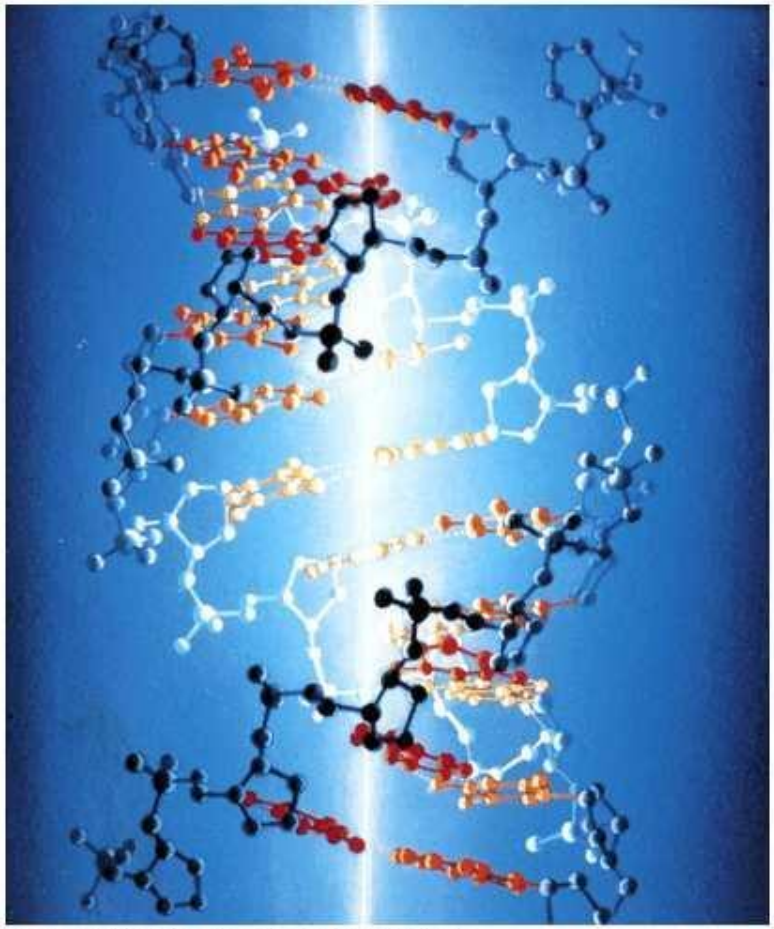
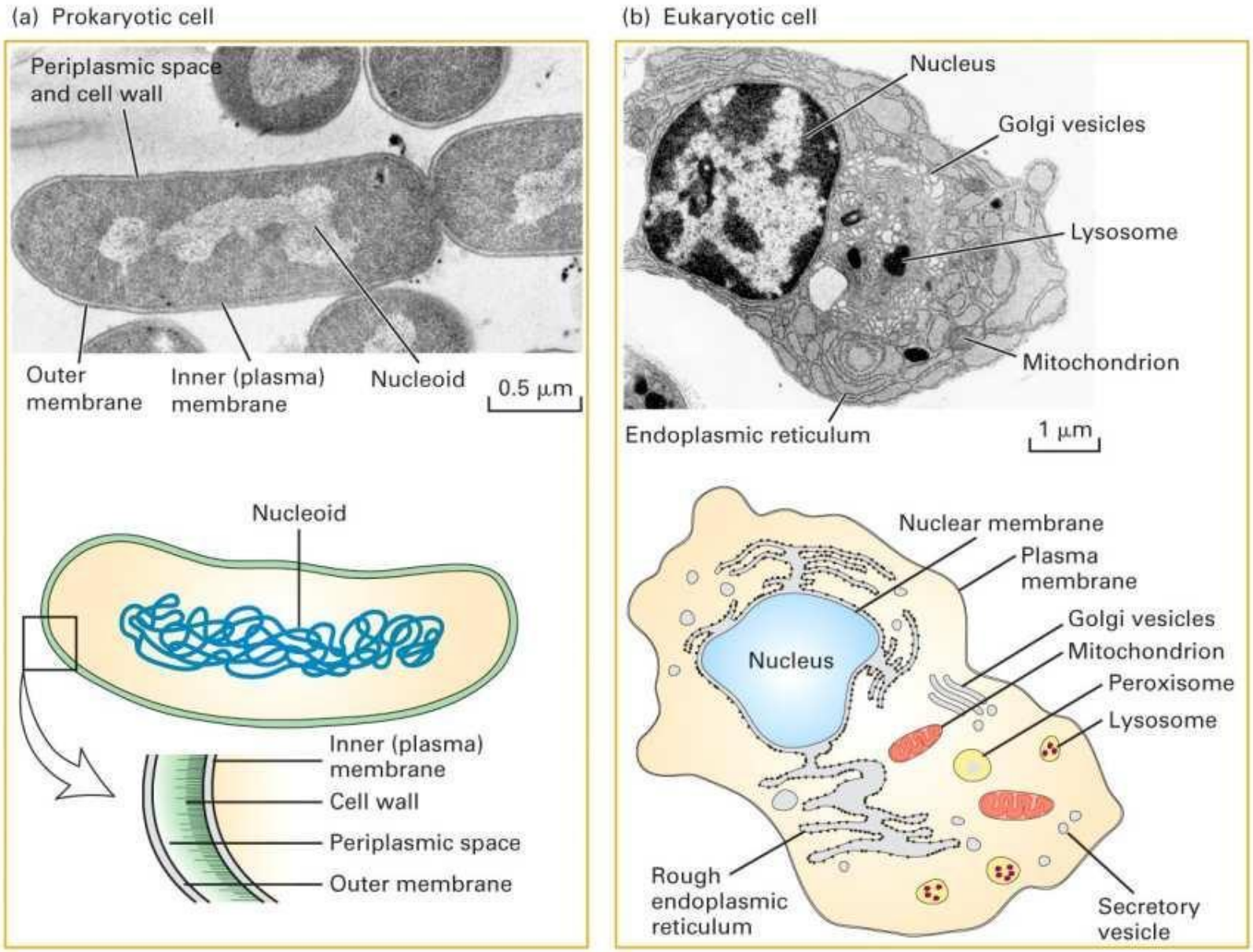
DNA,RNA, Recombinant DNA Technology

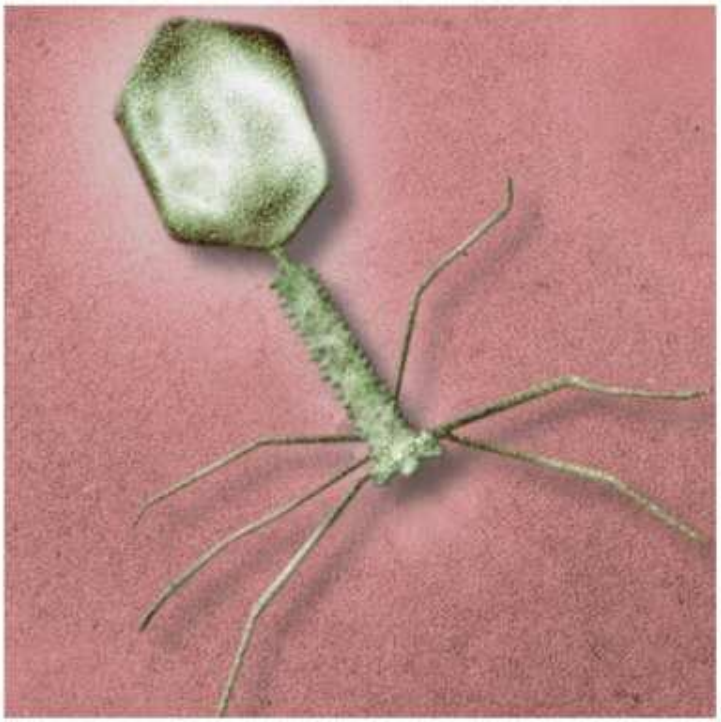


Chapter 3 Opener Fundamentals of Biochemistry, 2/e

“Metabolic pathways” expanded

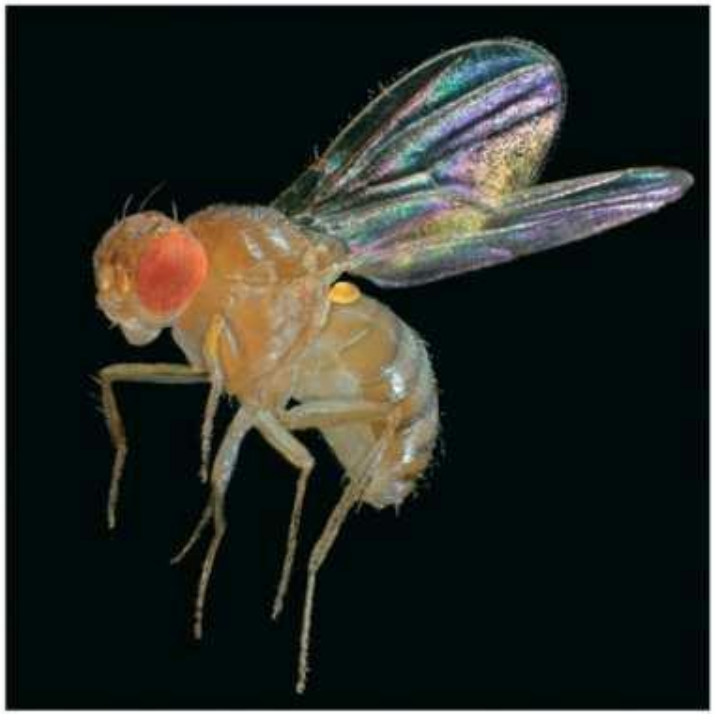


Model organisms: Cellular biology, biochemistry... molecular biology



Viruses

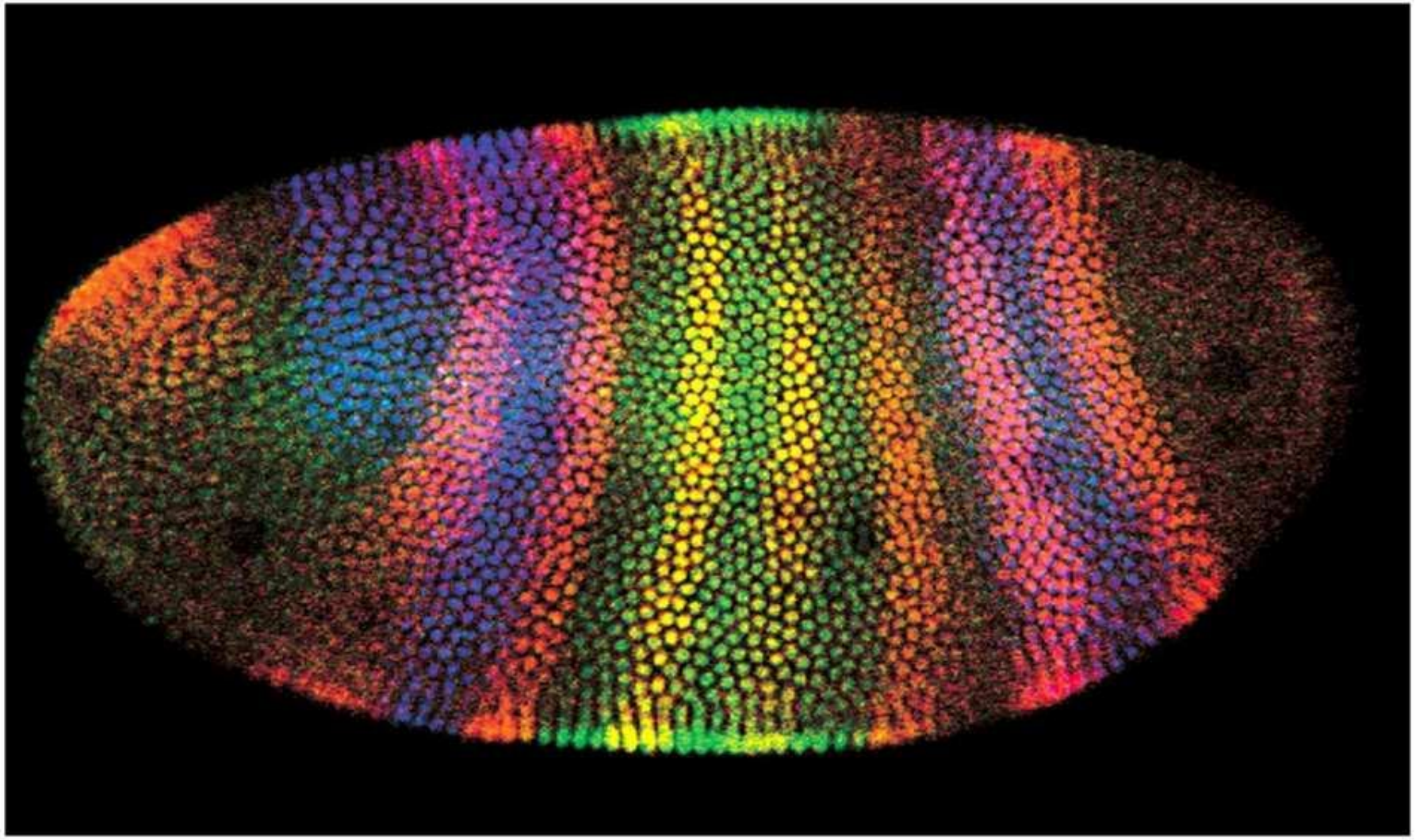
Proteins involved in DNA, RNA, protein synthesis
Gene regulation
Cancer and control of cell proliferation
Transport of proteins and organelles inside cells
Infection and immunity
Possible gene therapy approaches



Fruit fly (*Drosophila melanogaster*)

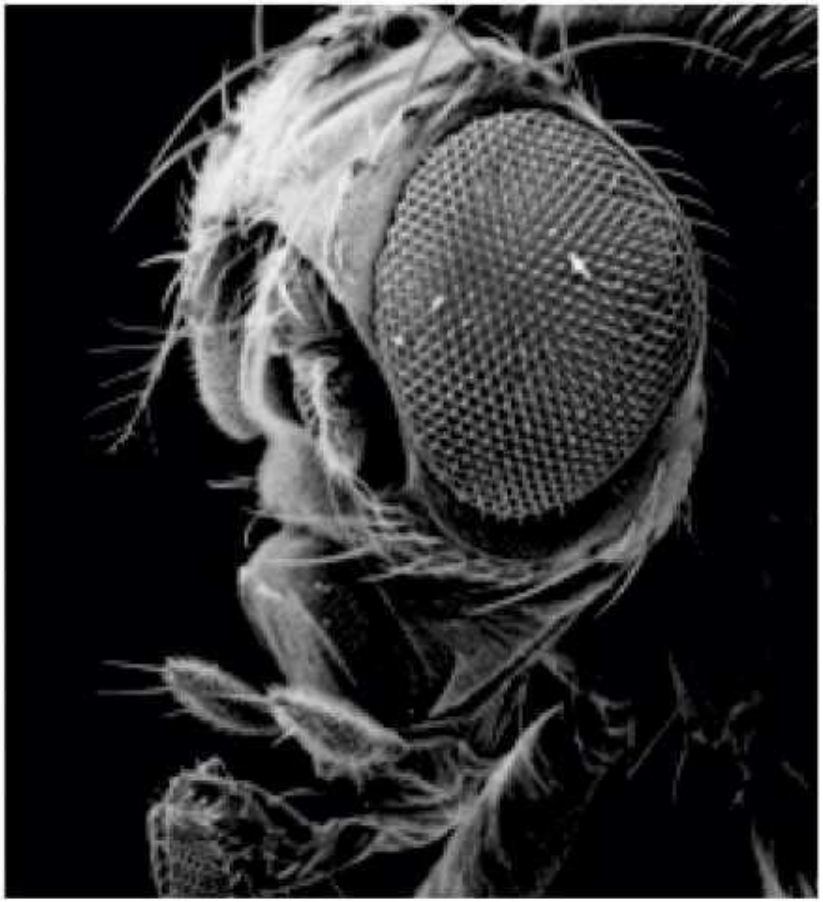
Development of the body plan
Generation of differentiated cell lineages
Formation of the nervous system, heart, and musculature
Programmed cell death
Genetic control of behavior
Cancer genes and control of cell proliferation
Control of cell polarization
Effects of drugs, alcohol, pesticides

Developmental biology.....



Fly mutation “eyeless”

(b)



(c)

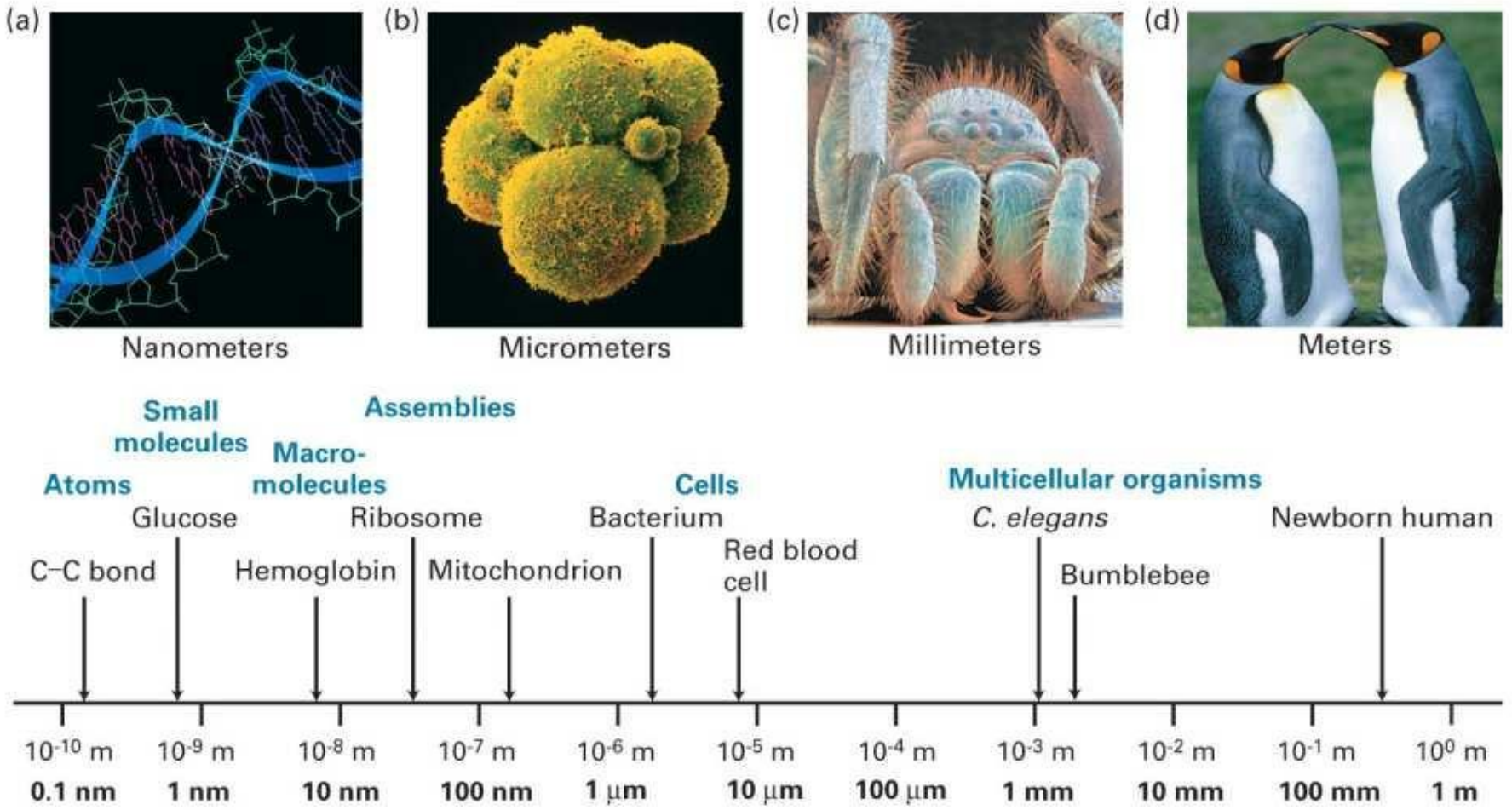
‘The fly and you are not much different.”

