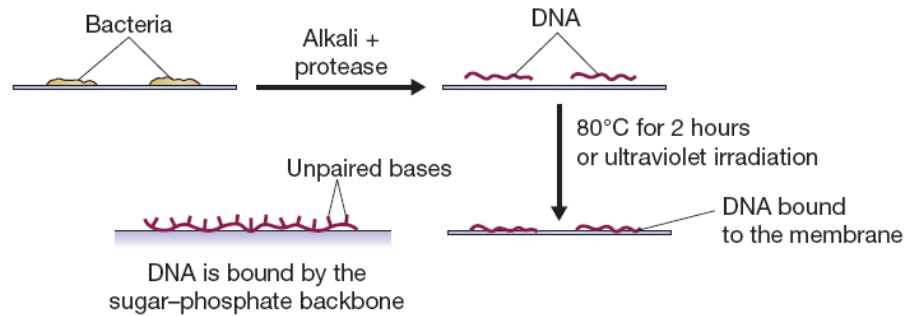


Πλάκα

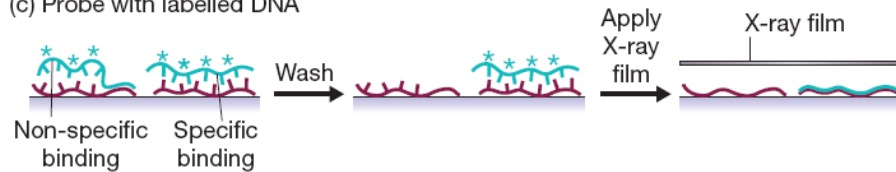
(a) Transfer colonies to nitrocellulose or nylon



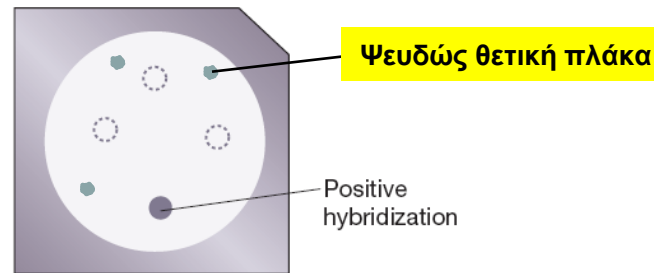
(b) Degrade cells, purify DNA

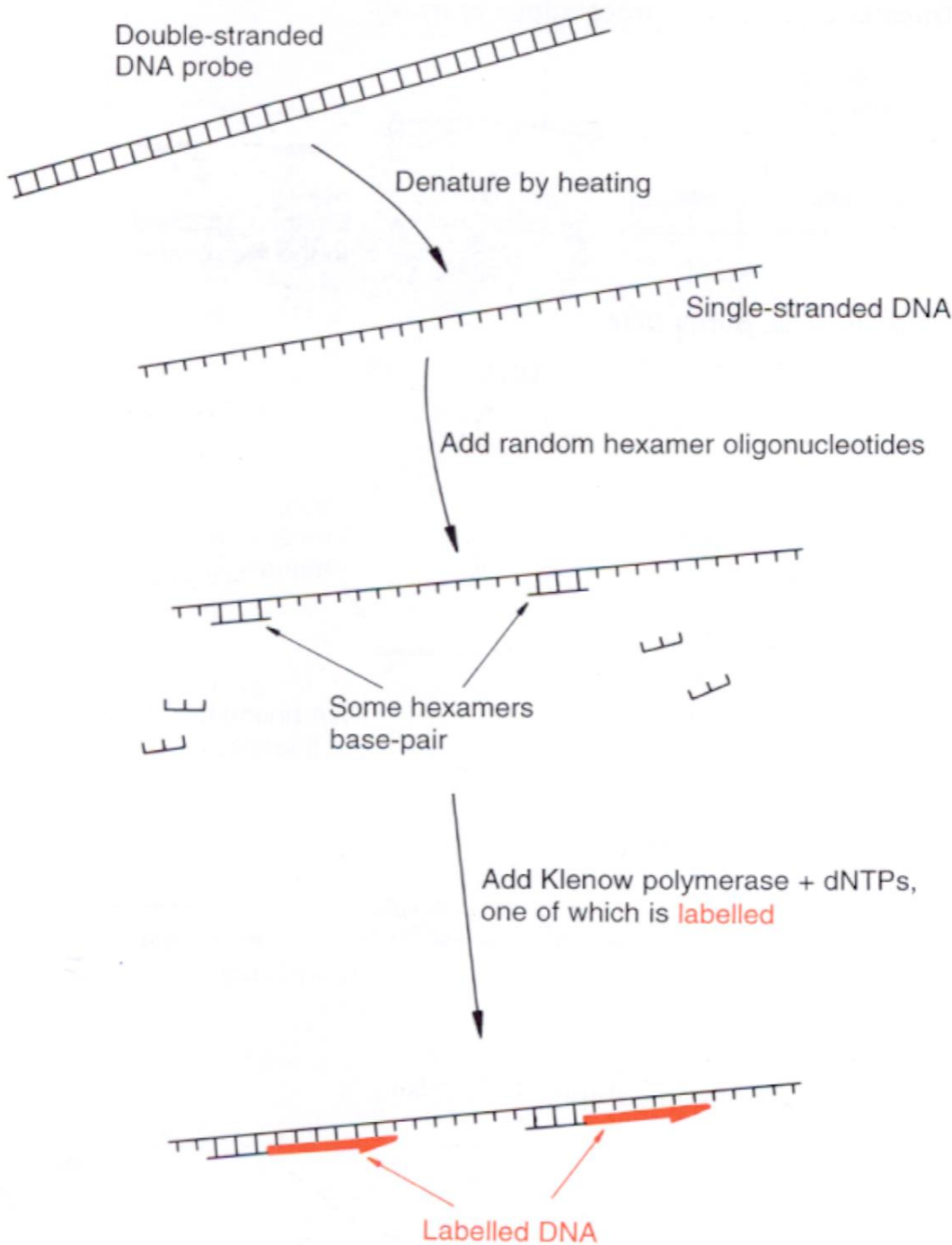


(c) Probe with labelled DNA



(d) The resulting autoradiograph





Σήμανση DNA - Ιχνηθέτης

Στοιχισή-Ιχνηθέτες PCR

```
BD      T--LNQGLINGLMSGVLGVSGGMQHNGLN--MQQHTPRSDSANSISSGRDDLSPSSSLNG 223
Cc      T--LNQGLINGLMSGVLGVSGGMQHNGLN--MQQHTPRSDSANSISSGRDDLSPSSSLNG 223
DS      ANGTPNGLIG-VVGGGGVGLGVGGGGVGGGLGMQHTPRSDSVNSISSGRDDLSPSSSLNG 237
DY      ANGTSNGLIG-IVGGGGSGVGVGGGGMGGLGMQHTPRSDSVNSISSGRDDLSPSSSLNG 236
Bm      Q-----LQPQQPHAPPTLPTMPLPMPPTTPKSENE-SMSSGREELSPASSING 184
          :                   :          **:*:.  *:*:*:*:*:*:*:*:*
BD      YSANDSCDVKKIKKGPAPRLQEELCLVCGDRASGYHYNALTCEGCKGFFRRSVTKNAVYC 283
Cc      YSANDSCDVKKIKKGPAPRLQEELCLVCGDRASGYHYNALTCEGCKGFFRRSVTKNAVYC 283
DS      YSANESCDAKKSKKGPAPRVQEELCLVCGDRASGYHYNALTCEGCKGFFRRSVTKSAVYC 297
DY      YSANESCDAKKSKKGPAPRVQEELCLVCGDRASGYHYNALTCEGCKGFFRRSVTKSAVYC 296
Bm      CSA-DADARRQKKGPAPRQOEELCLVCGDRASGYHYNALTCEGCKG----VTKNAVYI 242
          **  ..*.: : ***** ***********.***
BD      CKFGRSCEMDMYMRKCKQECRMKKCLAVGMRPECVVPENQCAMKRREKKAQKEKEKQTT- 342
Cc      CKFGRSCEMDMYMRKCKQECRMKKCLAVGMRPECVVPENQCAMKRREKKAQKEKEKQTT- 342
DS      CKFGRACEMDMYMRKCKQECRLKKCLAVGMRPECVVPENQCAMKRREKKAQKEKDKMTTS 357
DY      CKFGRACEMDMYMRKCKQECRLKKCLAVGMRPECVVPENQCAMKRREKKAQKEKDKMTTS 356
Bm      ----HACEMDMYMRKCKQECRLKKCLAVGMRPECVIQE---PSKNKDRQRQKK----- 292
          ****: :*****:*****: *  . * .: : : : **
BD      -----GNSPIICKTES--IKNEILELMNCEPPS-----HPTCP-LLPDDIVAKCKASN 387
Cc      -----GNSPIICKTES--IKNEILELMNCEPPS-----HPTCP-LLPDDIVAKCKASN 387
DS      PSSQHGGNGSLGSGGQDFVKKEILDLMTCEPPQ-----HATIP-LLPDEILAKCQARN 410
DY      PSSQHGGNGSLGSGSAHDFVKKEILDLMTCEPPQ-----HATIP-LLPDEILAKCQARN 409
Bm      -----DKGILLPVSTTTVEDHMPPIMQCDPPPEAARIHEVVPRYLSEKLMEQNRQKN 345
          :  :           :.:. : * *:* *  . * * .: : : : *
BD      IPPLTRNQLAVIYKLIWYQDGYEQPSEEDLKRIMS--TPDENESPNDISFRHITEITILT 445
Cc      IPPLTRNQLAVIYKLIWYQDGYEQPSEEDLKRIMS--TPDENESPNDISFRHITEITILT 445
DS      IPSLTYNQLAVIYKLIWYQDGYEQPSEEDLRRIMS--QPDENESQTDVSFRHITEITILT 468
DY      IPSLTYNQLAVIYKLIWYQDGYEQPSEEDLRRIMS--QPDENESQTDVSFRHITEITILT 467
Bm      IPPLSANQKSLIARLVWYQEGYEQPSDEDLKRVTQTWQSDEEDEESDLPFRQITEMTILT 405
          **.*: * * ::* :*:***:*****:***:*: .  .**:. . *.**:*:*:*:*
BD      VQLLIVEFAKGLPAFTKIPQEDQITLLKACSSSEVMMLRMARRYDHNSDSIFFANNRSYTRD 505
Cc      VQLLIVEFAKGLPAFTKIPQEDQITLLKACSSSEVMMLRMARRYDHNSDSIFFANNRSYTRD 505
DS      VQLLIVEFAKGLPAFTKIPQEDQITLLKACSSSEVMMLRMARRYDHSSDSIFFANNRSYTRD 528
DY      VQLLIVEFAKGLPAFTKIPQEDQITLLKACSSSEVMMLRMARRYDHSSDSIFFANNRSYTRD 527
Bm      VQLLIVEFAKGLPGFSKISQSDQITLLKASSSEVMMLRVARRYDAASDSVLFANNKAYTRD 465
          *****.*:*.*.*****.*****:*****  ***: :*****: :*****
```

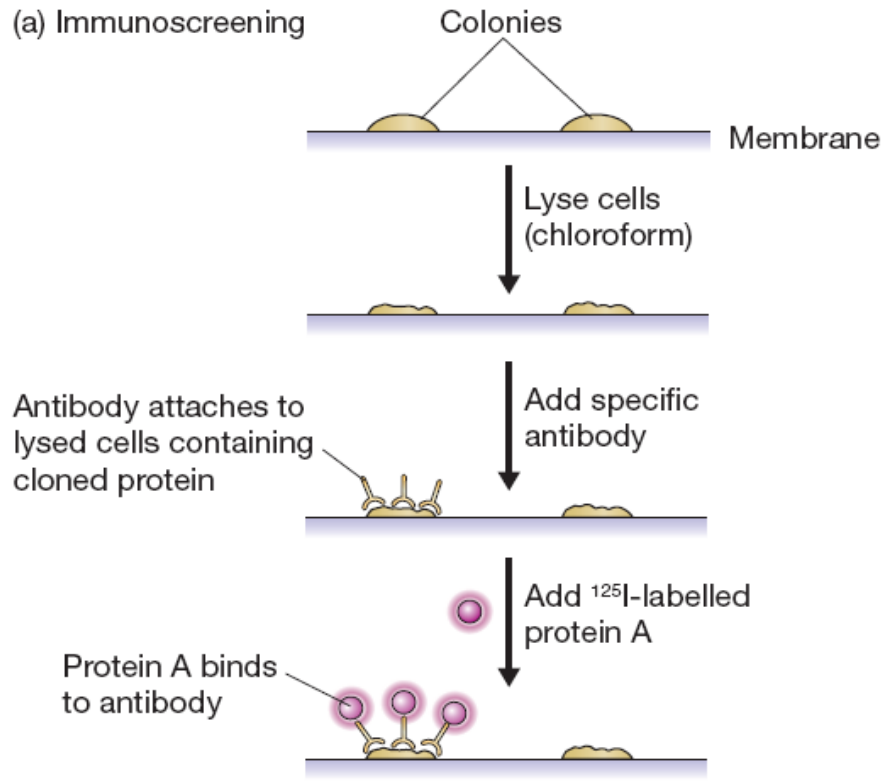
Εκφυλισμένα ολιγονουκλεοτίδια

F- 8.14

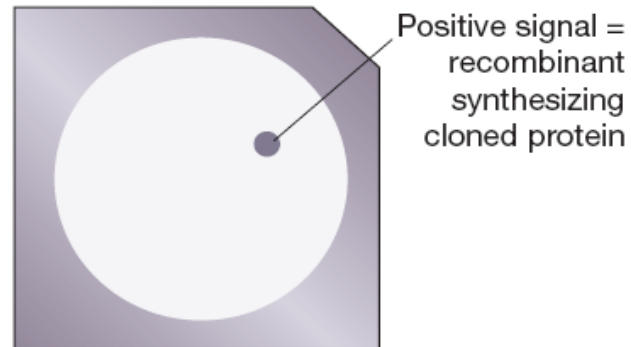
GLY-SER-ALA-LYS-LYS-GLY-ALA-THR-LEU-PHE-LYS-THR-ARG-CYS-GLU-15
LEU-CYS-HIS-THR-VAL-GLU-LYS-GLY-GLY-PRO-HIS-LYS-VAL-GLY-PRO-30
ASN-LEU-HIS-GLY-ILE-PHE-GLY-ARG-HIS-SER-GLY-GLN-ALA-GLN-GLY-45
TYR-SER-TYR-THR-ASP-ALA-ASN-ILE-LYS-LYS-ASN-VAL-LEU-TRP-ASP-60
GLU-ASN-ASN-MET-SER-GLU-TYR-LEU-THR-ASN-PRO-LYS-LYS-TYR-ILE-75
PRO-GLY-THR-LYS-MET-ALA-PHE-GLY-GLY-LEU-LYS-LYS-GLU-LYS-ASP-90
ARG-ASN-ASP-LEU-ILE-THR-TYR-LEU-LYS-LYS-ALA-CYS-GLU¹⁰³

Επιλογή κλώνων με αντισώματα Βιβλιοθήκες έκφρασης

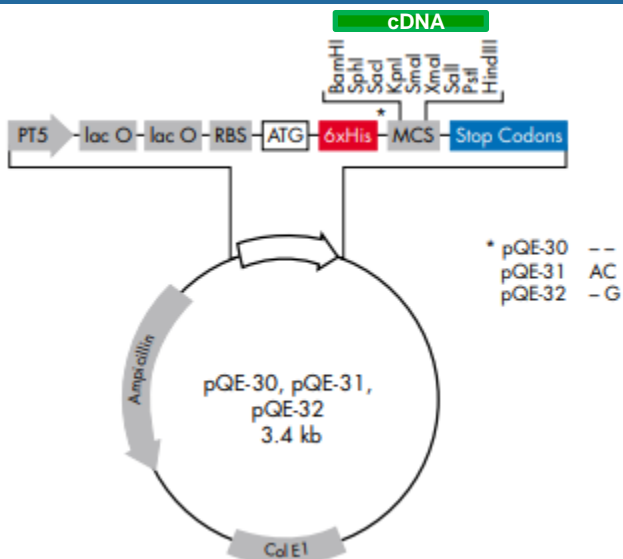
(a) Immunoscreening



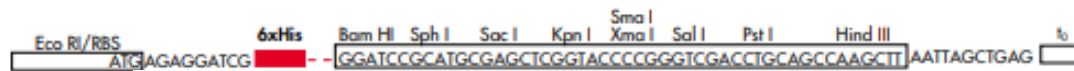
(b) The resulting autoradiograph



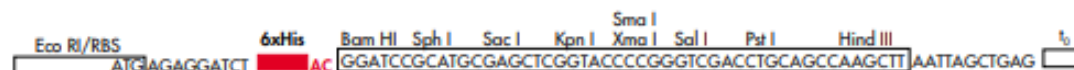
Πλασμίδια έκφρασης πρωτεϊνών



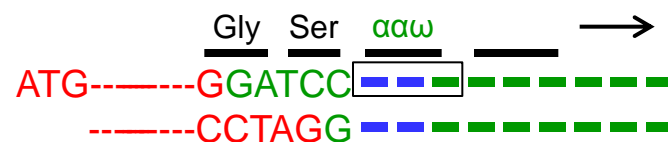
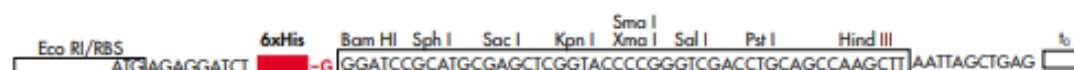
pQE-30



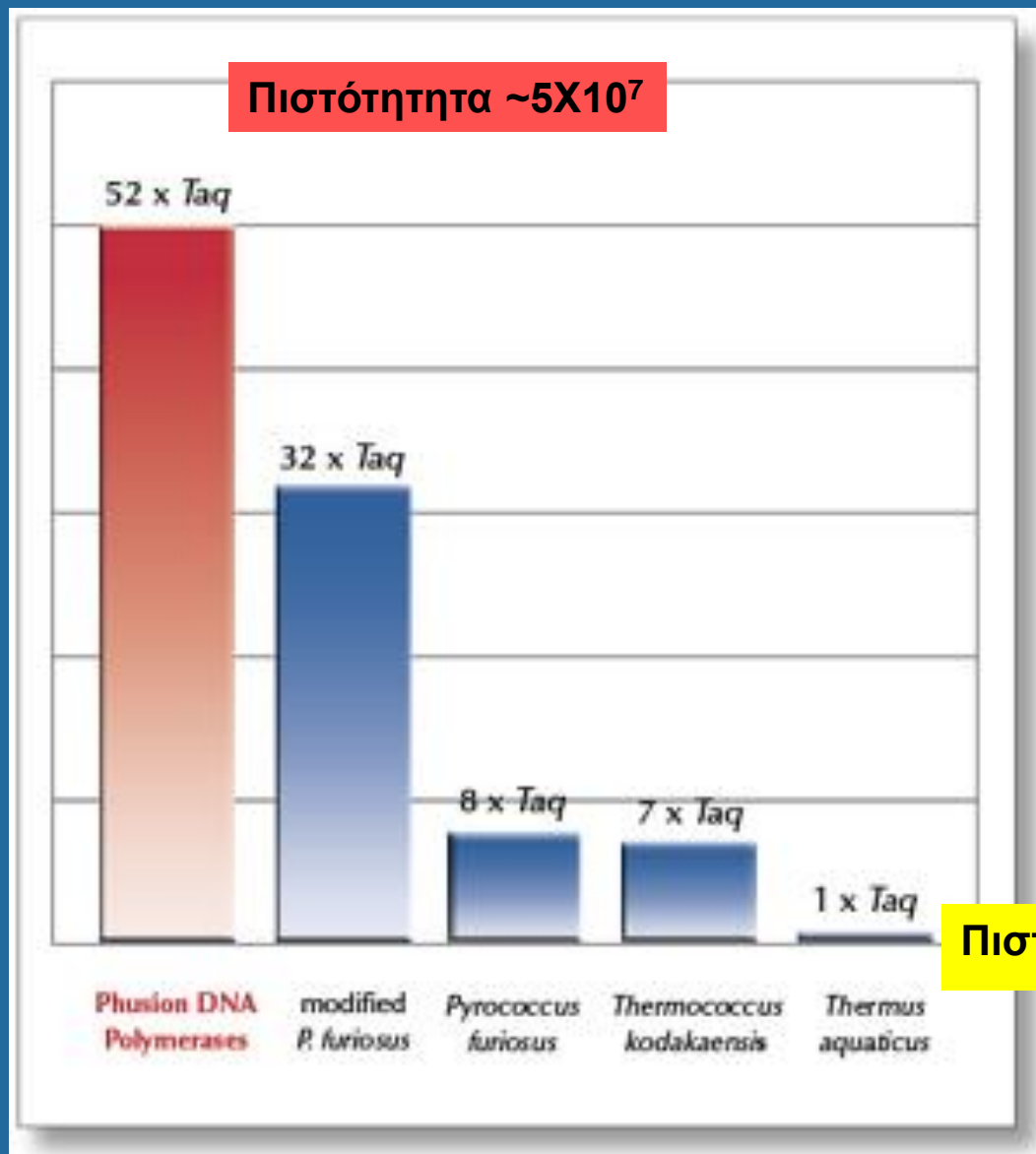
pQE-31



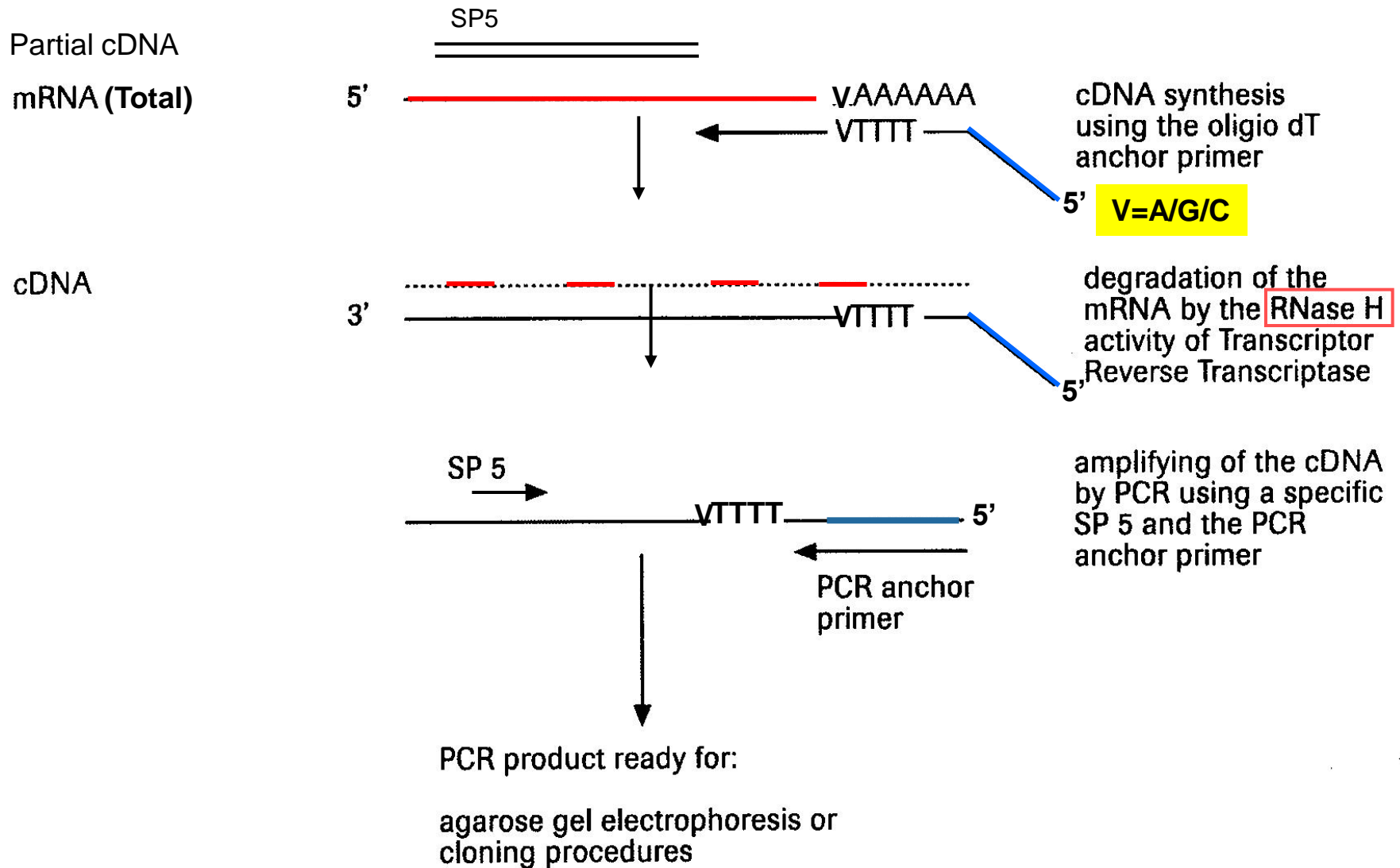
pQE-32



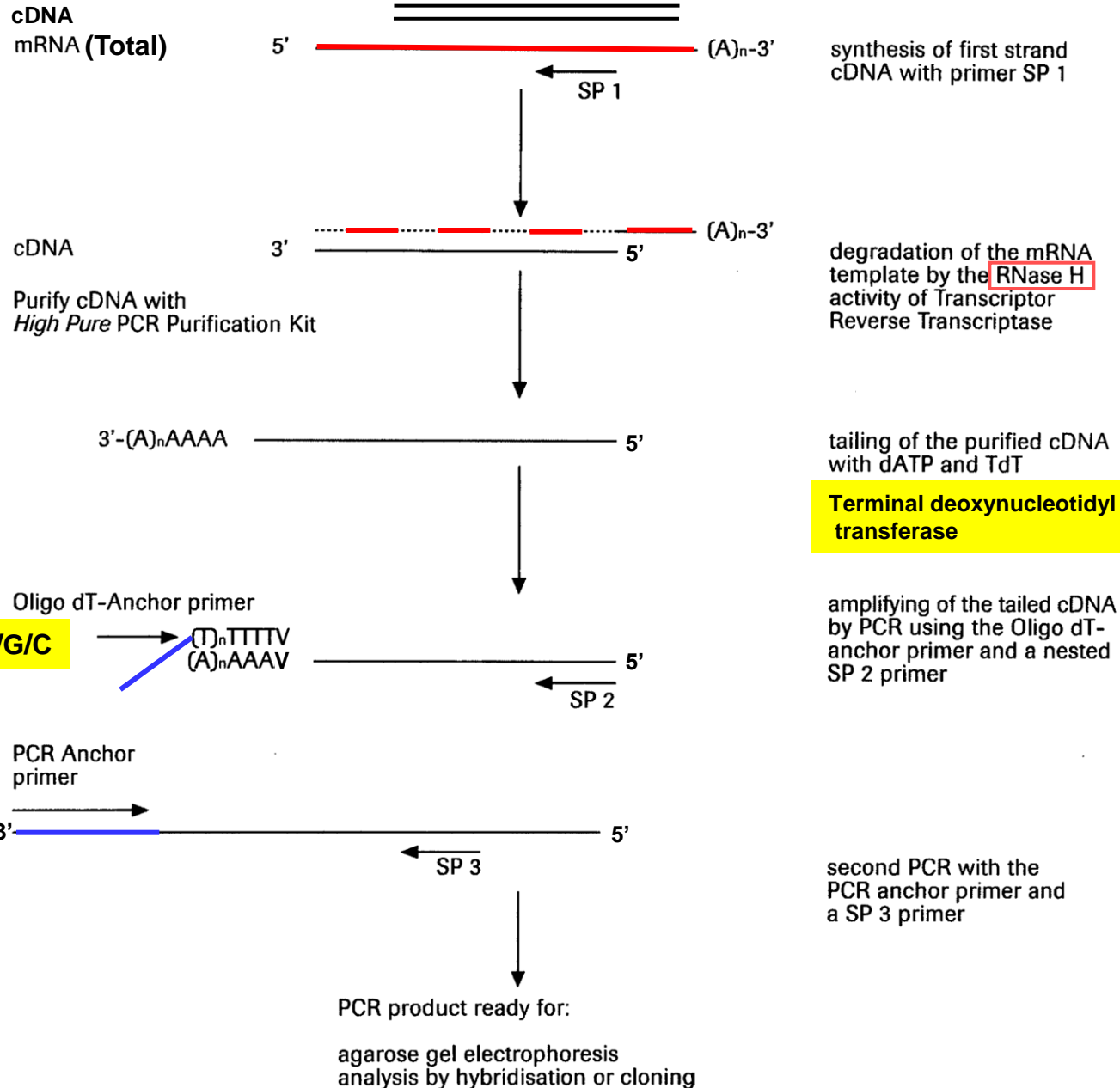
PCR Πολυμεράσες



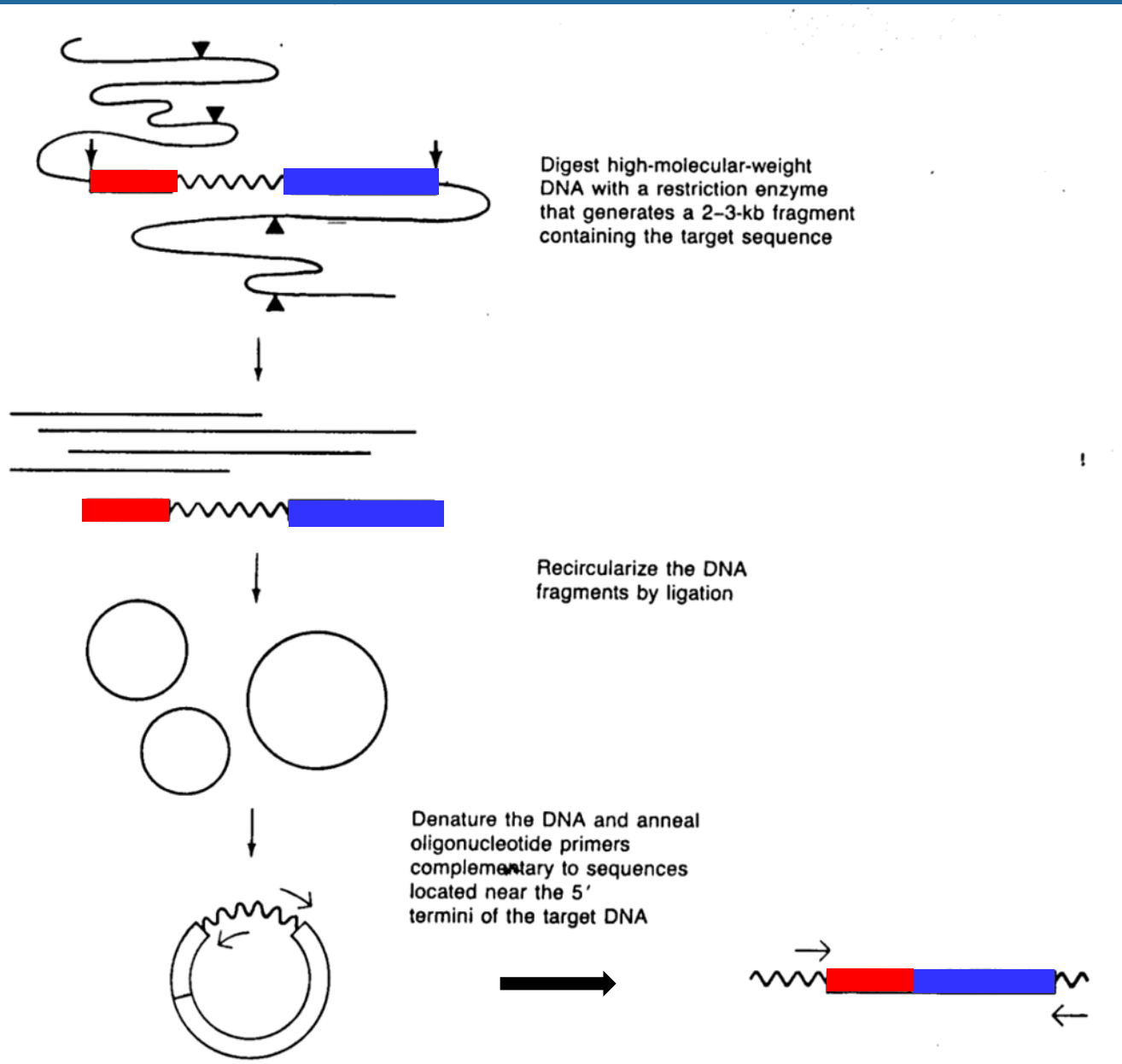
Rapid Amplification of cDNA Ends (3' RACE)