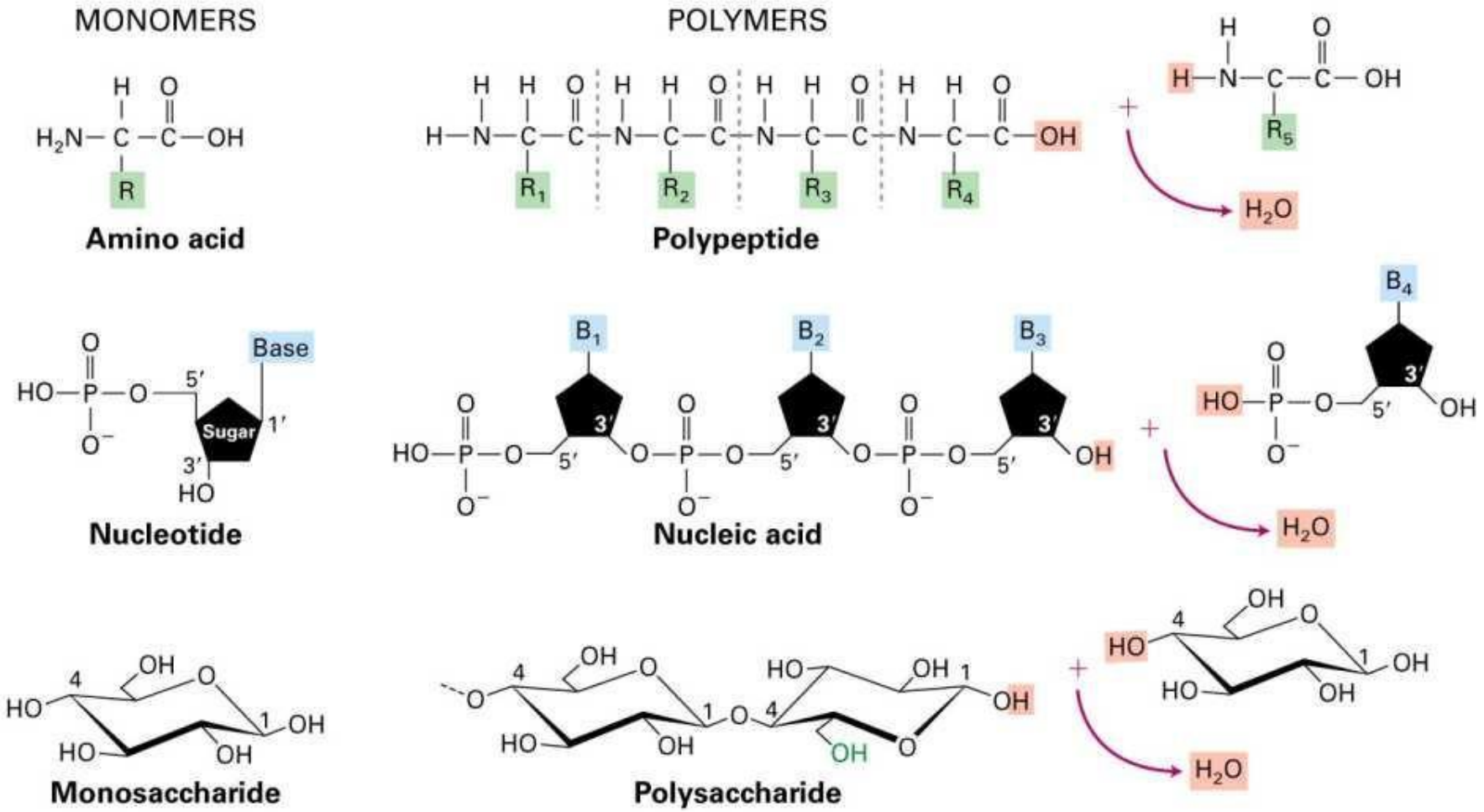
Mice, including cultured cells

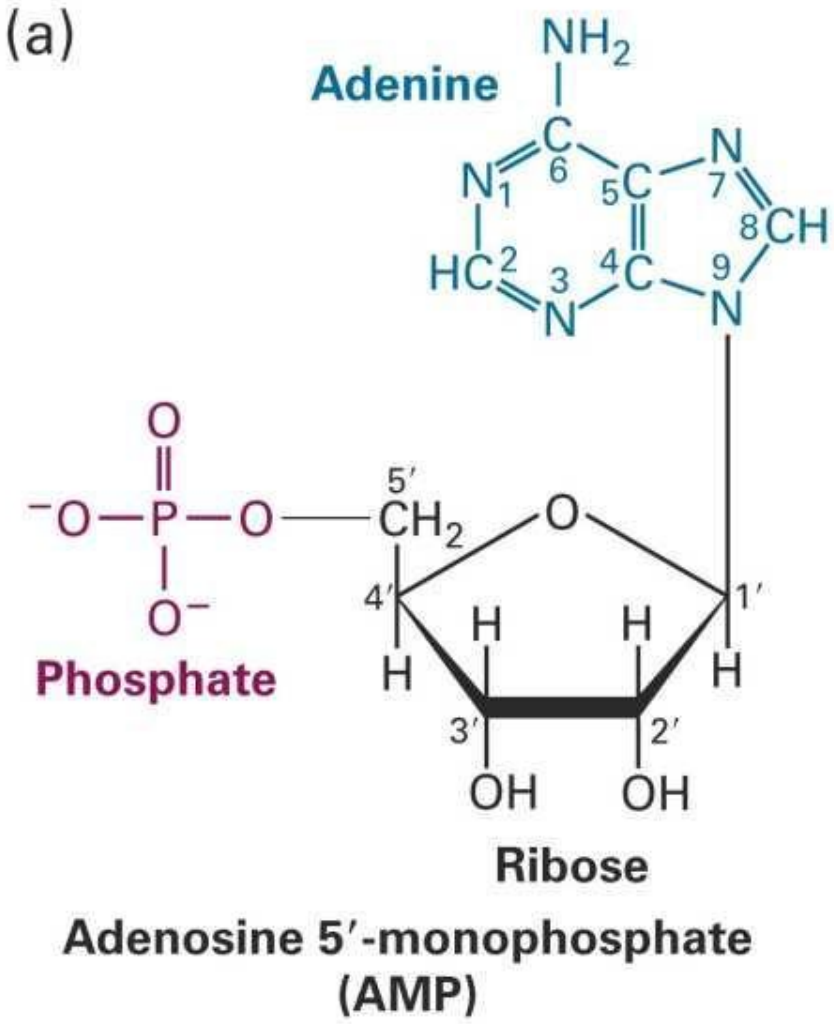
Development of body tissues
Function of mammalian immune system
Formation and function of brain and nervous system
Models of cancers and other human diseases
Gene regulation and inheritance
Infectious disease

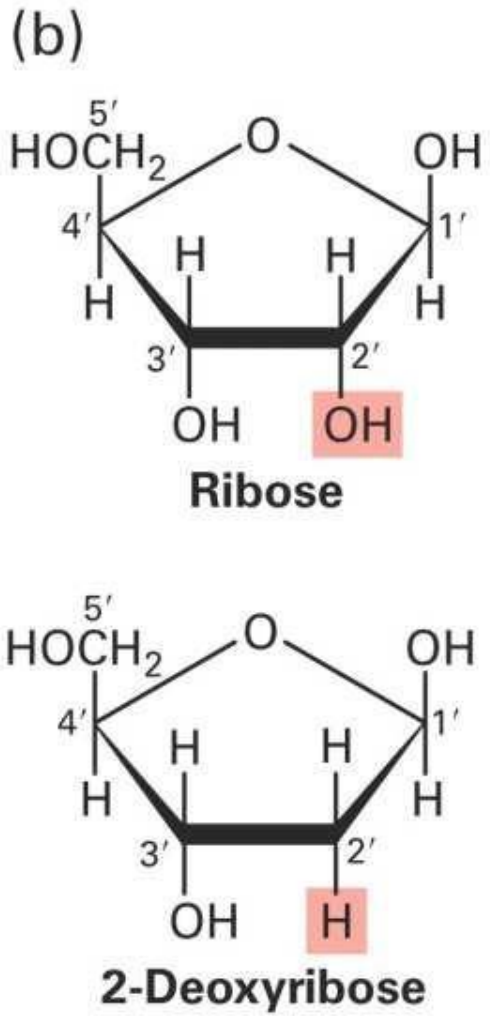
Jaenisch, R.Nat. Genet. 2001 27: 327-331

The ‘first’ science: “technology drives research drives technology dri...

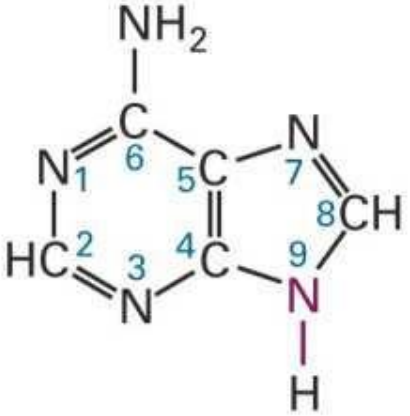



MONOMERS
POLYMERS
Amino acid
Polypeptide
H_2O
Base
Sugar
Nucleotide
Nucleic acid
H_2O
Monosaccharide
Polysaccharide
H_2O

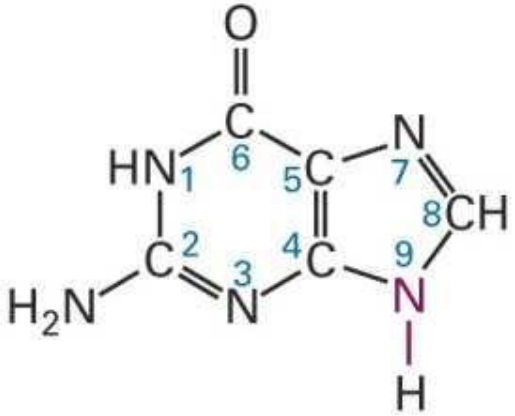

(a)
Adenine
Phosphate
Ribose
Adenosine 5′-monophosphate
(AMP)


(b)
Ribose
2-Deoxyribose

PURINES

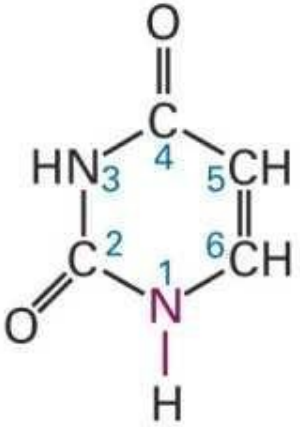


Adenine (A)

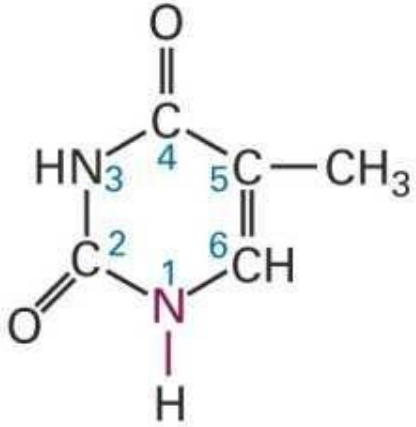


Guanine (G)

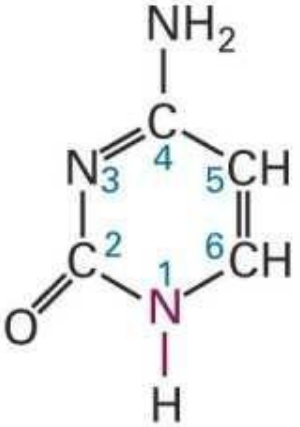
PYRIMIDINES



Uracil (U)

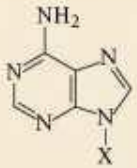
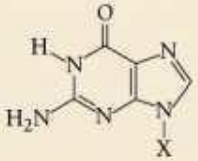
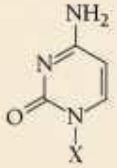
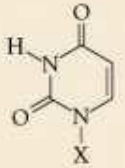
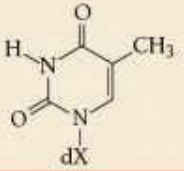


Thymine (T)



Cytosine (C)

Table 3-1 **Names and Abbreviations of Nucleic Acid Bases, Nucleosides, and Nucleotides**

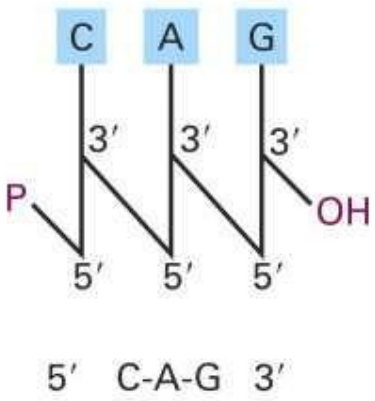
Base Formula	Base (X = H)	Nucleoside (X = ribose[a])	Nucleotide[b] (X = ribose phosphate[a])	
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP	DNA + RNA
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP	DNA + RNA
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP	DNA + RNA
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP	RNA
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP	DNA

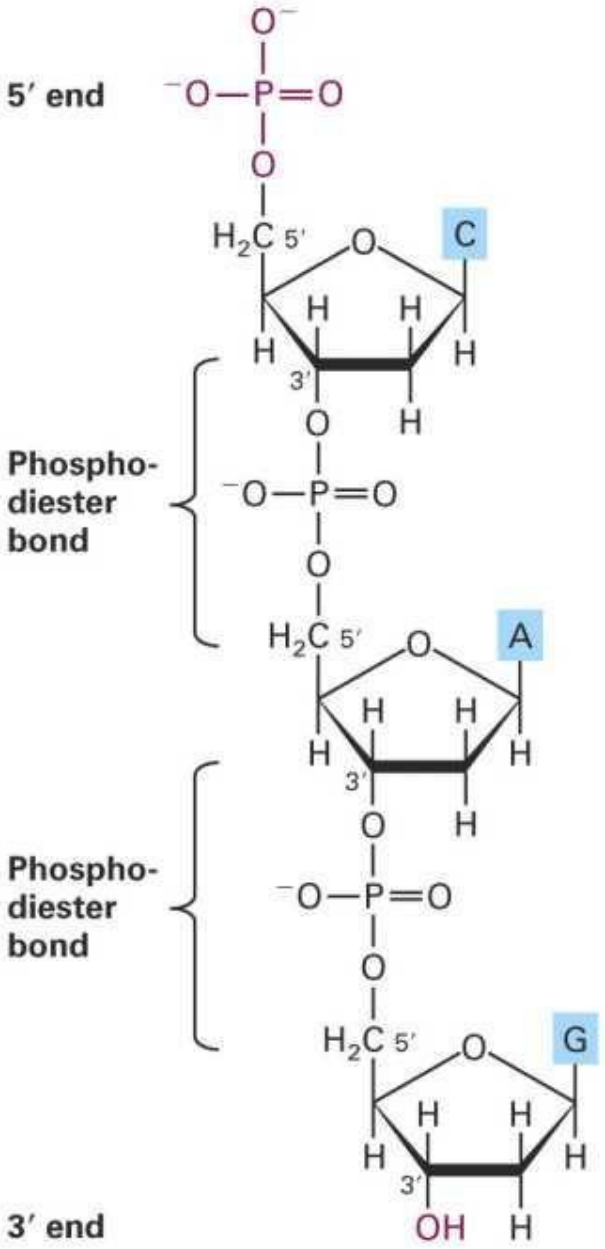
[a]The presence of a 2′-deoxyribose unit in place of ribose, as occurs in DNA, is implied by the prefixes "deoxy" or "d." For example, the deoxynucleoside of adenine is deoxyadenosine or dA. However, for thymine-containing residues, which rarely occur in RNA, the prefix is redundant and may be dropped. The presence of a ribose unit may be explicitly implied by the prefix "ribo."

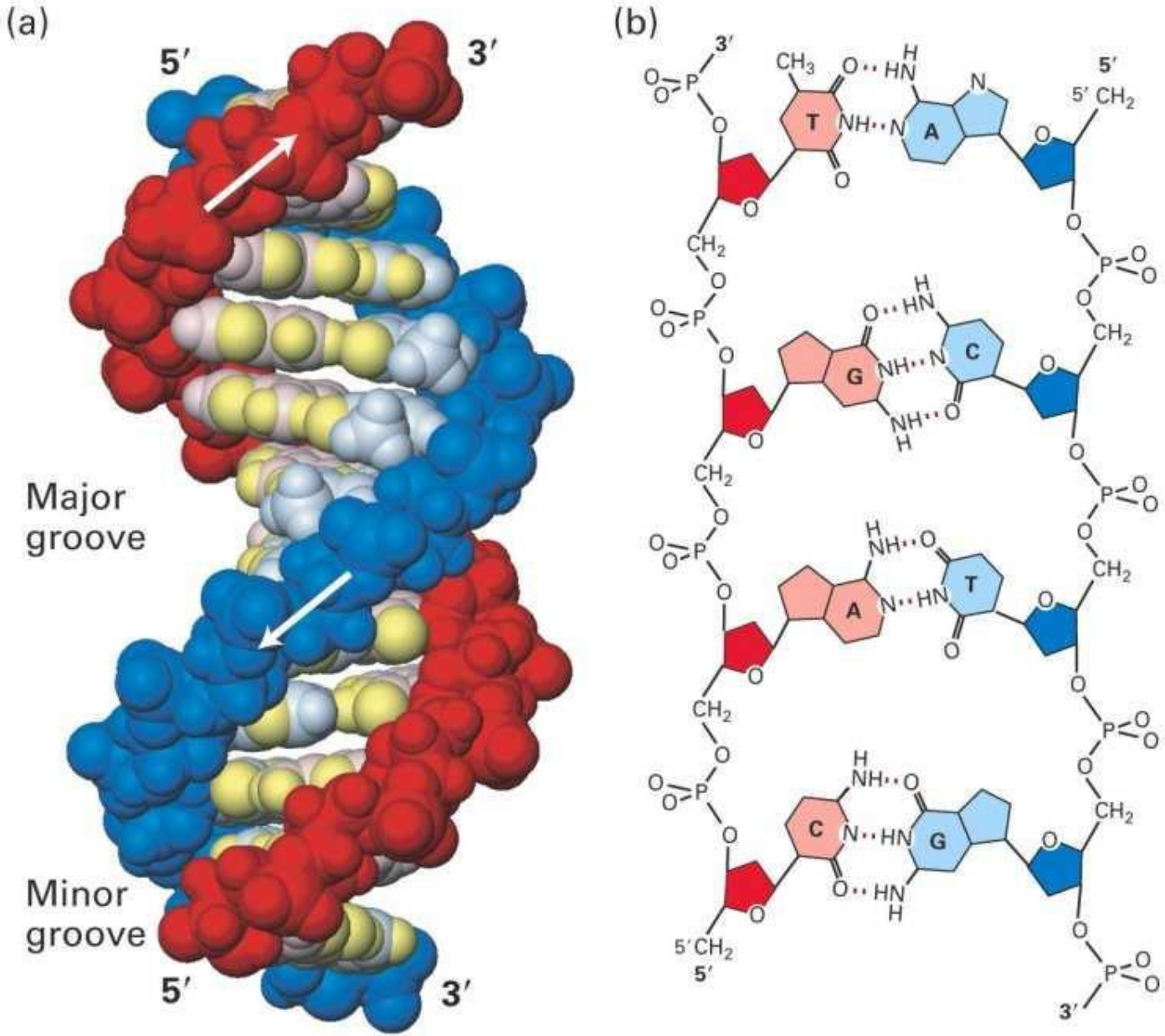
[b]The position of the phosphate group in a nucleotide may be explicitly specified as in, for example, 3′-AMP and 5′-GMP.