Rapid Amplification of cDNA Ends (5' RACE)

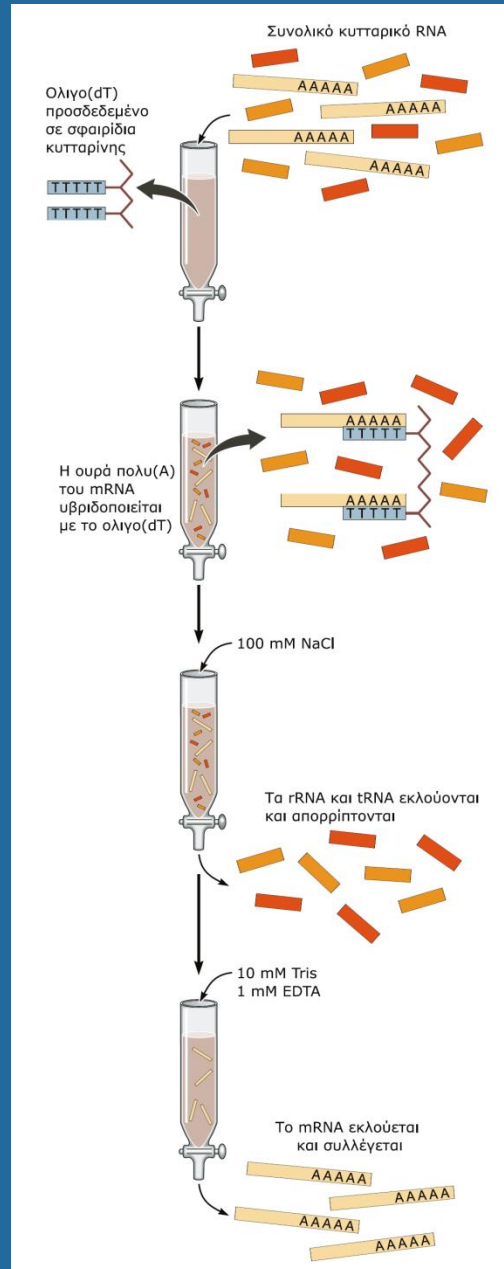


Inverted PCR



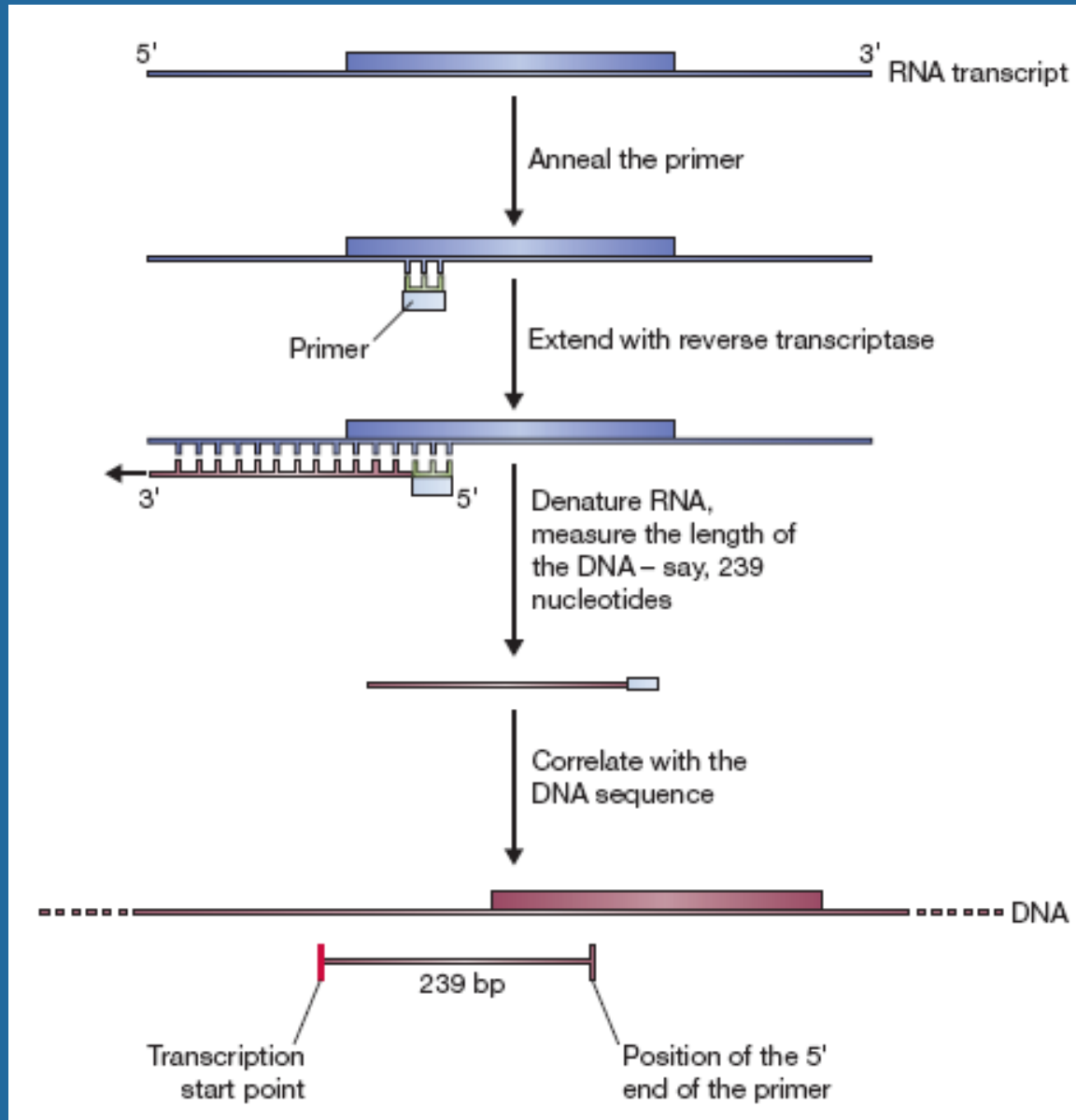
Απομόνωση πολυ(A+) RNA

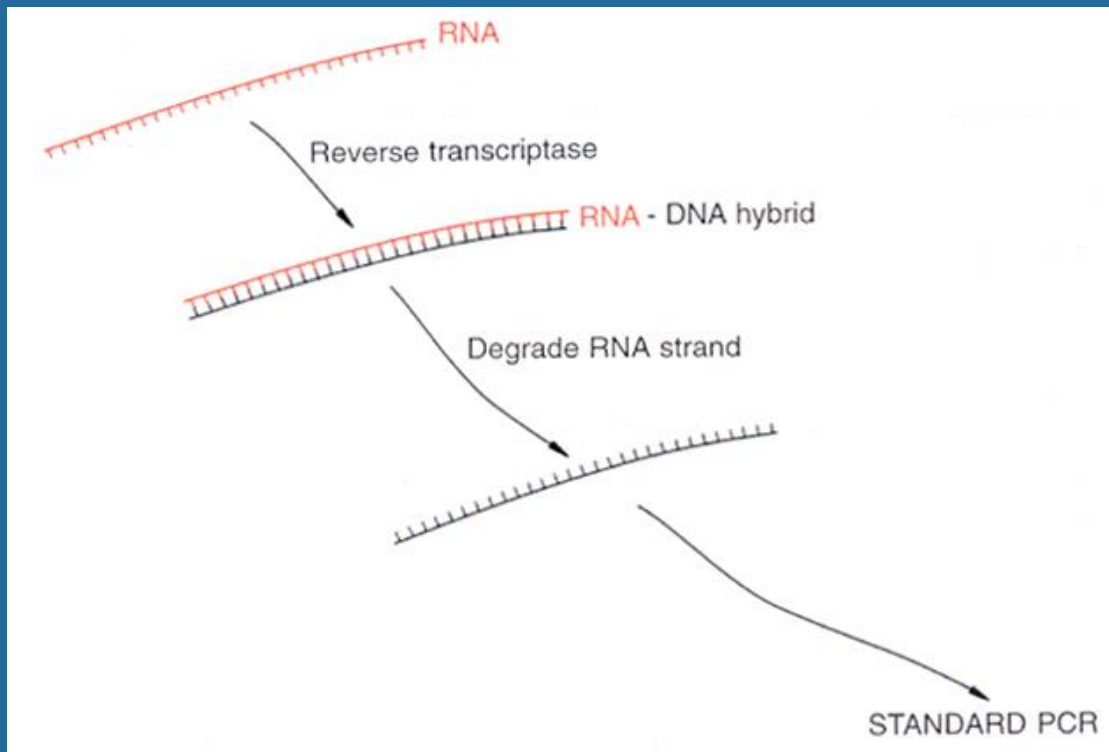
ΕΙΚΟΝΑ 4-6



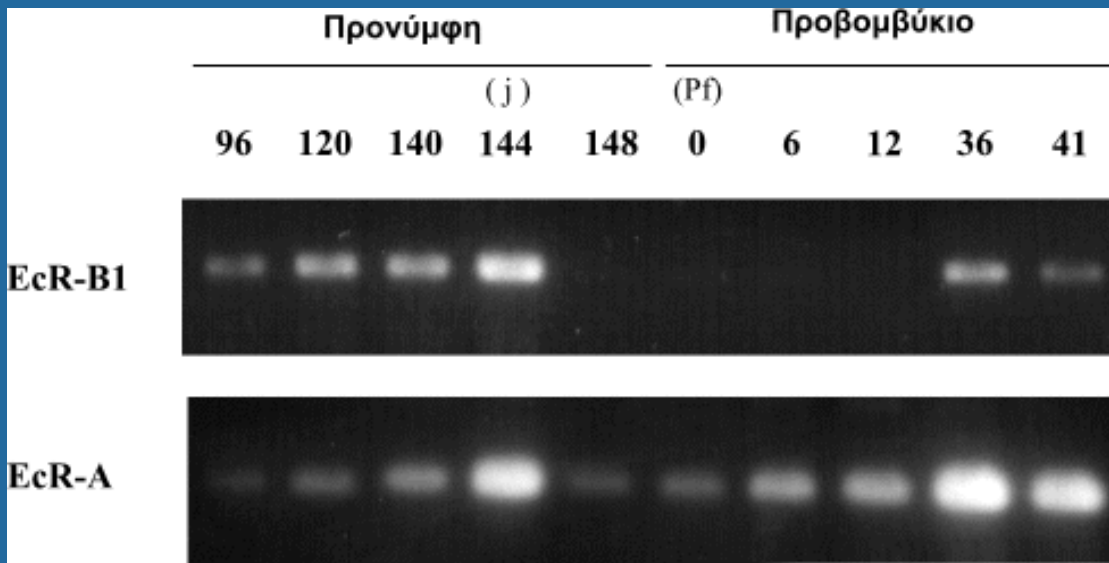
Προσδιορισμός του +1 (Primer extension)

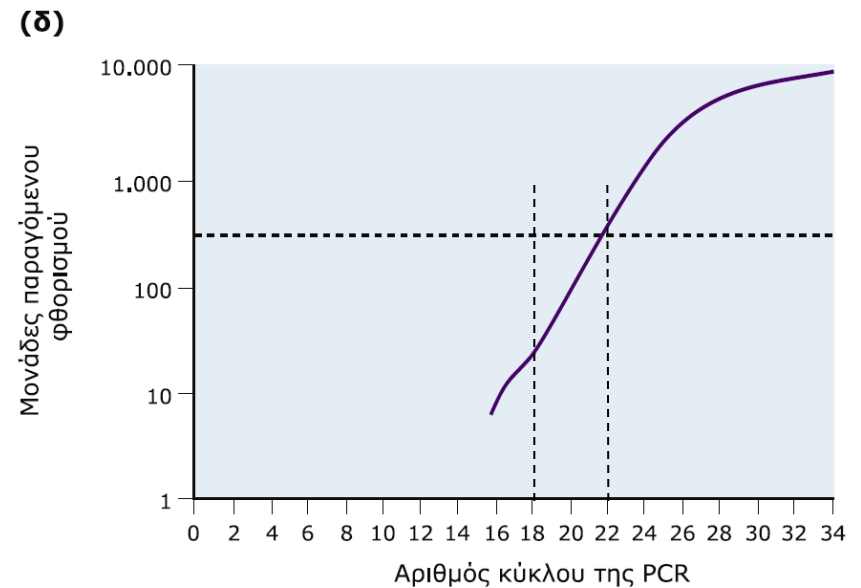
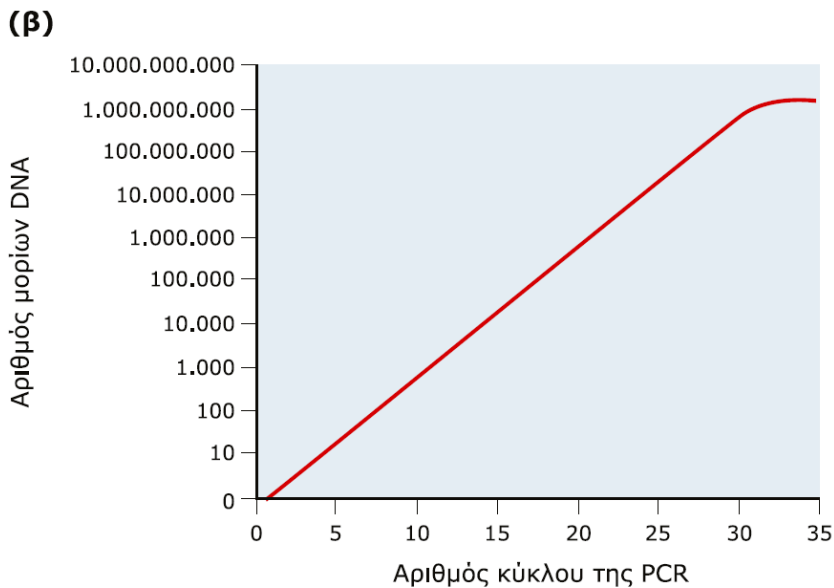
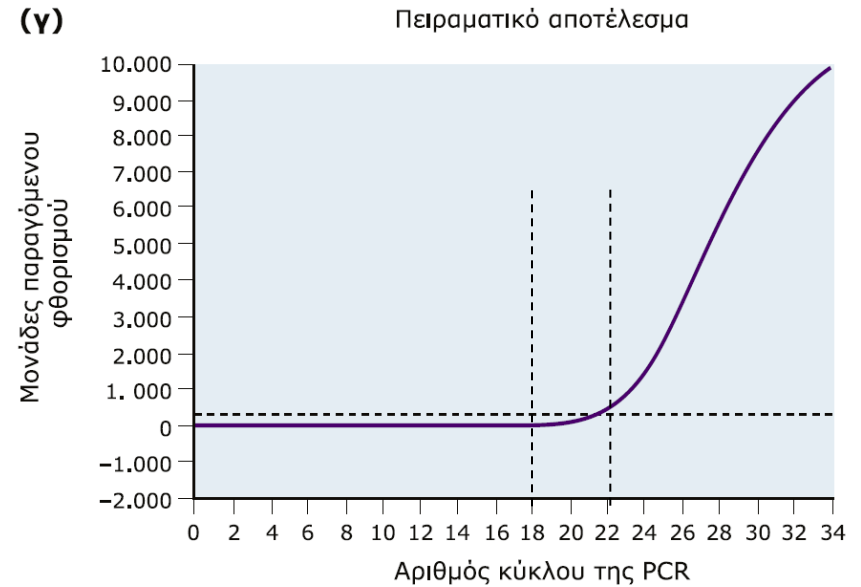
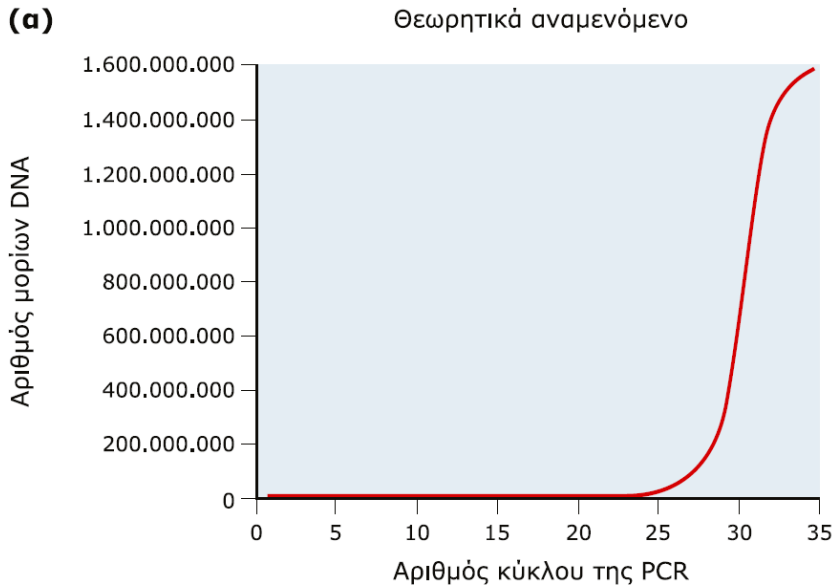
F- 11.6





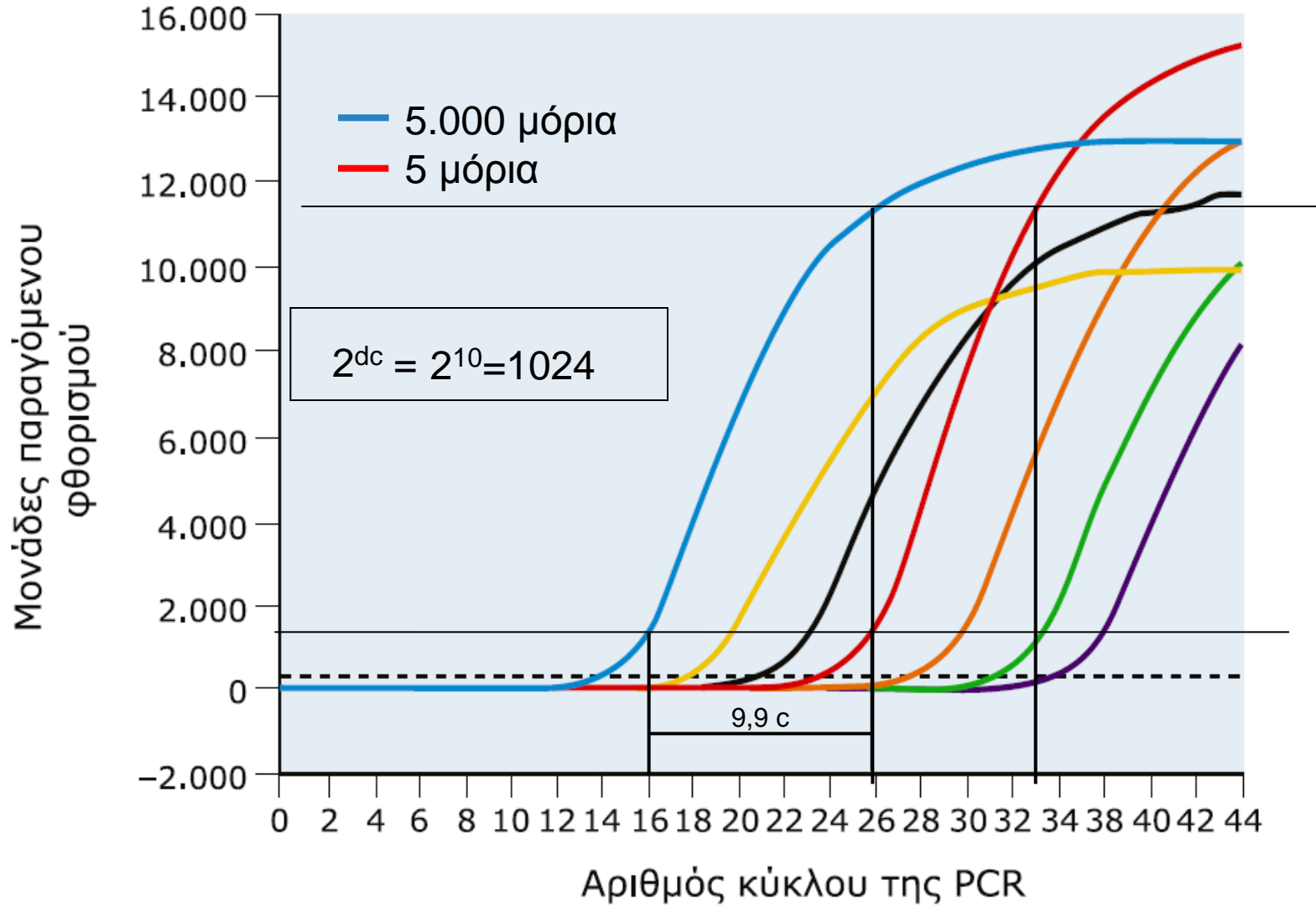
RT-PCR



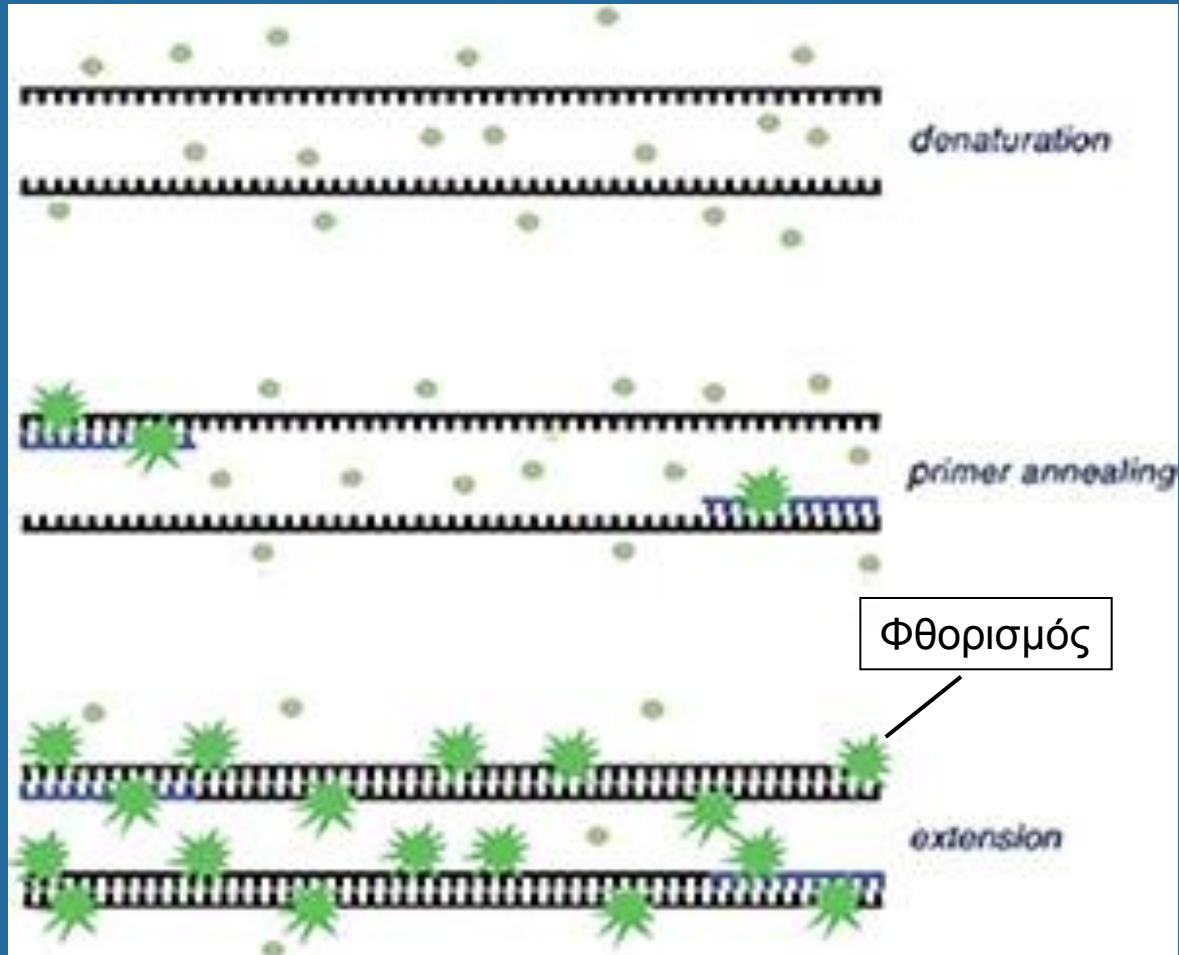


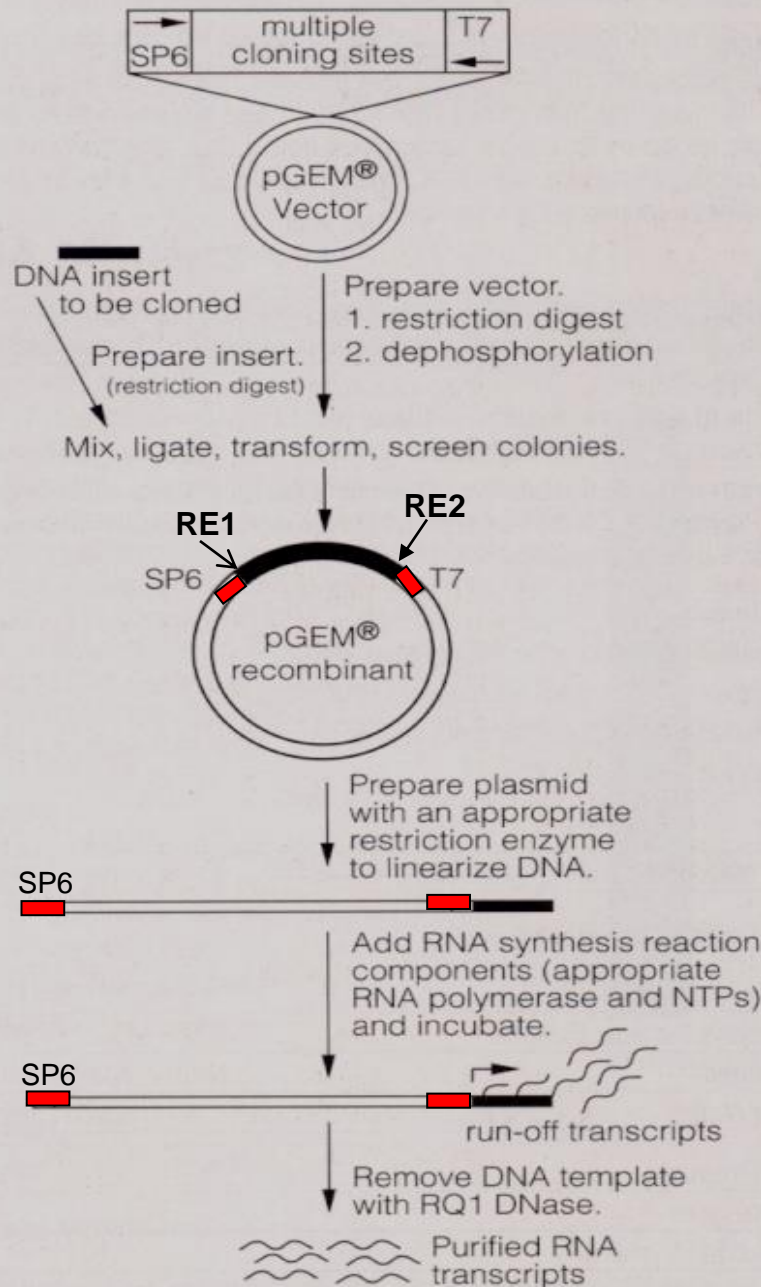
(ε)

Διαδοχικές αραιώσεις



SYBR GREEN





Sense or anti-sense RNA



In vitro μετάφραση
In situ υβρυδοποίηση
Καταστολή μετάφρασης

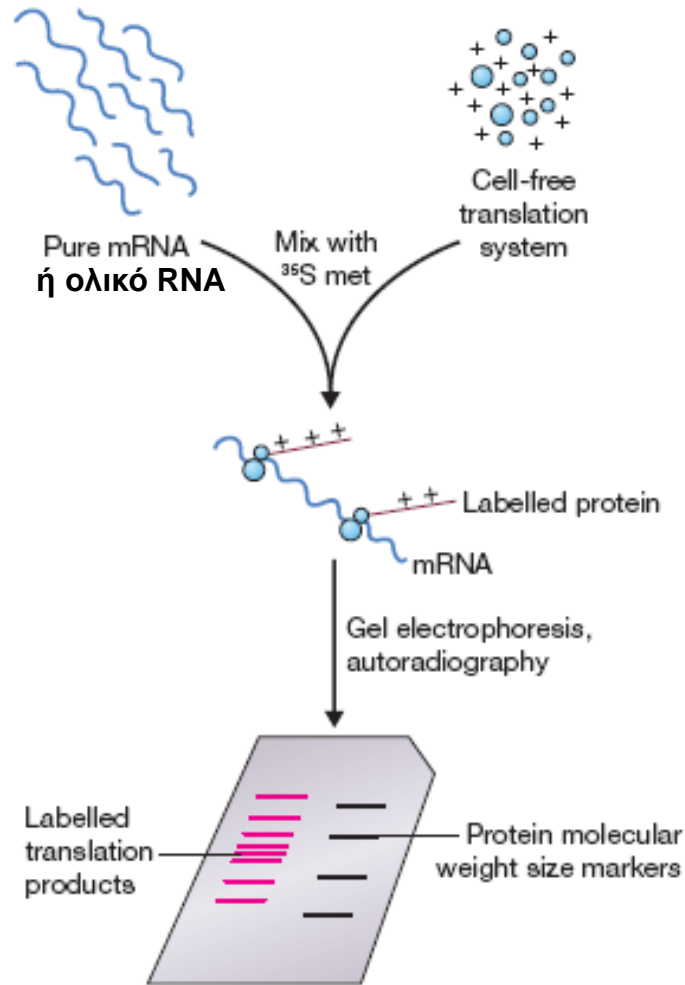


Figure 11.18
Cell-free translation.