Table 3-1 Fundamentals of Biochemistry, 2/e

DNA structure -> sequence

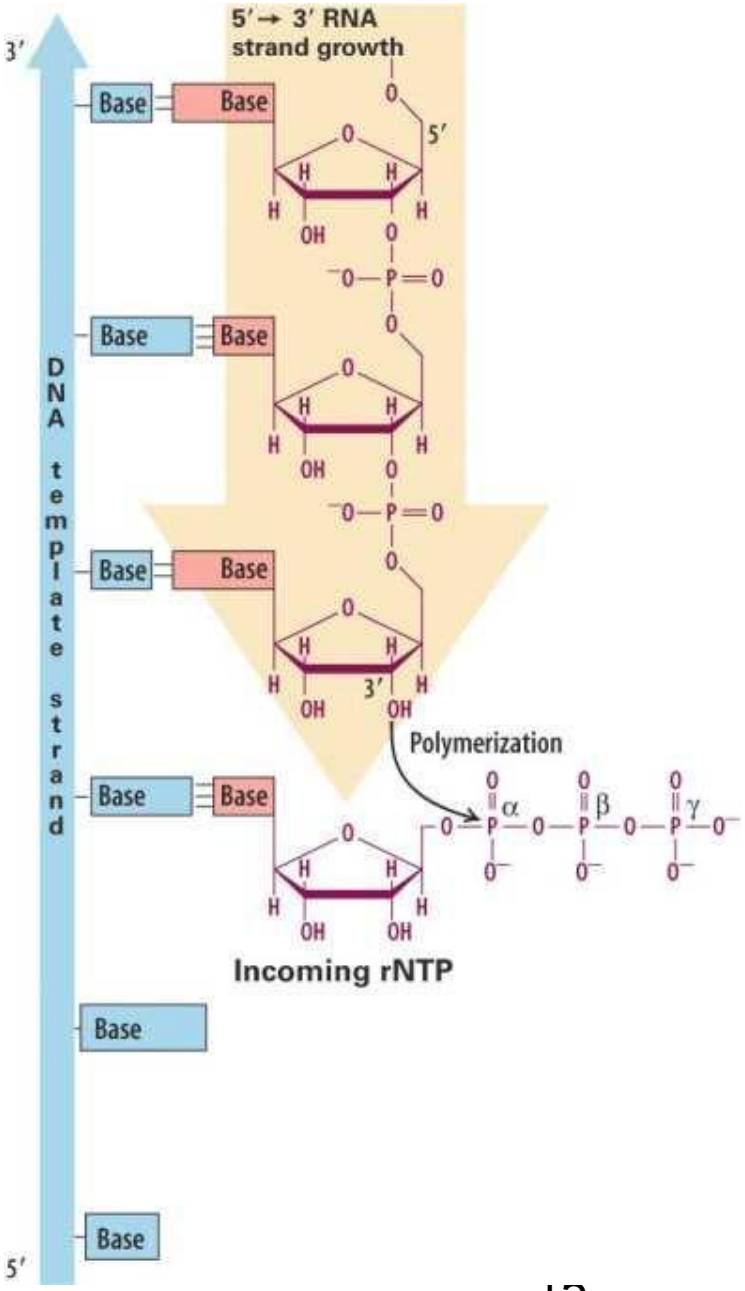




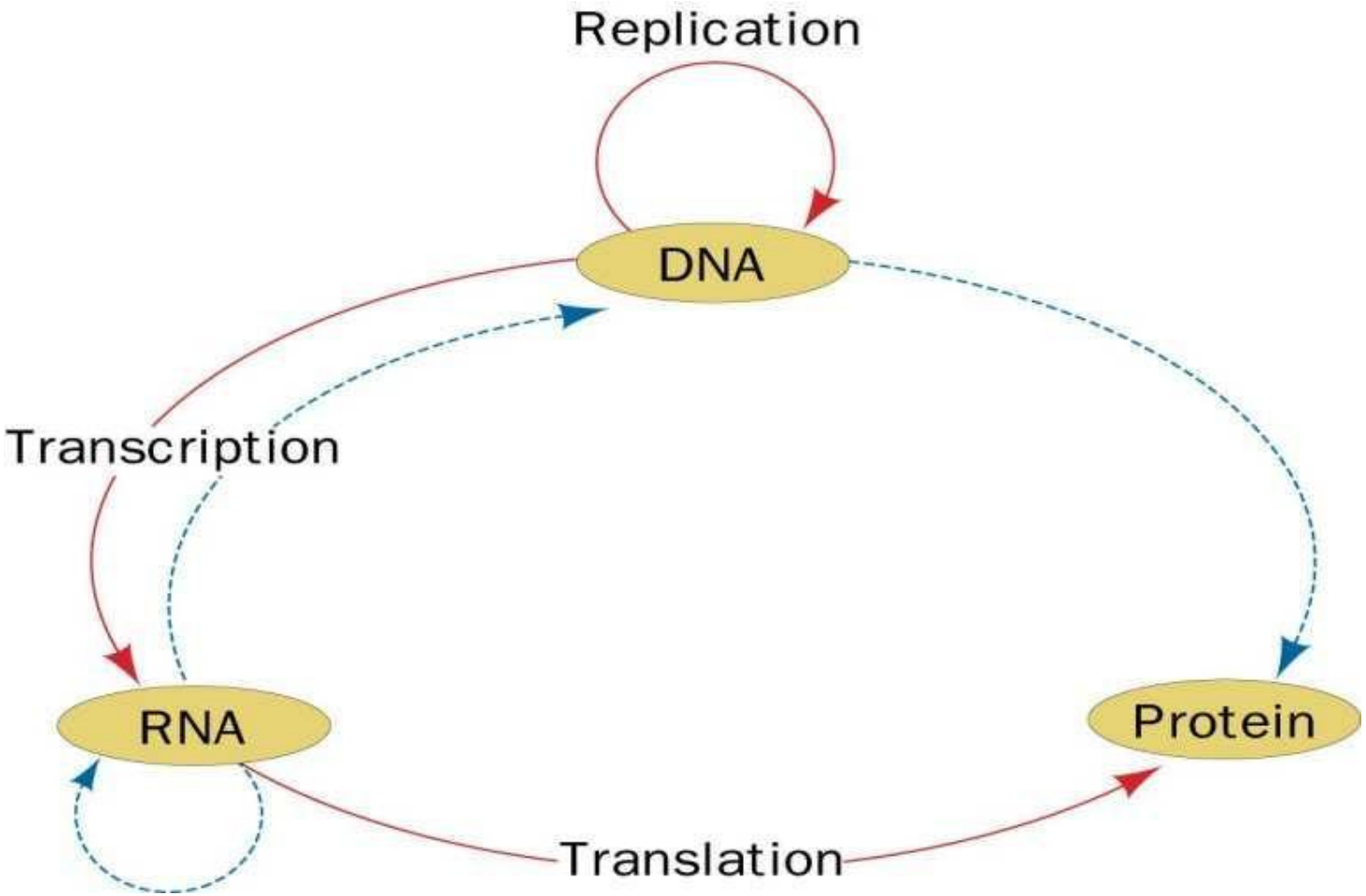

(a)
5′
3′
Major groove
Minor groove
5′
3′
(b)
3′
5′
5′ CH2
CH3
T
A
G
C
A
T
C
G
5′ CH2
5′
3′

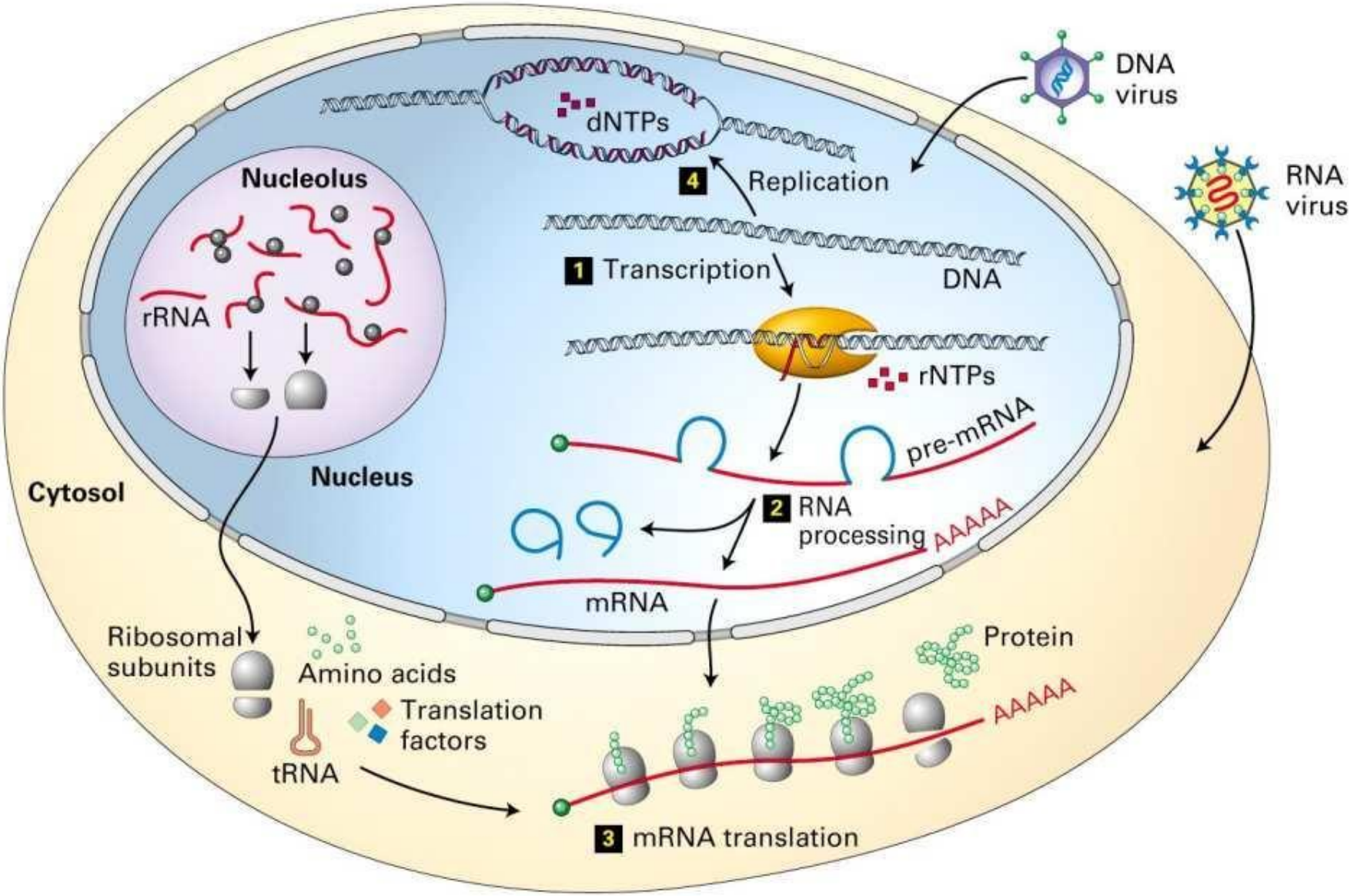
Polymerase reaction:

5′-> 3′

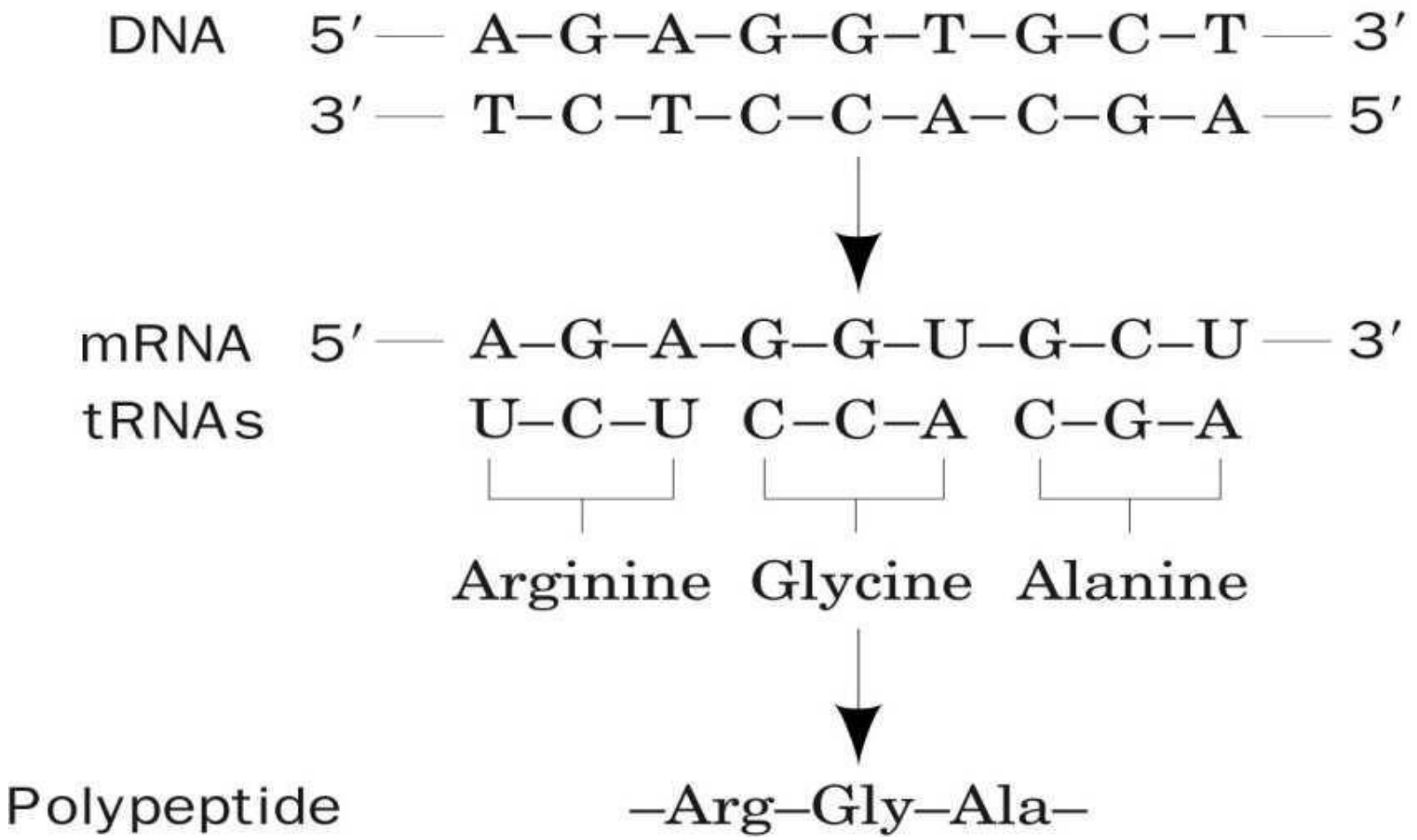


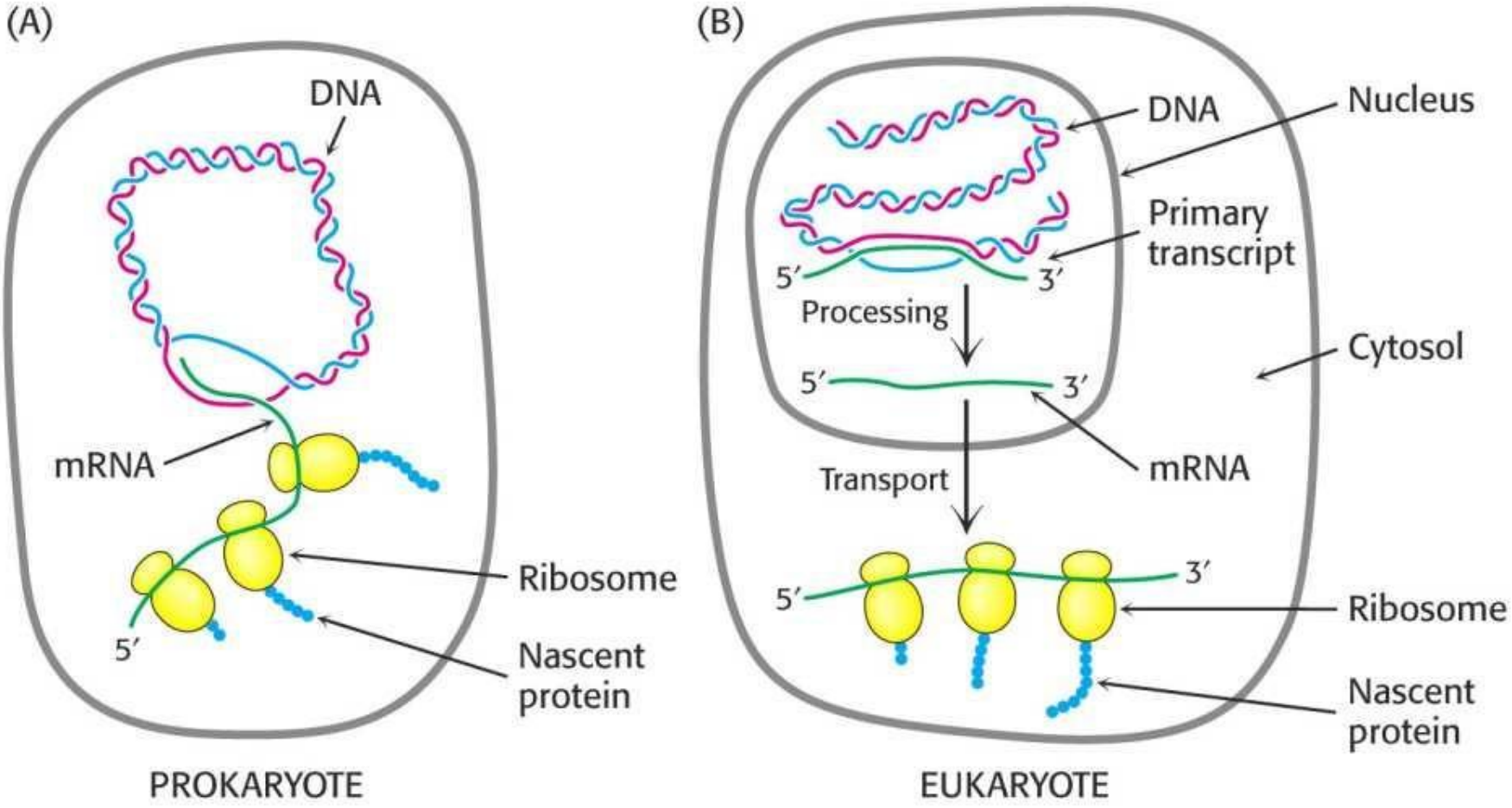
The central dogma



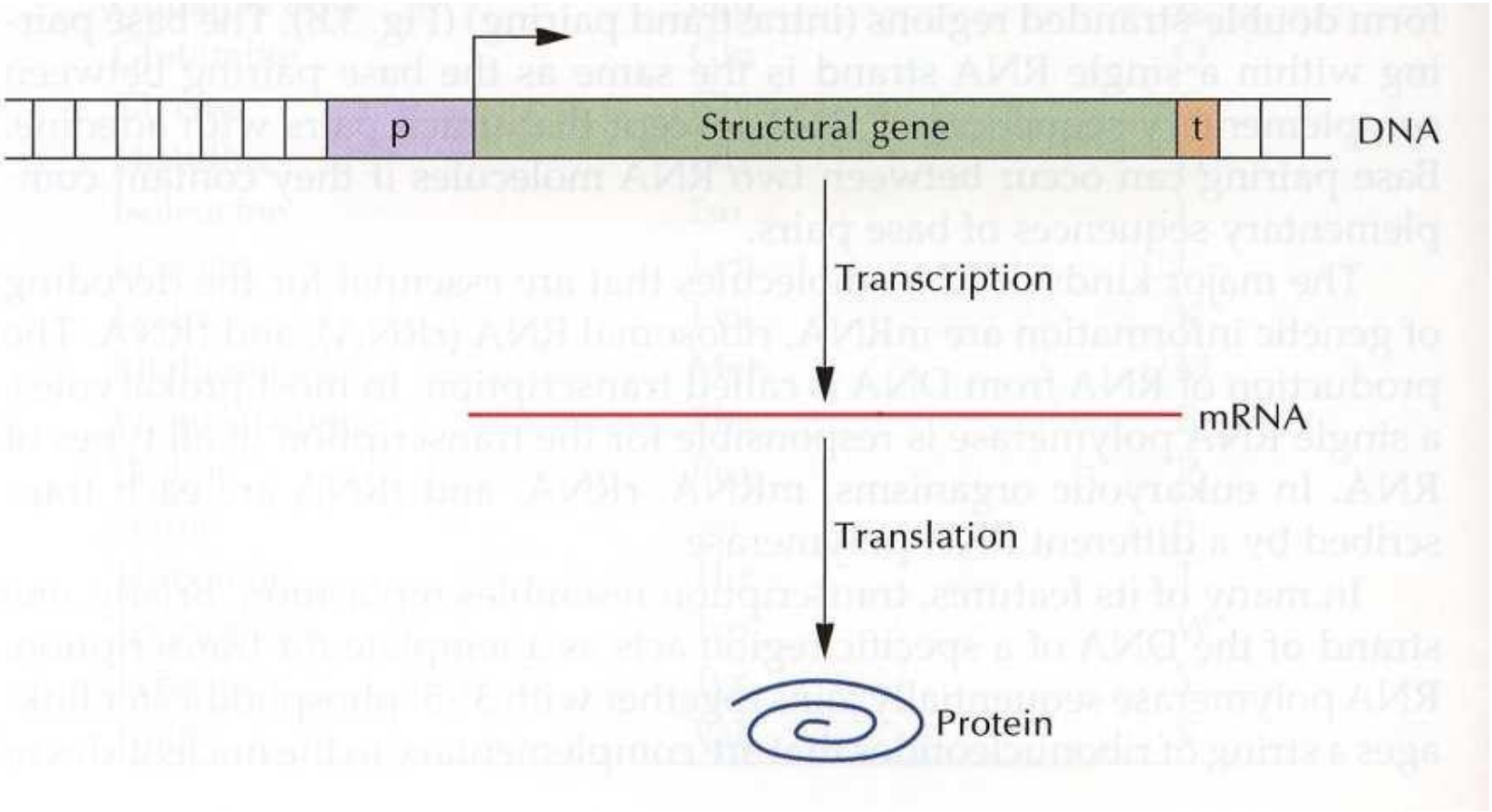

DNA virus
RNA virus
dNTPs
4 Replication
Nucleolus
rRNA
1 Transcription
DNA
rNTPs
pre-mRNA
Nucleus
Cytosol
2 RNA processing
AAAAA
mRNA
Ribosomal subunits
Amino acids
Translation factors
tRNA
Protein
AAAAA
3 mRNA translation

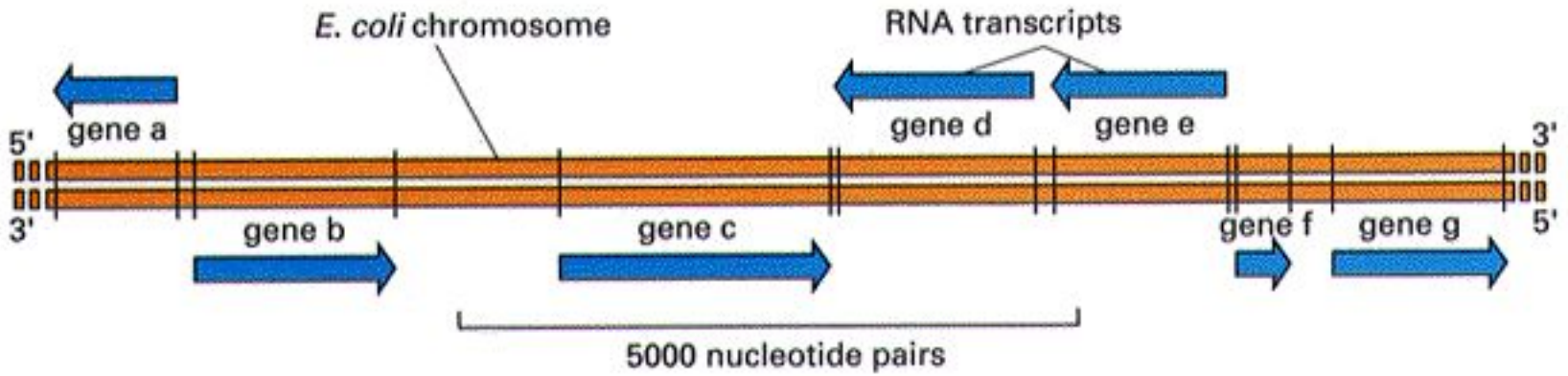
Gene expression.




(A)
DNA
mRNA
Ribosome
5′
Nascent protein
PROKARYOTE
(B)
DNA
Nucleus
Primary transcript
5′
3′
Processing
Cytosol
5′
3′
mRNA
Transport
5′
3′
Ribosome
Nascent protein
EUKARYOTE

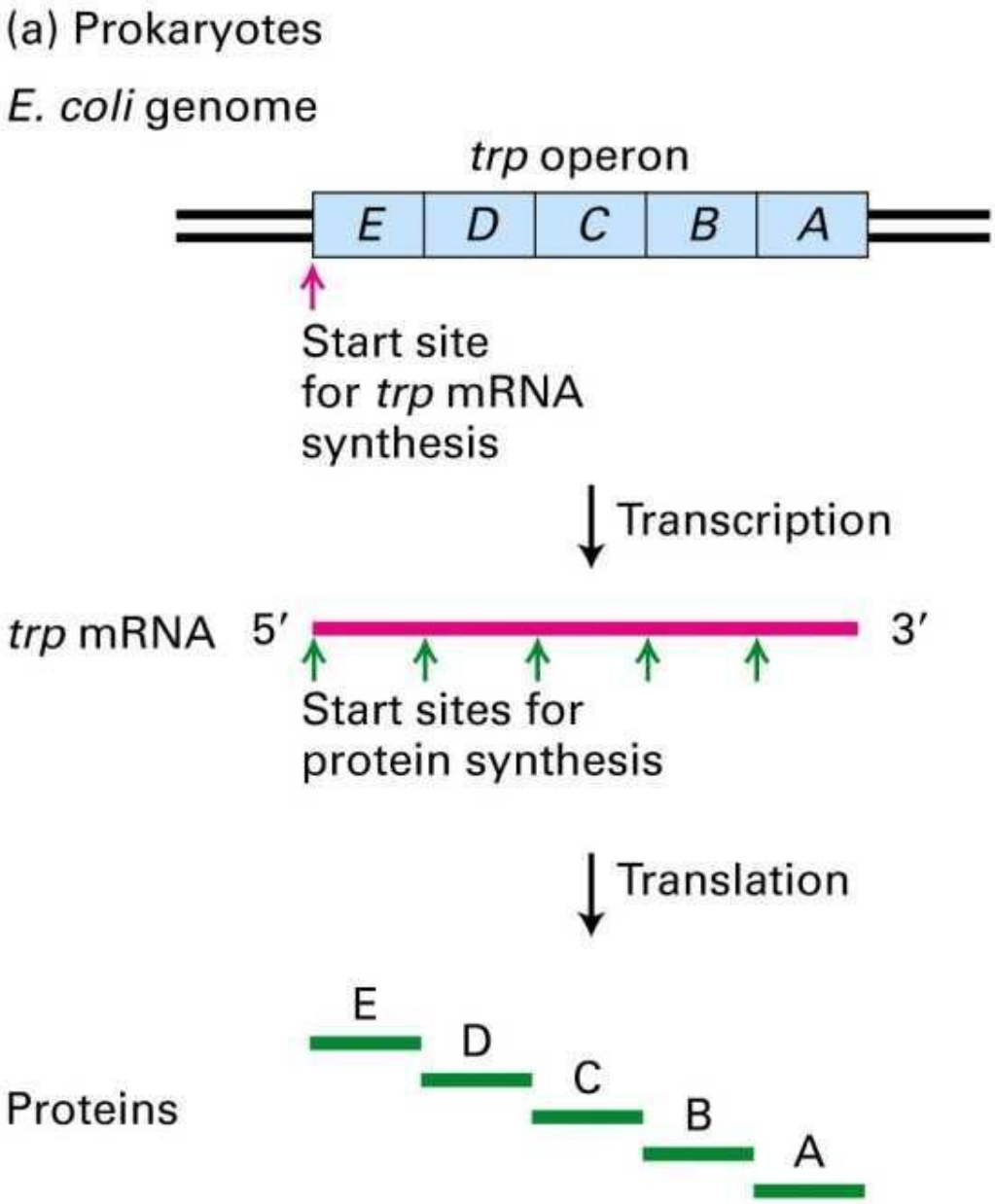
What is a gene?



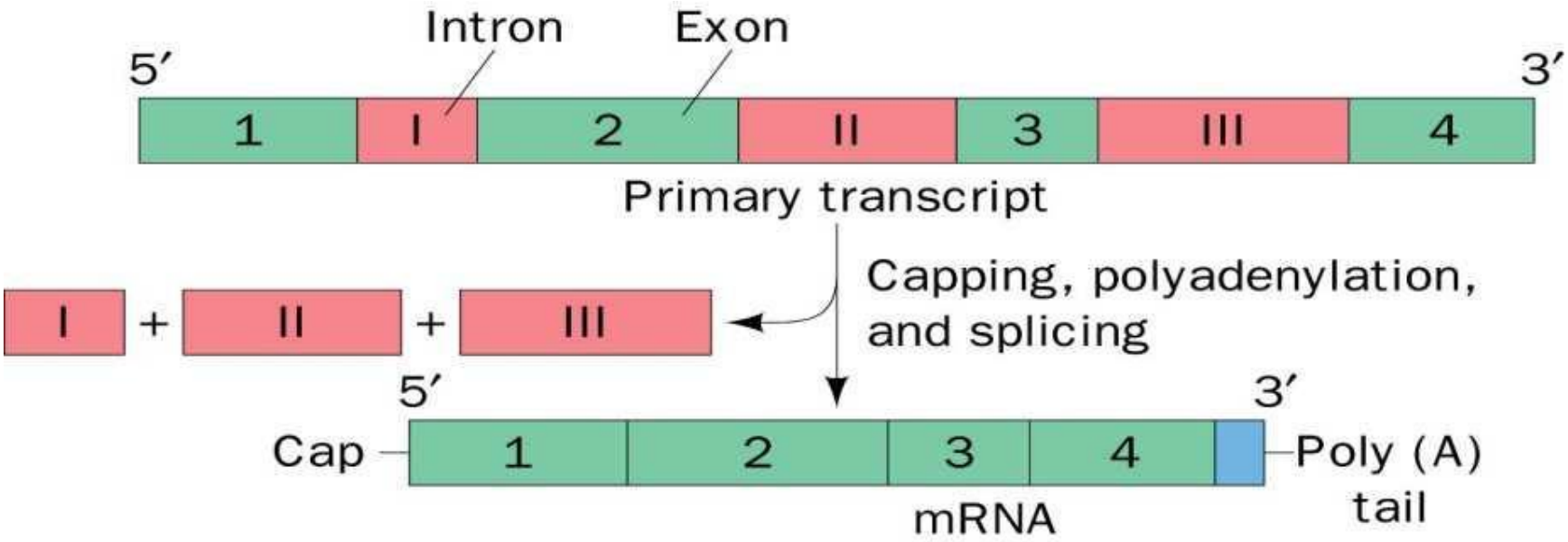


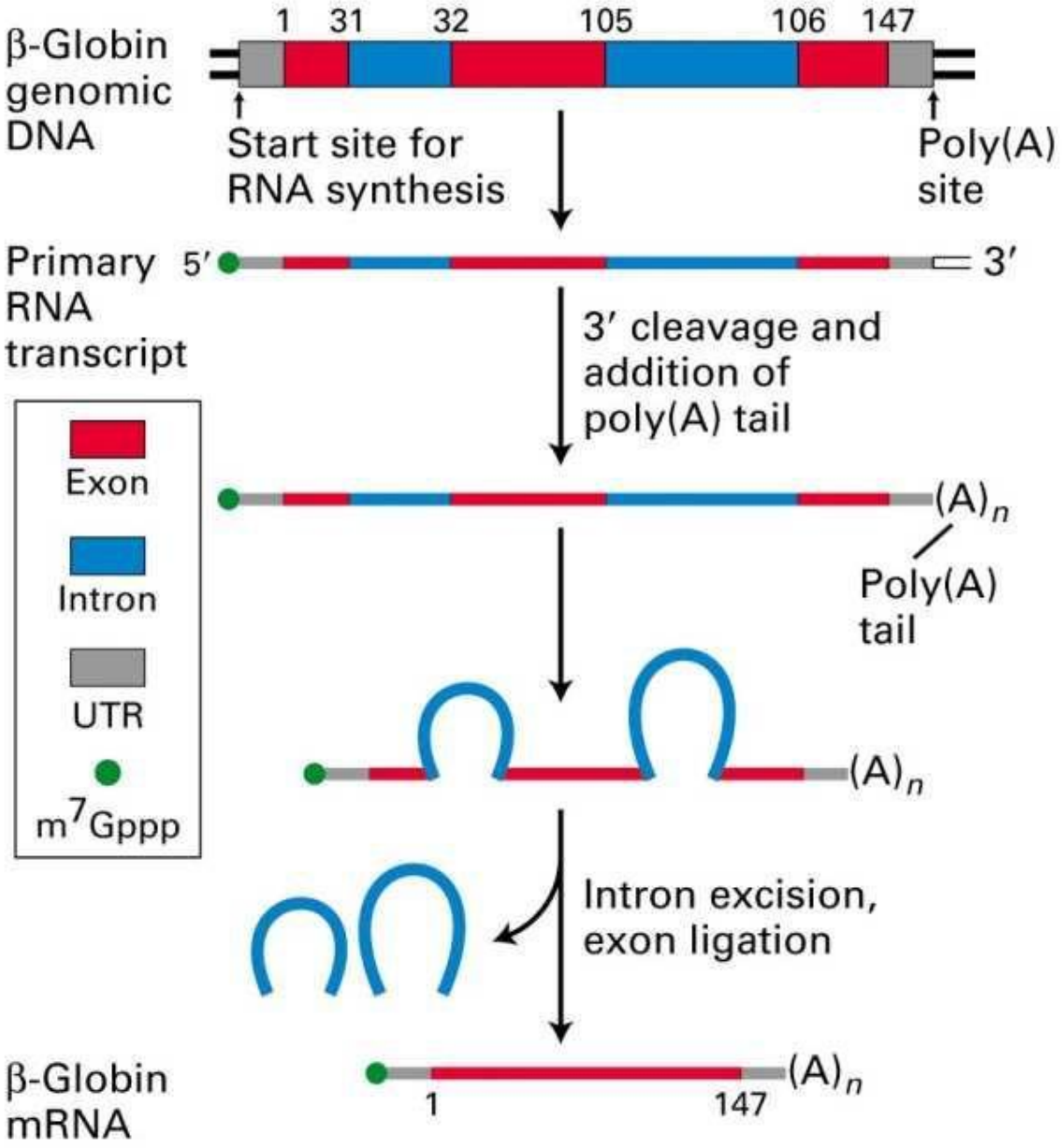
(a) Monocistronic

(b) Polycistronic


(a) Prokaryotes
E. coli genome
trp operon
E
D
C
B
A
Start site
for trp mRNA
synthesis
Transcription
trp mRNA
5′
3′
Start sites for
protein synthesis
Translation
Proteins
E
D
C
B
A

Eukaryotes – Intron-Exon concept




β-Globin genomic DNA
1
31
32
105
106
147
Start site for RNA synthesis
Poly(A) site
Primary RNA transcript
5′
3′
3′ cleavage and addition of poly(A) tail
Exon
Intron
UTR
m7Gppp
(A)n
Poly(A) tail
(A)n
Intron excision, exon ligation
β-Globin mRNA
(A)n
1
147

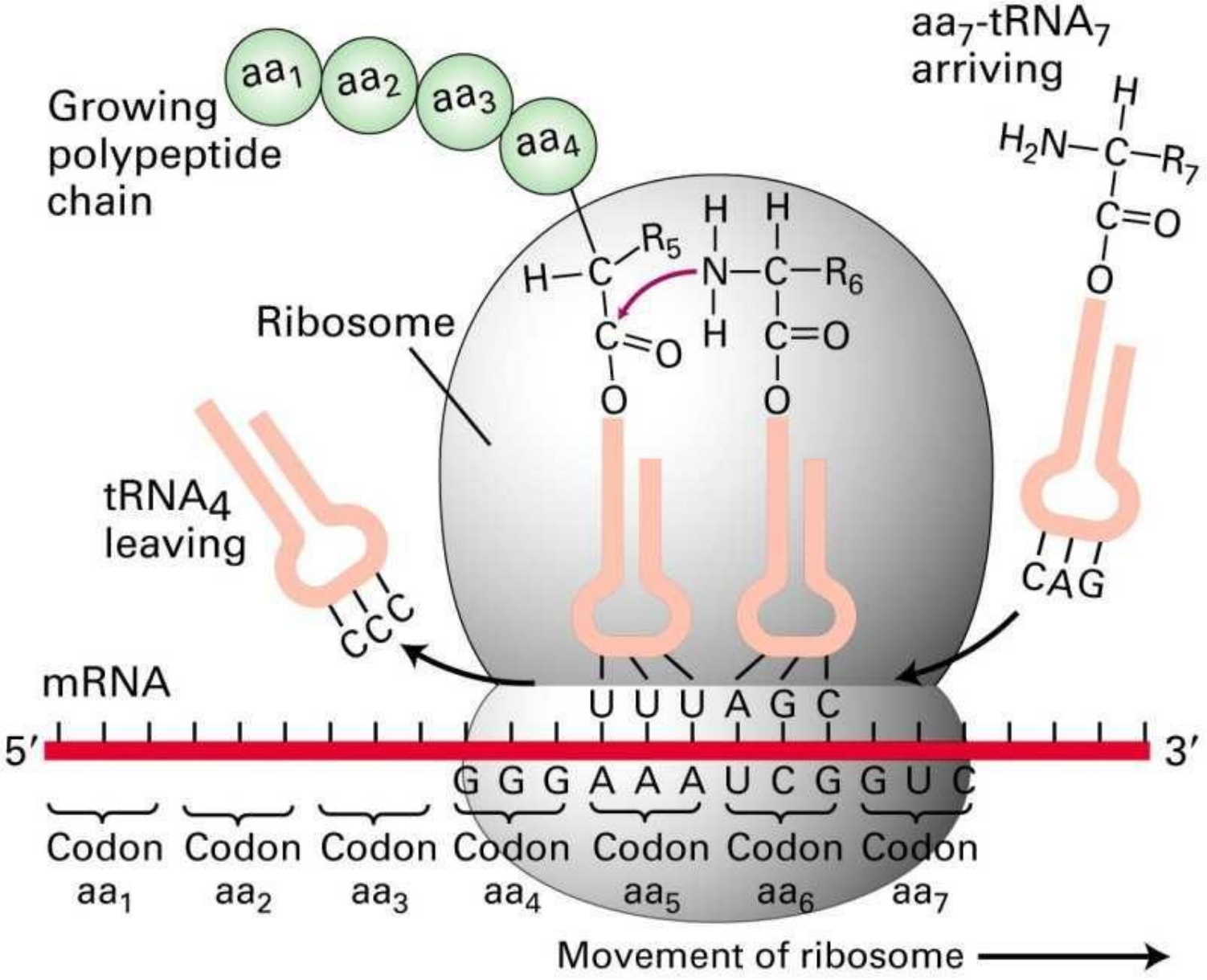
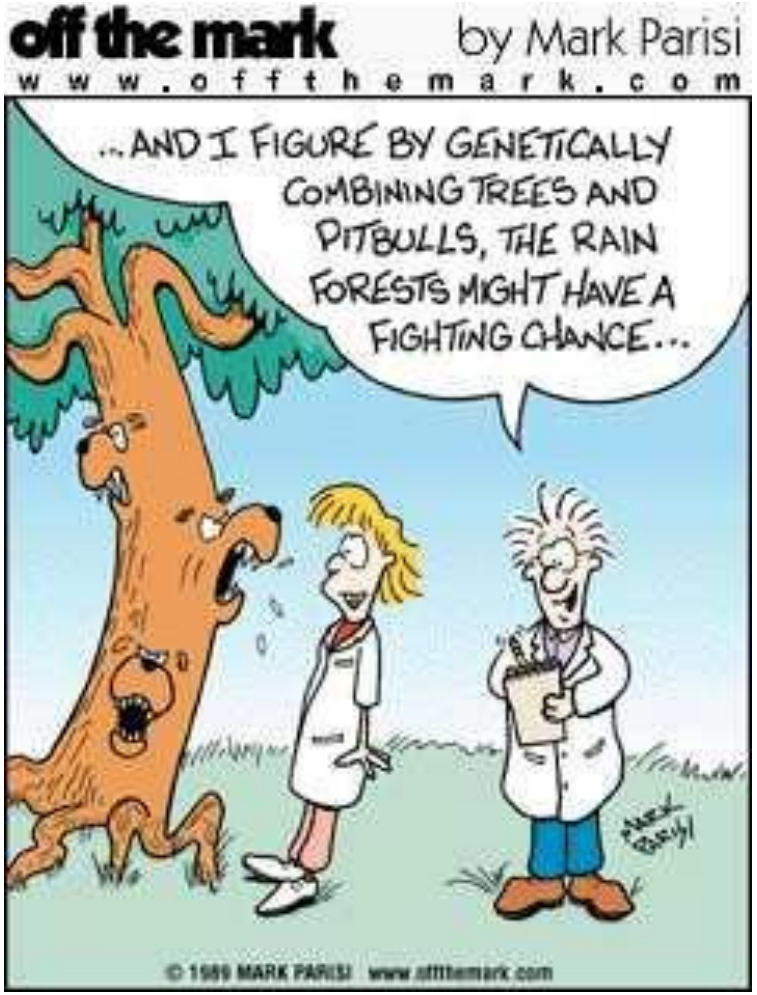

Growing polypeptide chain
aa1
aa2
aa3
aa4
aa7-tRNA7 arriving
Ribosome
tRNA4 leaving
CCC
CAG
mRNA
5′
3′
U U U A G C
G G G A A A U C G G U C
Codon aa1
Codon aa2
Codon aa3
Codon aa4
Codon aa5
Codon aa6
Codon aa7
Movement of ribosome

TABLE 5.4 The genetic code

First position (5′ end)	Second position				Third position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5′ AUG 3′ on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

Recombinant DNA Technology



Definitions

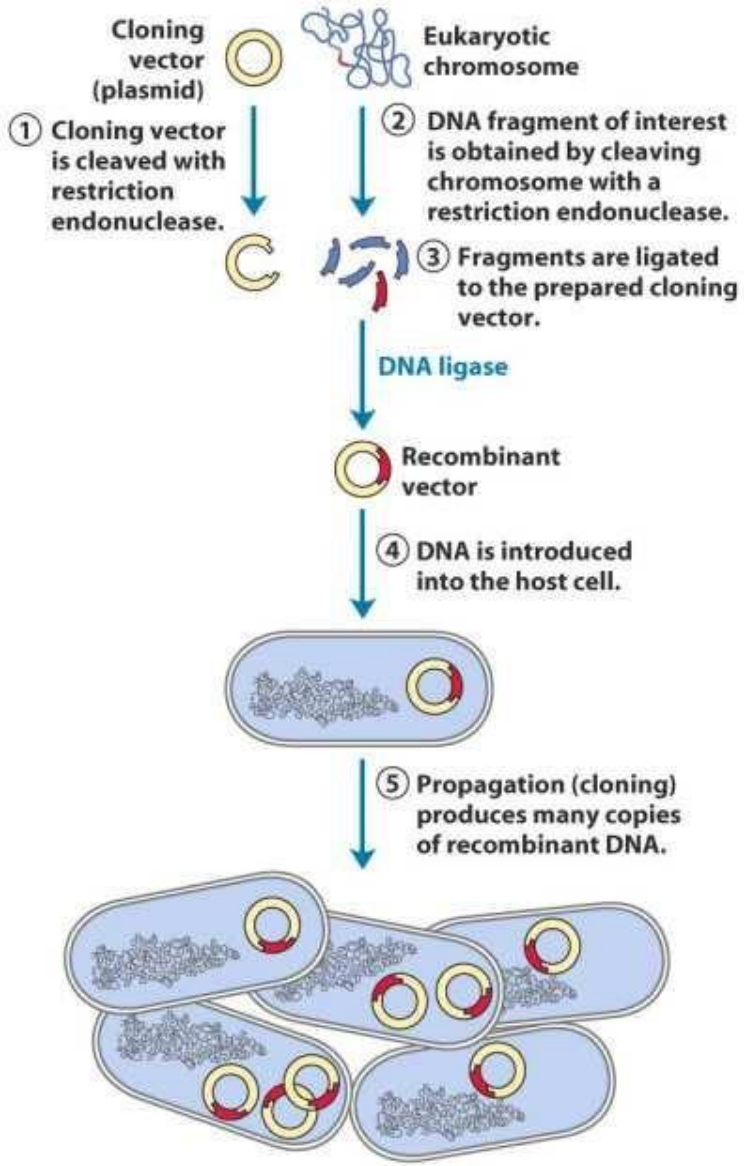
Recombinant DNA, a DNA construct created by fusing different fragments of DNA

Genetic Engineering, the deliberate alteration of DNA through the creation of recombinant DNA

Genetically Modified Organism, a living entity modified through genetic engineering