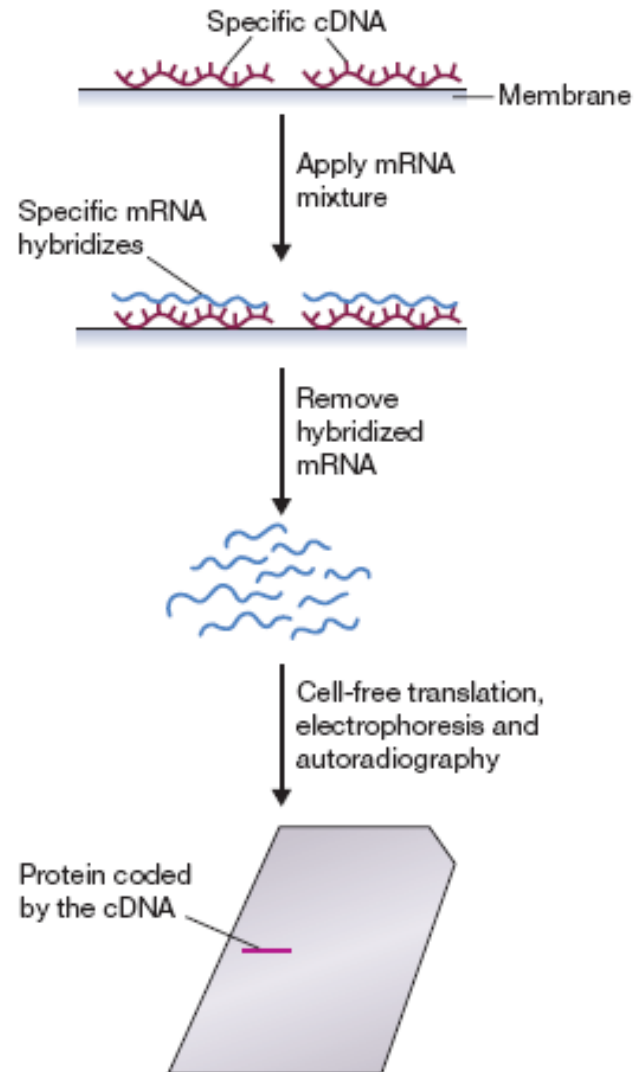


Figure 11.19
Hybrid-release translation.

In vivo μεταγραφή-μετάφραση παραγωγή πρωτεΐνης

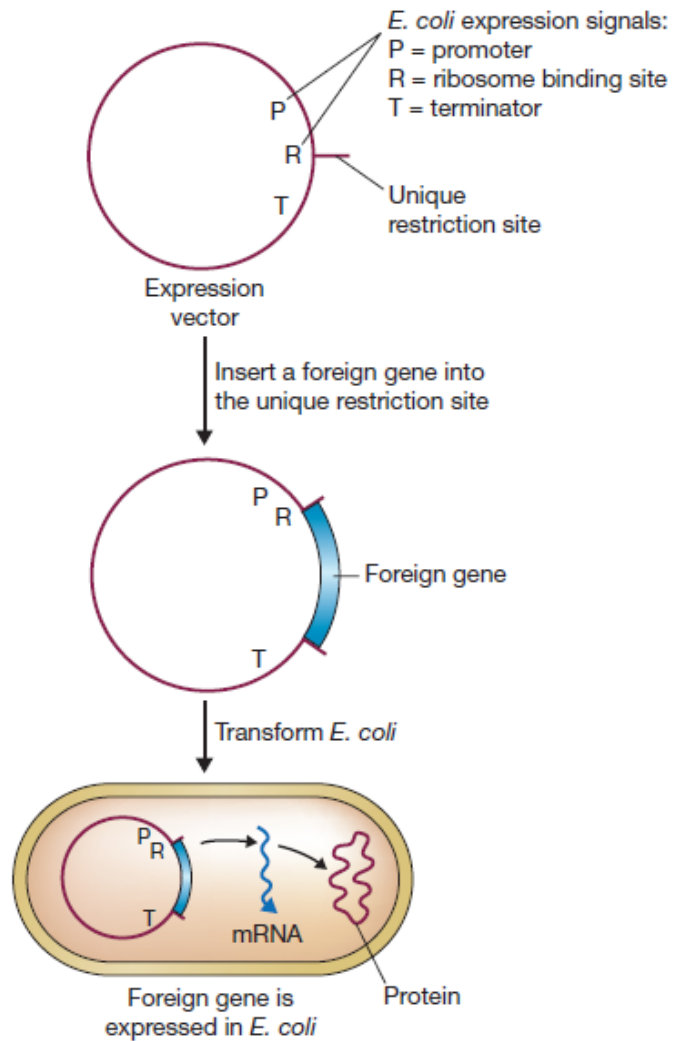
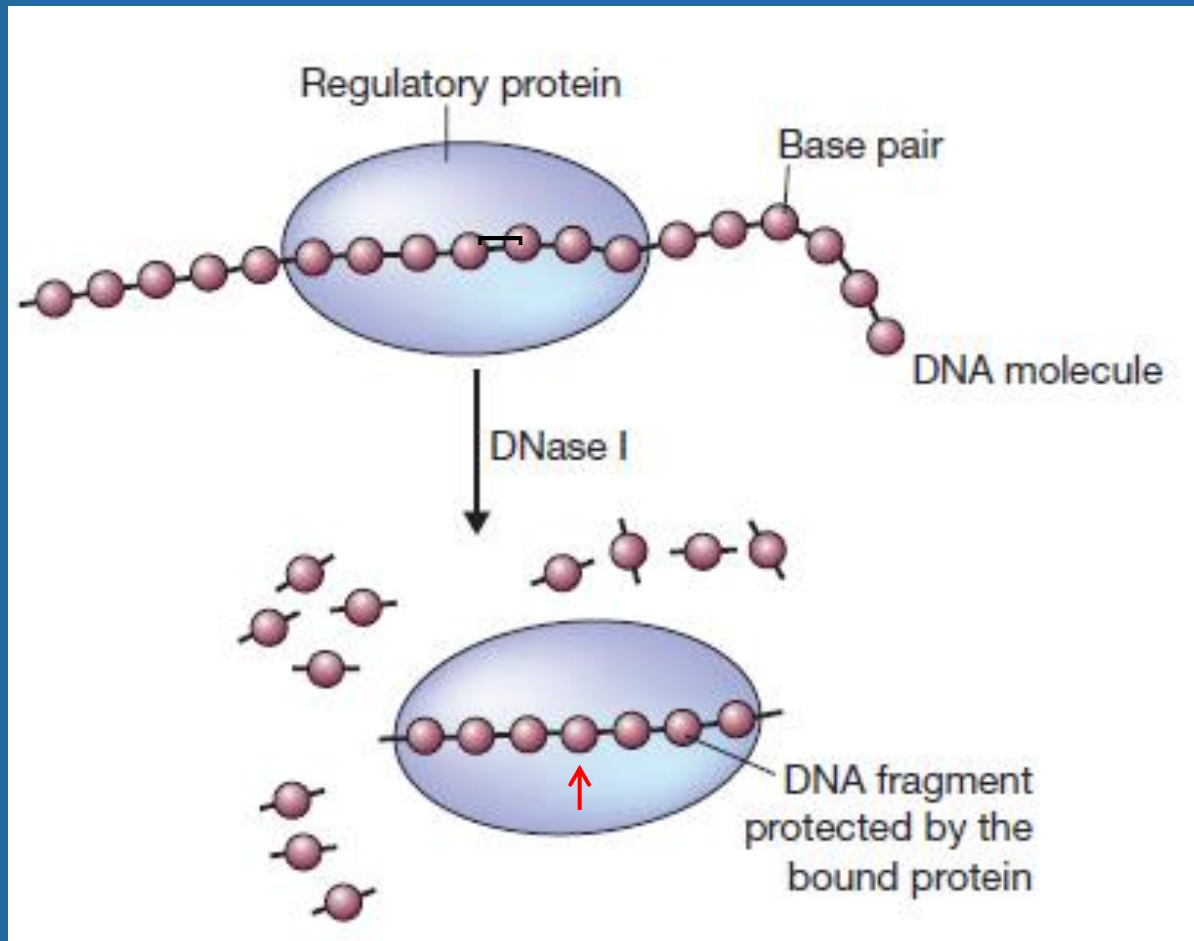


Figure 13.5

The use of an expression vector to achieve expression of a foreign gene in *E. coli*.

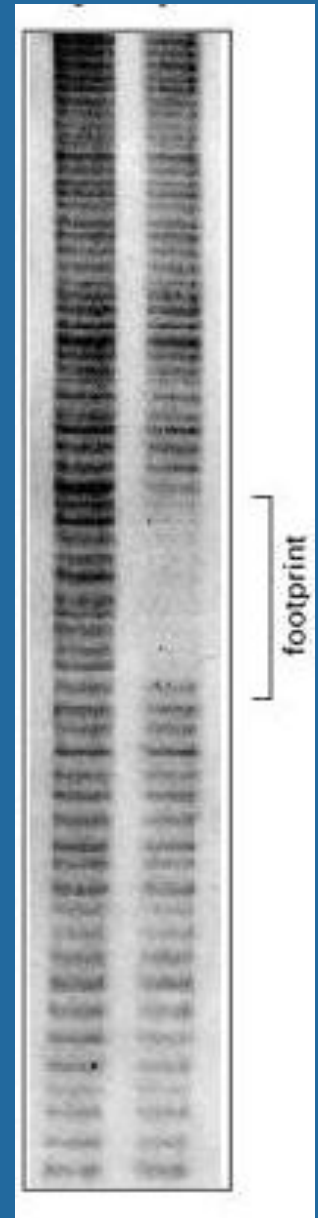
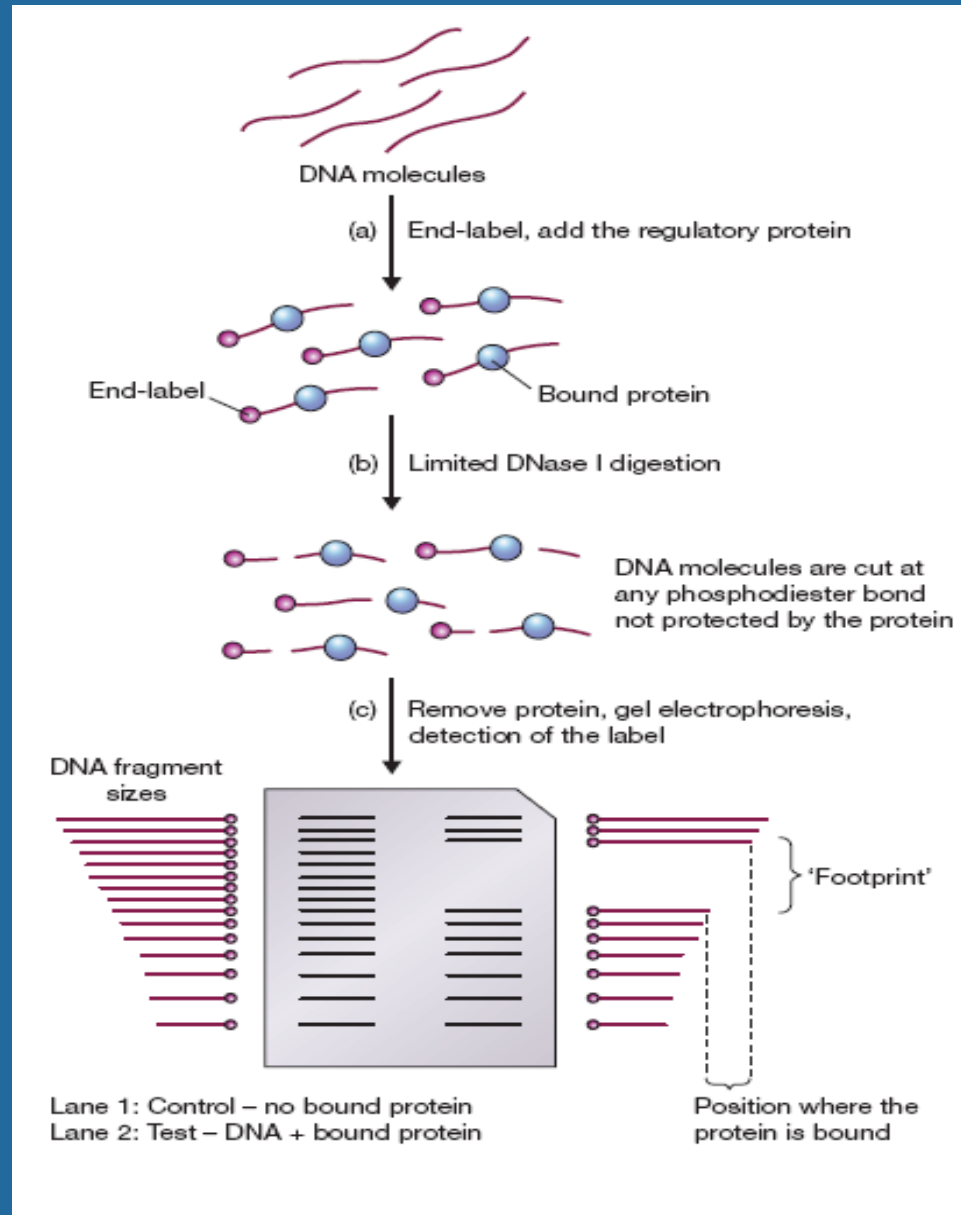
Πρόσδεση πρωτεϊνών στο DNA



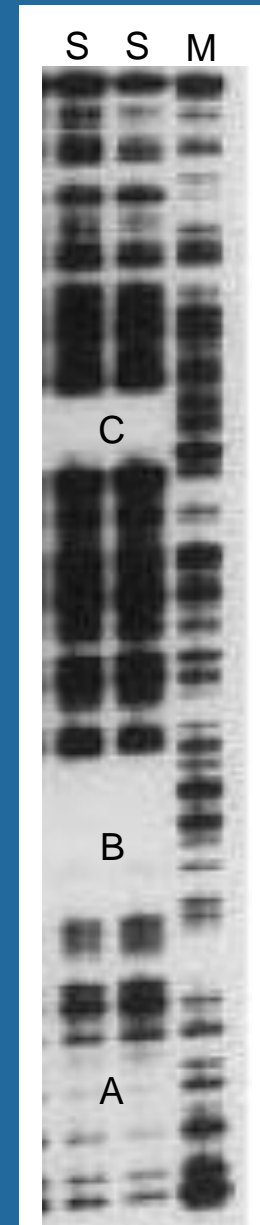
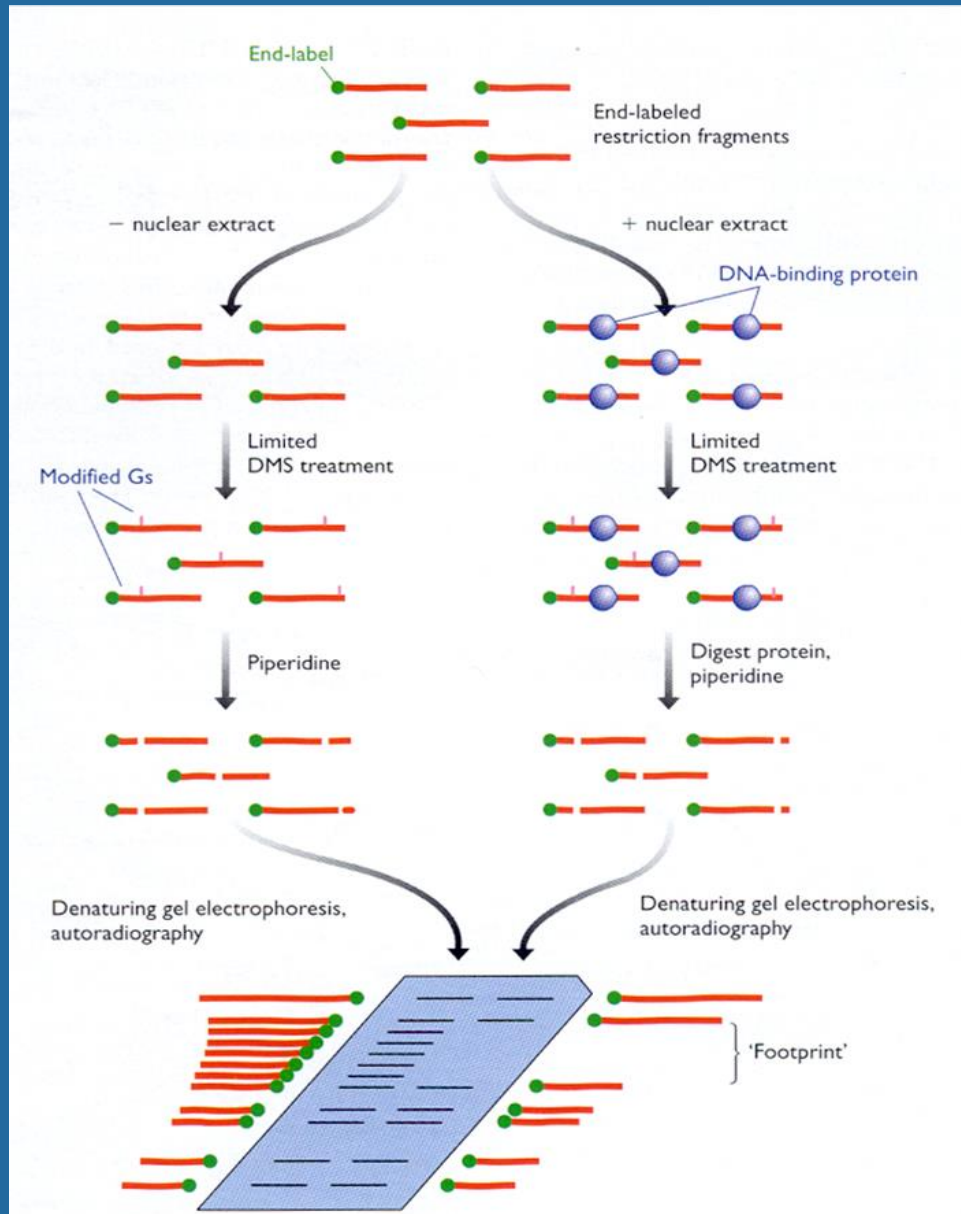
Αλληλεπιδράσεις Πρωτεϊνών-DNA

Ιχνηλάτηση περιοχής πρόσδεσης

Fig.11.12, 11.13

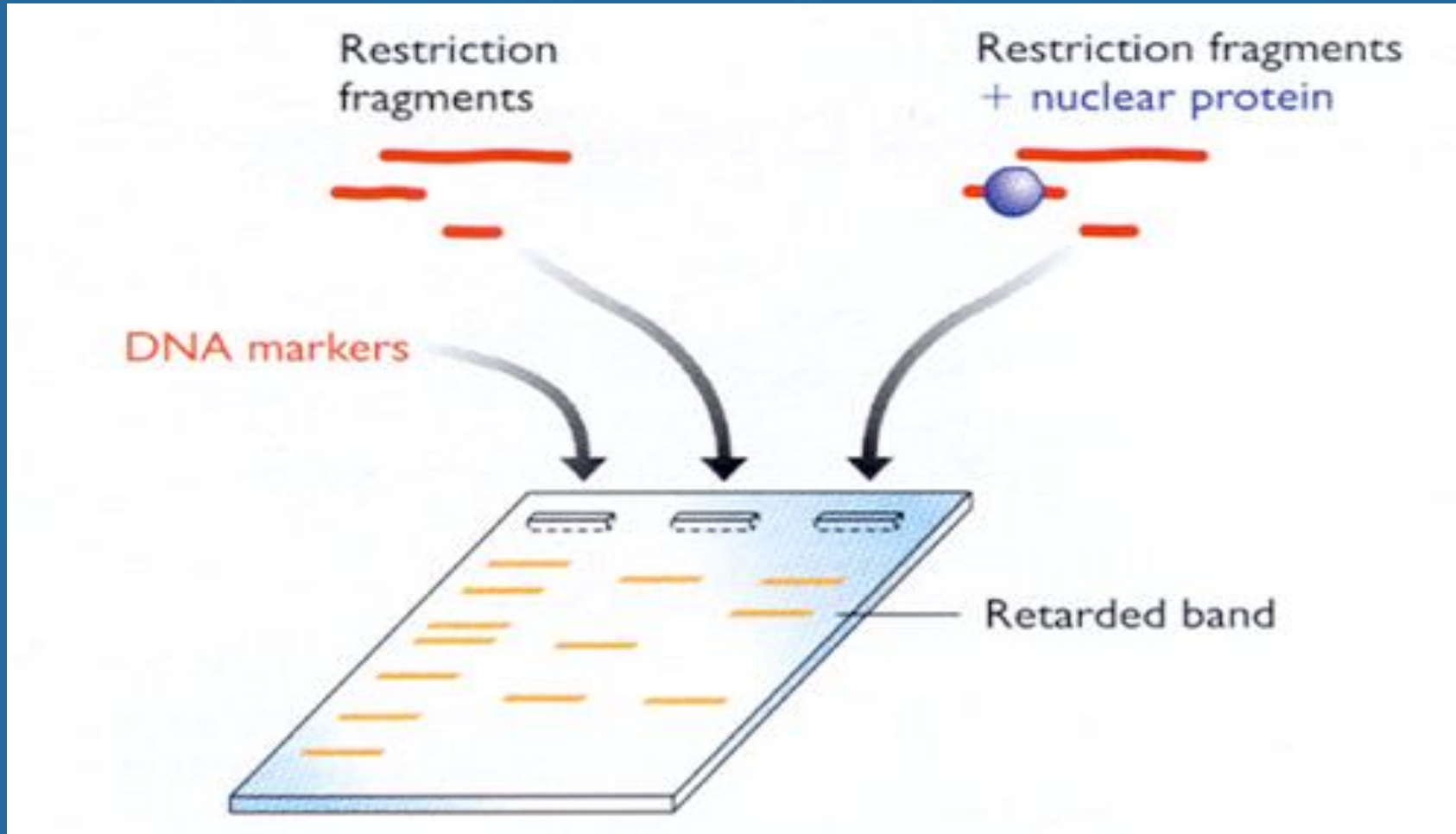


Αλληλεπιδράσεις Πρωτεϊνών-DNA Ιχνηλάτηση Θέσεων Πρόσδεσης



Αλληλεπιδράσεις πρωτεϊνών-DNA
Electrophoretic mobility shift assay (EMSA)

Fig.-11.9



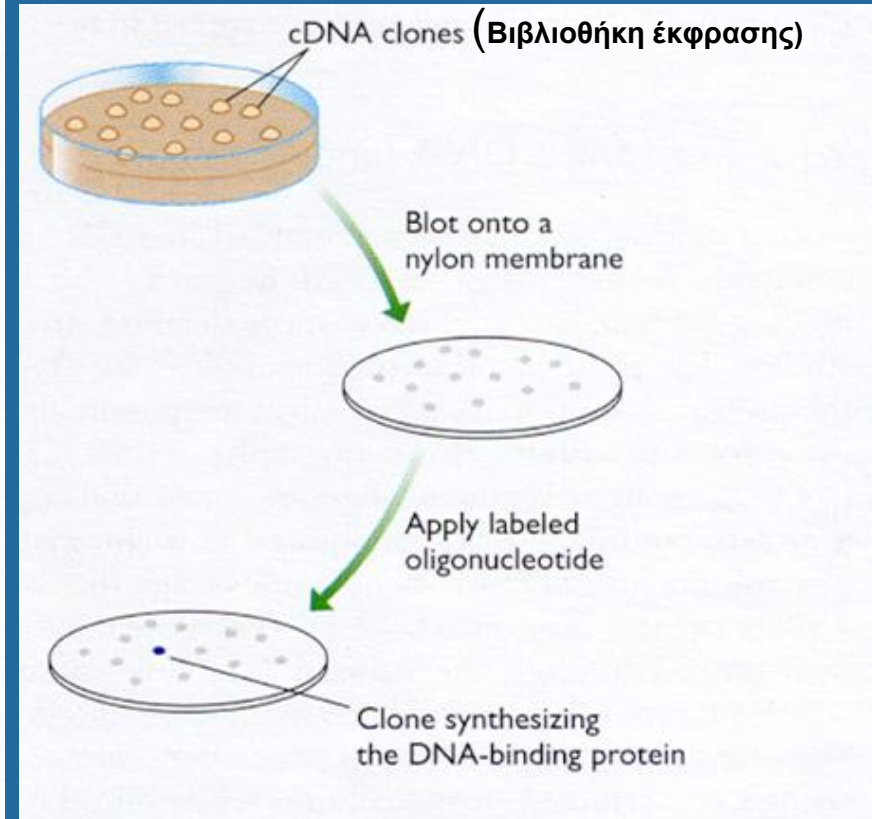
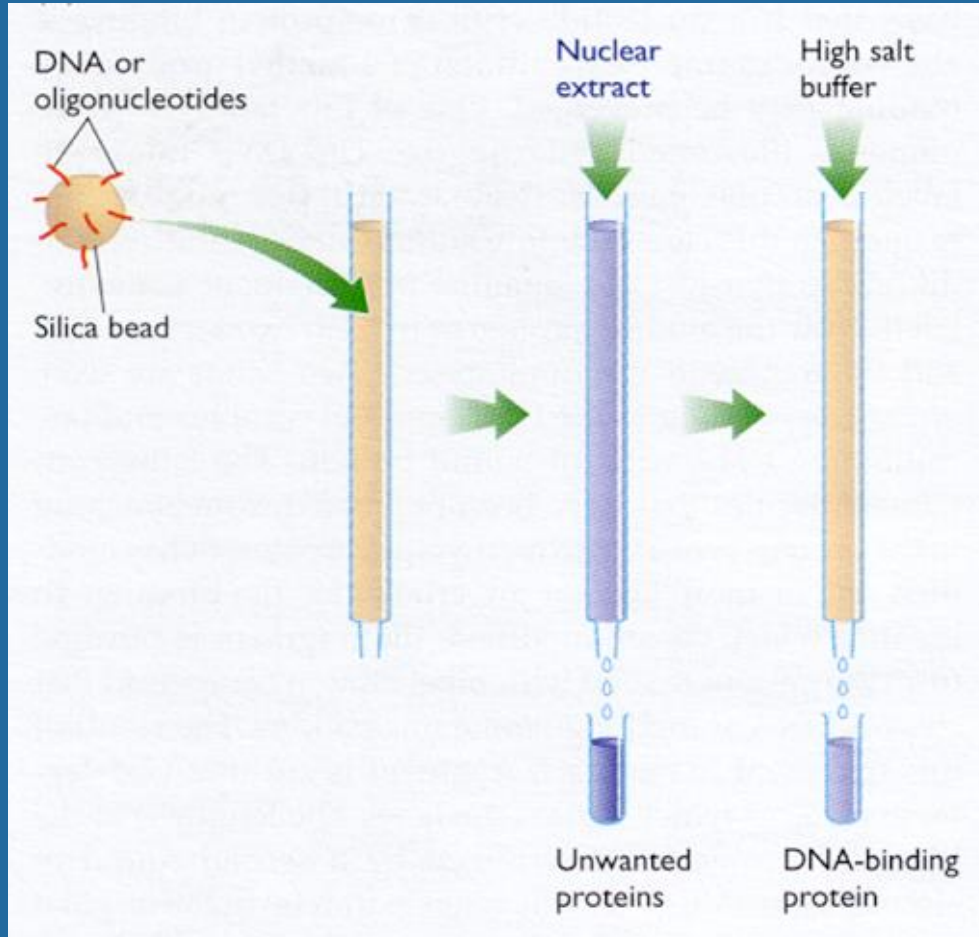
Αλληλεπιδράσεις Πρωτεϊνών-DNA

Electrophoretic mobility shift assay (EMSA)

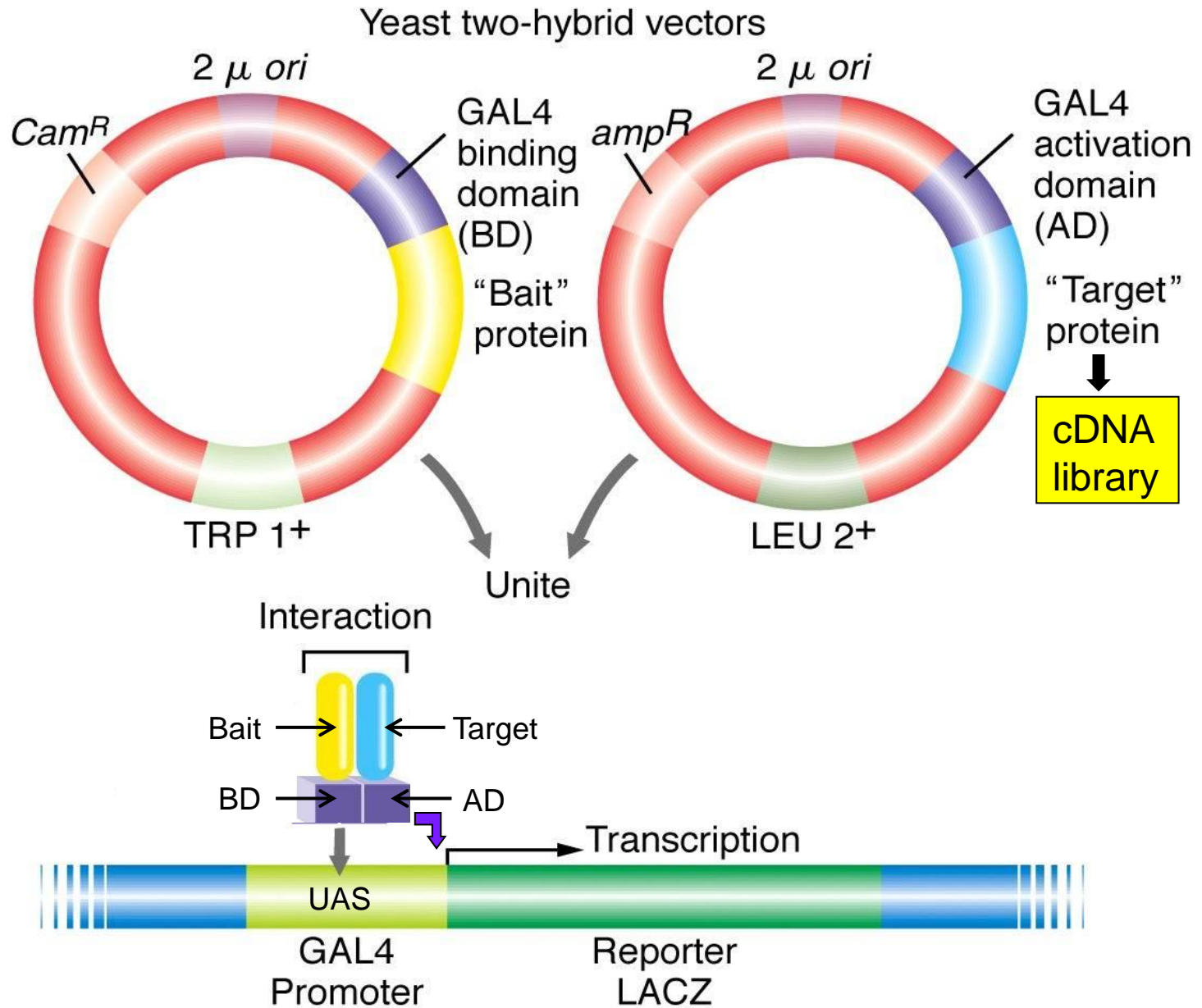
Nuclear extract or protein	-	+	+	+
Antibody	-	-	+	-
Competitor 100X	-	-	-	+