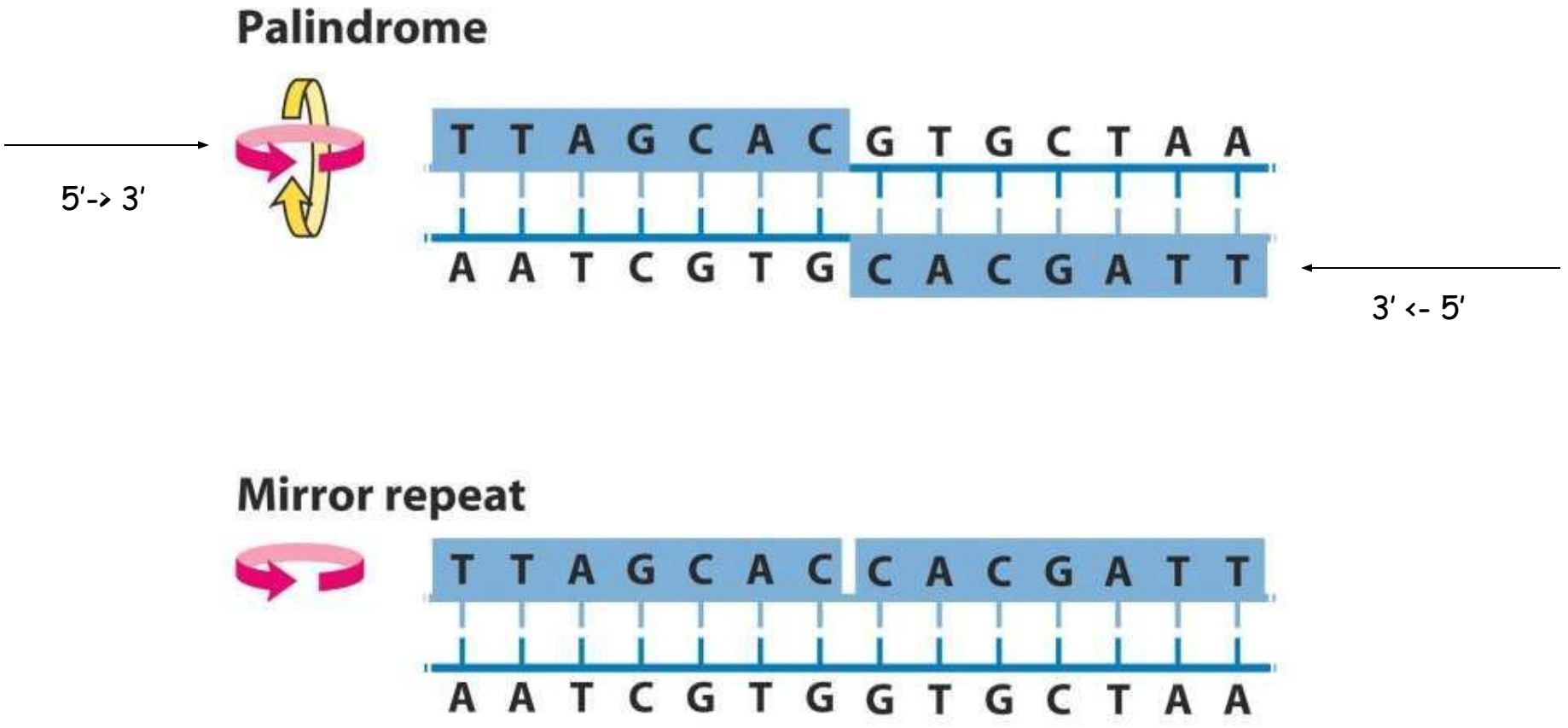
Transgenic, a genetically modified organism containing DNA from another source

Recombinant DNA Technology



Clones -> Cells or organisms with identical DNA

Restrictionendonucleases



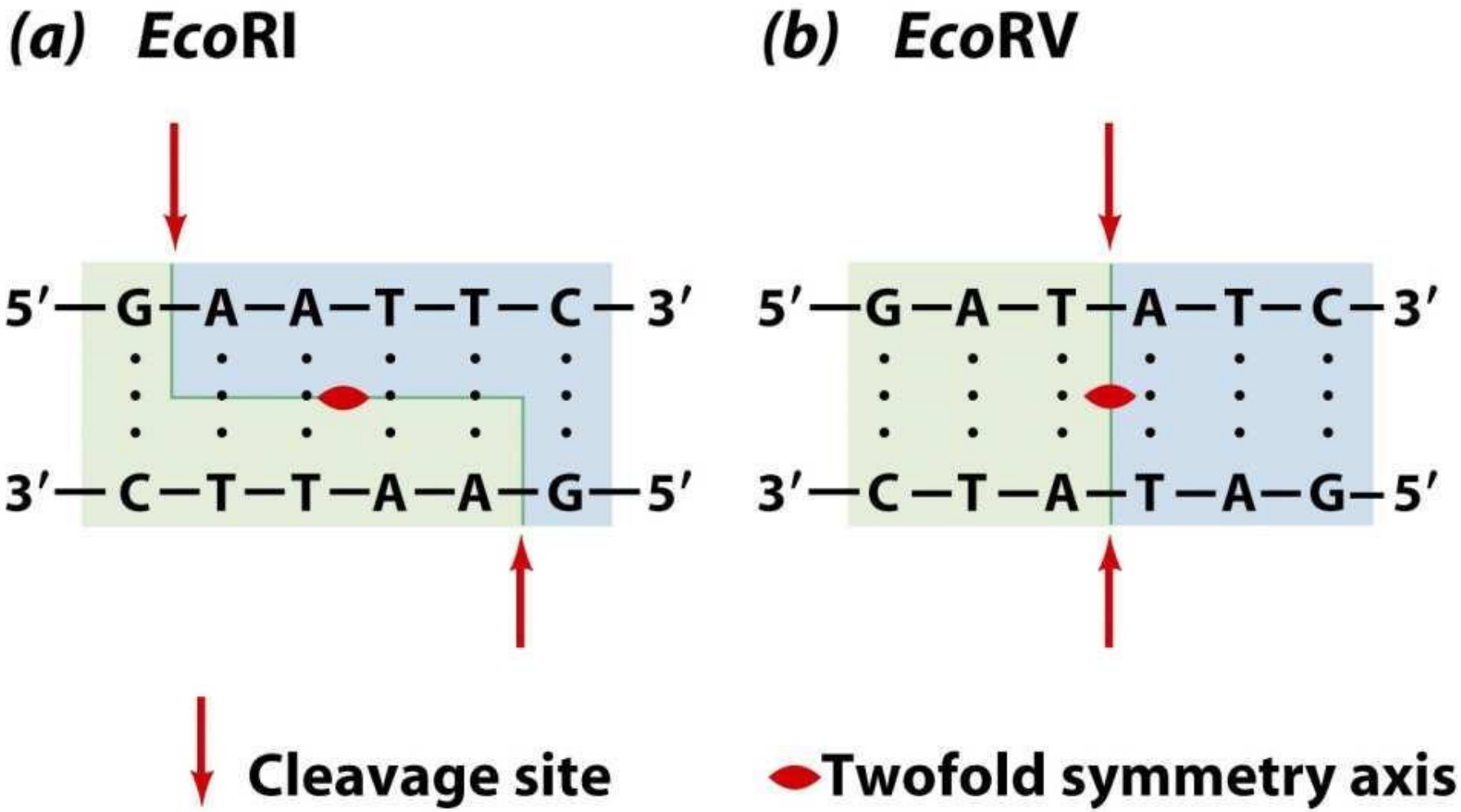


Figure 3-16 Fundamentals of Biochemistry, 2/e

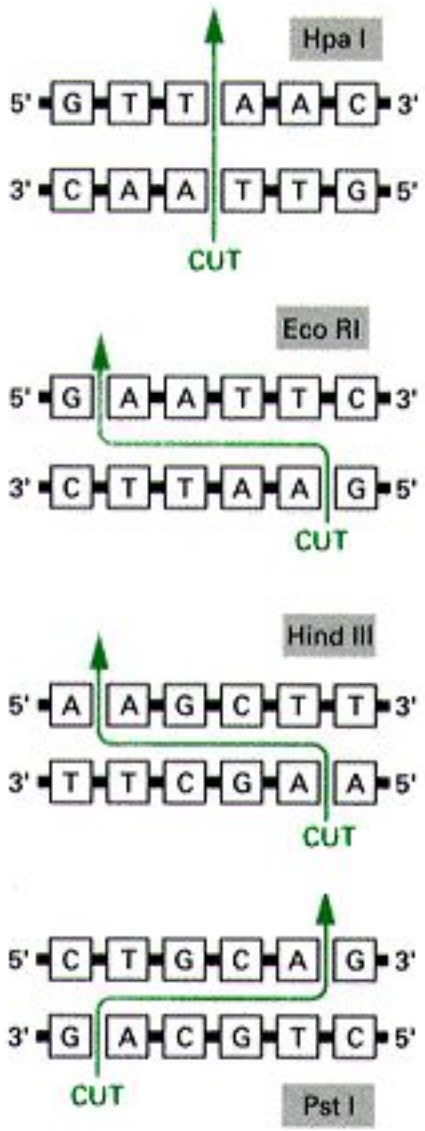


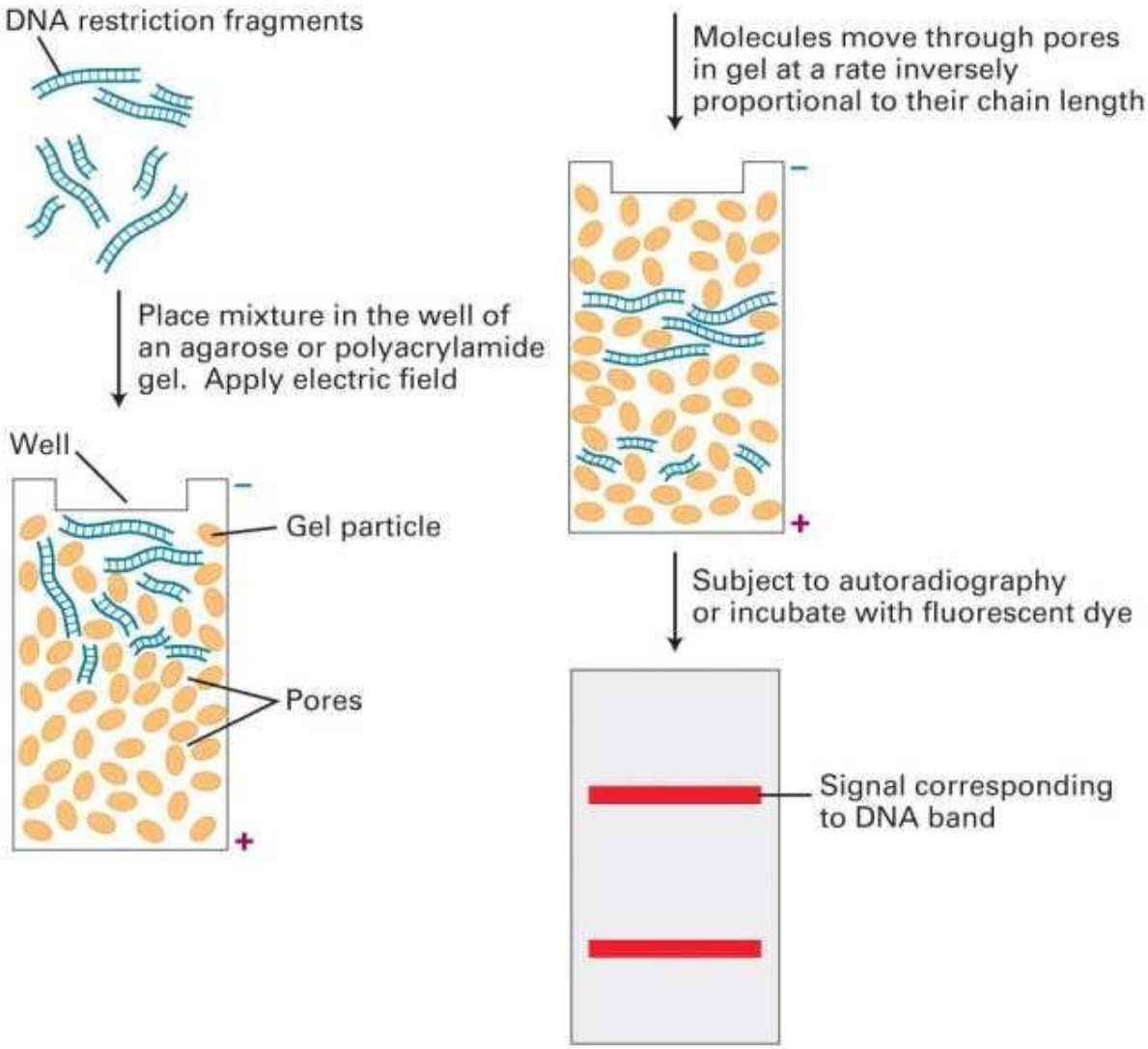
Table 3-2 Recognition and Cleavage Sites of Some Restriction Enzymes

Enzyme	Recognition Sequence[a]	Microorganism
*Alu*I	AG↓CT	*Arthrobacter luteus*
*Bam*HI	G↓GATCC	*Bacillus amyloliquefaciens* H
*Bgl*I	GCCNNNNN↓NGGC	*Bacillus globigii*
*Bgl*II	A↓GATCT	*Bacillus globigii*
*Eco*RI	G↓AATTC	*Escherichia coli* RY13
*Eco*RII	↓CC($^{A}_{T}$)GG	*Escherichia coli* R245
*Eco*RV	GAT↓ATC	*Escherichia coli* J62 pLG74
*Hae*II	RGCGC↓Y	*Haemophilus aegyptius*
*Hae*III	GG↓CC	*Haemophilus aegyptius*
*Hin*dIII	A↓AGCTT	*Haemophilus influenzae* R_d
*Hpa*II	C↓CGG	*Haemophilus parainfluenzae*
*Msp*I	C↓CGG	*Moraxella* species
*Pst*I	CTGCA↓G	*Providencia stuartii* 164
*Pvu*II	CAG↓CTG	*Proteus vulgaris*
*Sal*I	G↓TCGAC	*Streptomyces albus* G
*Taq*I	T↓CGA	*Thermus aquaticus*
*Xho*I	C↓TCGAG	*Xanthomonas holcicola*

[a]The recognition sequence is abbreviated so that only one strand, reading 5′ to 3′, is given. The cleavage site is represented by an arrow (↓). R, Y, and N represent a purine nucleotide, a pyrimidine nucleotide, and any nucleotide, respectively.

Source: Roberts, R.J. and Macelis, D., REBASE—the restriction enzyme database, http://rebase.neb.com.

Gel Electrophoresis



Gel Electrophoresis

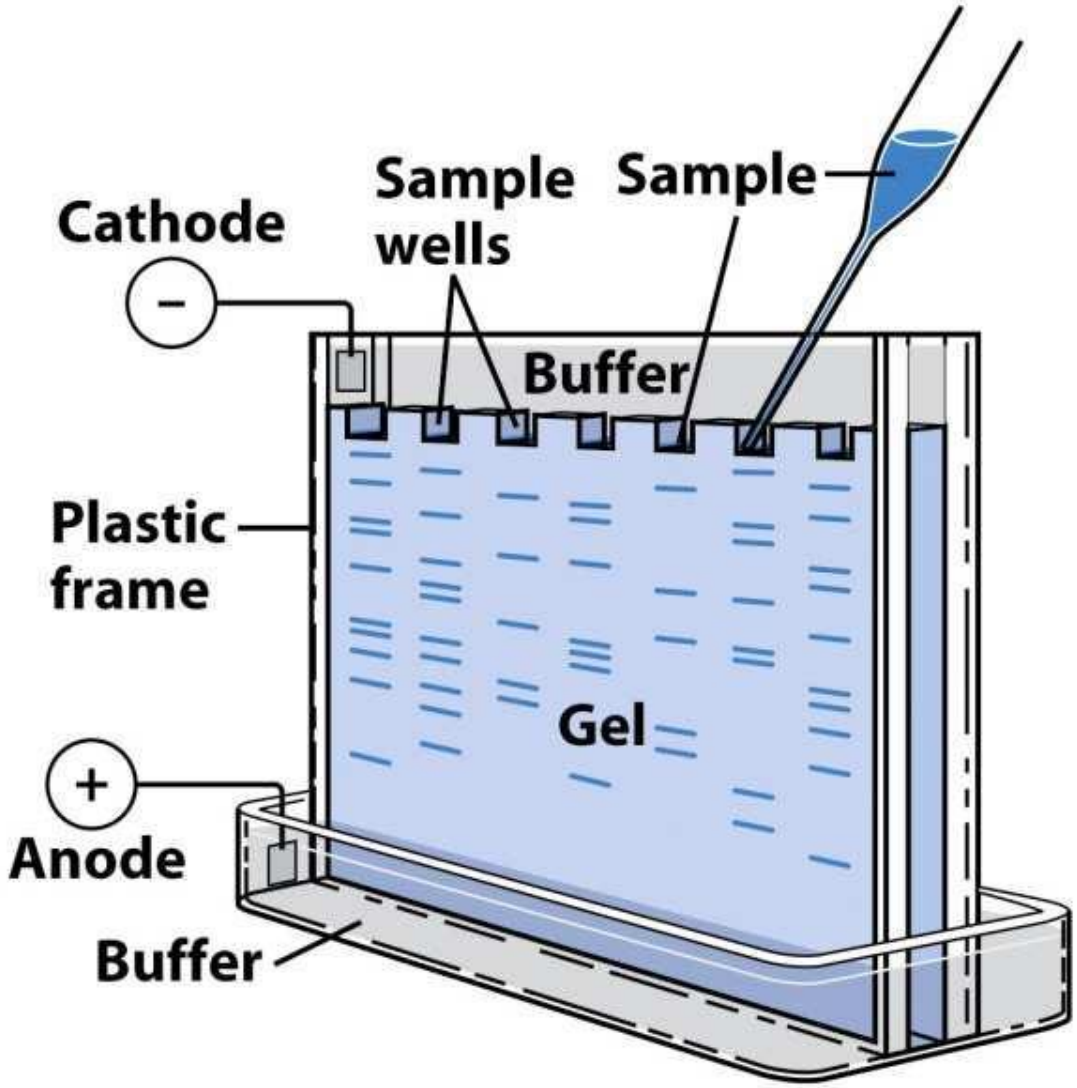
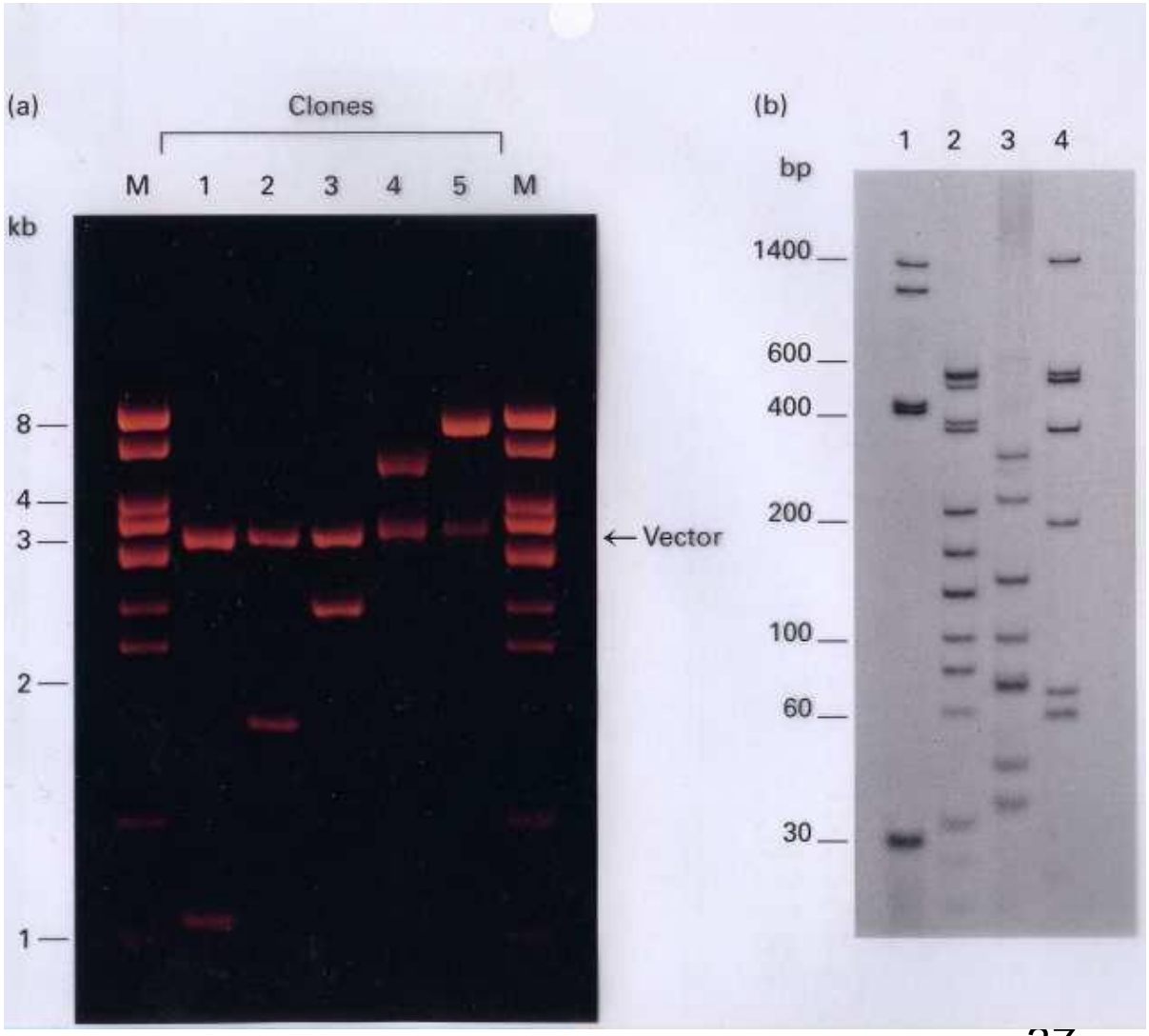
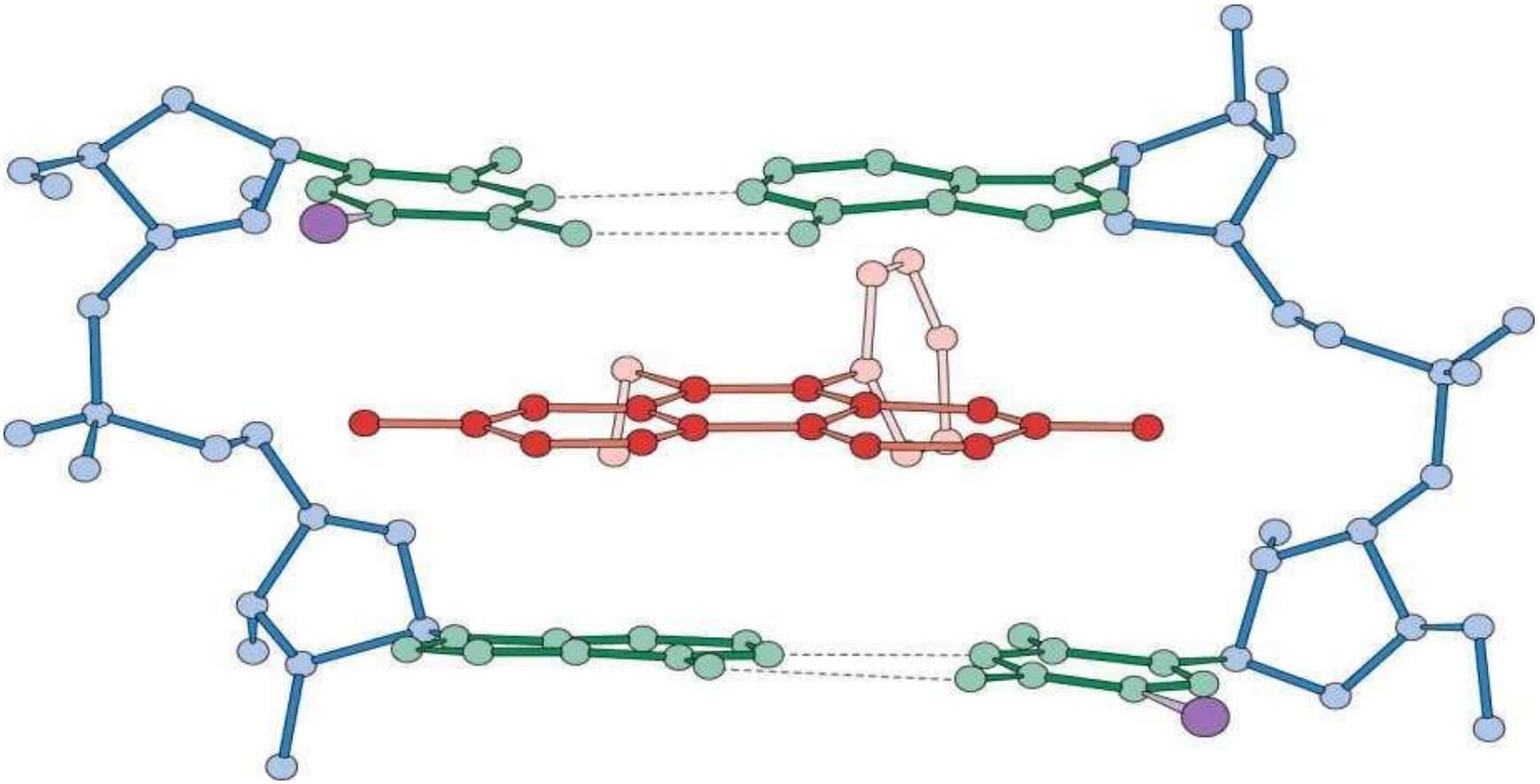


Figure 3-17 Fundamentals of Biochemistry, 2/e

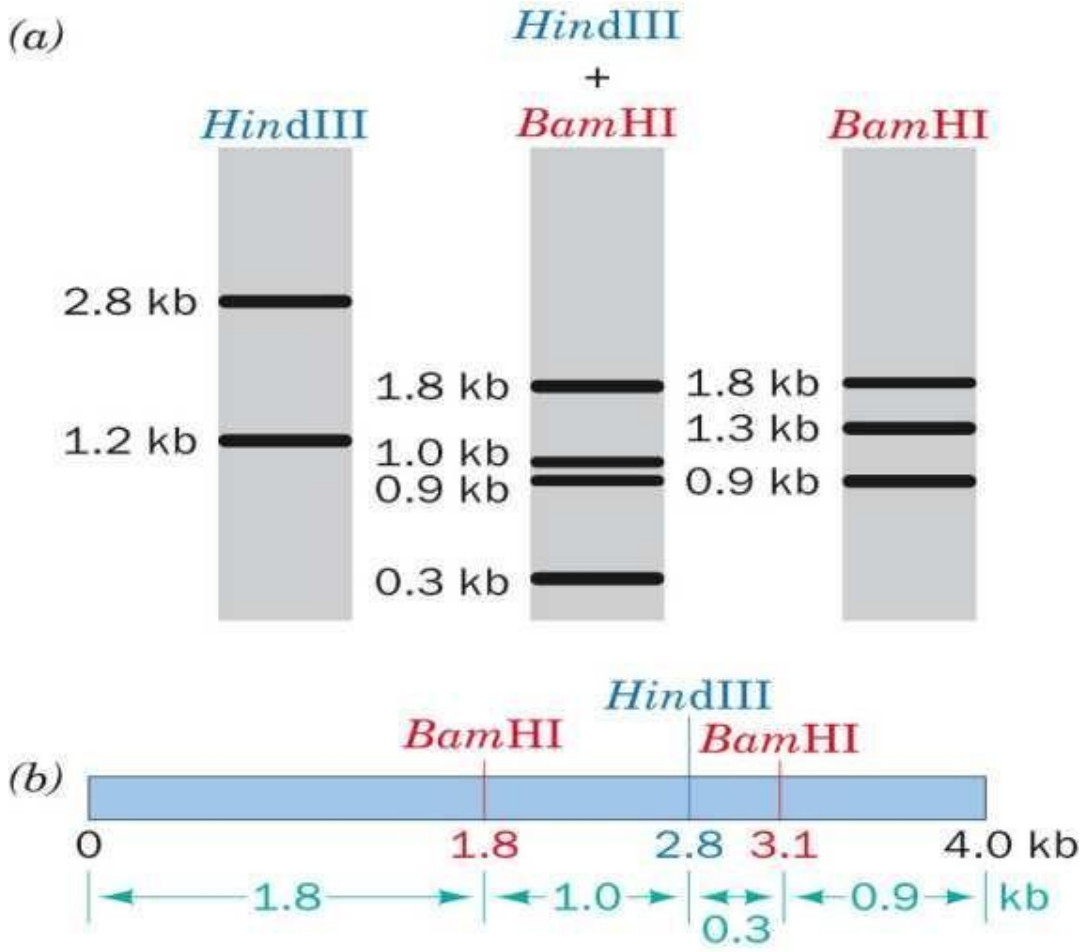
Gel Electrophoresis



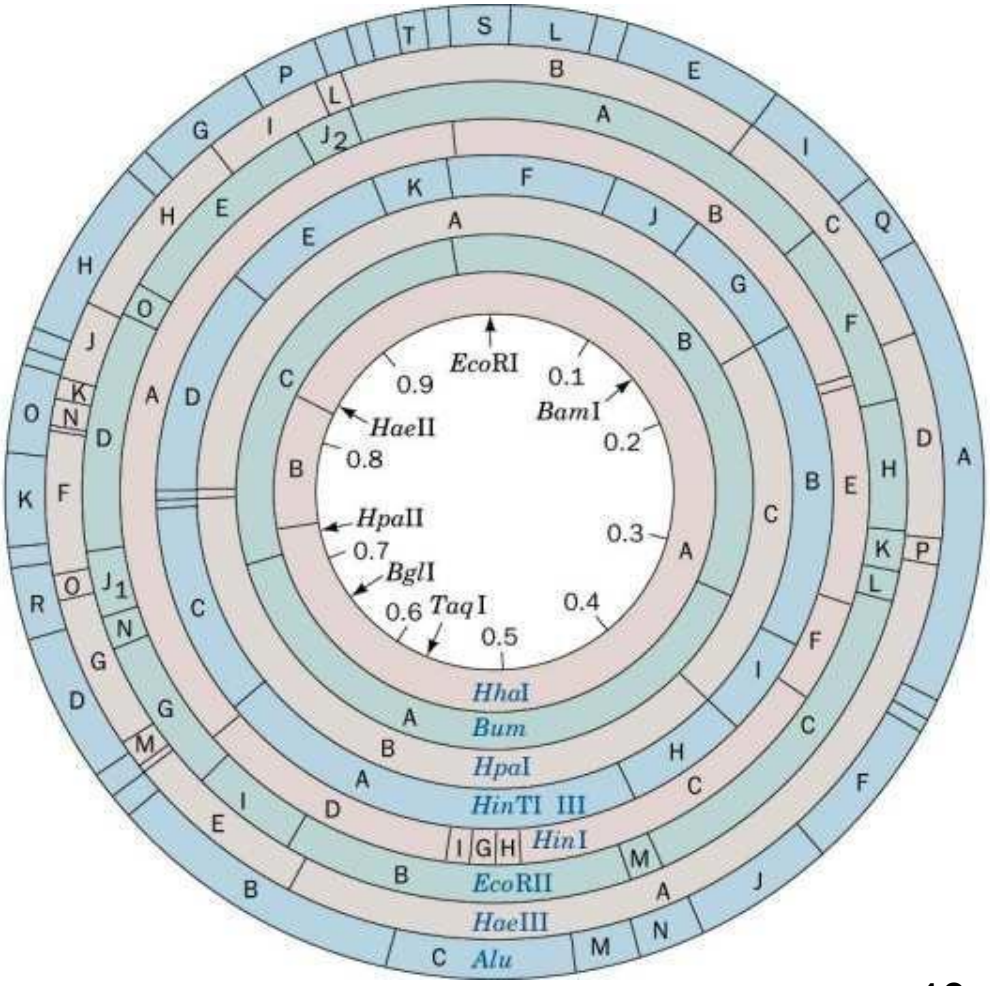
X-Ray structure of a complex of ethidium bromid with DNA.

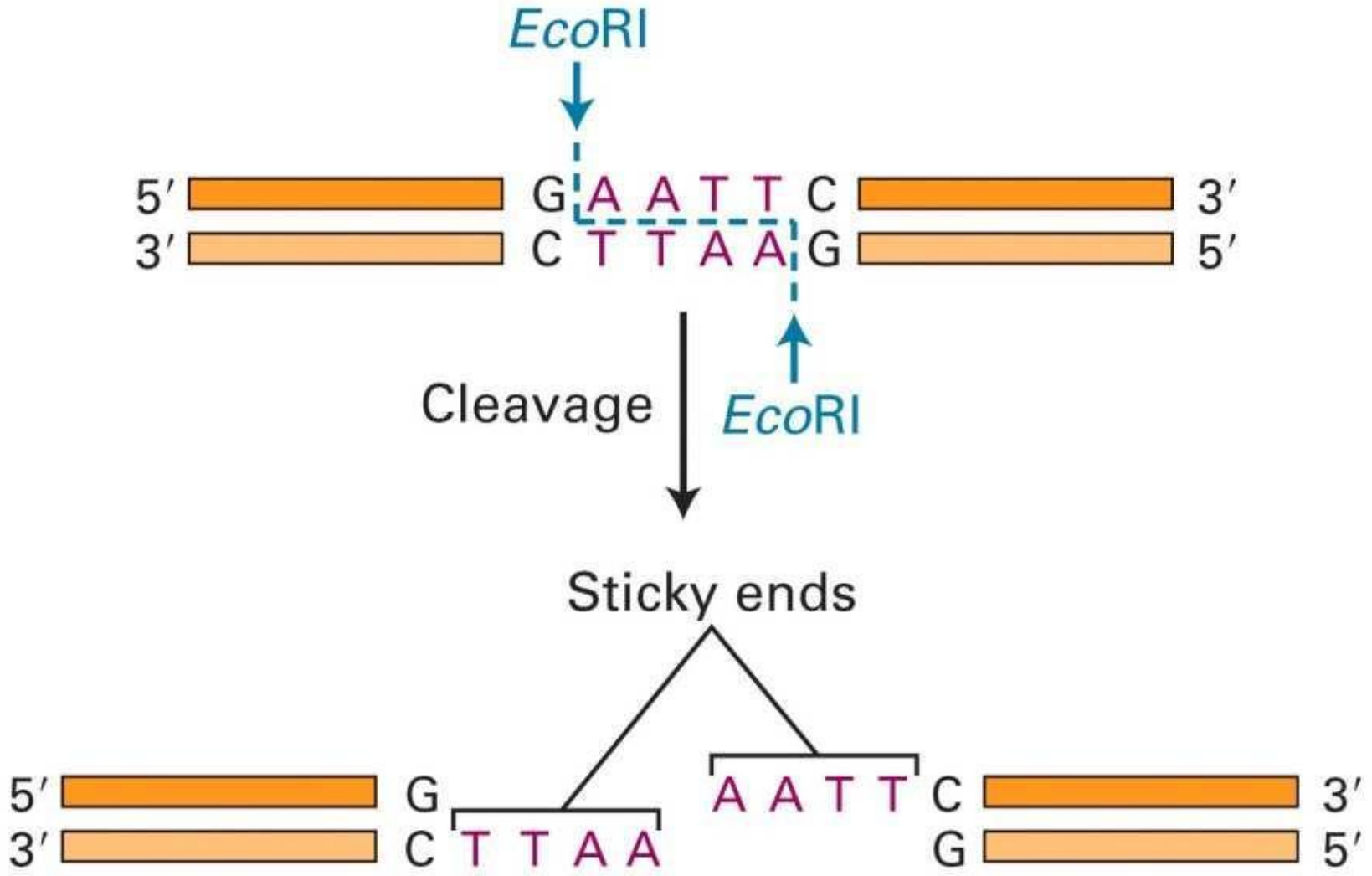


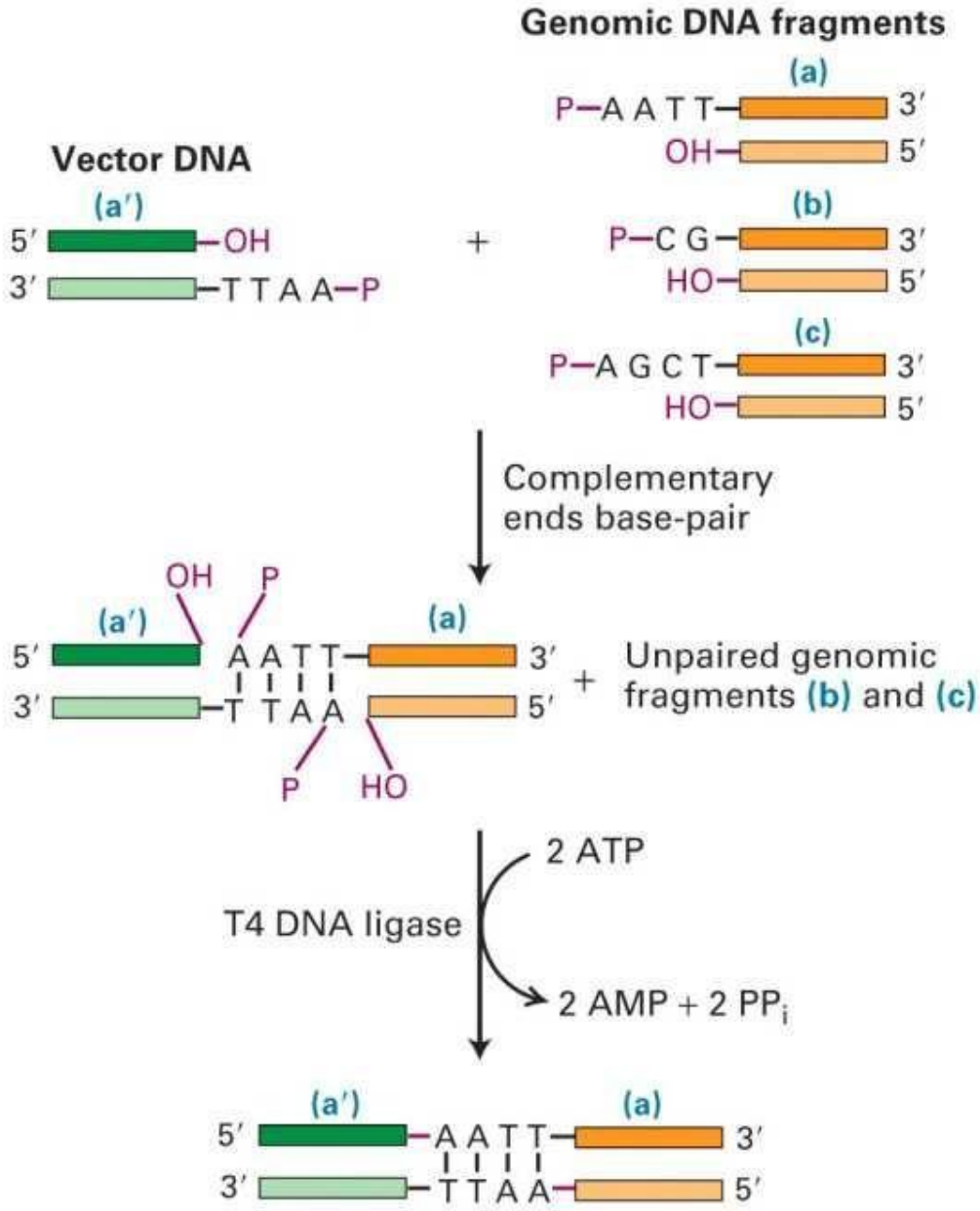
Construction of a restriction map.



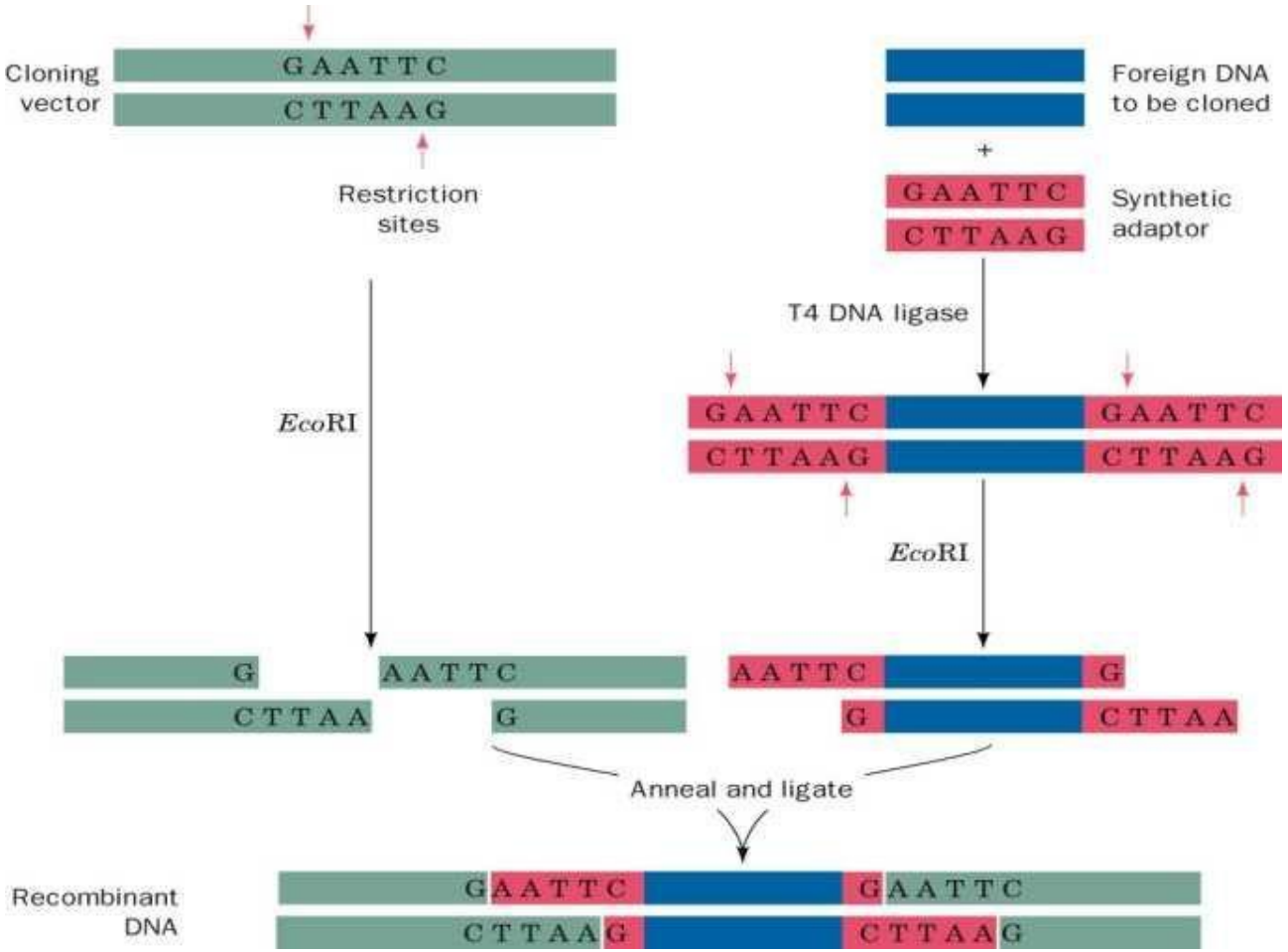
Restriction map for the 5243-bp circular DNA of SV40.