Απομόνωση ρυθμιστικών παραγόντων

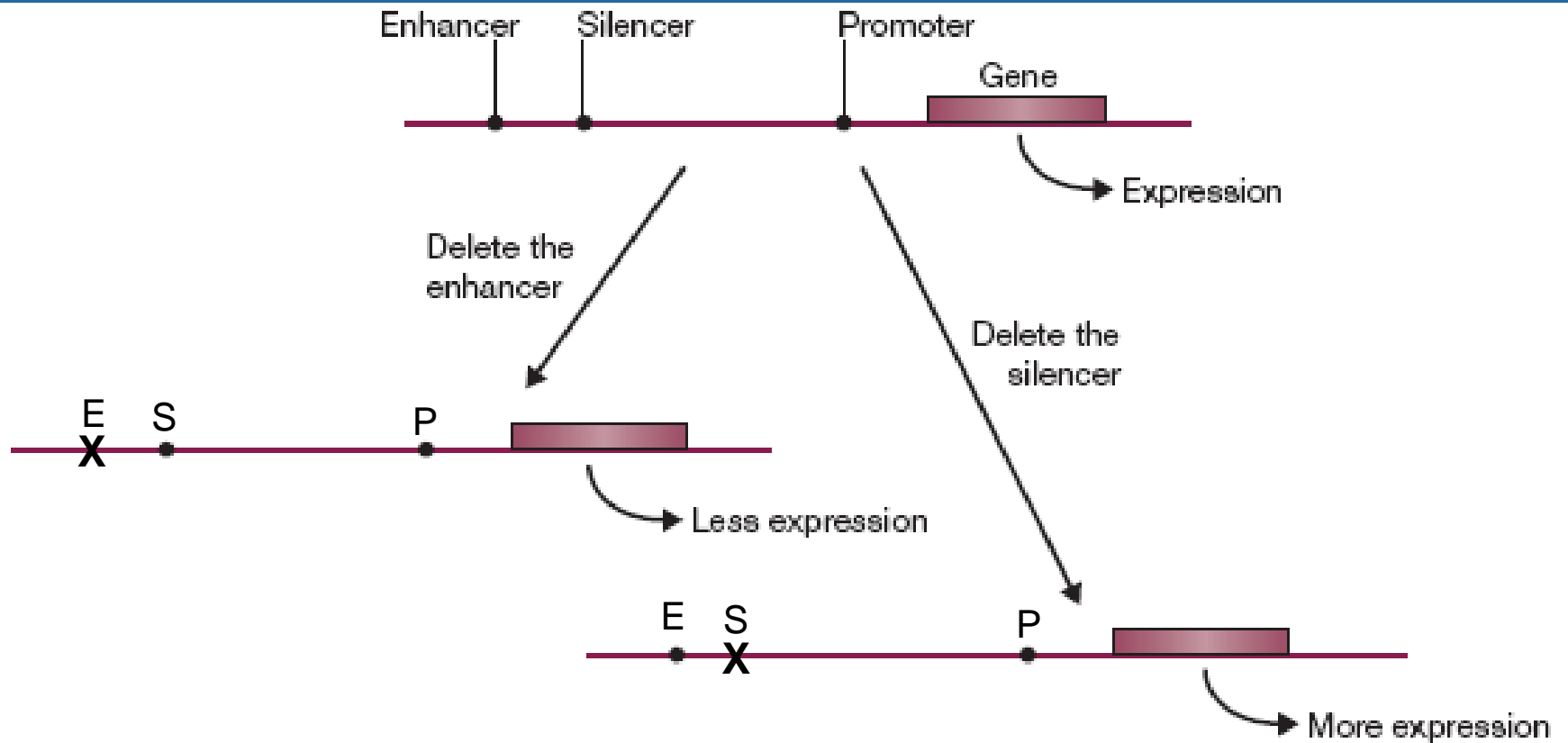


Αλληλεπιδράσεις πρωτεϊνών



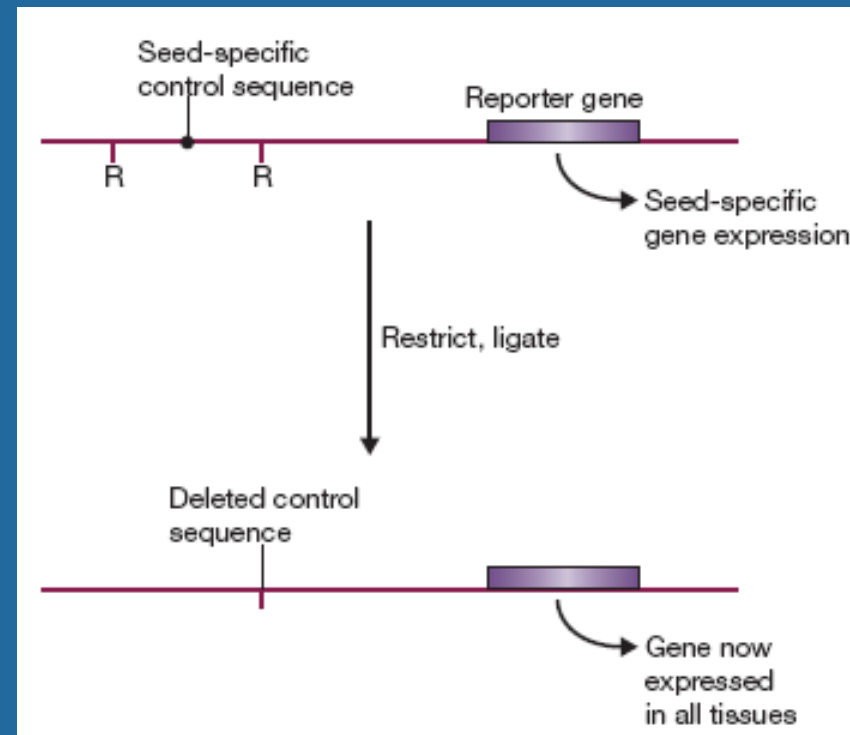
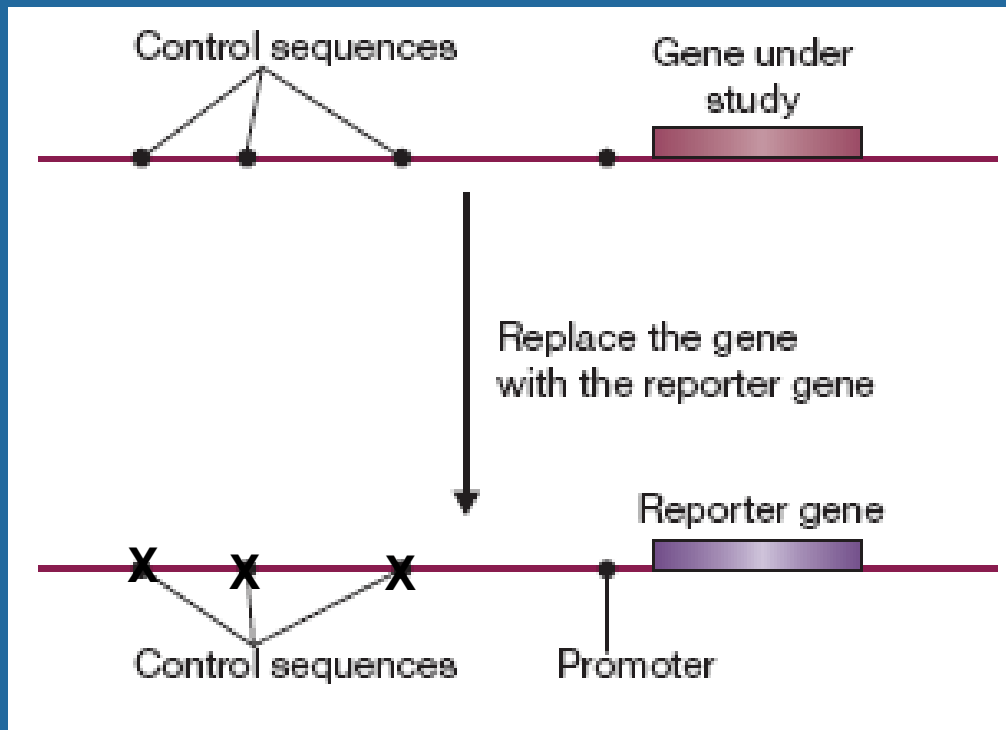
Ρύθμιση των γονιδίων

F-11.15



Ρύθμιση των γονιδίων

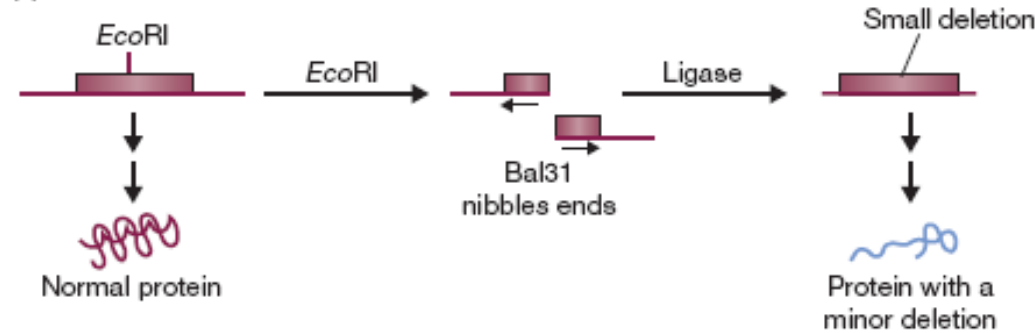
F-11.16, 11.17



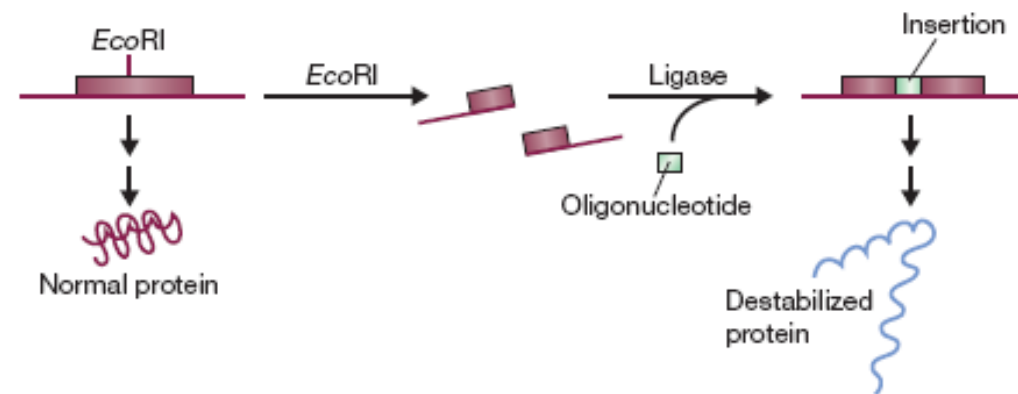
(a) Restriction fragment deletion



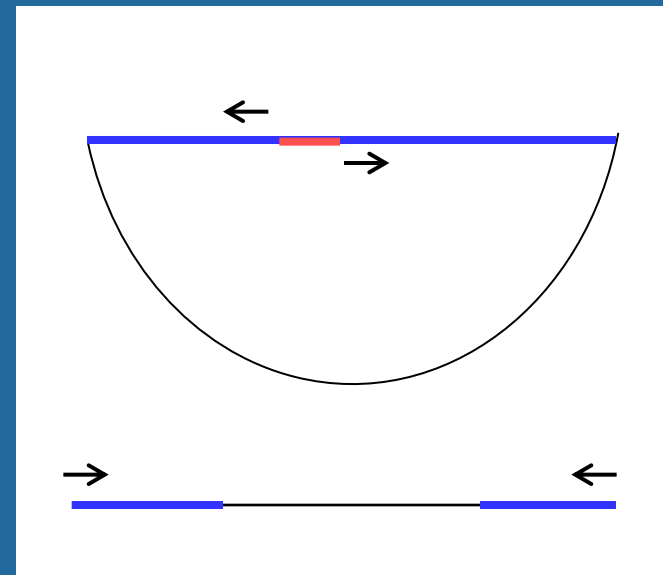
(b) Nucleotide removal at restriction site



(c) Insertion of an oligonucleotide



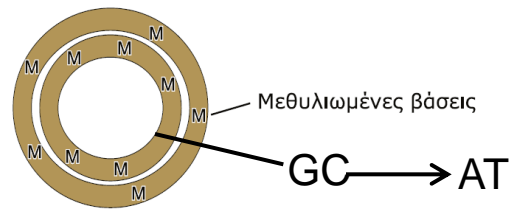
Inverted PCR Ελείματα



Σημειακές μεταλλάξεις

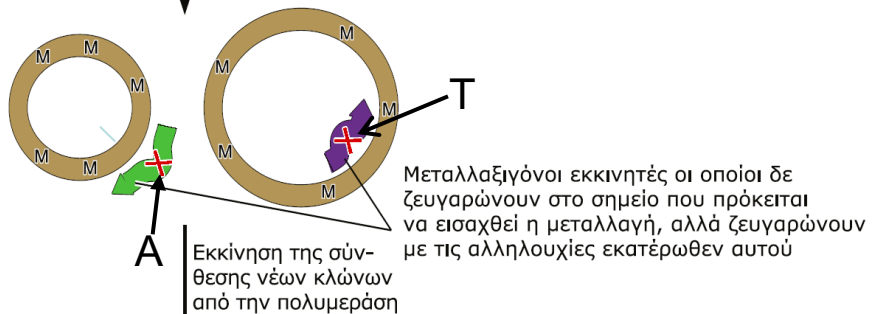
ΕΙΚΟΝΑ 6.3

(α) Απομόνωση πλασμιδιακού DNA από ένα βακτηριακό στέλεχος με ενεργότητα μεθυλάσης *dam*

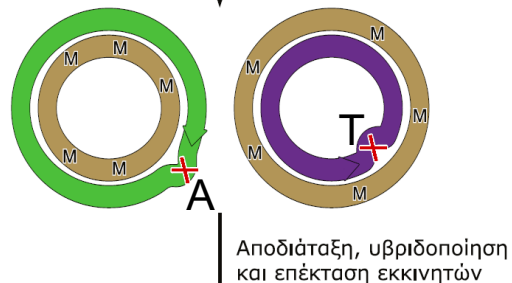


Αποδιάταξη του πλασμιδίου και υβριδοποίηση των μεταλλαξιγόνων ολιγονουκλεοτιδίων-εκκινήτων

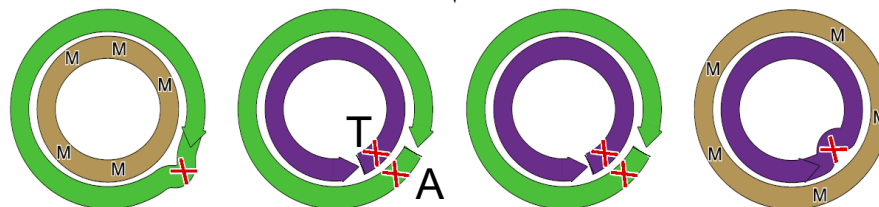
(β) Αποδιάταξη του πλασμιδιακού DNA, προσθήκη των μεταλλαξιγόνων εκκινήτων και υβριδοποίησή τους στους κλώνους του πλασμιδίου



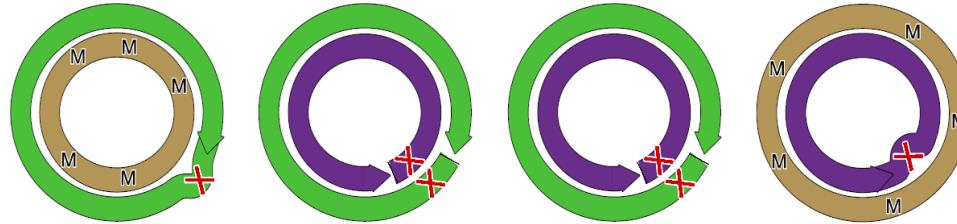
(γ) Επέκταση μεταλλαξιγόνων εκκινήτων



(δ) Διαδοχικές επαναλήψεις των β και γ σε ένα θερμοκυκλοποιητή



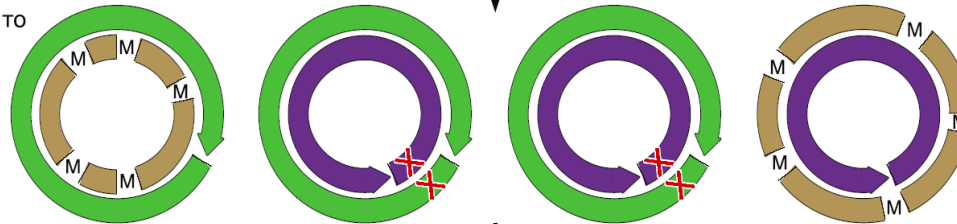
(δ) Διαδοχικές επαναλήψεις των β και γ σε ένα θερμοκυκλοποιητή



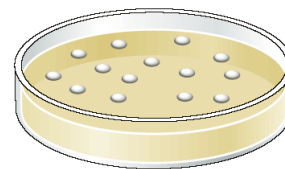
Αποδιάταξη, υβριδοποίηση και επέκταση εκκινητών

Πολλαπλοί κύκλοι αποδιάταξης, υβριδοποίησης και επέκτασης των εκκινητών

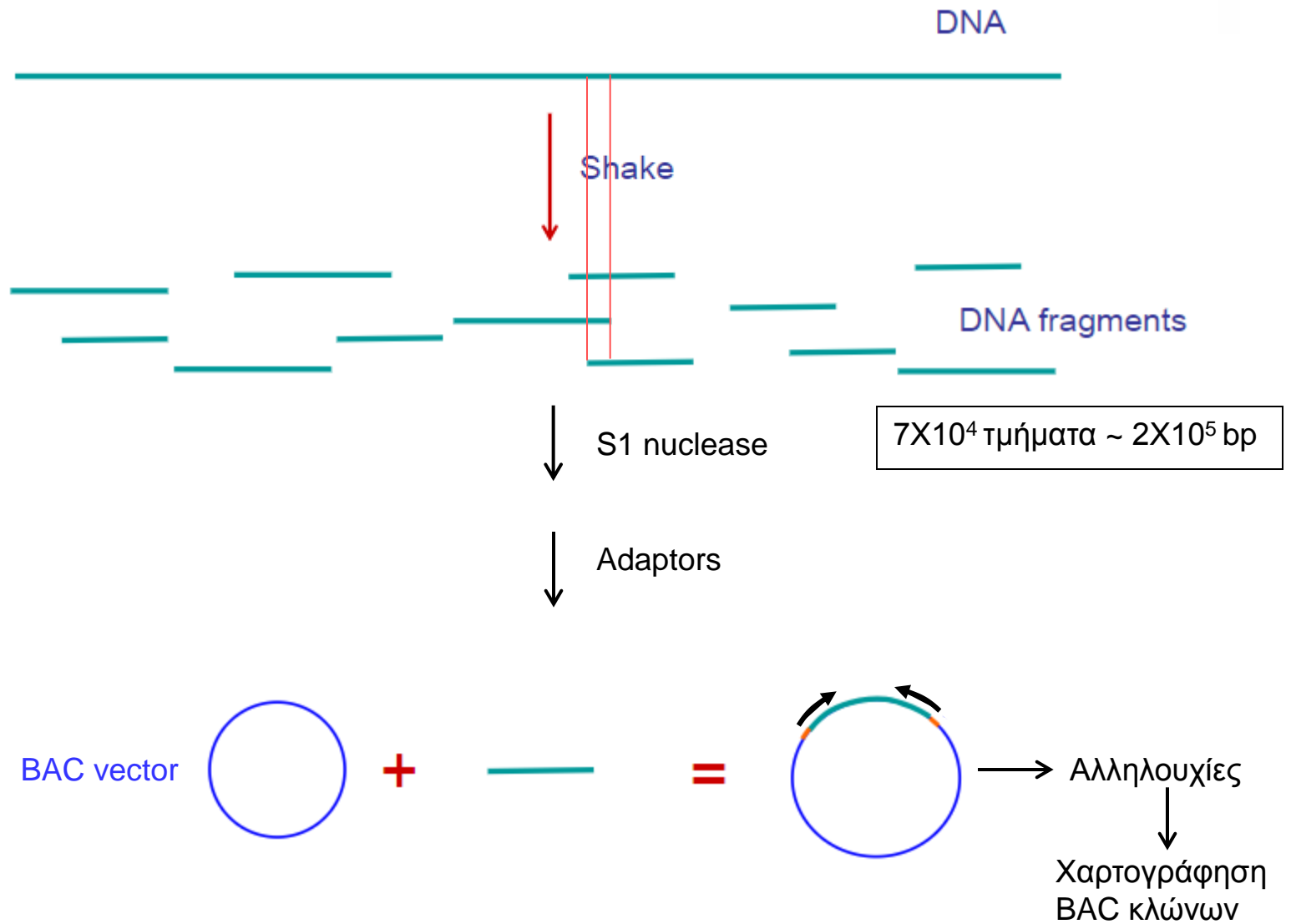
(ε) Πέψη των προϊόντων με το ένζυμο DpnI, το οποίο κόβει μεθυλιωμένες και ημιμεθυλιωμένες θέσεις GATC



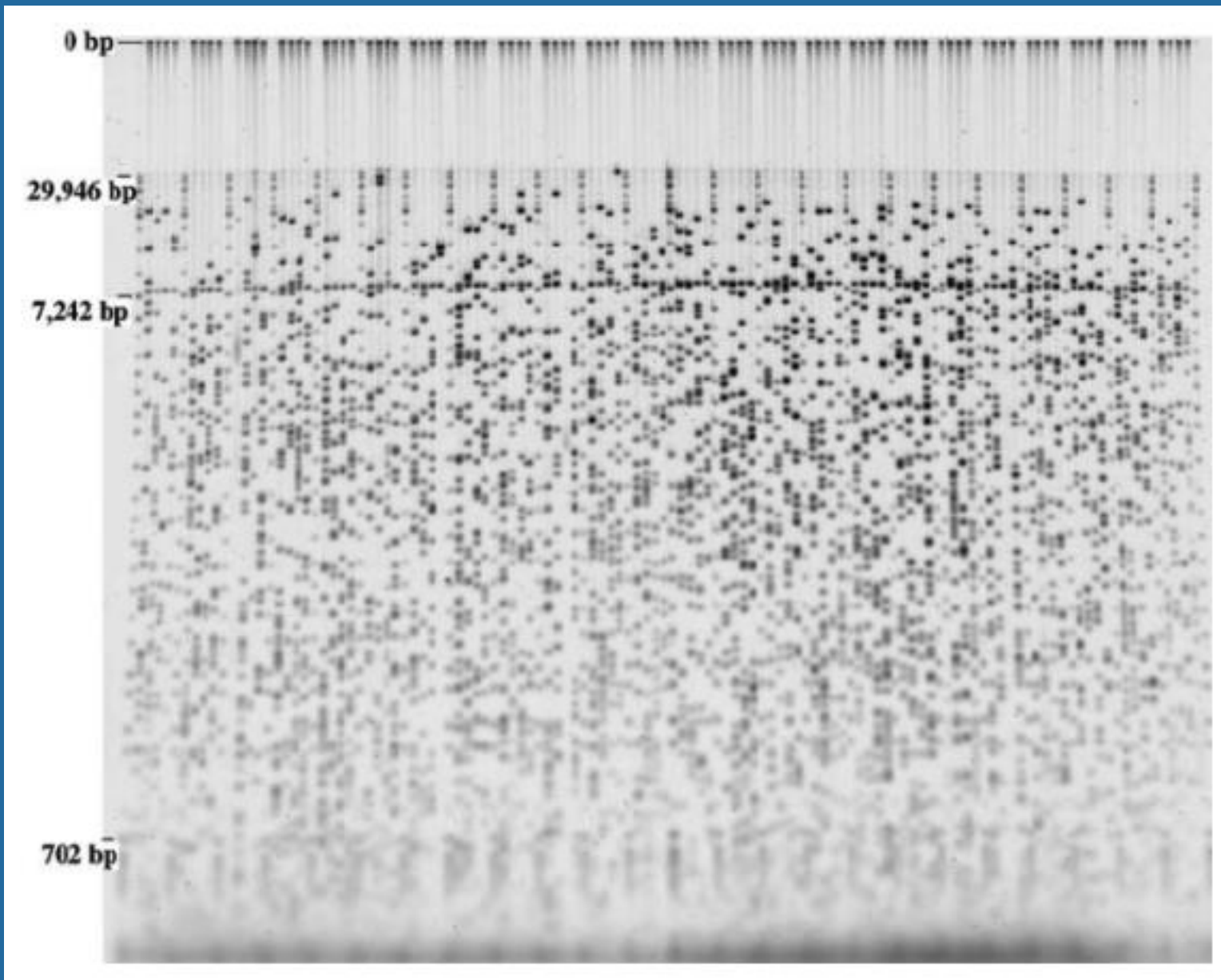
(στ) Μετασχηματισμός βακτηρίων, απομόνωση πλασμιδιακού DNA από τυχαίες αποικίες και ανάλυση της νουκλεοτιδικής αλληλουχίας του, προκειμένου να επιβεβαιωθεί η παρουσία της επιθυμητής μεταλλαγής



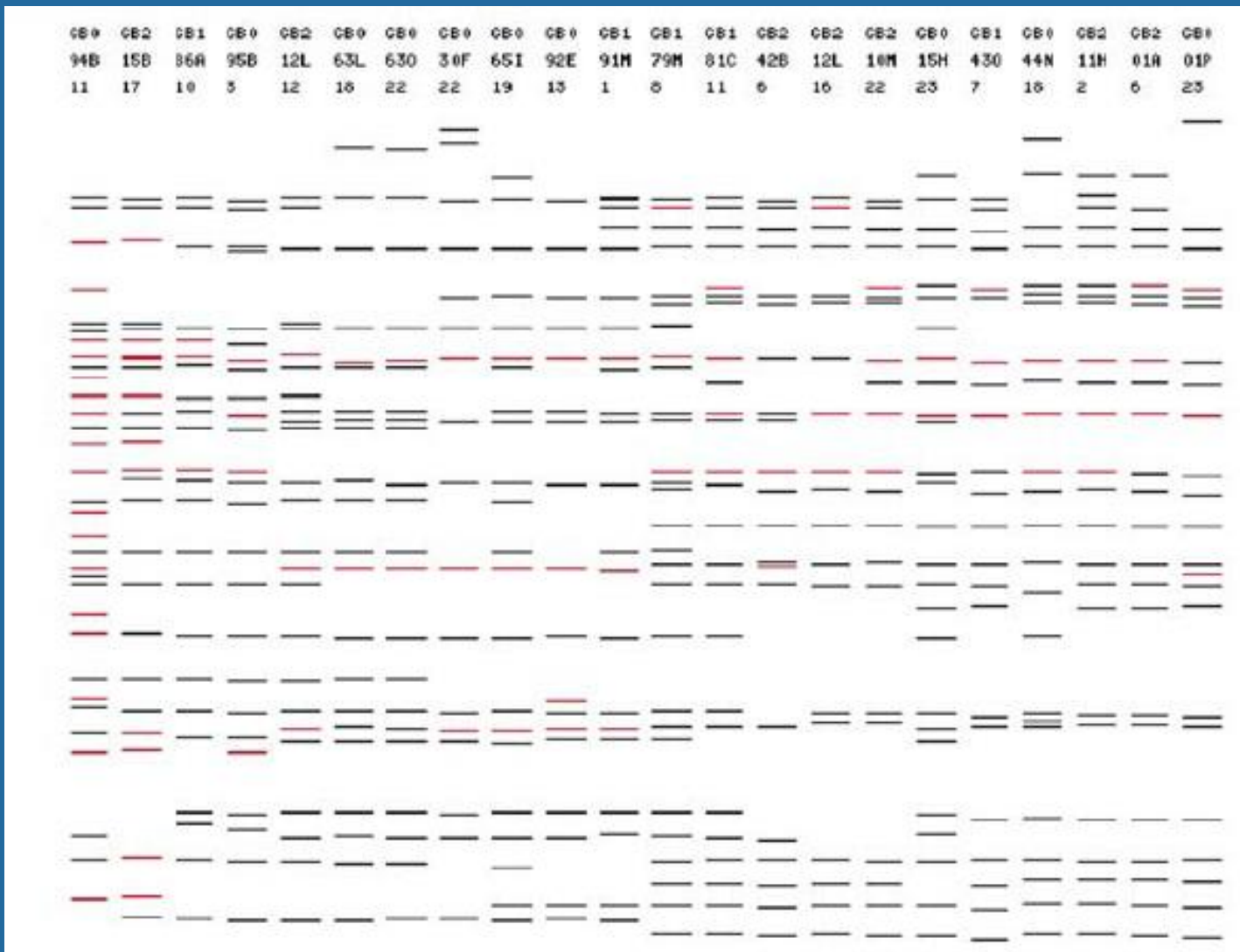
Κλωνοποίηση σε βακτηριακά τεχνητά χρωμοσώματα (BACs)



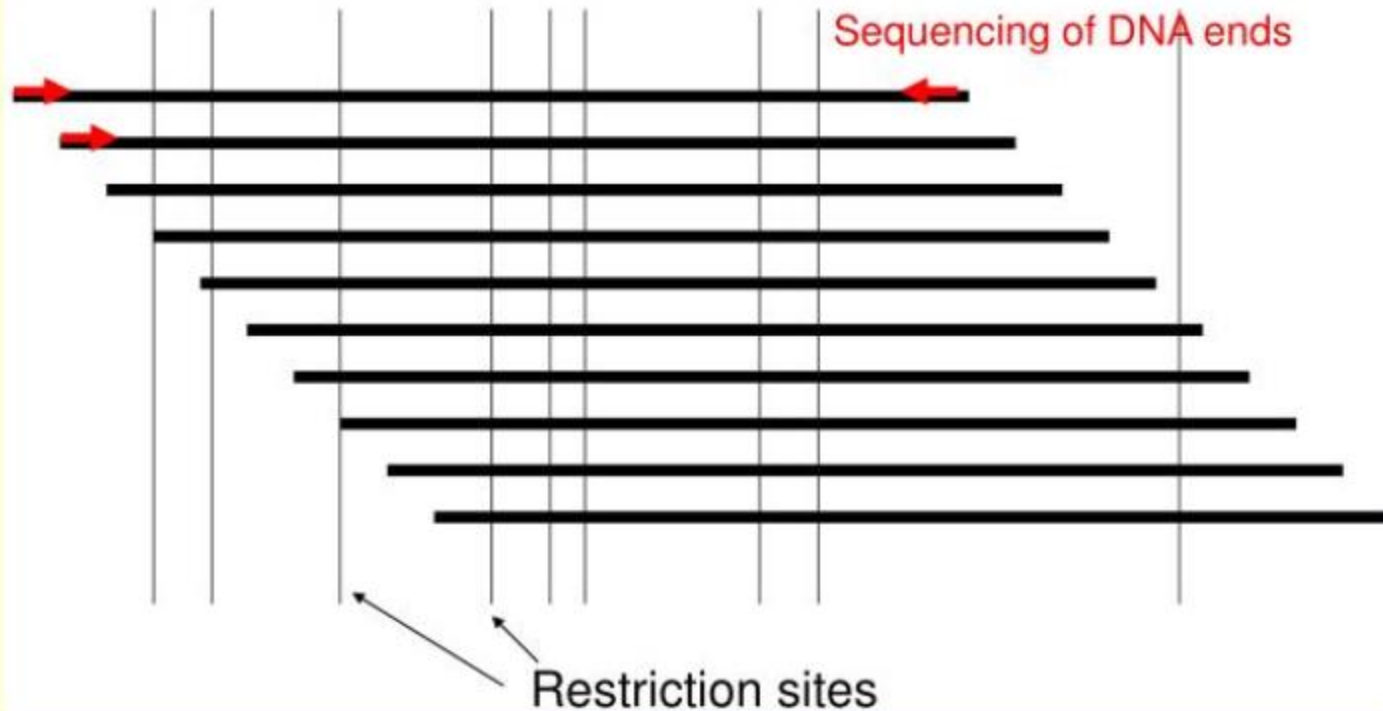
Χαρτογράφηση με ένζυμα περιορισμού



Χαρτογράφηση με ένζυμα περιορισμού



Map construction - BAC fingerprinting

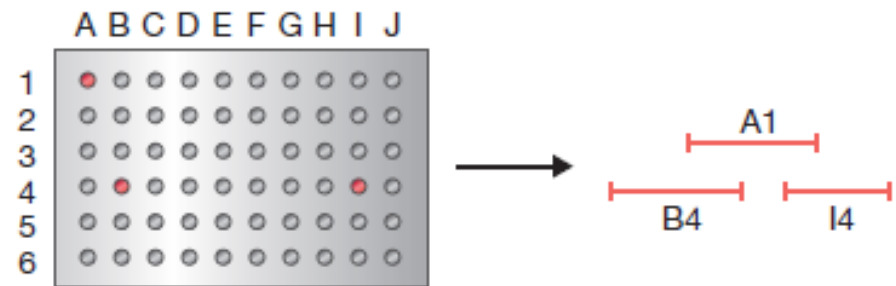


- 10-20x more bp in BACs than in the genome for map construction (Arabidopsis – 20 000, rice - 70 000)

Figure 10.13

Chromosome walking.