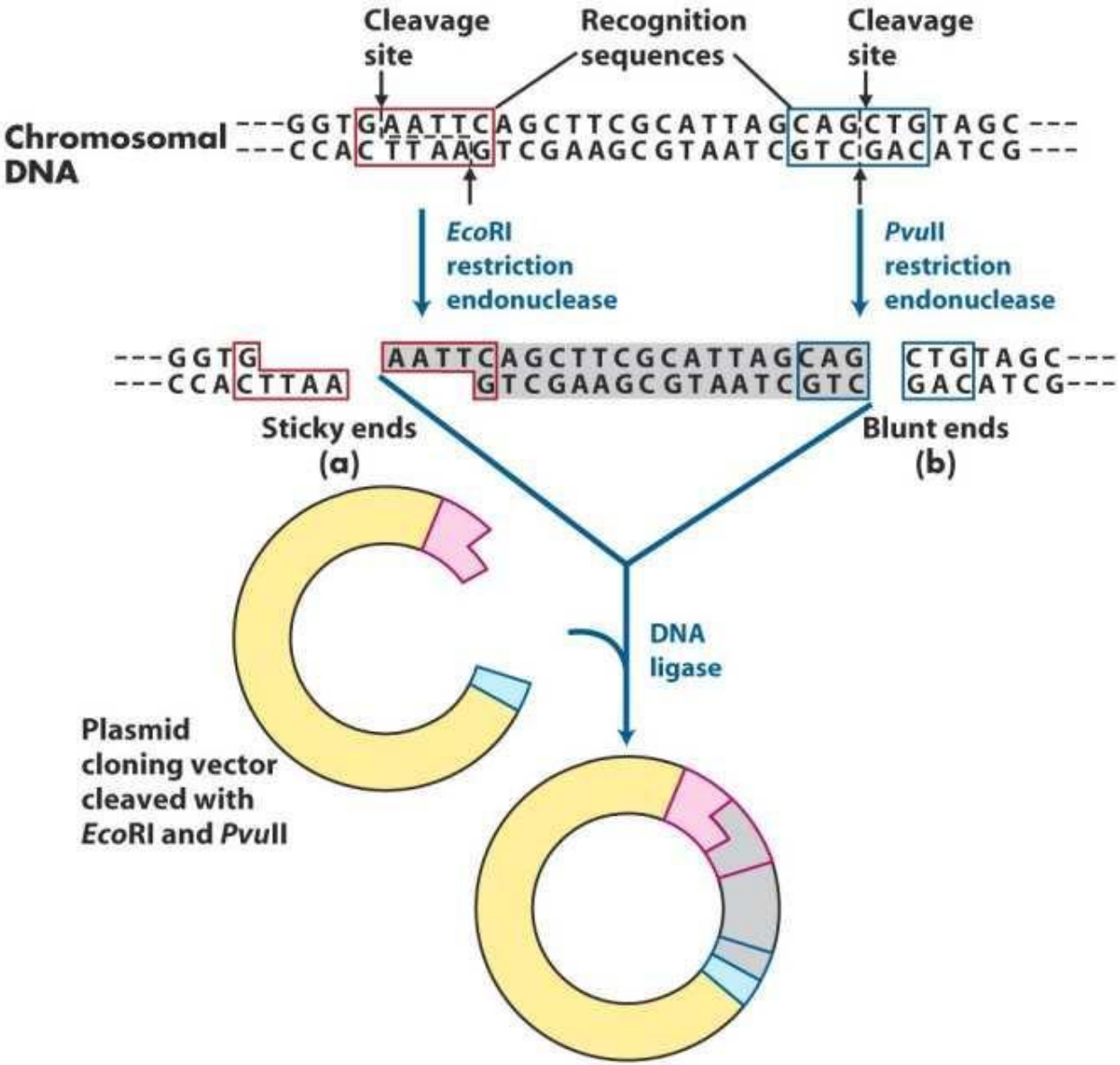



EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

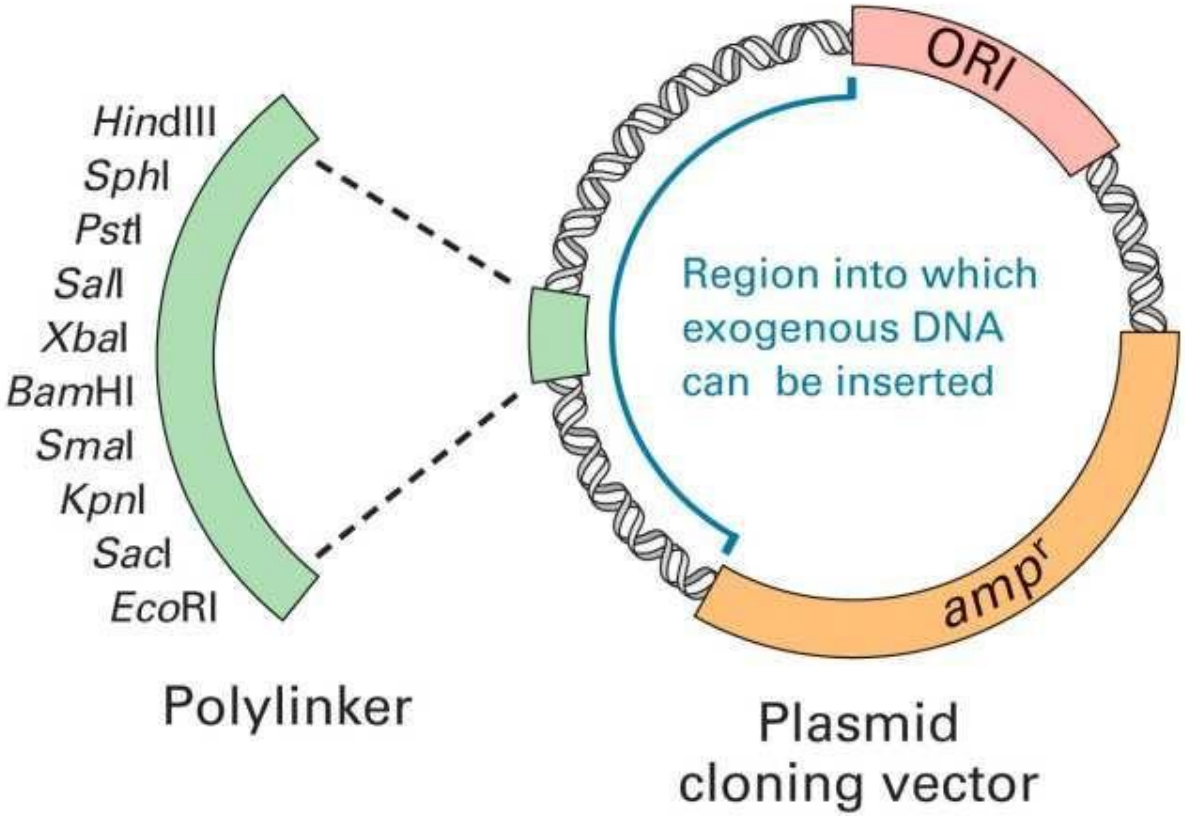

Genomic DNA fragments
(a)
P—A A T T—
3′
OH—
5′
Vector DNA
(a′)
5′
—OH
3′
—T T A A—P
+
(b)
P—C G—
3′
HO—
5′
(c)
P—A G C T—
3′
HO—
5′
Complementary ends base-pair
OH
P
(a′)
(a)
5′
A A T T—
3′
3′
—T T A A
5′
+
Unpaired genomic fragments (b) and (c)
P
HO
2 ATP
T4 DNA ligase
2 AMP + 2 PPi
(a′)
(a)
5′
—A A T T—
3′
3′
—T T A A—
5′

Construction of a recombinant DNA molecule through the use of synthetic oligonucleotide adaptors




Cleavage site
Recognition sequences
Cleavage site
Chromosomal DNA
---GGTGAATTCAGCTTCGCATTAGCAGCTGTAGC---
---CCACTTAAGTCGAAGCGTAATCGTCGACATCG---
EcoRI restriction endonuclease
PvuII restriction endonuclease
---GGTG
---CCACTTAA
AATTCAGCTTCGCATTAGCAG
GTCGAAGCGTAATCGTC
CTGTAGC---
GACATCG---
Sticky ends
(a)
Blunt ends
(b)
DNA ligase
Plasmid cloning vector cleaved with EcoRI and PvuII

Plasmid Cloning Vectors



Plasmid Cloning Vectors

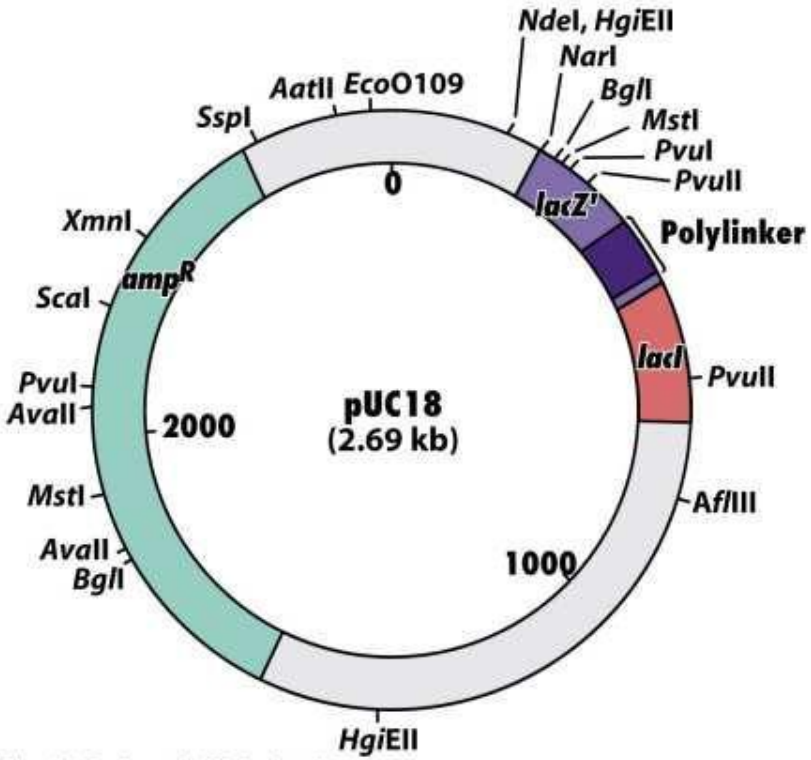
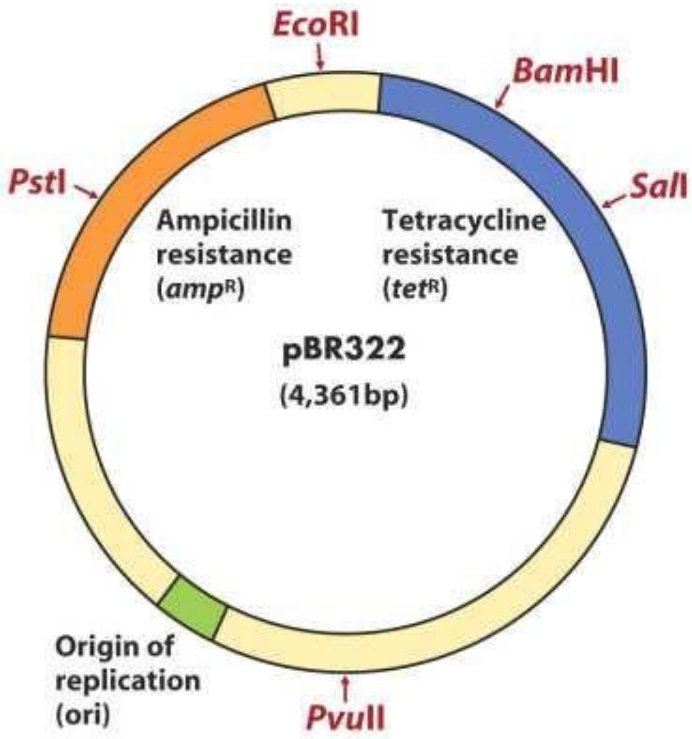


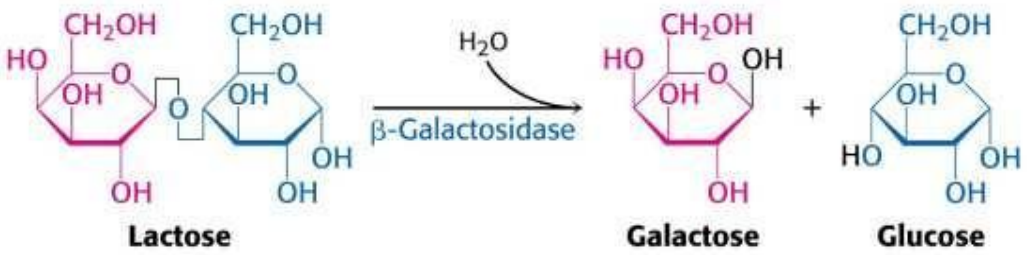
Figure 3-25 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

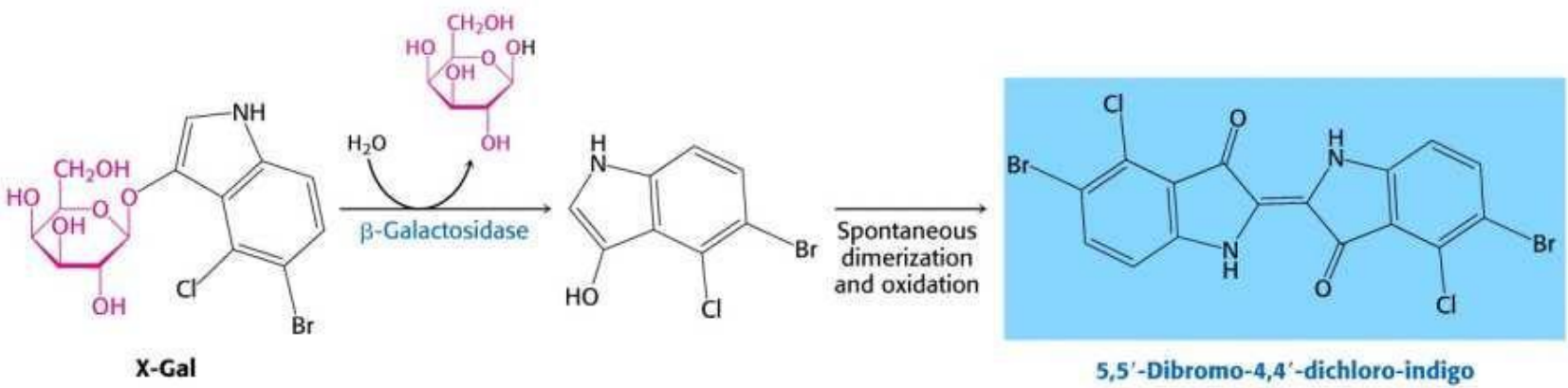


Insertional inactivation

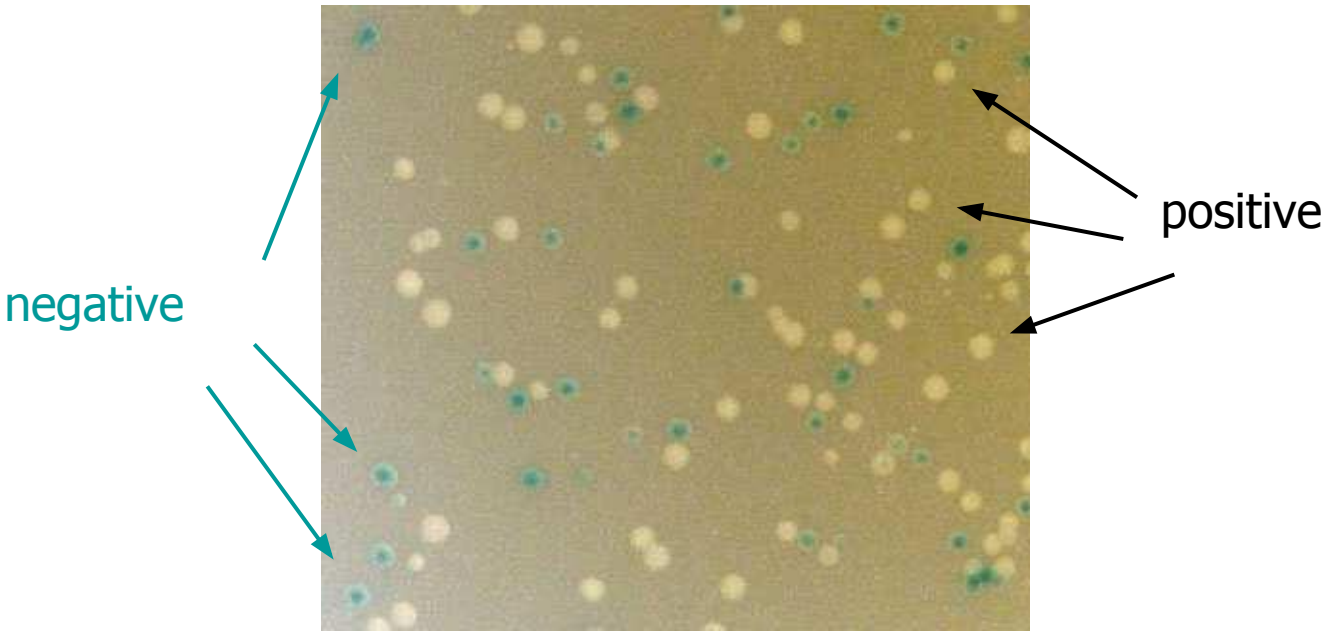
Gene in cloning site:

- *LacZ* -> pUC18 (*lacZ* complements the host defect in *lacZ*)
  -> pUC18 into host organism -> active *lacZ* (β-galactosidase) from plasmid-> cleavage of X-gal (blue colonies)
  -> gene cloned into polylinker -> *lacZ* gene disrupted -> no cleavage of X-gal (white colonies)





Blue/White Selection

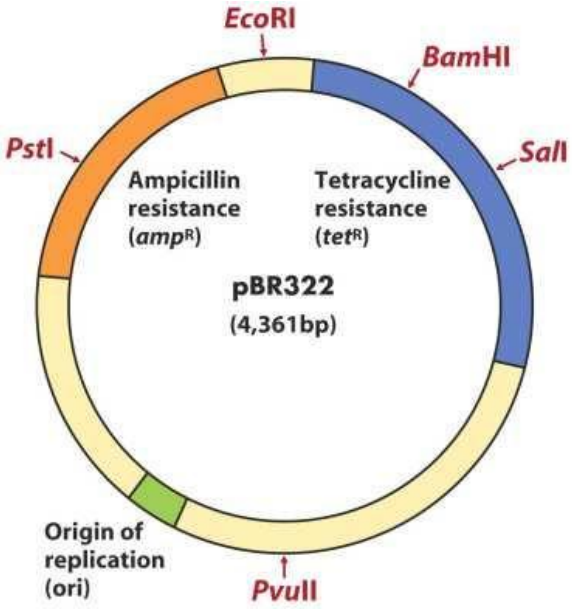


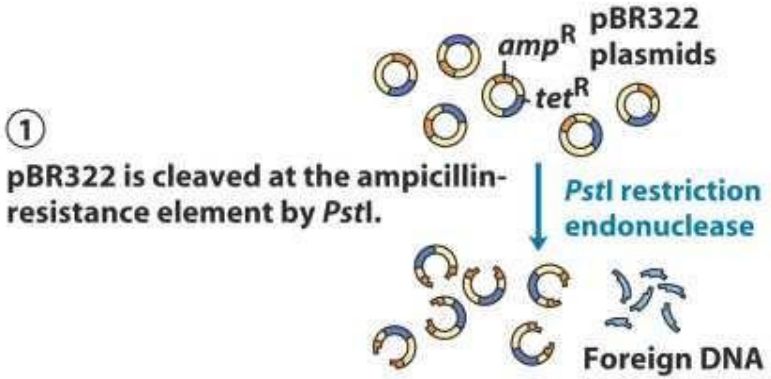
http://www.bio.davidson.edu/Courses/genomics/method/reporters.html

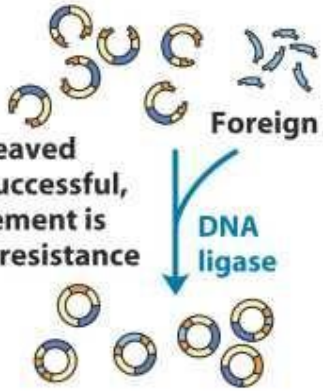
Insertional inactivation

Gene in cloning site:

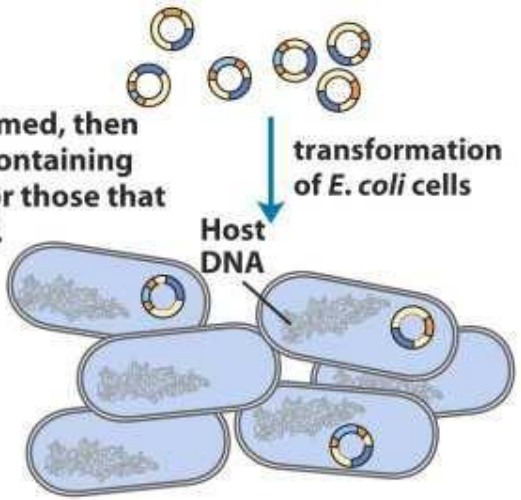
- Resistance marker -> pBR322 (cloning sites within antibiotica resistence marker)
  -> plasmid into host -> resistance against 2 antibiotica
  -> gene cloned within one resistance marker -> gene for antibiotica resistance marker disrupted -> sensitive against one antibioticum



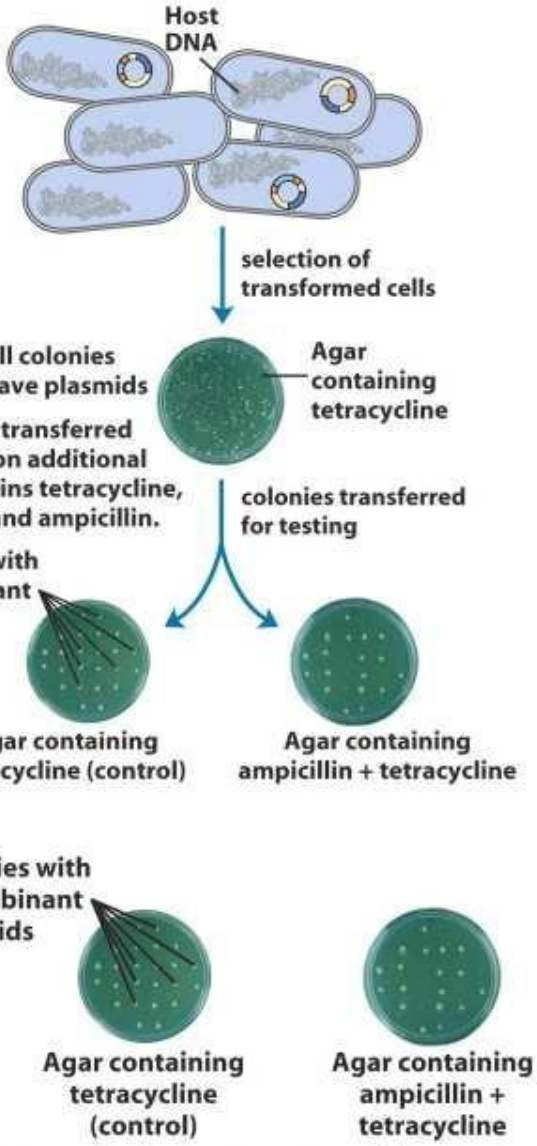




② Foreign DNA is ligated to cleaved pBR322. Where ligation is successful, the ampicillin-resistance element is disrupted. The tetracycline-resistance element remains intact.