(a) Probe the library with clone A1



(b) Probe the library with clone I4

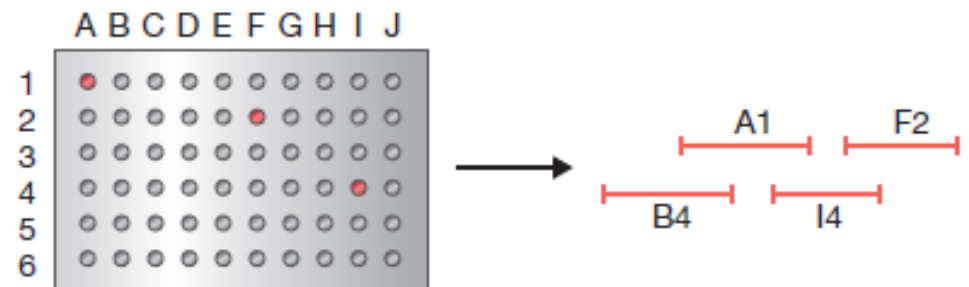
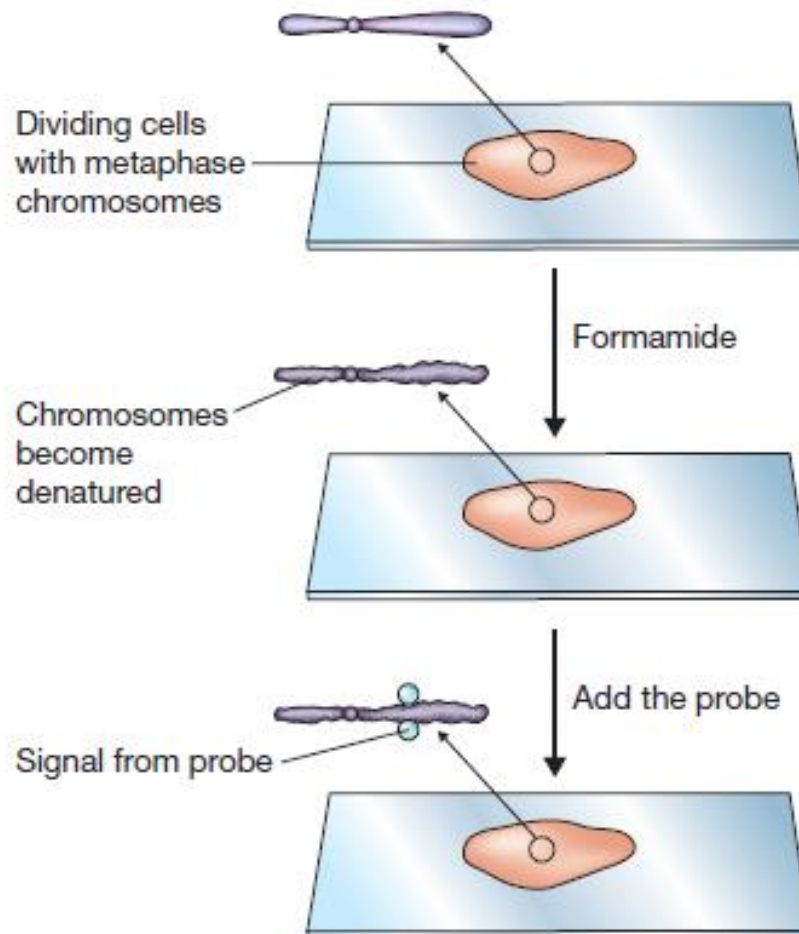


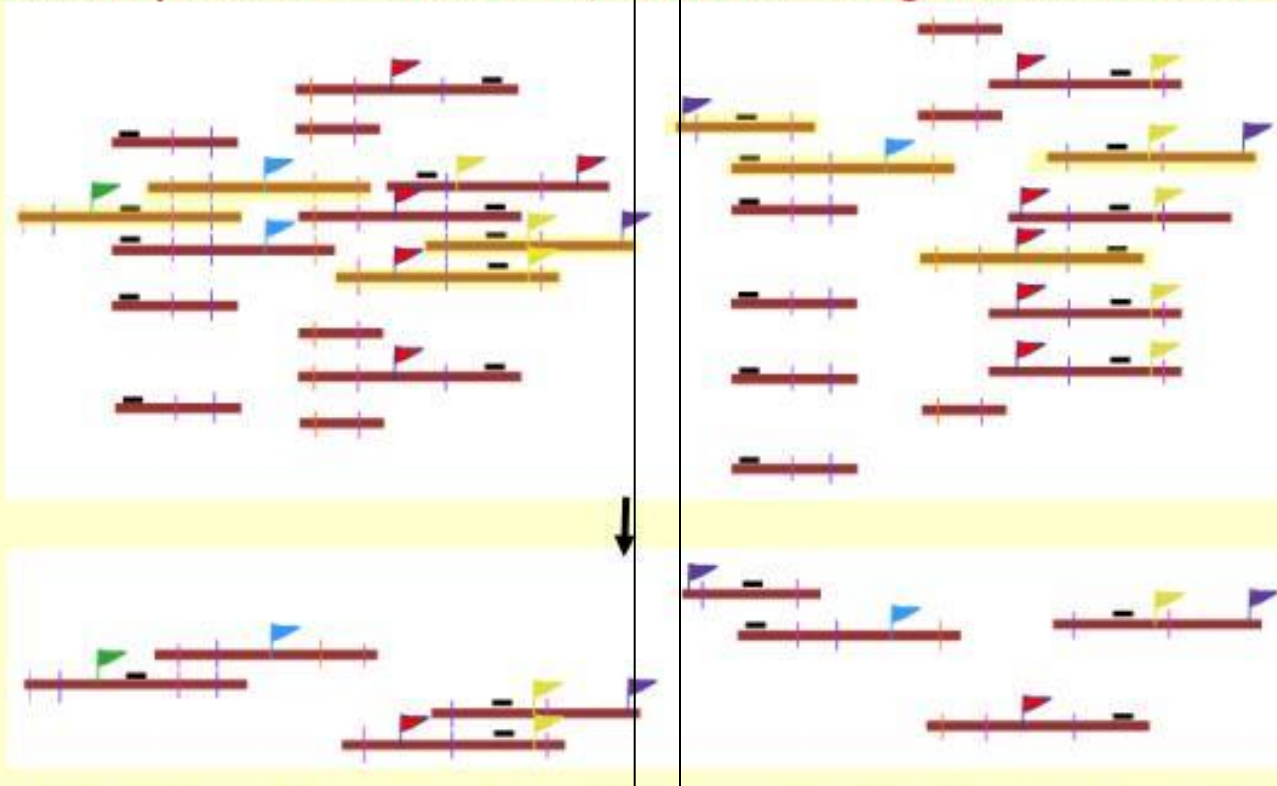
Figure 10.20

Fluorescent *in situ* hybridization.



Minimum tiling path

= the lowest possible set of BACs covering the whole sequence

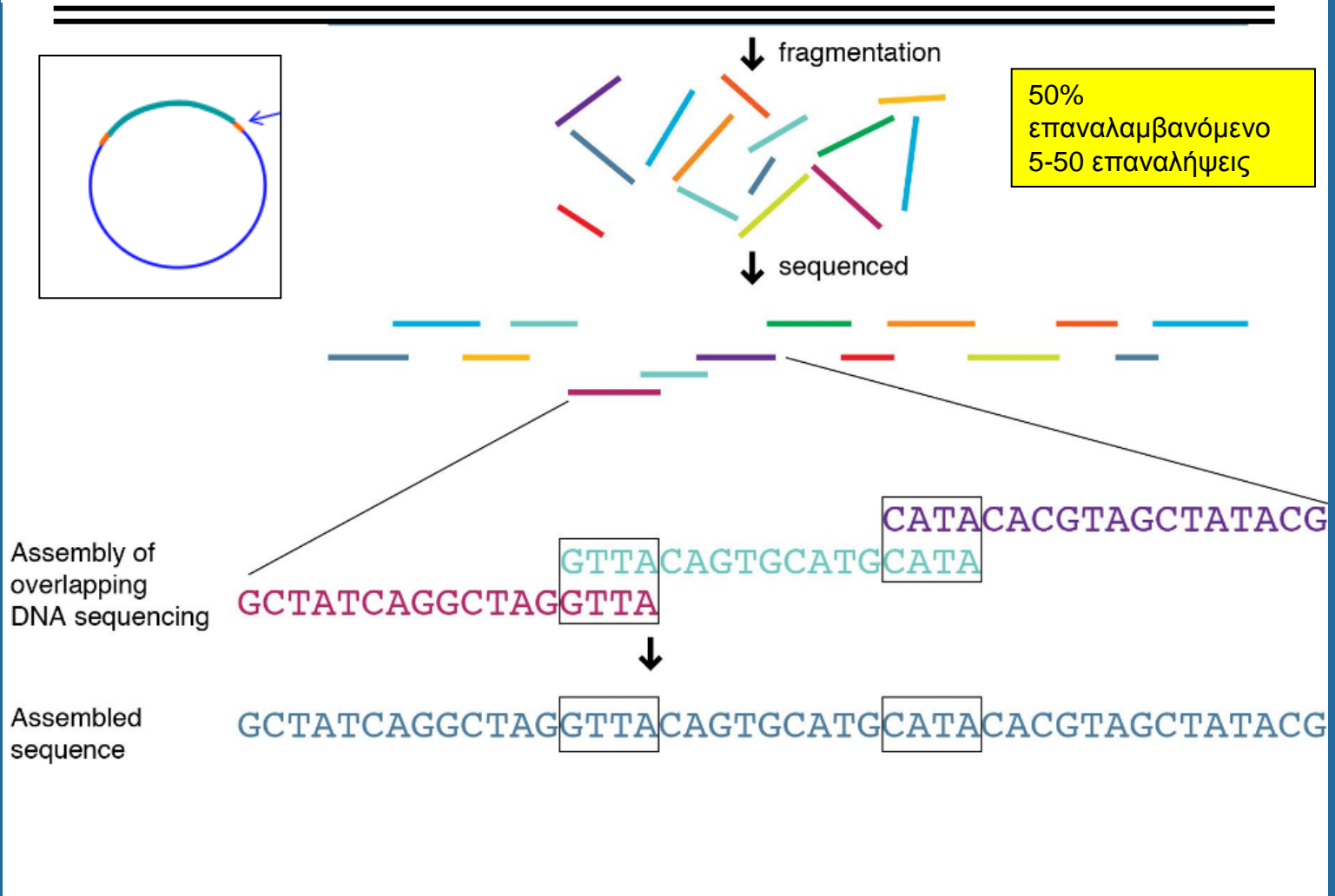


physical map arrangement and mapping and clone selection

- by restriction fragment analysis
- using terminal sequences and hybridization
- by hybridization with markers with known position in genetic map

Αλληλούχιση και χαρτογράφηση γονιδιωμάτων

Large DNA ή γονιδίωμα (Shotgun sequencing)



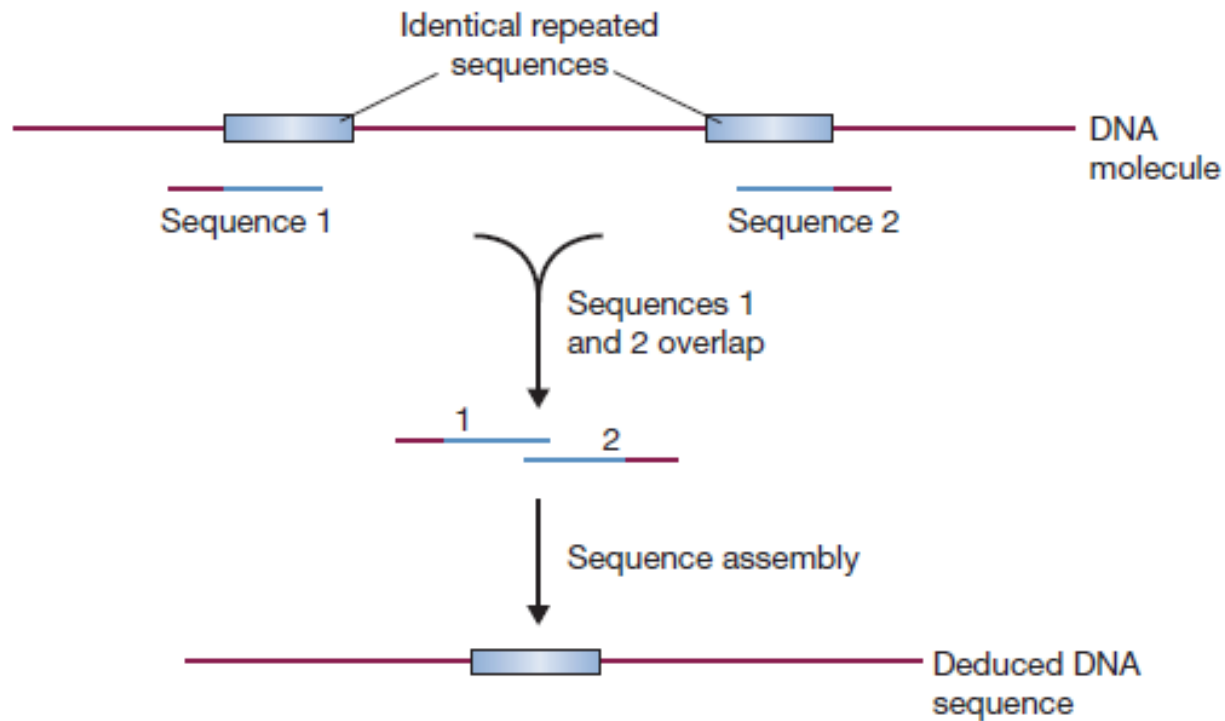
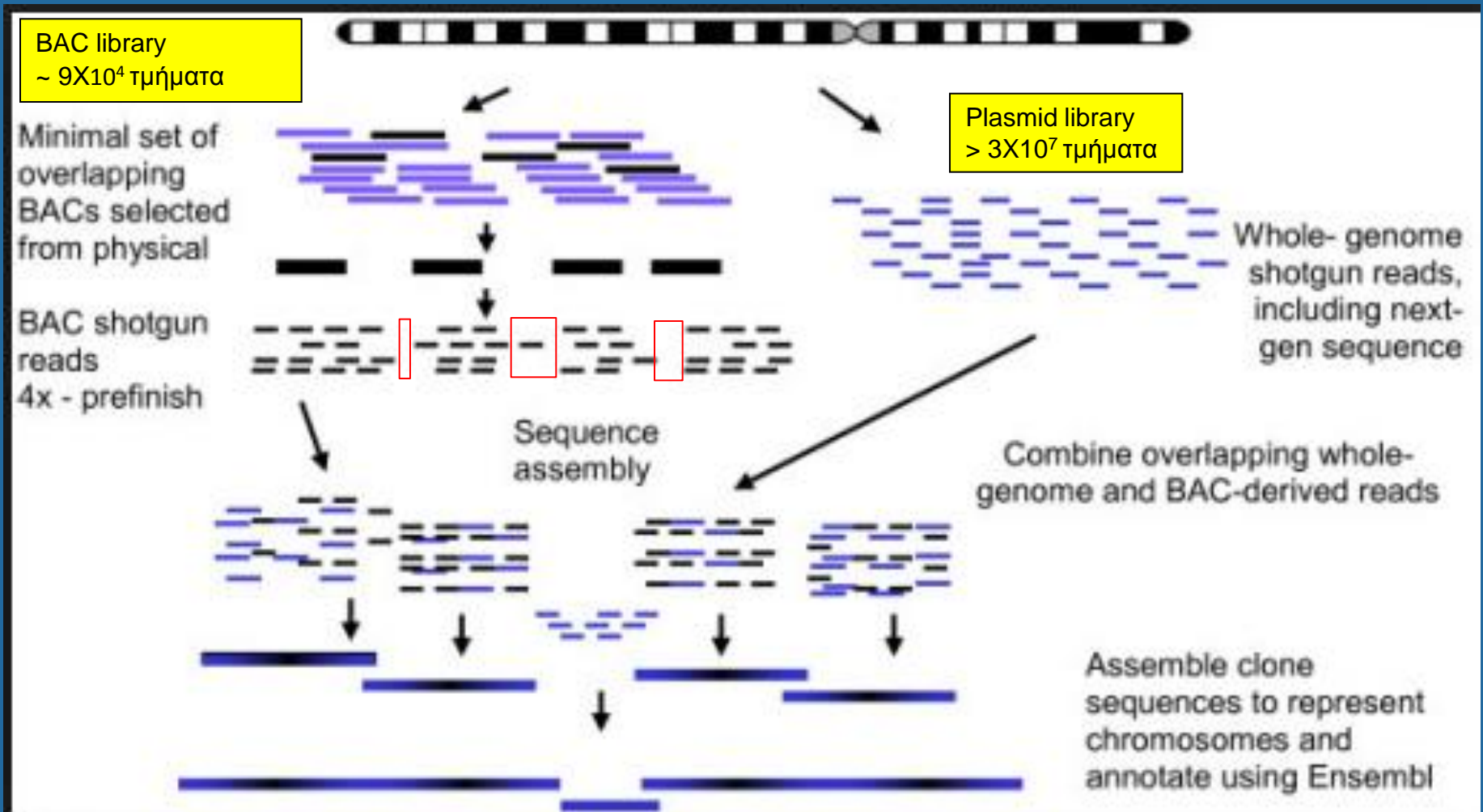
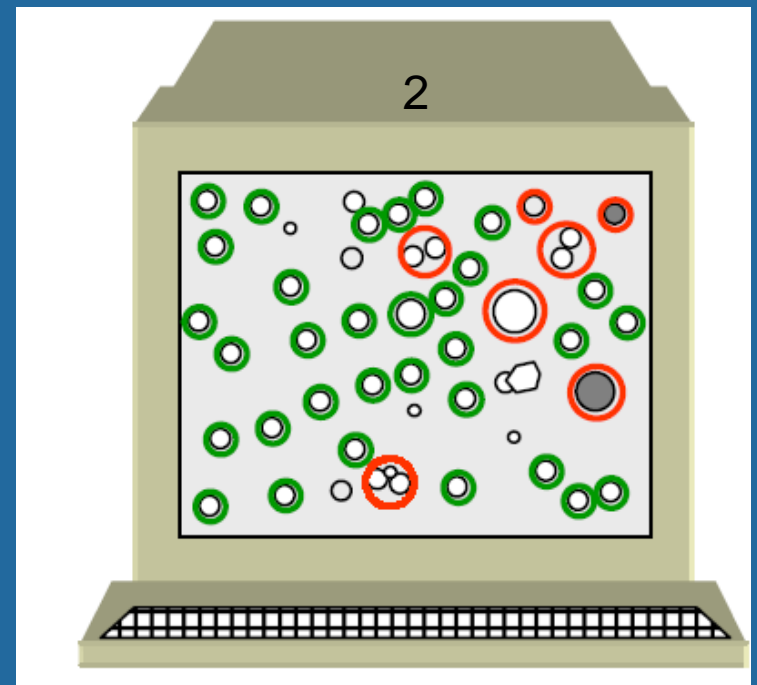
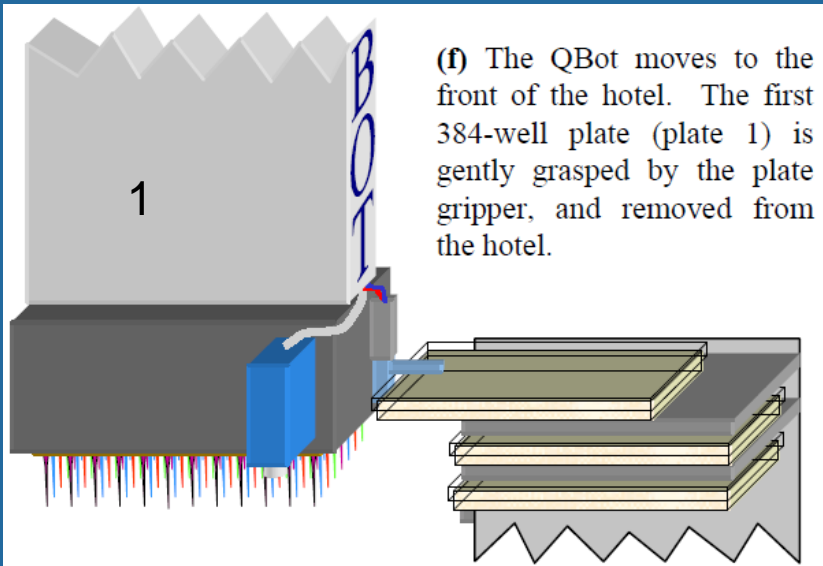


Figure 10.12

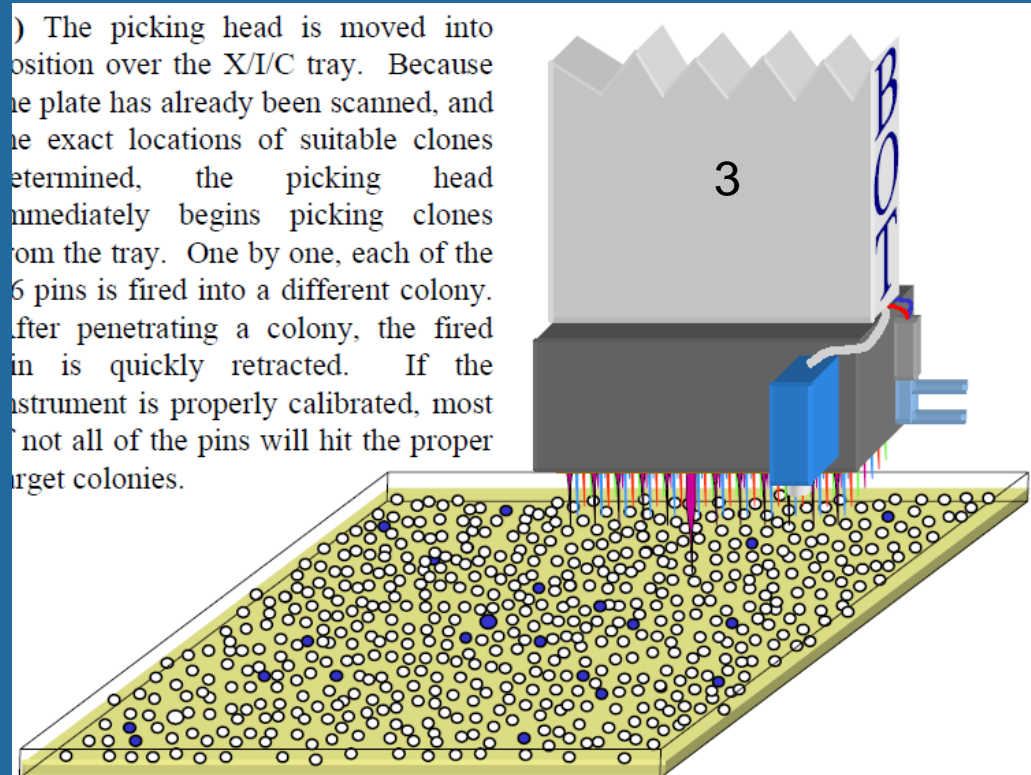
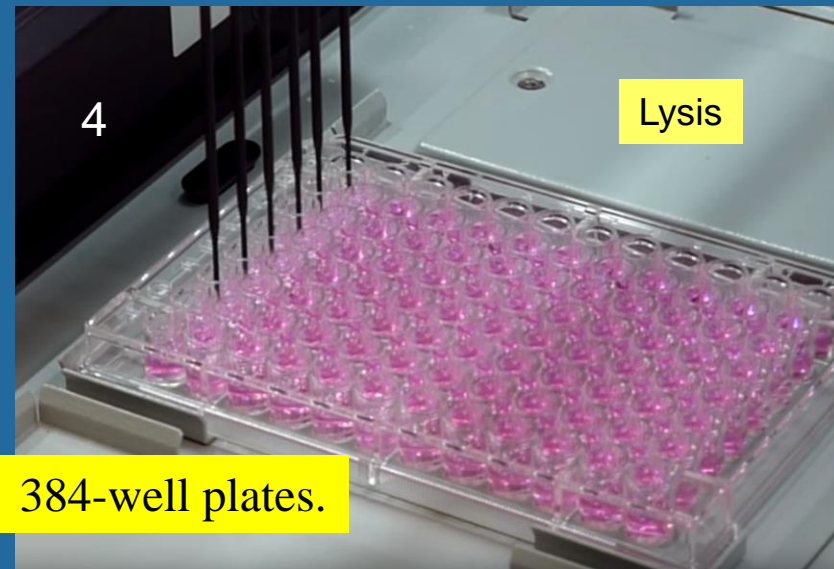
One problem with the shotgun approach. An incorrect overlap is made between two sequences that both terminate within a repeated element. The result is that a segment of the DNA molecule is absent from the DNA sequence.

Συνδιασμένη αλληλούχηση





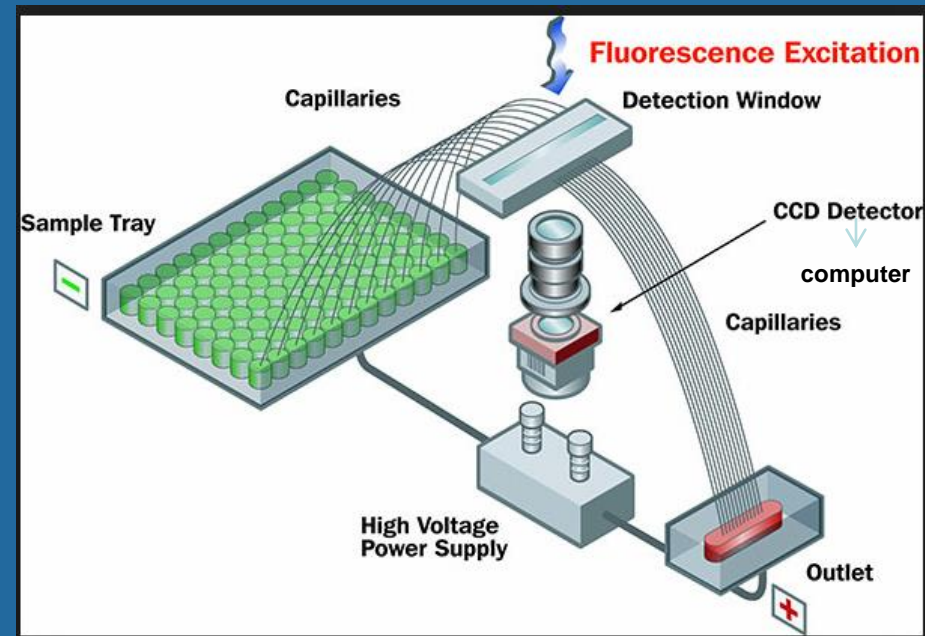
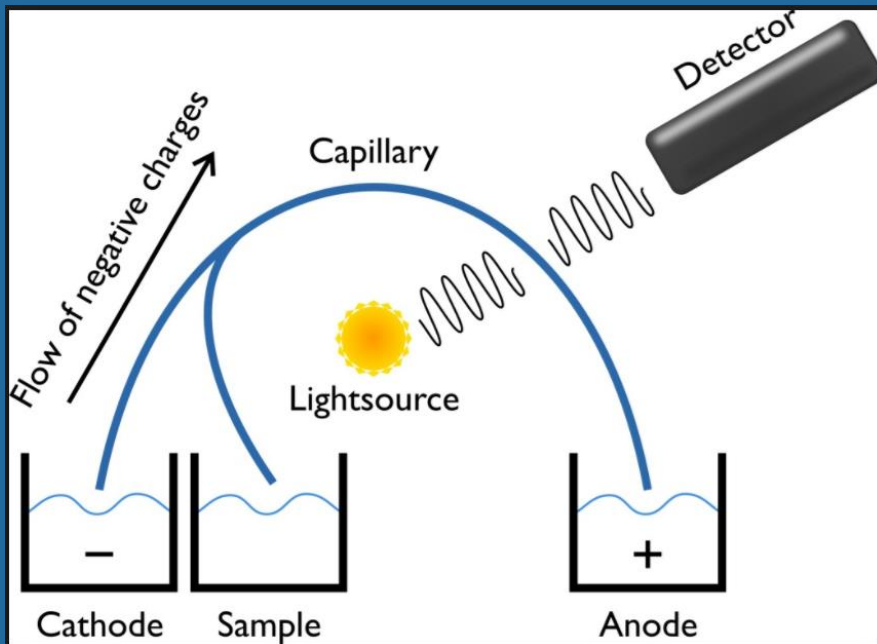
Microliter plate



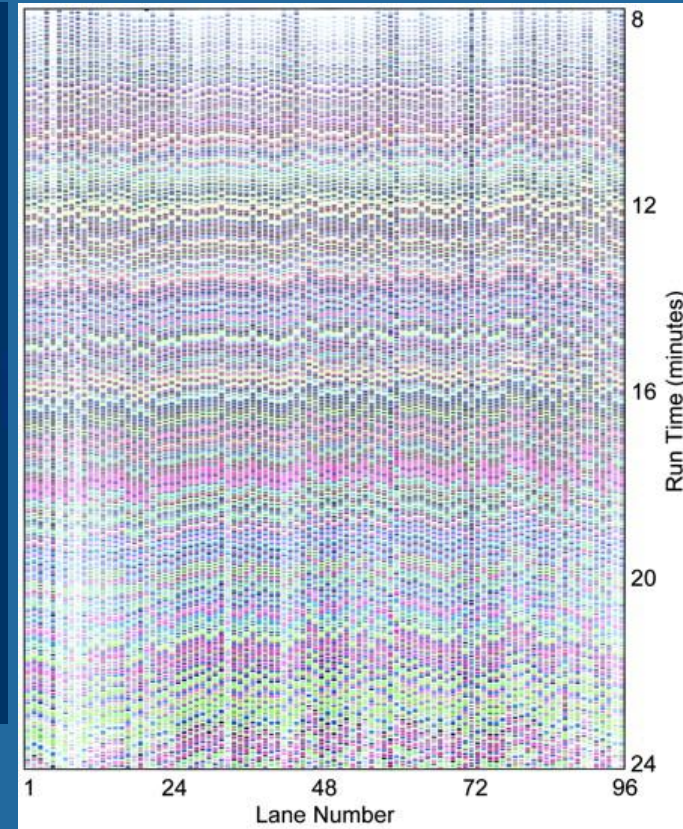
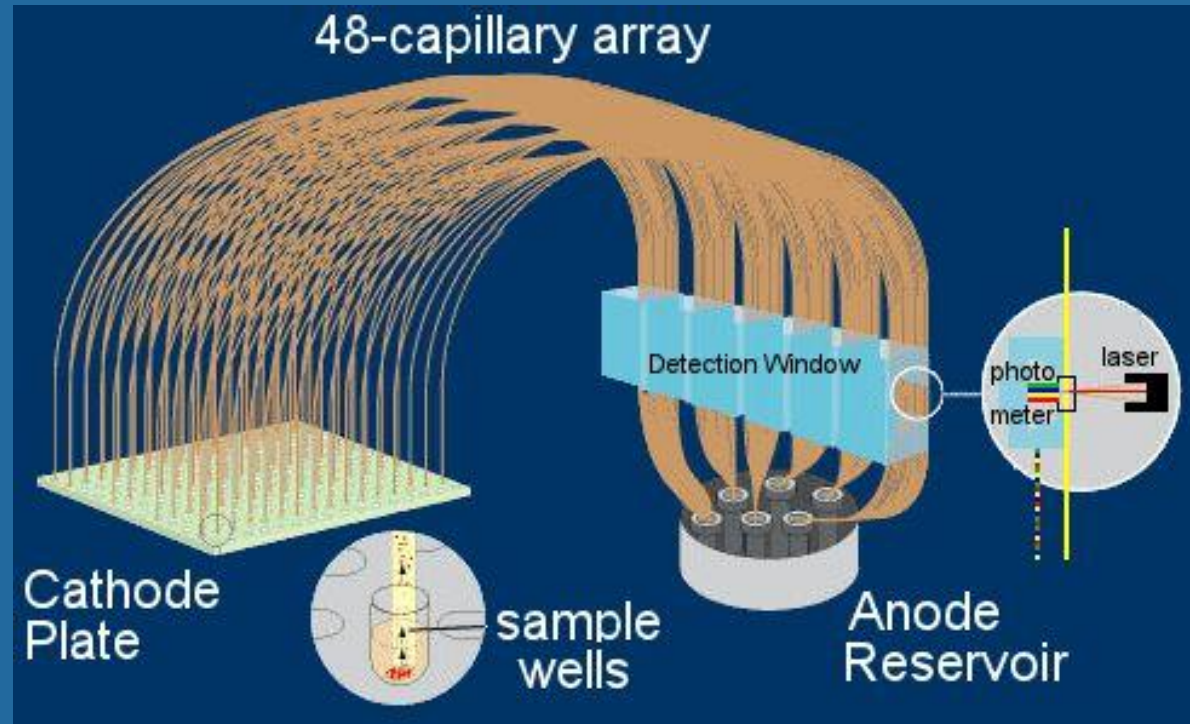
Αυτόματη αλληλούχηση

DNA isolation

Sequencing reactions

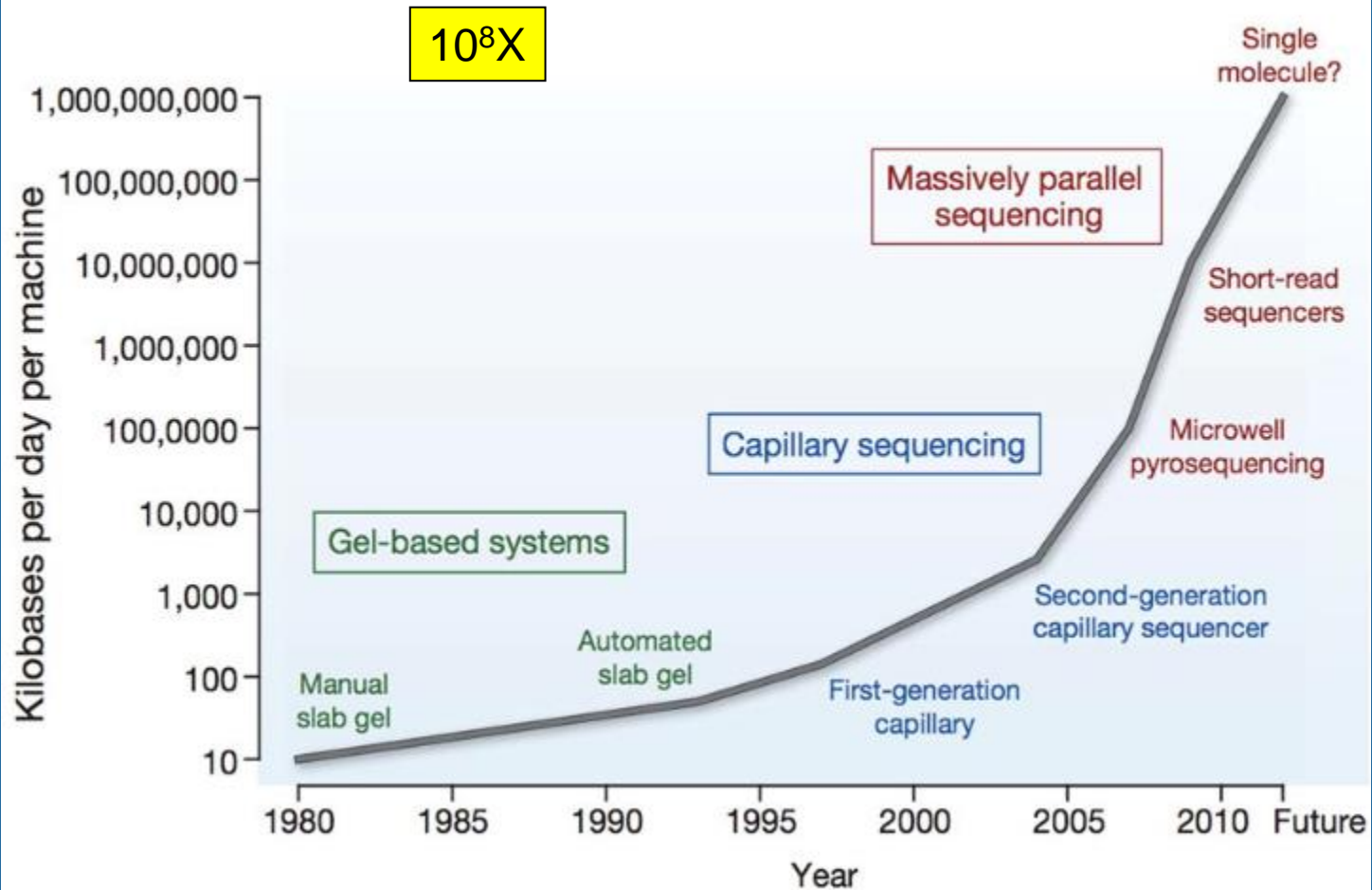


Αυτόματη αλληλούχηση

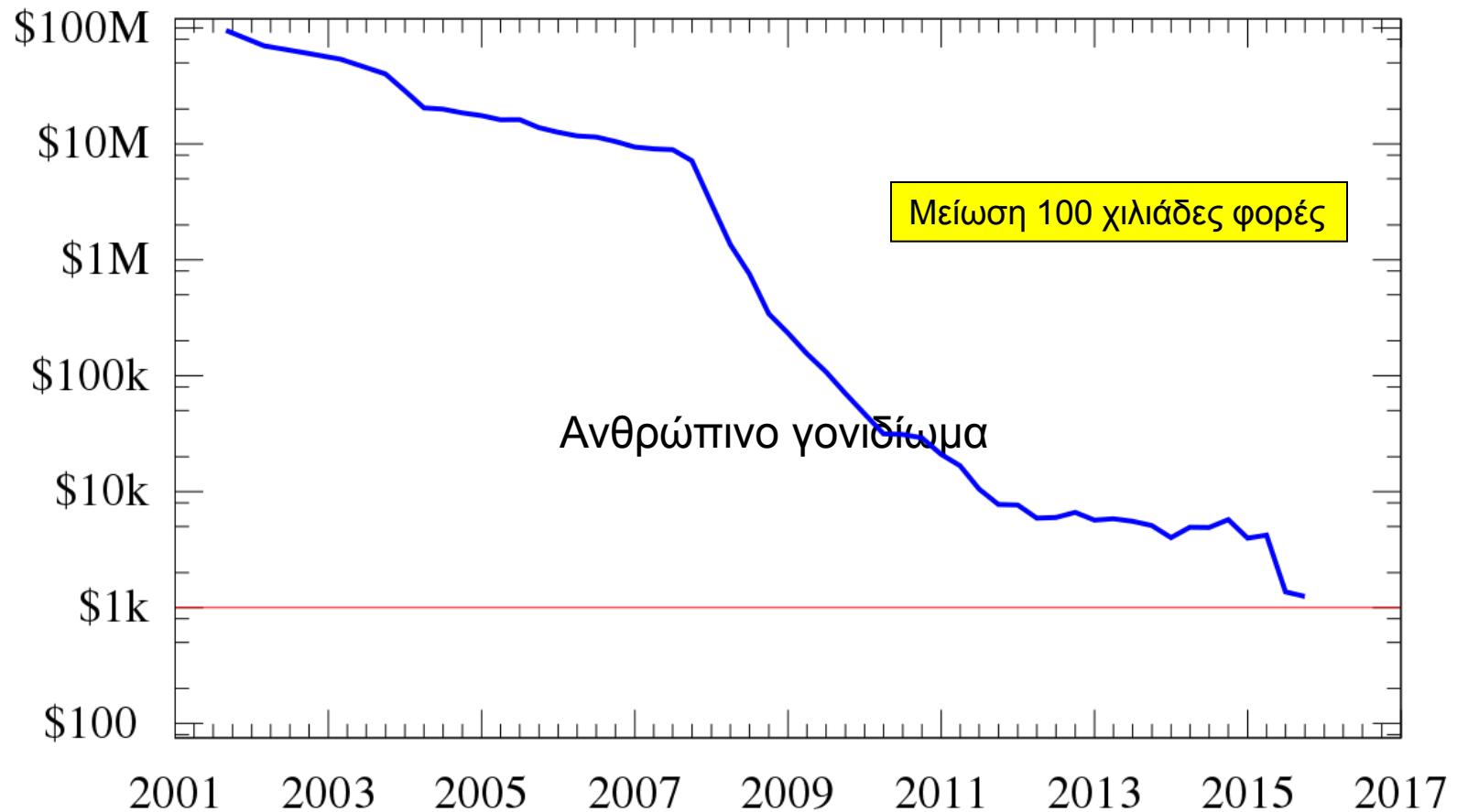


10^3 Kb/day

The History of DNA Sequencing Technology

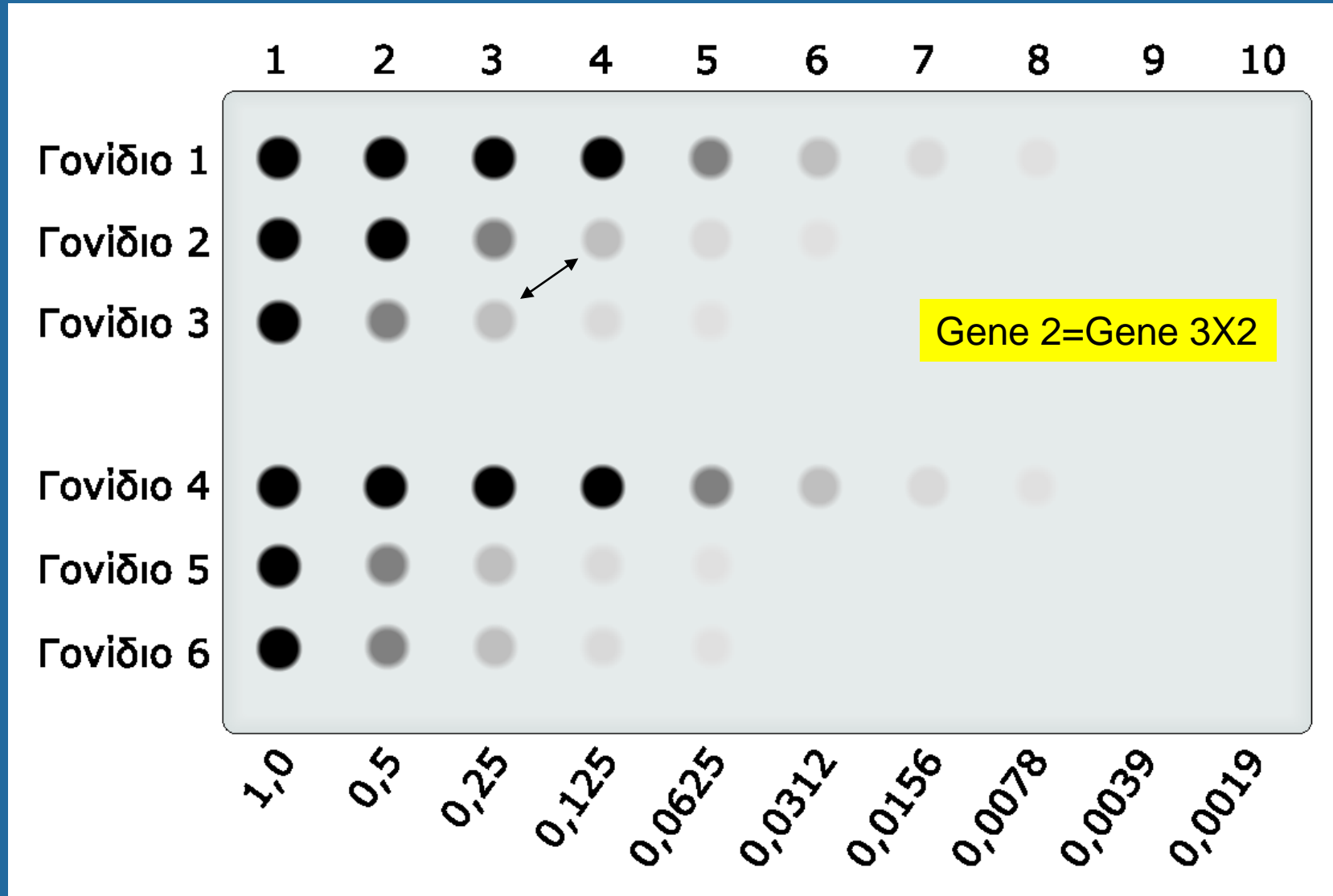


Cost to sequence a human genome (USD)



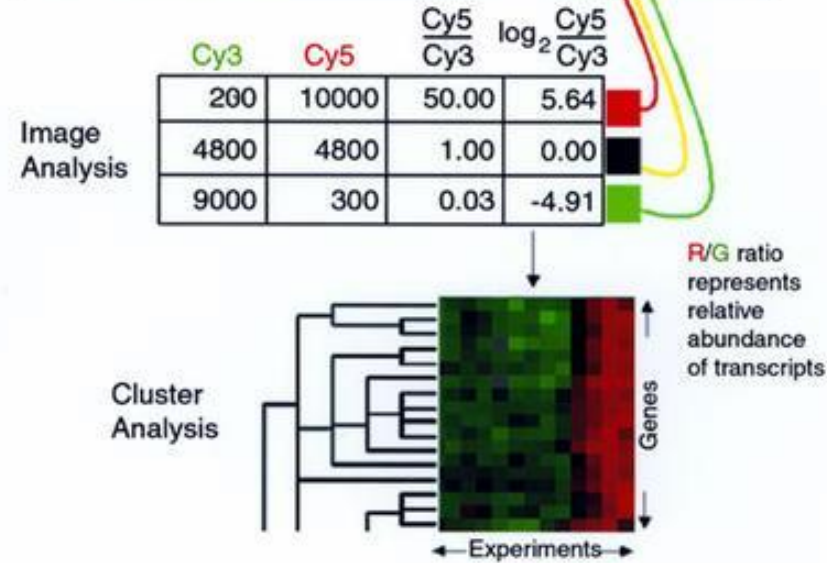
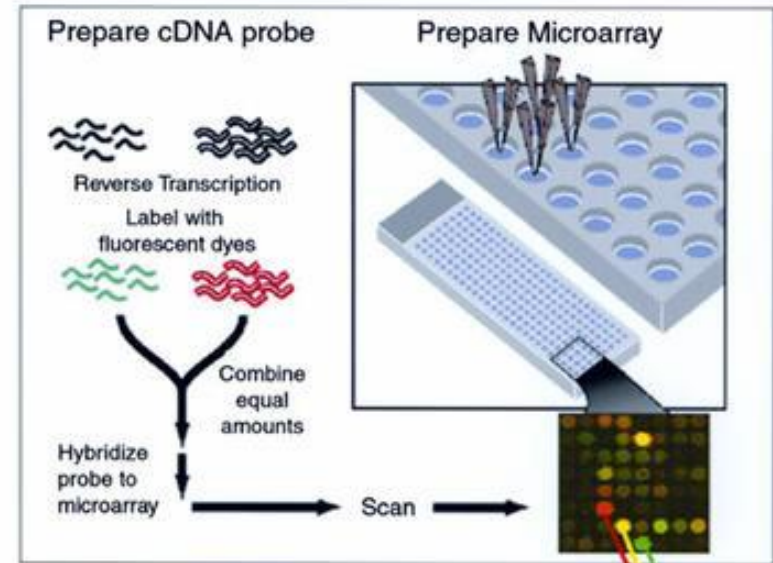
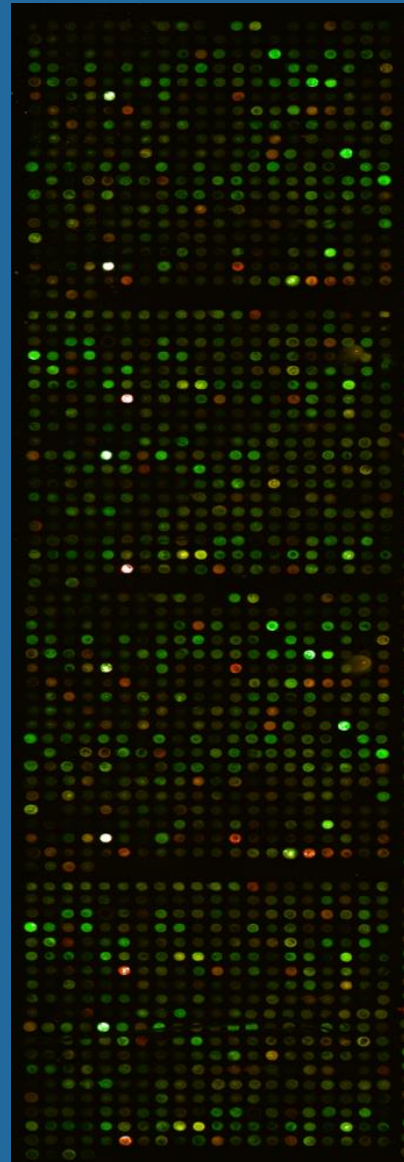
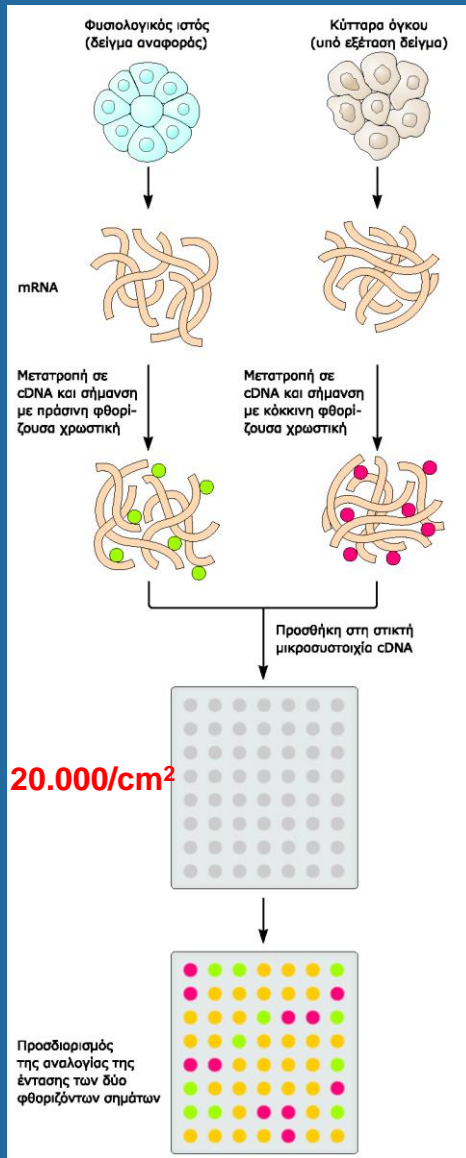
Μέτρηση των επιπέδων mRNA με στύπωμα κουκκίδας και υβριδοποίηση.

ΕΙΚΟΝΑ 13.1

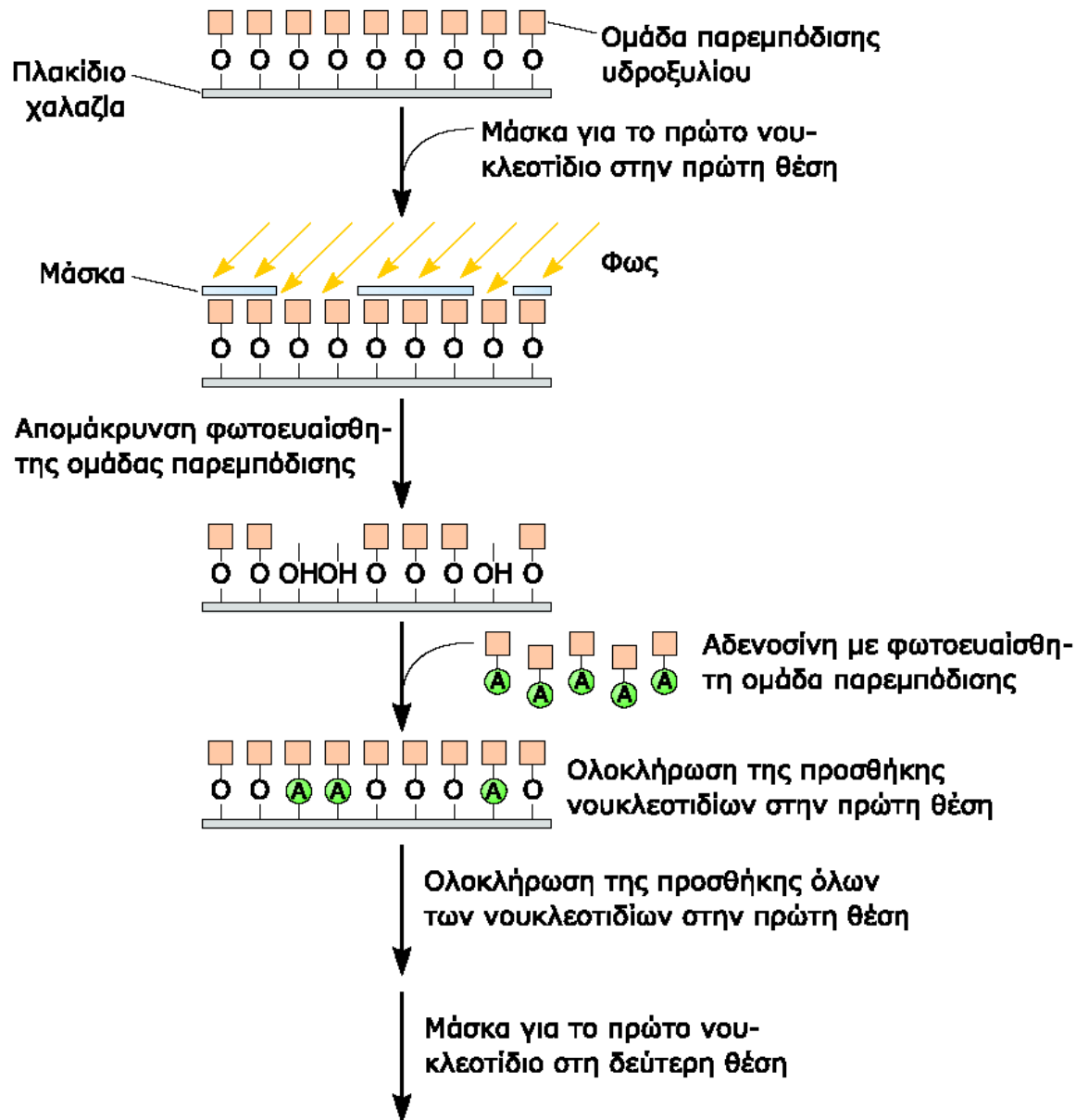


Μικροσυστοιχίες DNA

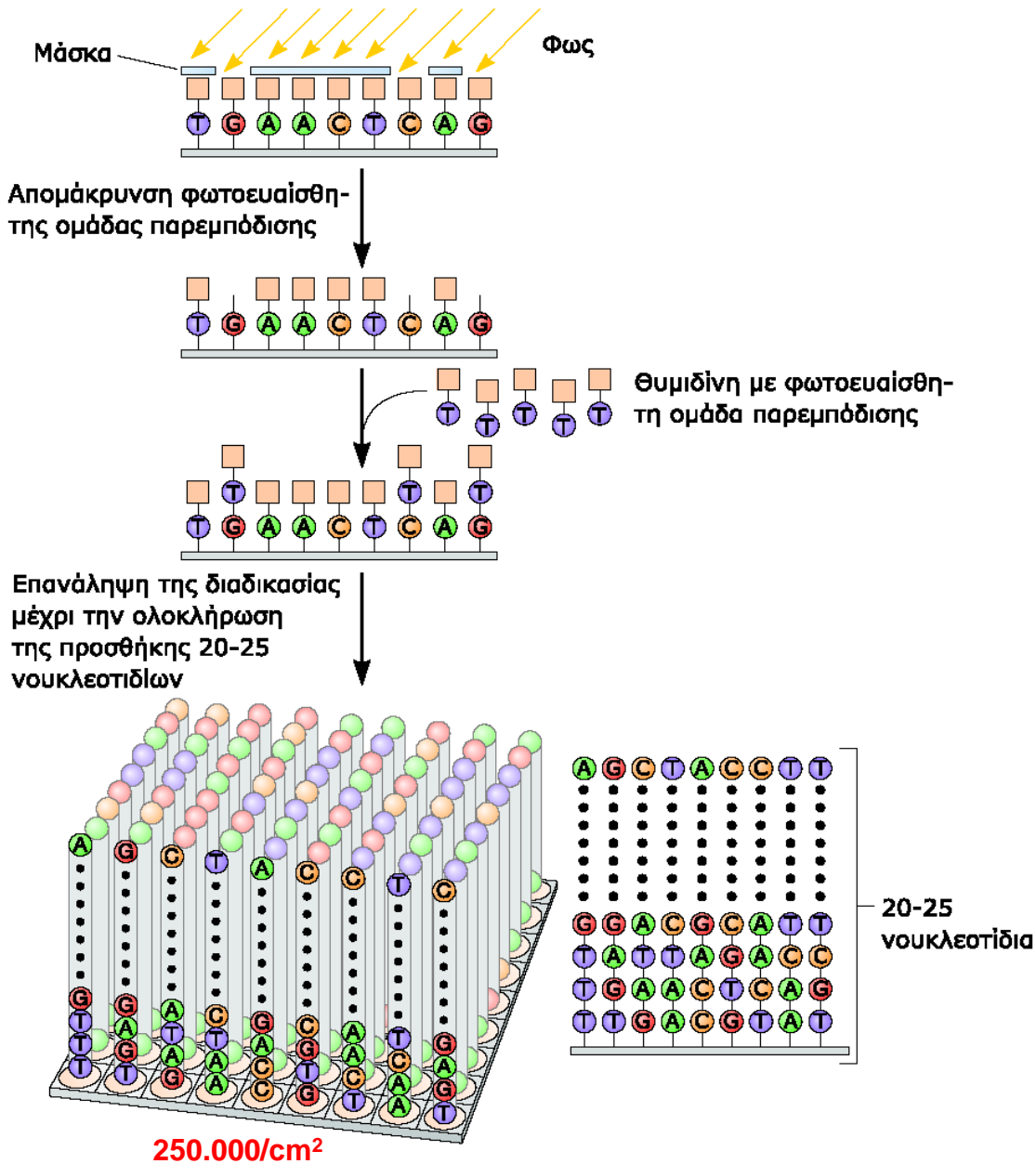
EIKONA 13.5



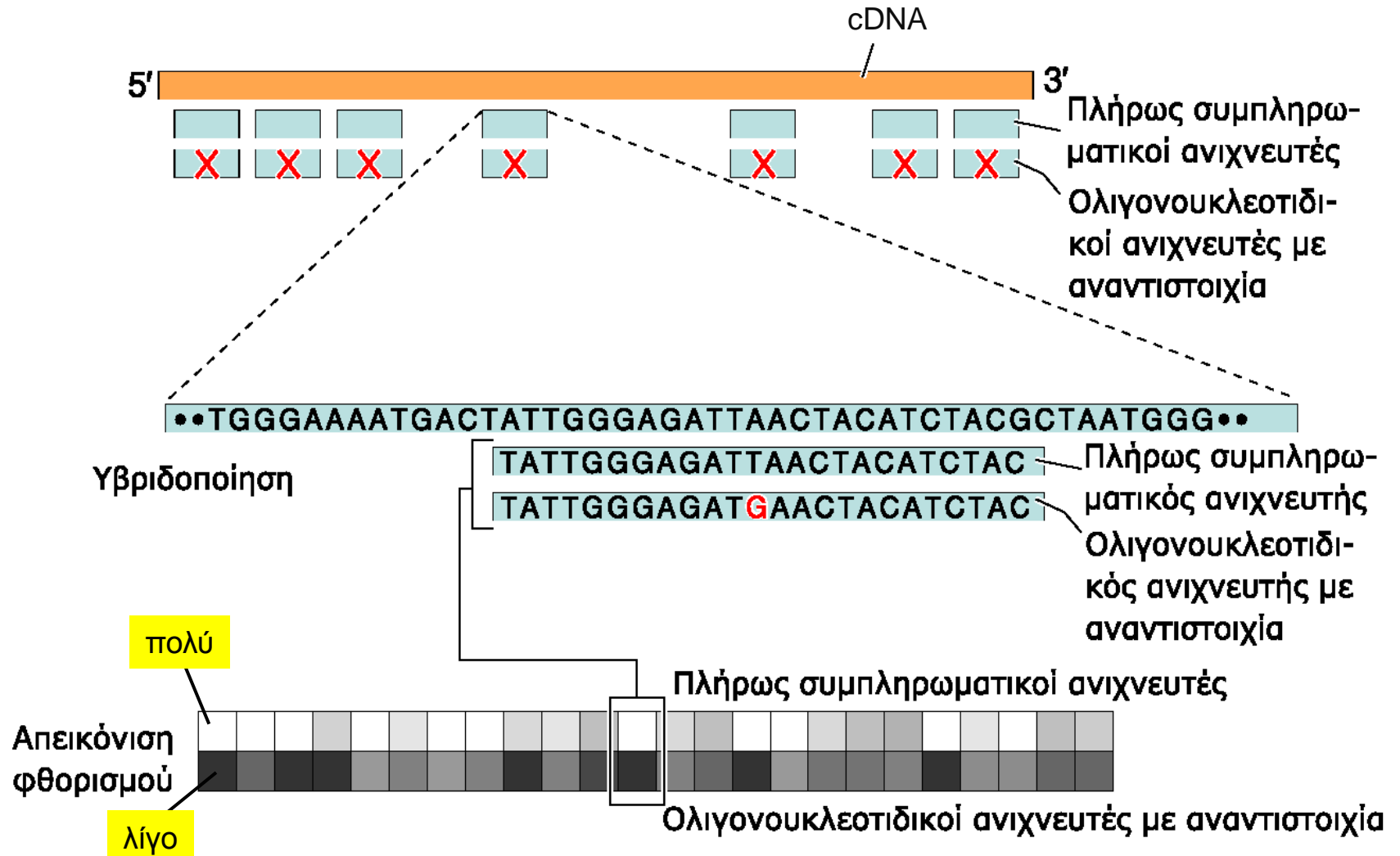
Ανάλυση μεταγραφμάτων Μικροσυστοιχίες ολιγονουκλεοτιδίων



Μικροσυστοιχίες ολιγονουκλεοτιδίων



Μικροσυστοιχίες ολιγονουκλεοτιδίων



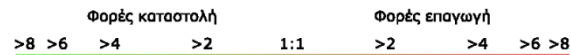
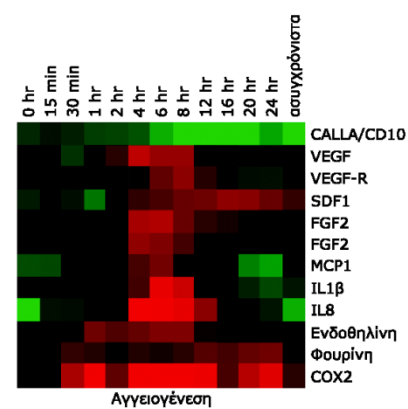
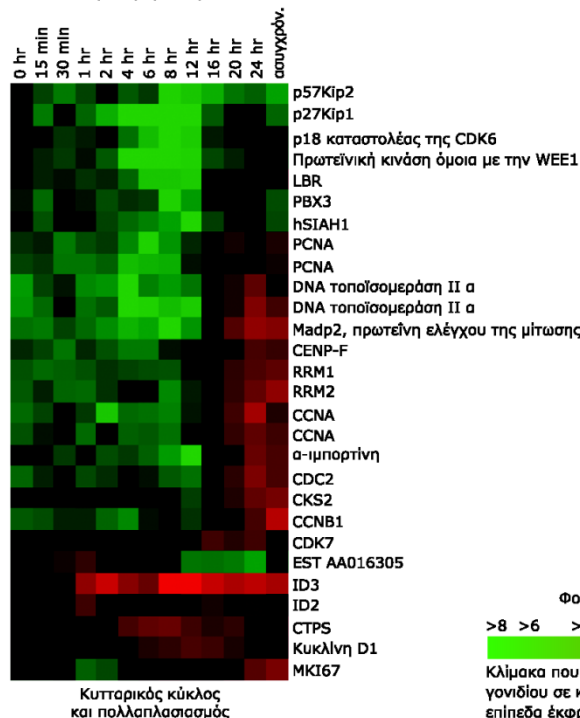
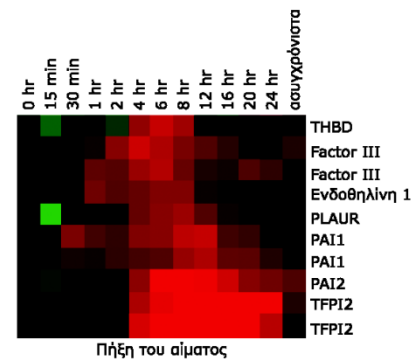
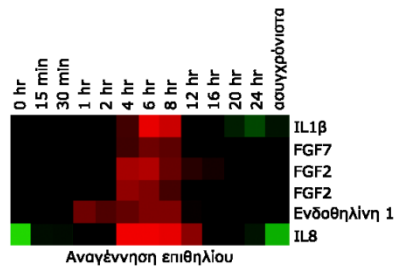
Ανάλυση μικροσυστοιχιών DNA



Μη πολλαπλασιαζόμενοι ανθρώπινοι ινοβλάστες
(διατηρούνται σε καλλιέργεια απουσία ορού)

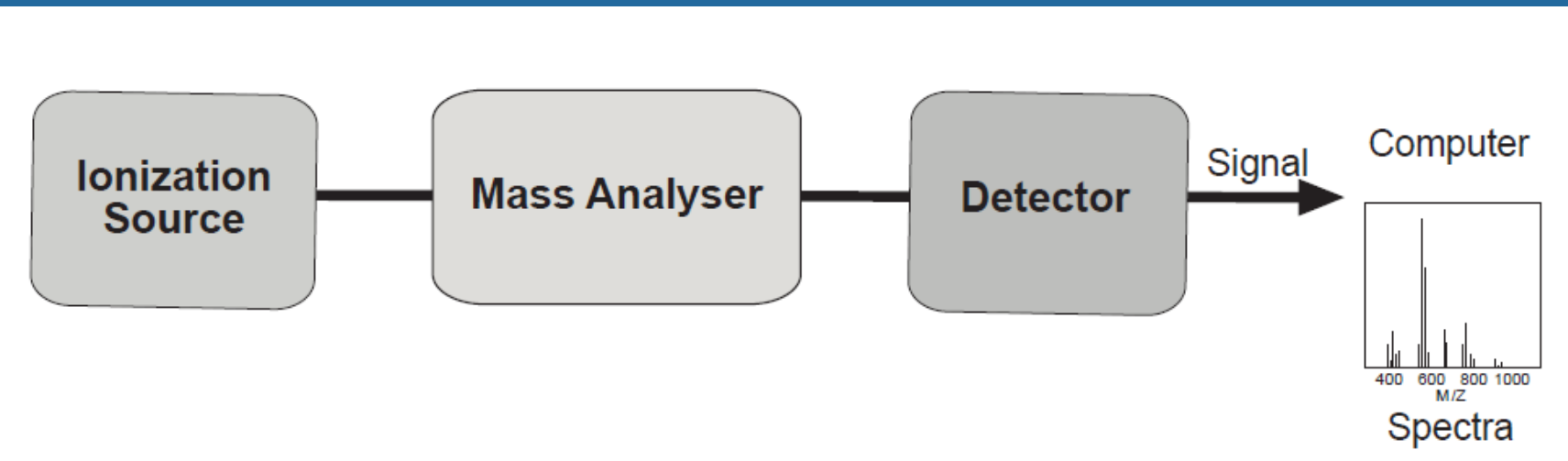
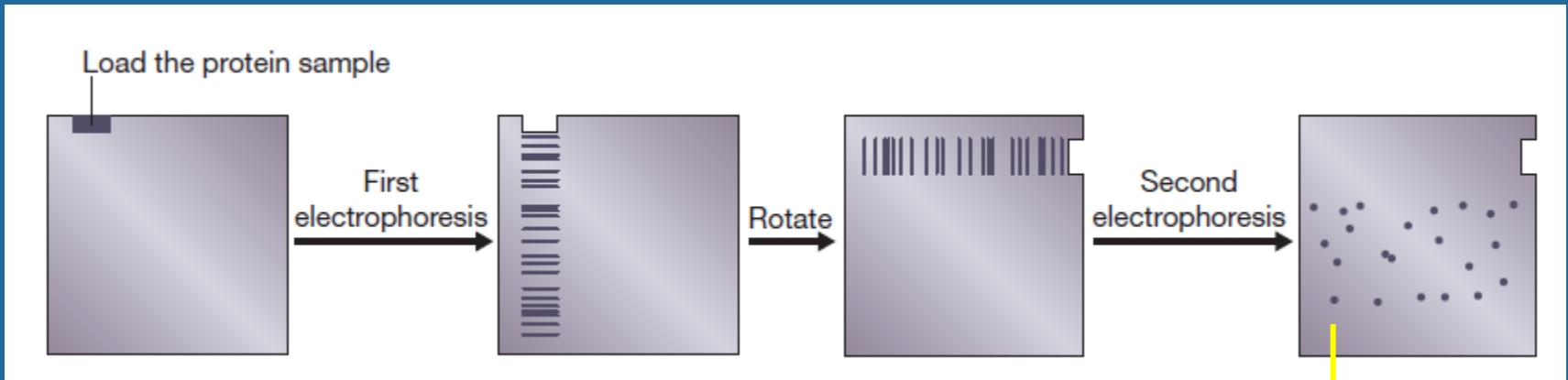
Προσθήκη ορού και επώαση,
λήψη δειγμάτων σε δώδεκα χρονικές στιγμές

Απομόνωση mRNA και προσδιορισμός του προτύπου της
γονιδιακής έκφρασης με τη χρήση μιας στικτής μικροσυστοιχίας

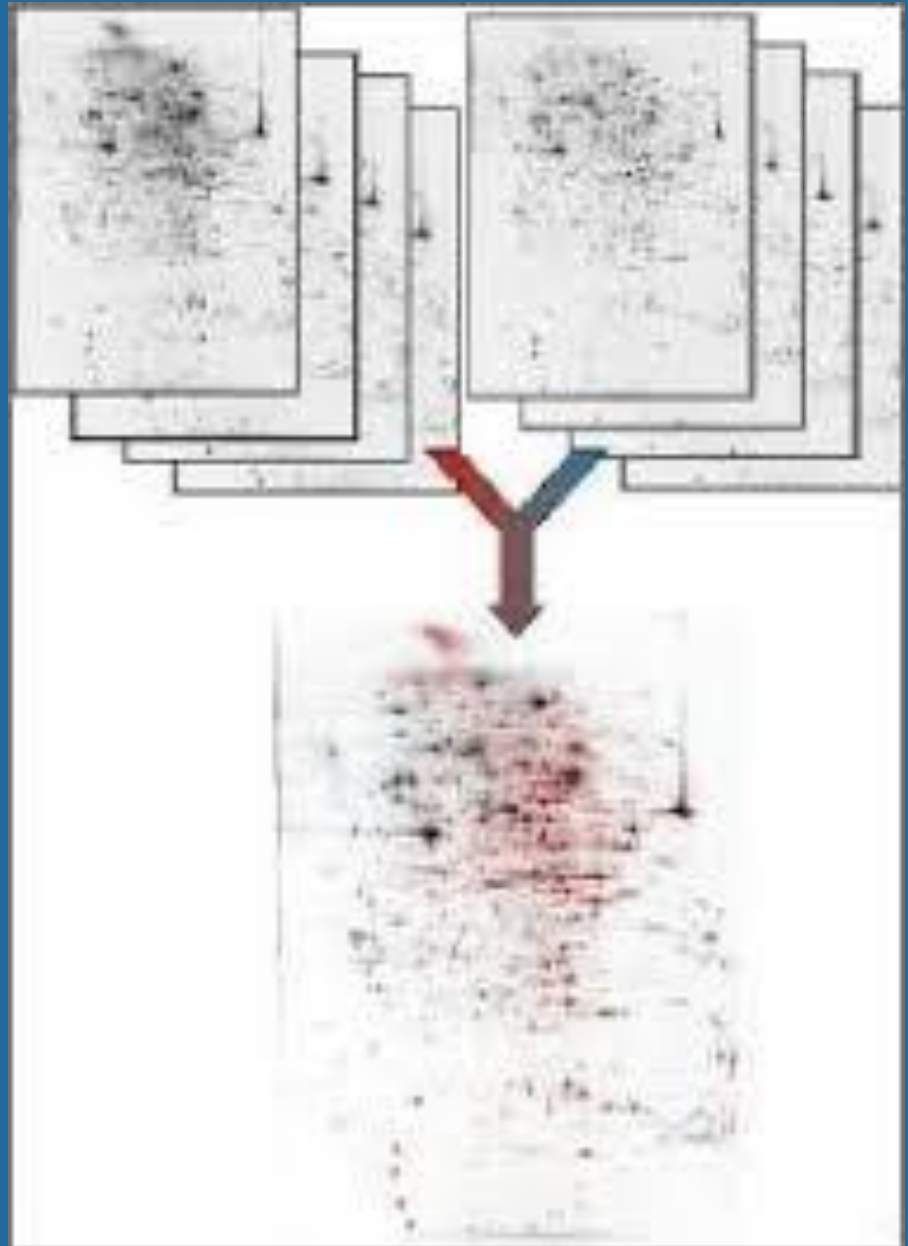
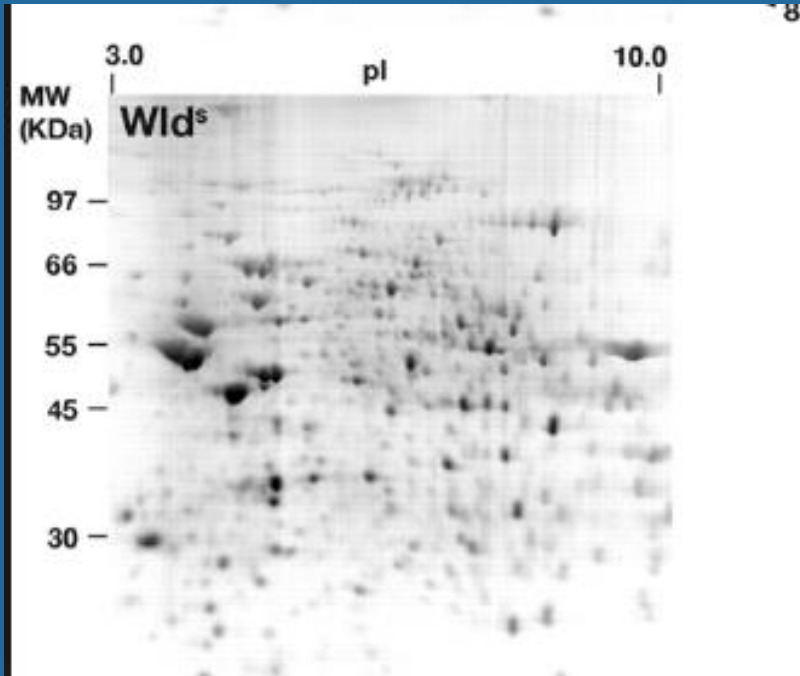


Κλίμακα που απεικονίζει το βαθμό επαγωγής ή καταστολής κάθε
γονιδίου σε κύτταρα που έχουν διεγερθεί με ορό σε σχέση με τα
επίπεδα έκφρασης πριν από τη διέγερση

Πρωτεομική



Πρωτεομική



CONCEPT: RADICAL INITIATORS

□ Chemical bonds can be cleaved in two ways: Heterolytically (ionic cleavage) and homolytically (radical cleavage).

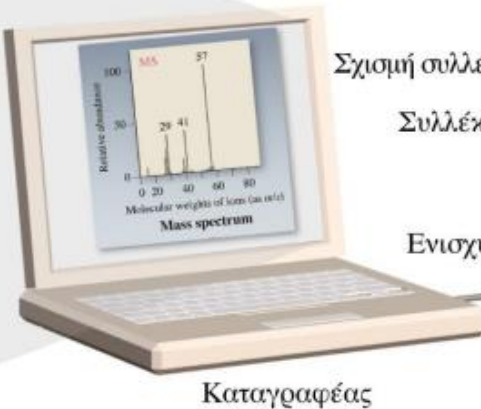
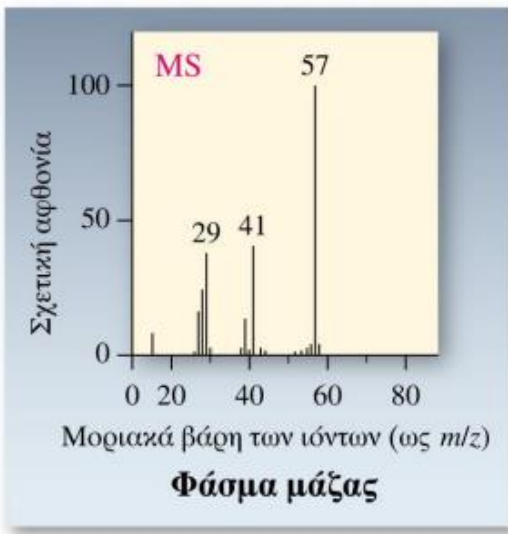
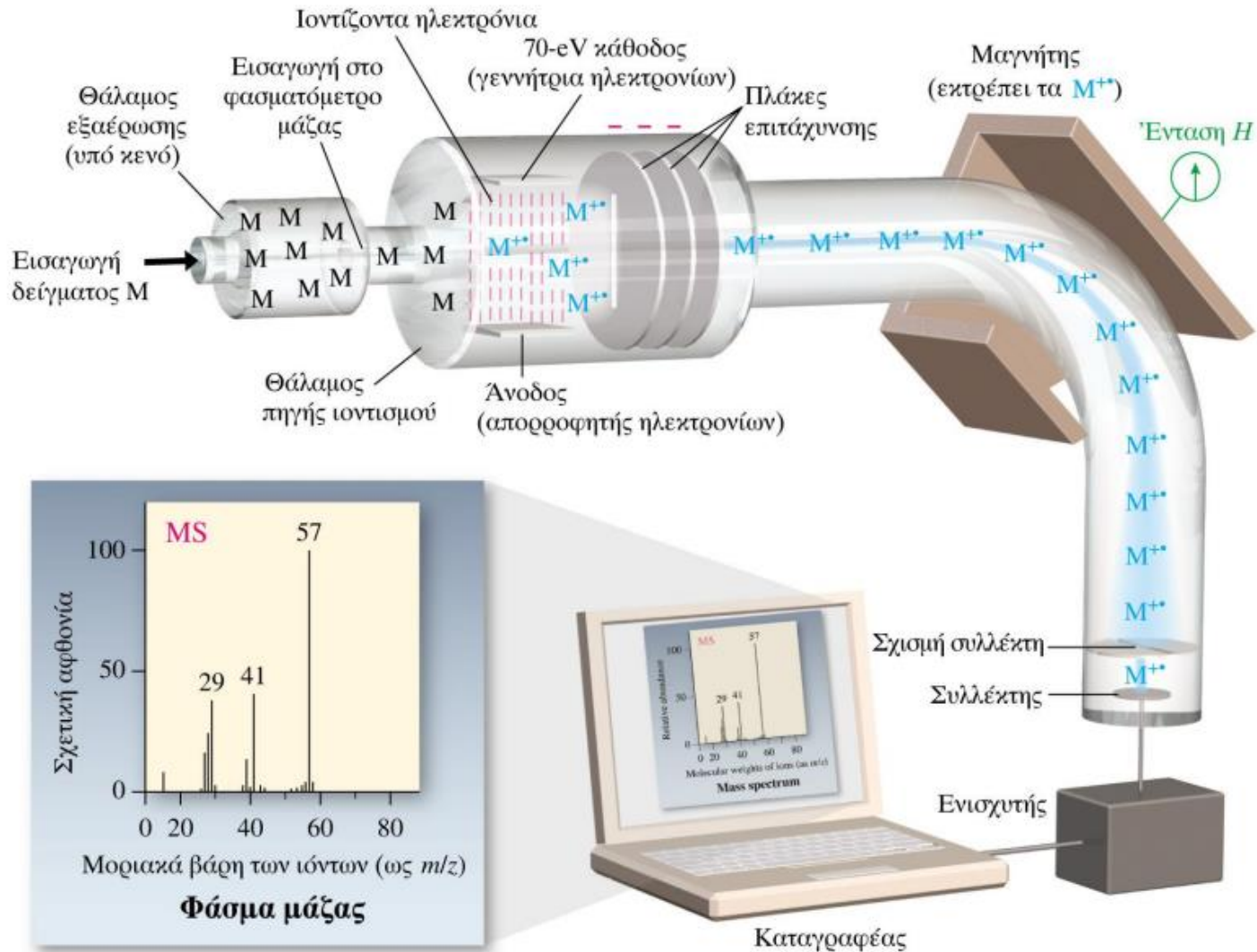


Heterolytic Cleavage



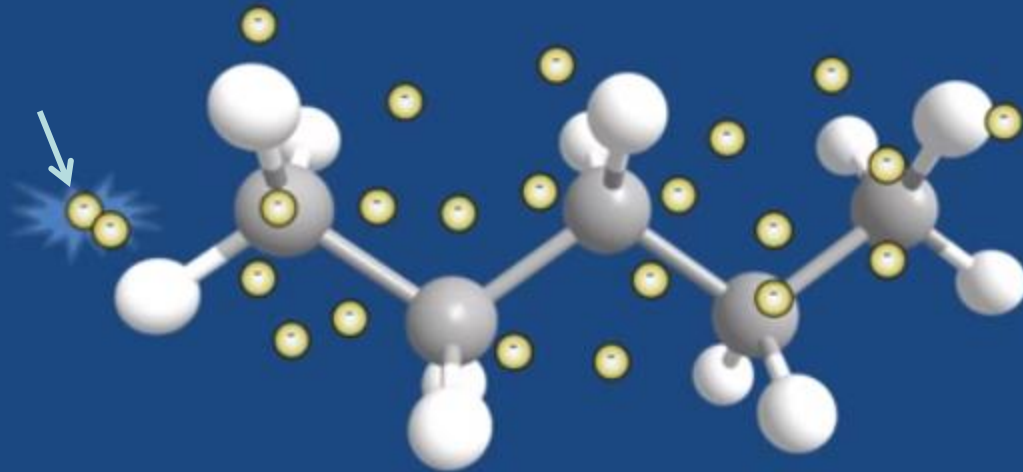
Homolytic Cleavage

Το φασματόμετρο μάζας διακρίνει τα ιόντα με βάση τη μάζα του



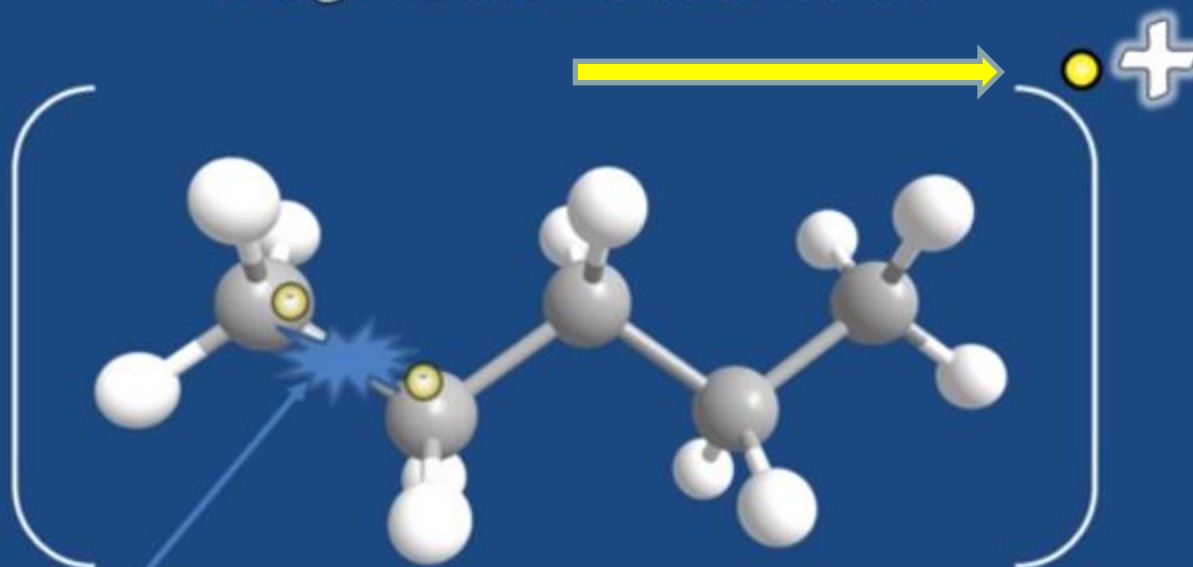
Ionization and Fragmentation in M.S.

Ionization of Pentane (Electron Impact)



Ionization and Fragmentation in M.S.

Fragmentation of Pentane

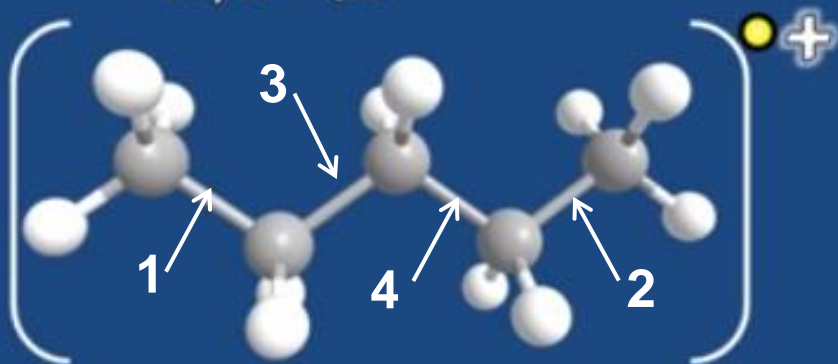


Homolytic Bond
Cleavage

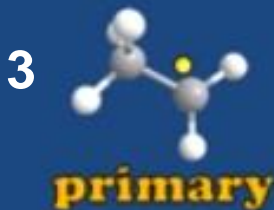
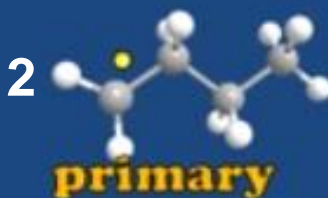
Ionization and Fragmentation in M.S.

Mass Spectrum of Pentane

$m/z = 72$



Radical
Fragment



Cation
Fragment



Cation
 m/z

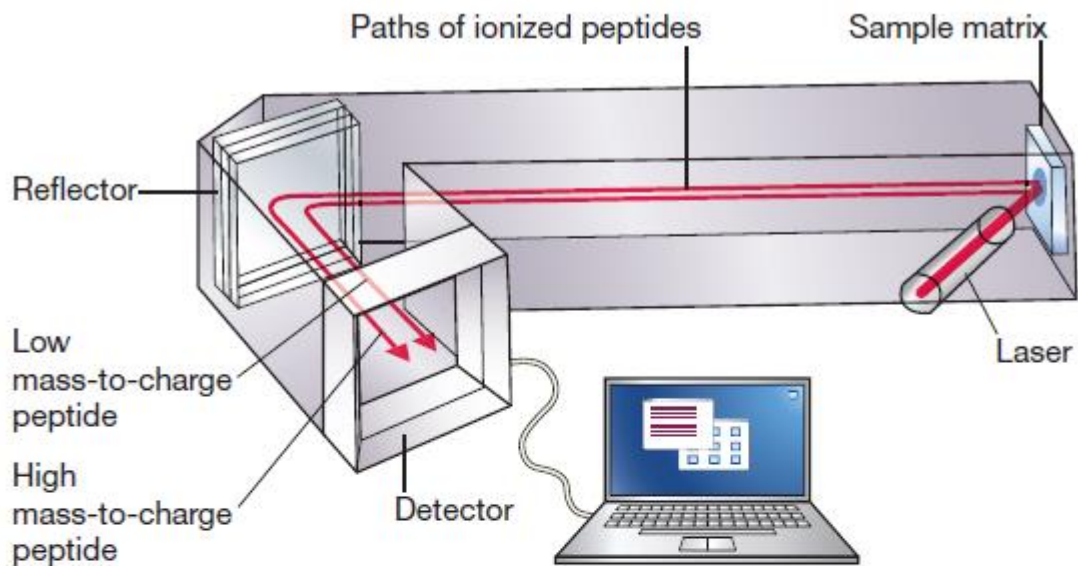
$m/z = 57$

$m/z = 15$

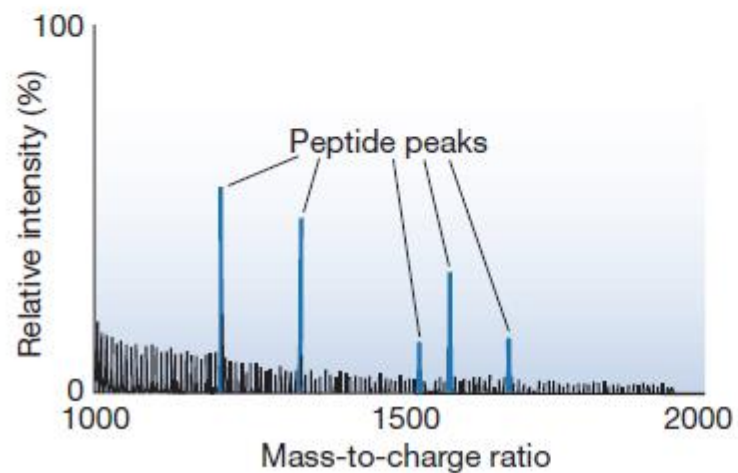
$m/z = 43$

$m/z = 29$

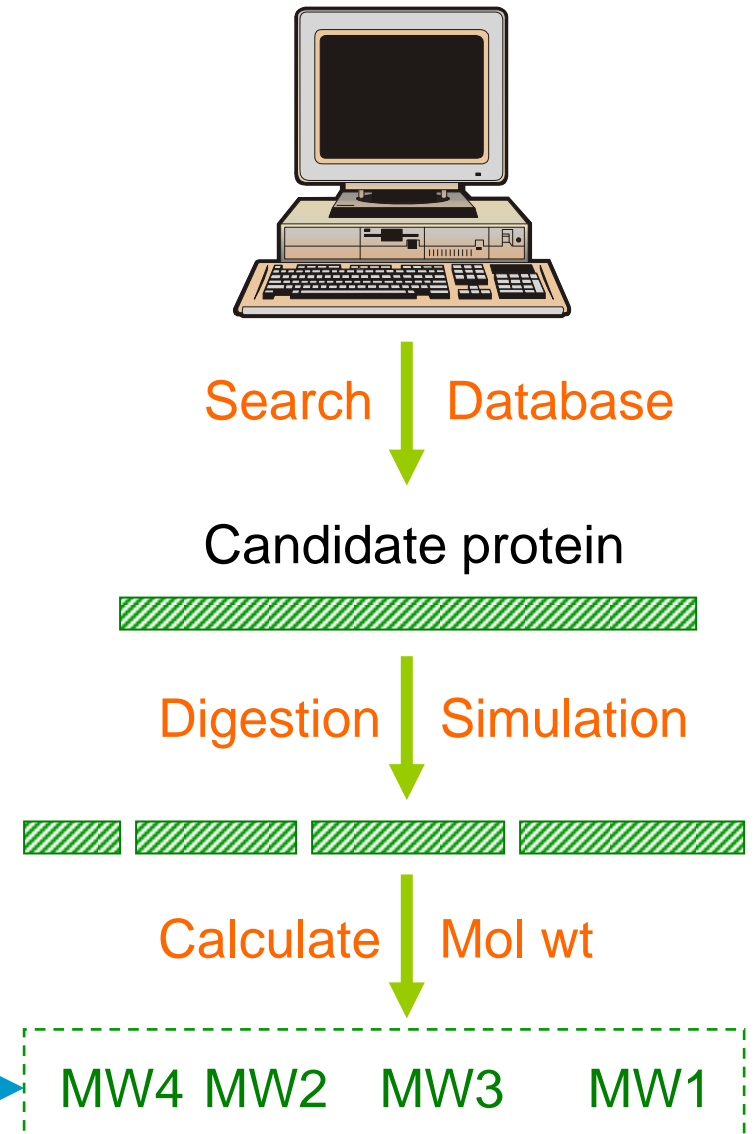
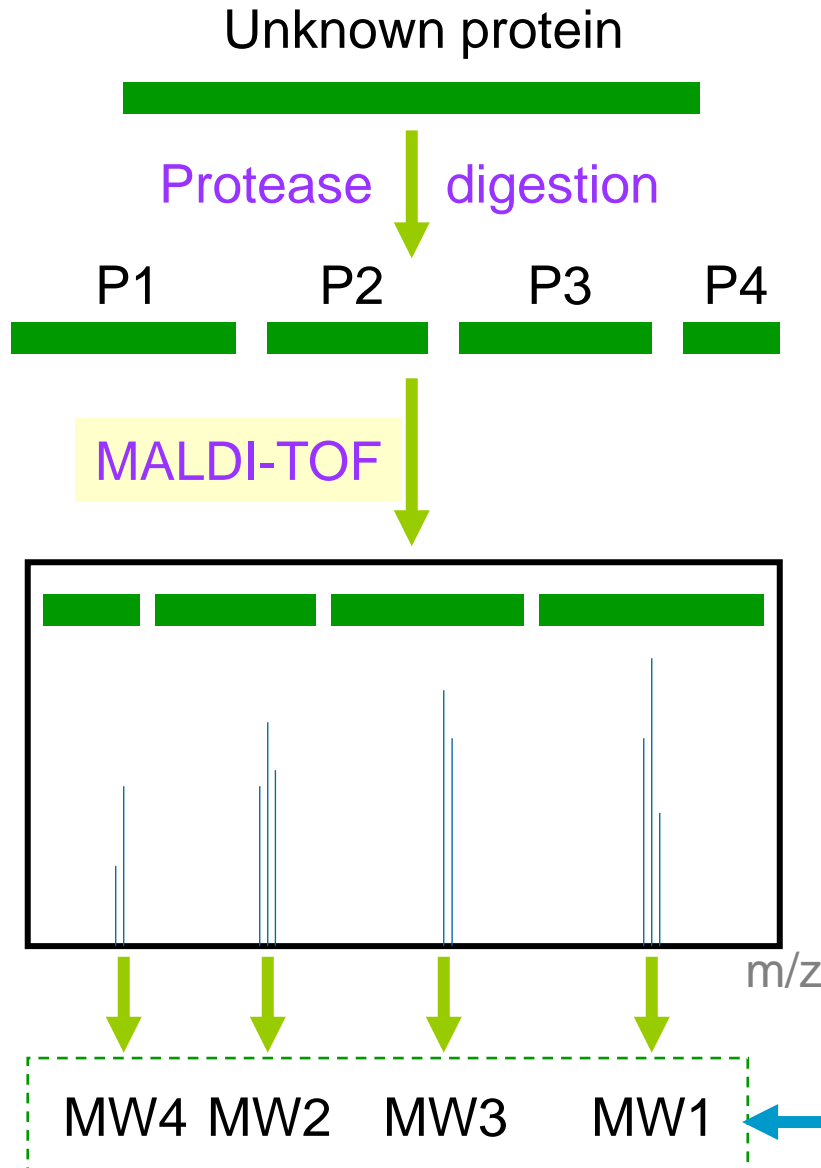
(a) MALDI-TOF mass spectrometry



(b) MALDI-TOF spectrum



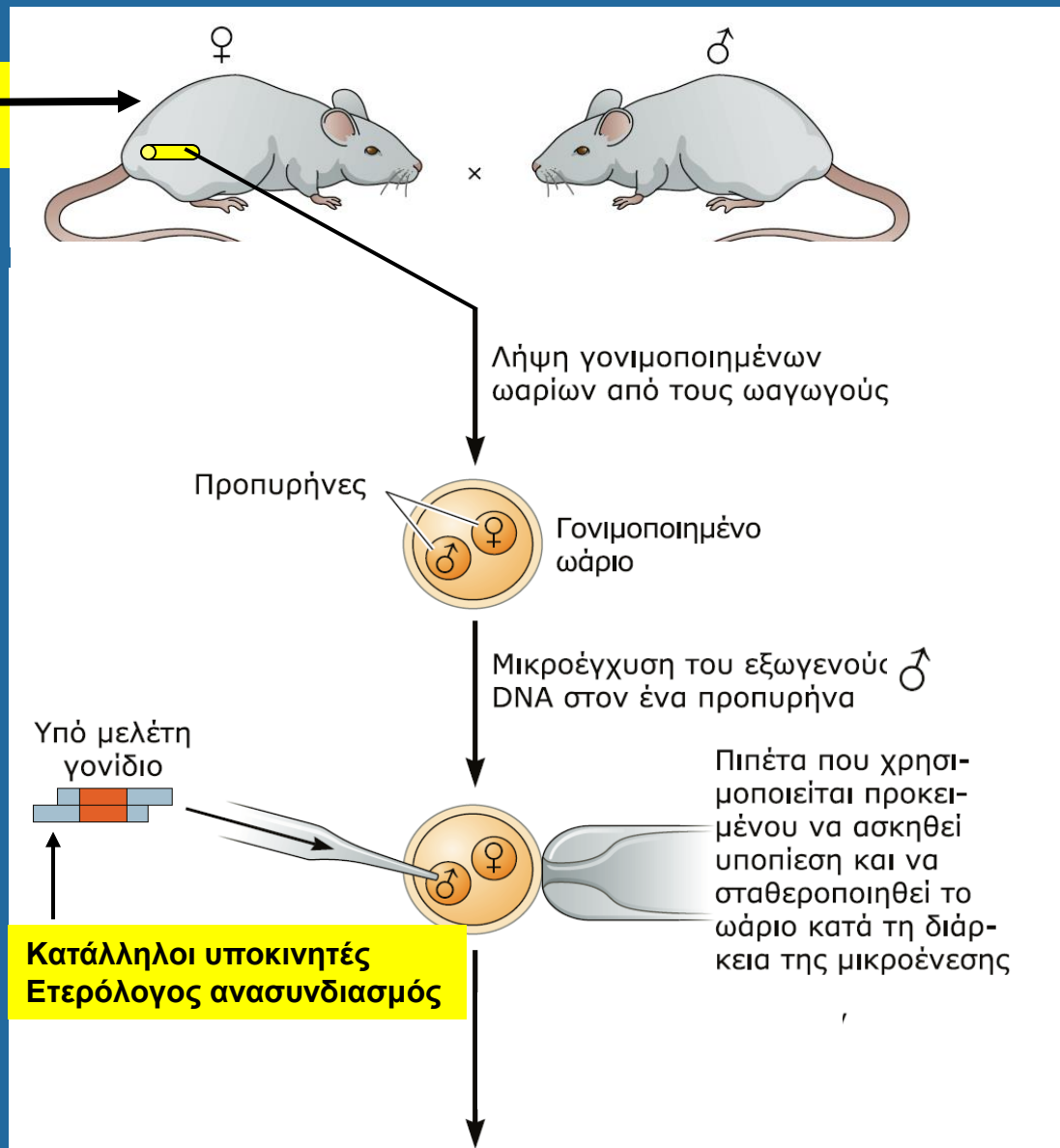
Πρωτεομική



Μετασχηματισμός σε θηλαστικά (ποντικούς)

ΕΙΚΟΝΑ 6.14

Ορμονική θεραπεία-Υπερωορρηξία



Υπό μελέτη γονίδιο

Κατάλληλοι υποκινητές
Ετερόλογος ανασυνδιασμός

Πιπέτα που χρησιμοποιείται προκειμένου να ασκηθεί υποπίεση και να σταθεροποιηθεί το ωάριο κατά τη διάρκεια της μικροένεσης

Εμφύτευση των εμβρύων
σε ορμονικά διεγερμένη
θετή μητέρα

(Ορμονική θεραπεία -
Διασταυρωμένη
με στείρα αρσενικά)



Απόγονοι

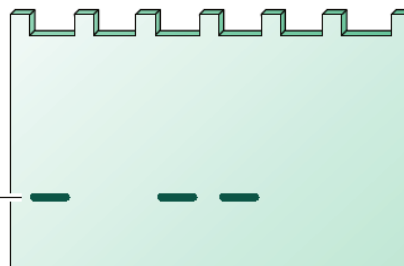
Ιδρυτής (+/-)



Ανάλυση του DNA
των απογόνων

Διασταύρωση ενός ιδρυτή
με κανονικά θηλυκά (-/-) και
επιλογή (+/-) απογόνων με PCR

Προϊόν PCR που
υποδεικνύει την
παρουσία του
διαγονιδίου

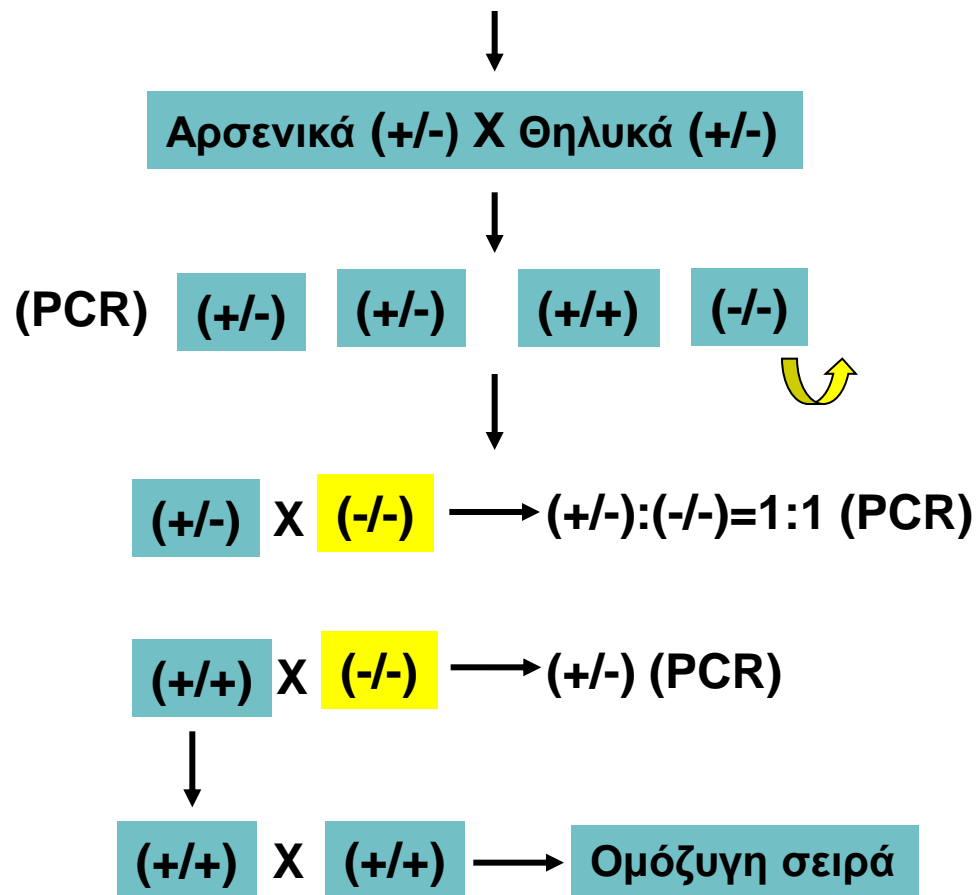


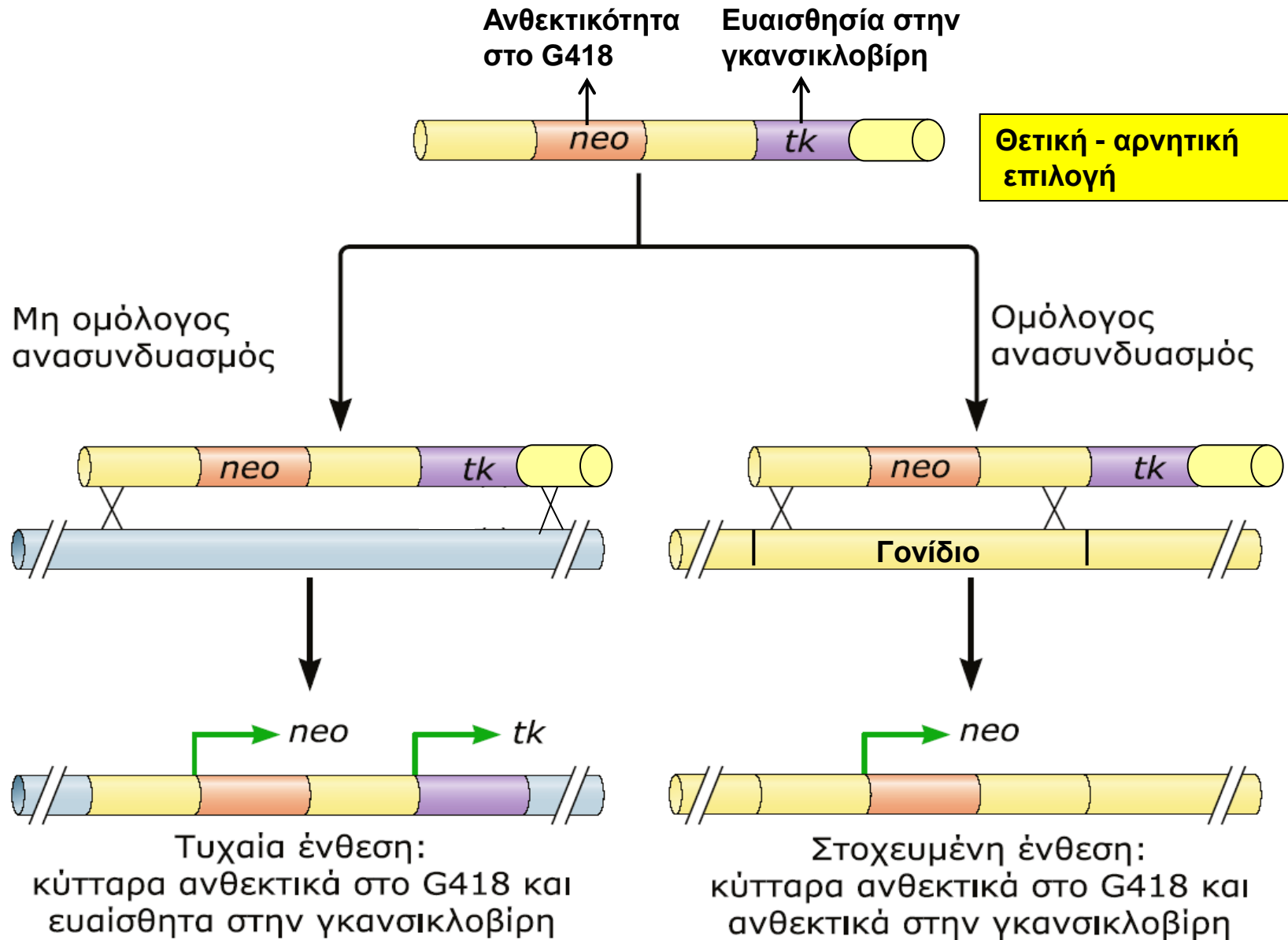
Ετερόζυγη σειρά -1 (+/-)



Μετασχηματισμός σε θηλαστικά (ποντικούς)

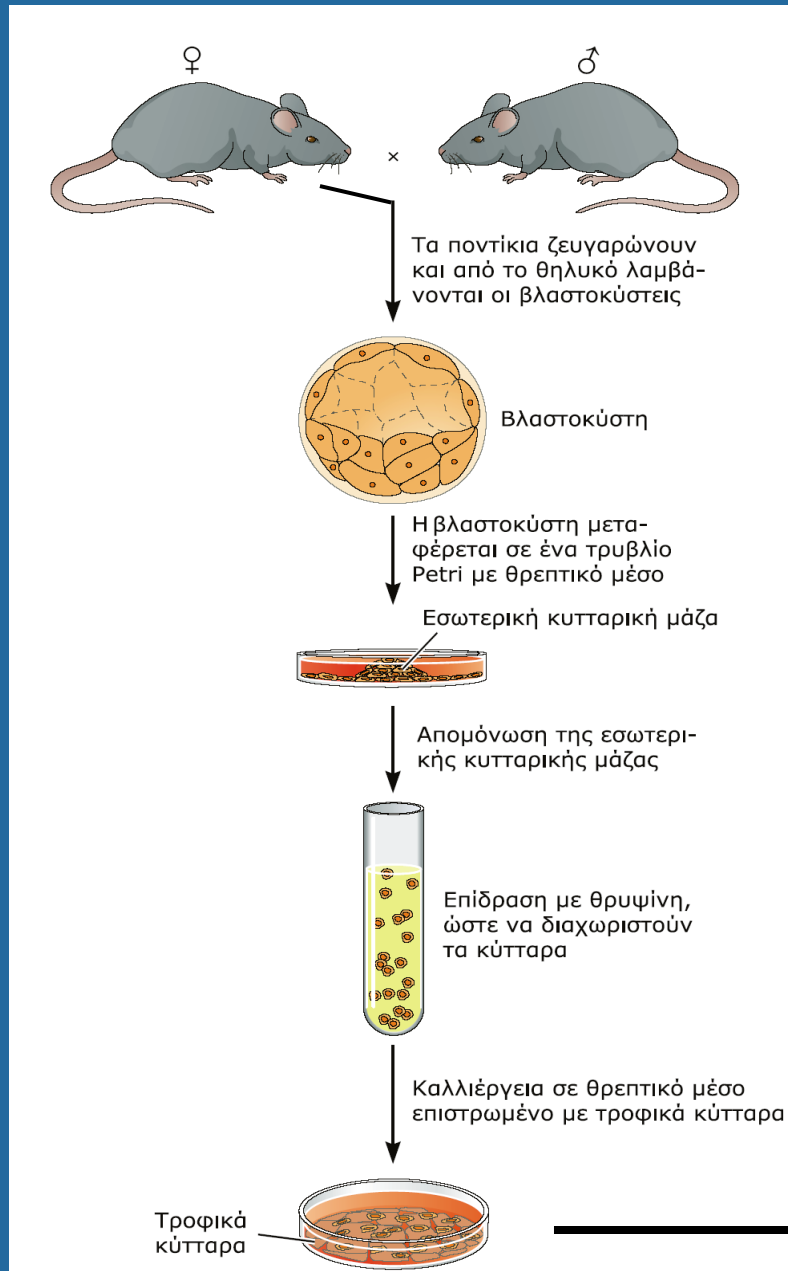
ΕΙΚΟΝΑ 6.14





Μετασχηματισμός σε θηλαστικά (ποντικούς) Μέθοδος βλαστοκυττάρων

ΕΙΚΟΝΑ 6.16

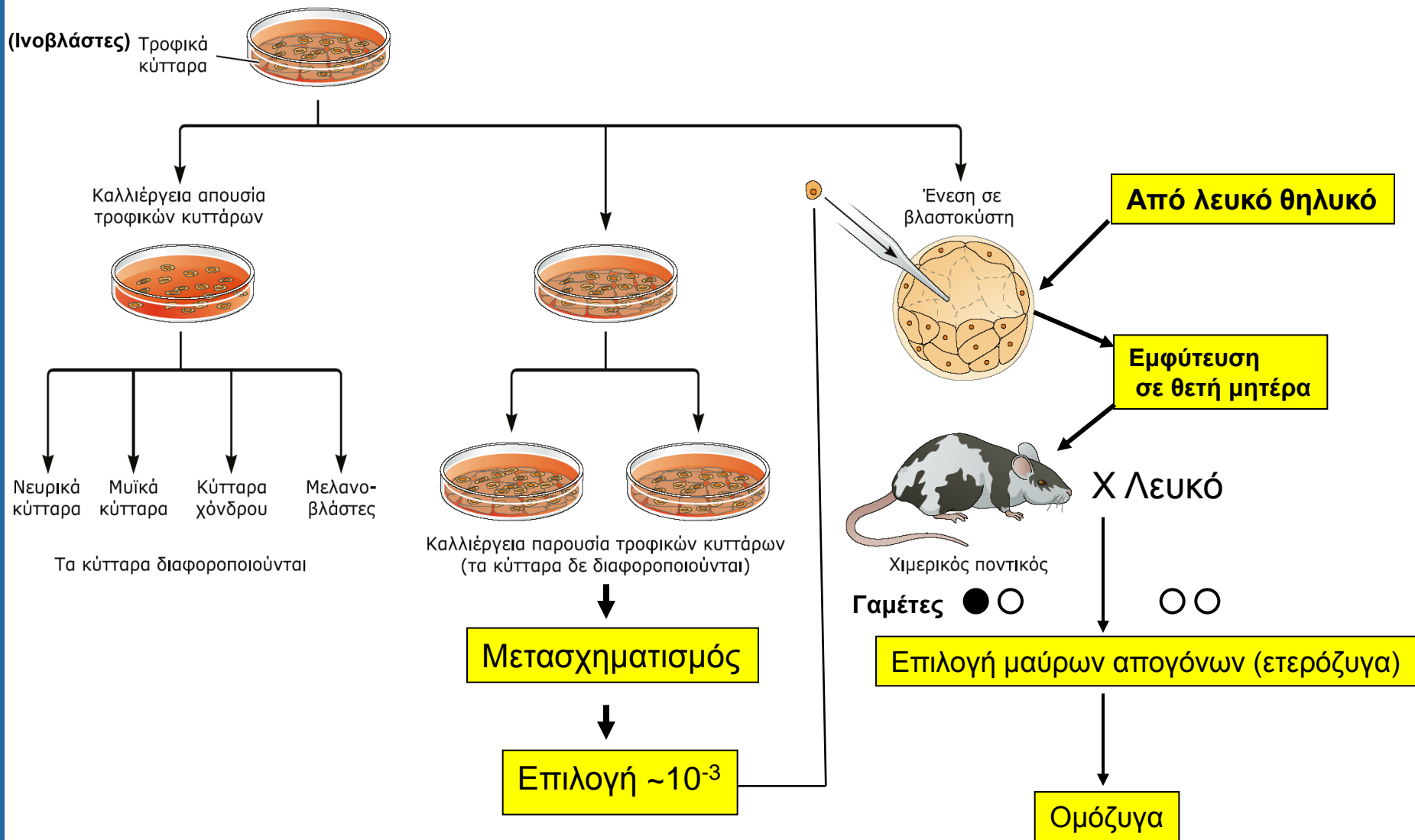


Μαύρα ποντίκια

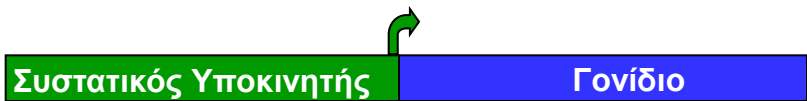
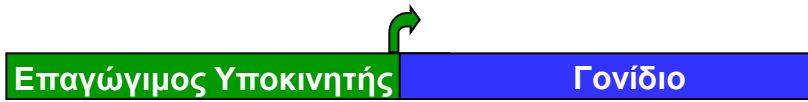
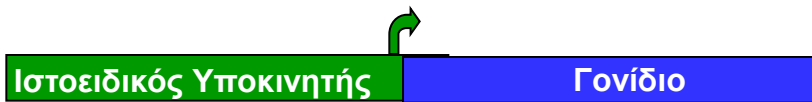
Μετασχηματισμός επιλογή

Μετασχηματισμός στα θηλαστικά (ποντικούς) Μέθοδος βλαστοκυττάρων

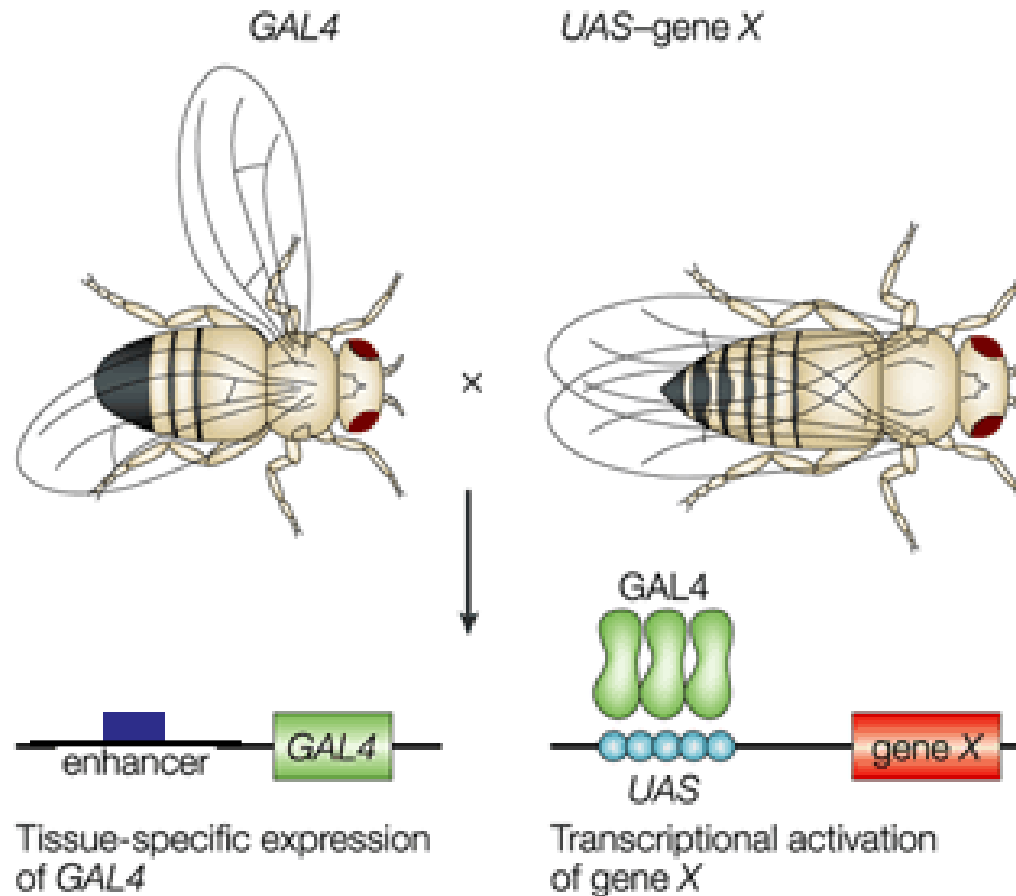
ΕΙΚΟΝΑ 6.16



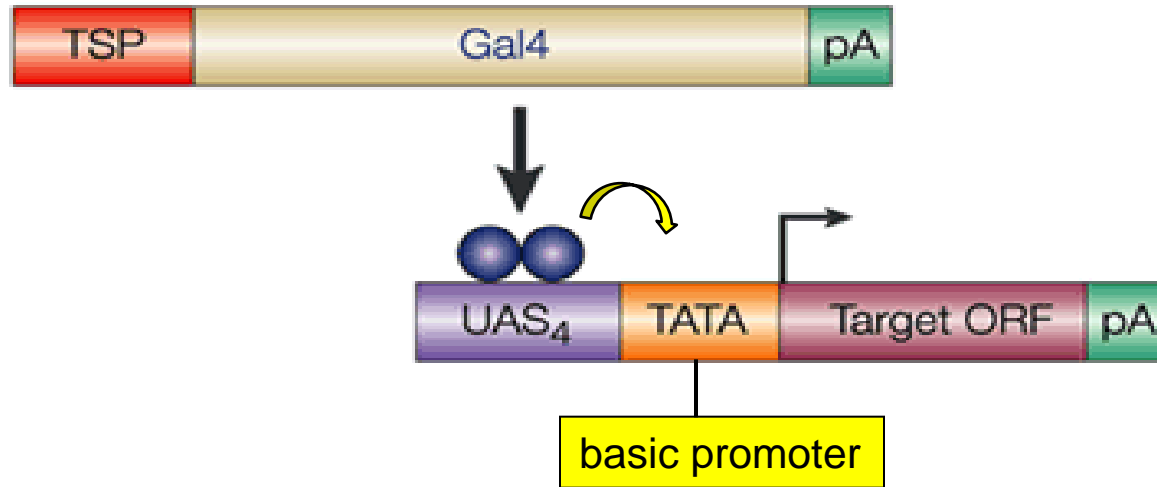
Συστήματα έκφρασης διαγονιδίων

- Συστατικά συστήματα  (Υπ. Ακτίνης)
- Επαγωγίμα συστήματα  (Υπ. Hsp70)
- Ιστο-ειδικά συστήματα  (GAL4)
- Επαγωγίμα συστήματα με ελεγχόμενη έκφραση (Συστήματα Tet-off/on)

Συστήματα ιστοειδικής έκφρασης (GAL4/UAS)

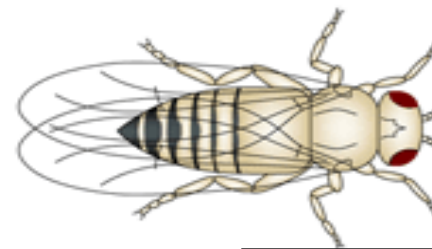


GAL4



Enhancer-trap GAL4

UAS-gene X



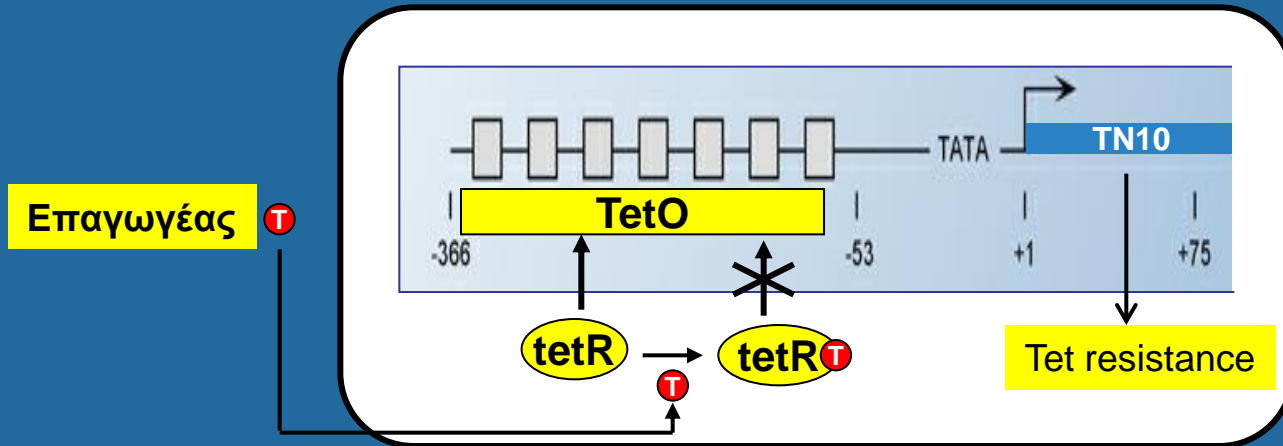
x

Εκατοντάδες

Genomic enhancer
basic promoter
GAL4
Tissue-specific expression of GAL4

GAL4
UAS
basic promoter
Reporter gene
Transcriptional activation of gene X

Συστήματα χρονοειδικής έκφρασης (Tet-off/on)



Tet-Off