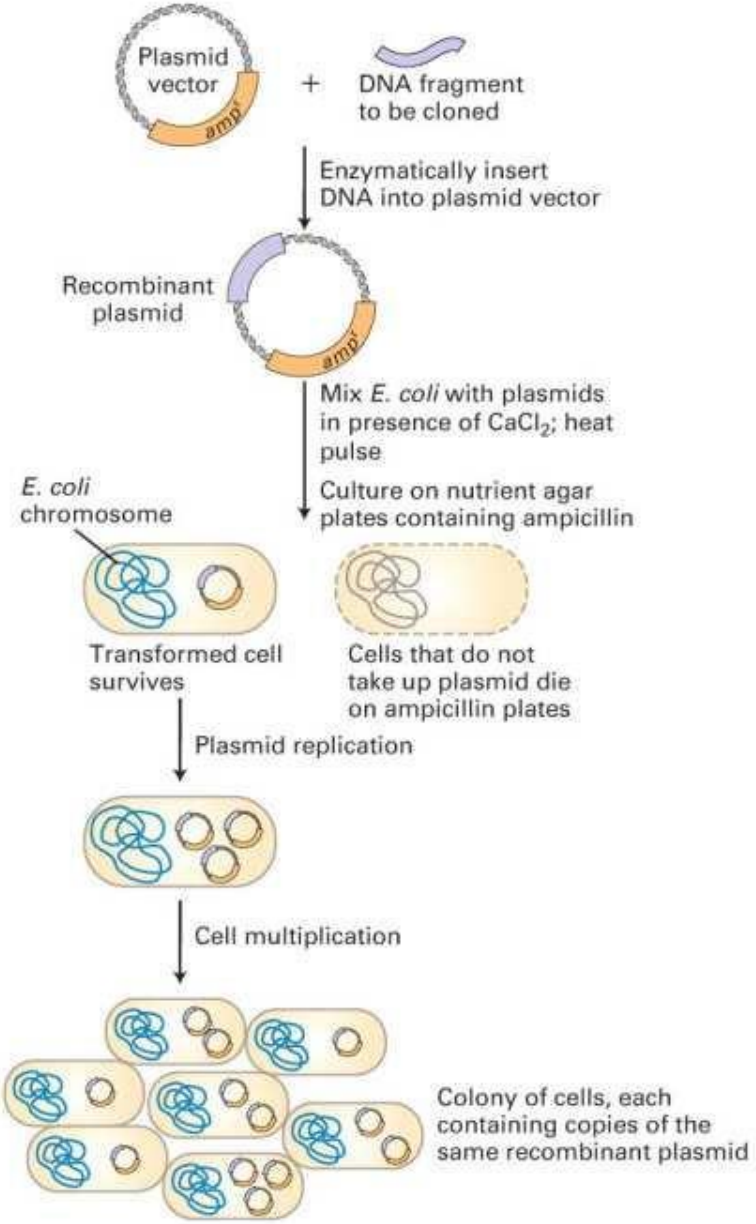
③ *E. coli* cells are transformed, then grown on agar plates containing tetracycline to select for those that have taken up plasmid.



④ Individual colonies are transferred to matching positions on additional plates. One plate contains tetracycline, the other tetracycline and ampicillin.

⑤ Cells that grow on tetracycline but not on tetracycline + ampicillin contain recombinant plasmids with disrupted ampicillin resistance, hence the foreign DNA. Cells with pBR322 without foreign DNA retain ampicillin resistance and grow on both plates.

Transformation and Selection



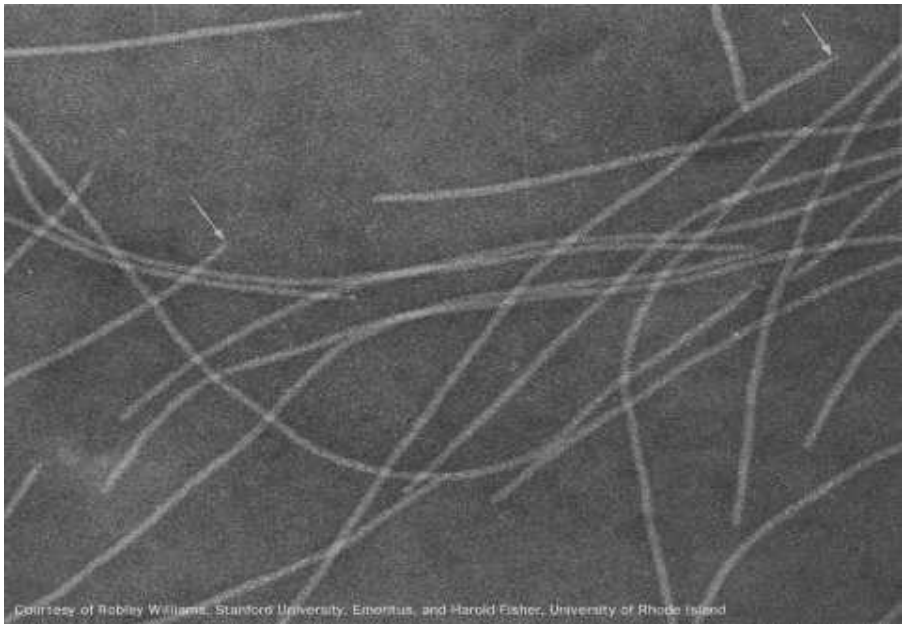
Horizontal gene transfer

- Transformation -> uptake of naked DNA (chemical transformation, electroporation)
- Conjugation -> DNA transfer by cell – cell contact
- Transduction -> DNA transfer by bacteriopage infection

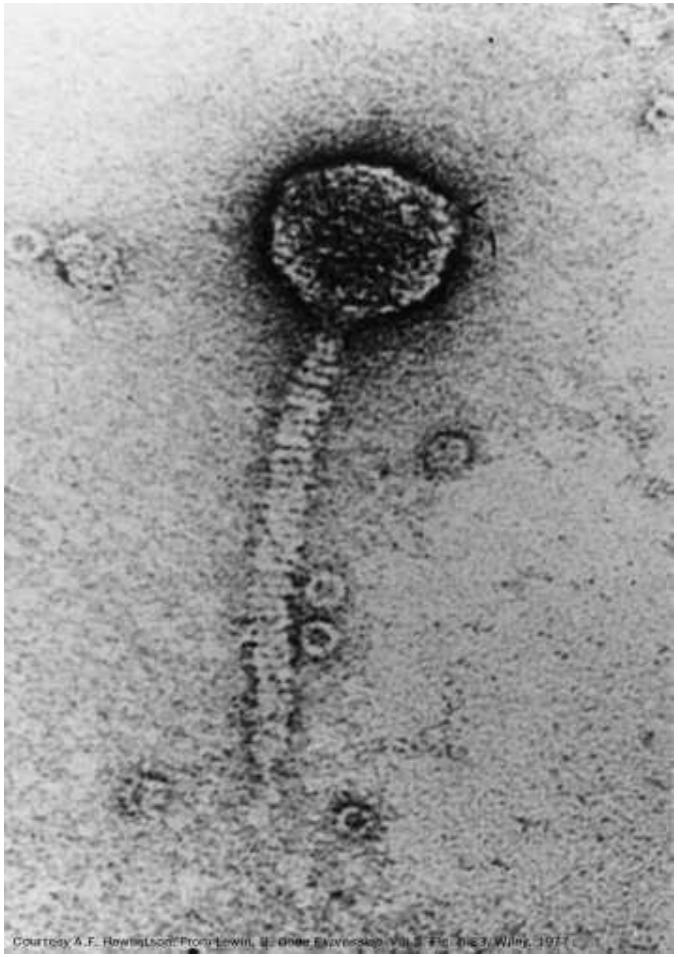
Other methods of Gene transfer -> used with fungi, animal and plant cells:

- Microinjection
- protoplasts

Bacteriophages

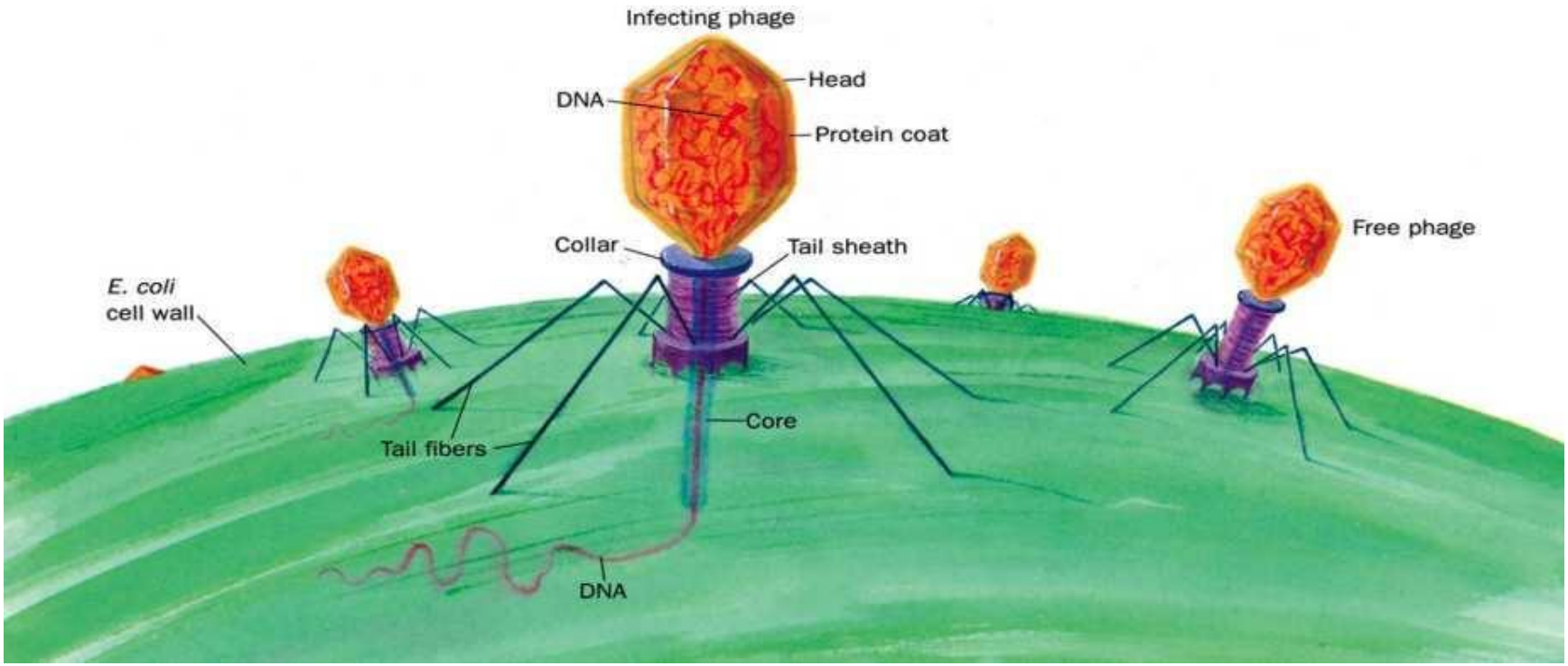


Electron micrograph of the filamentous bacteriophage M13.

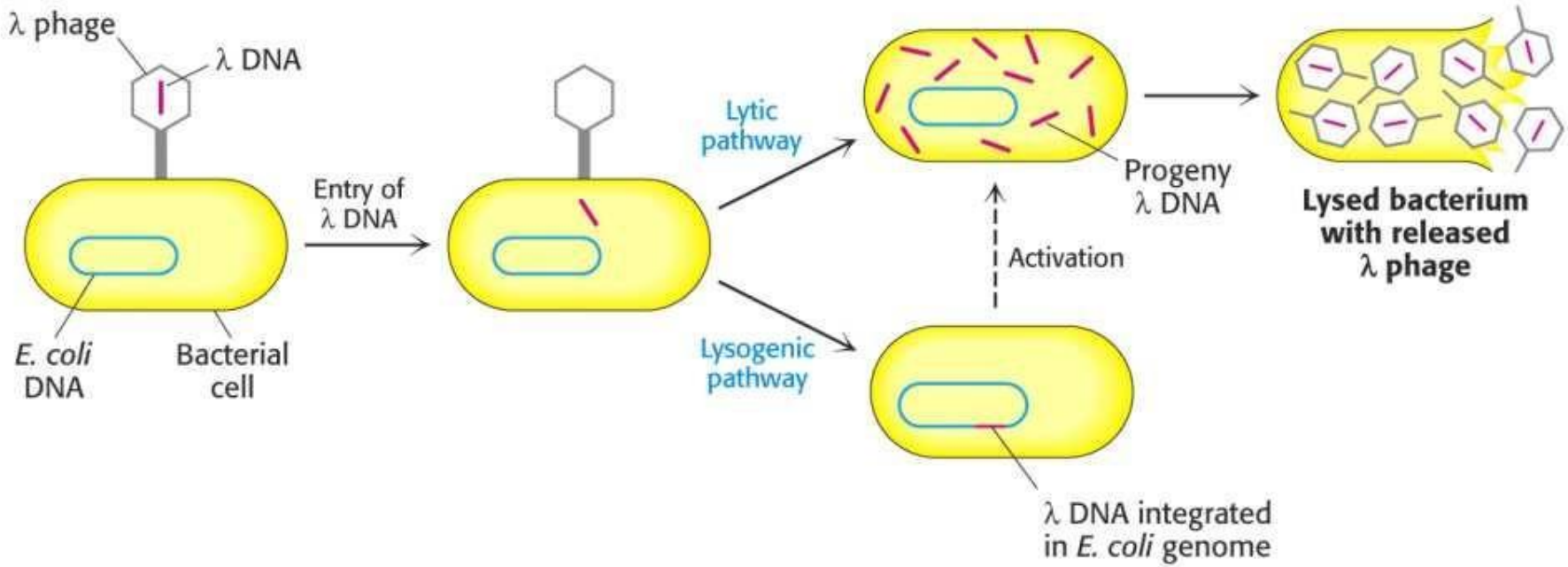


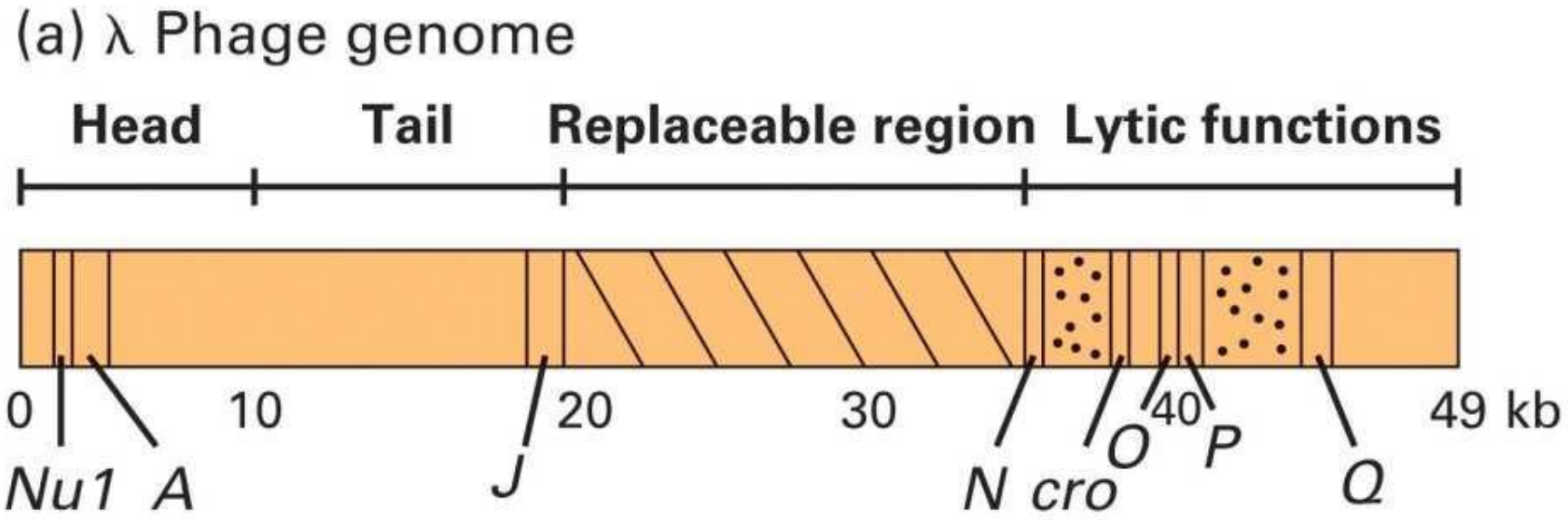
Electron micrograph of bacteriophage λ.

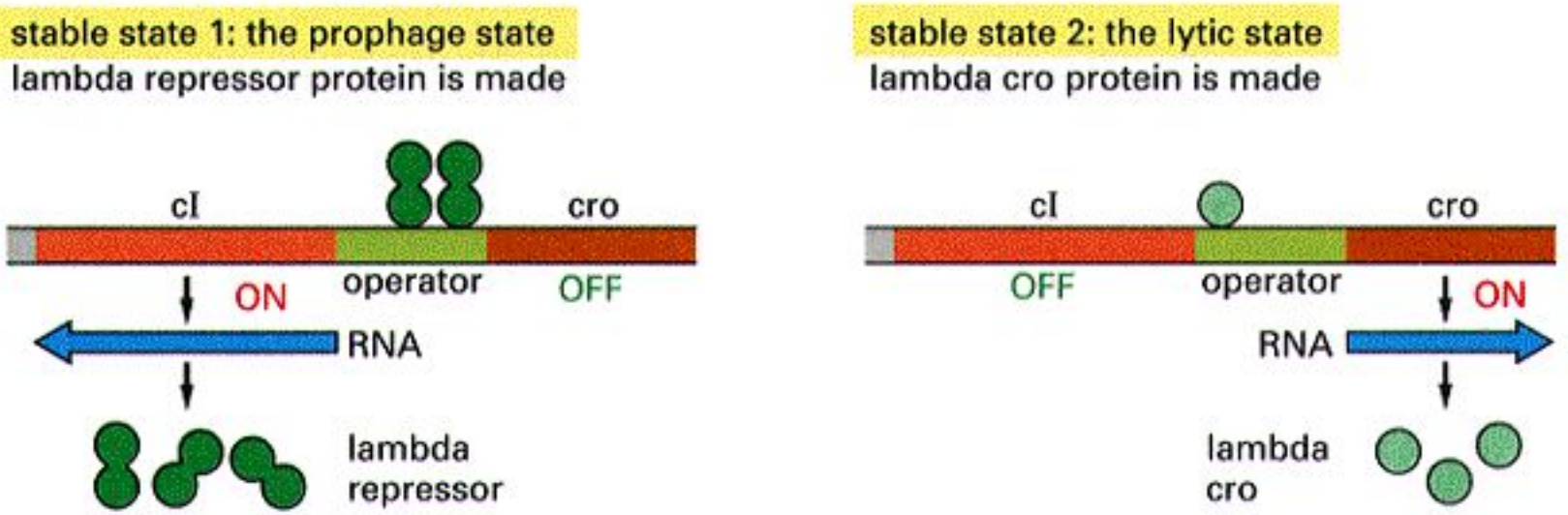
Bacteriophage T2 injecting its DNA into an *E. coli*



Life Cycle of Bacteriophage




(a) λ Phage genome
Head
Tail
Replaceable region
Lytic functions
0
10
20
30
40
49 kb
Nu1
A
J
N
cro
O
P
Q



Replication of bacteriophage upon infection of a cell

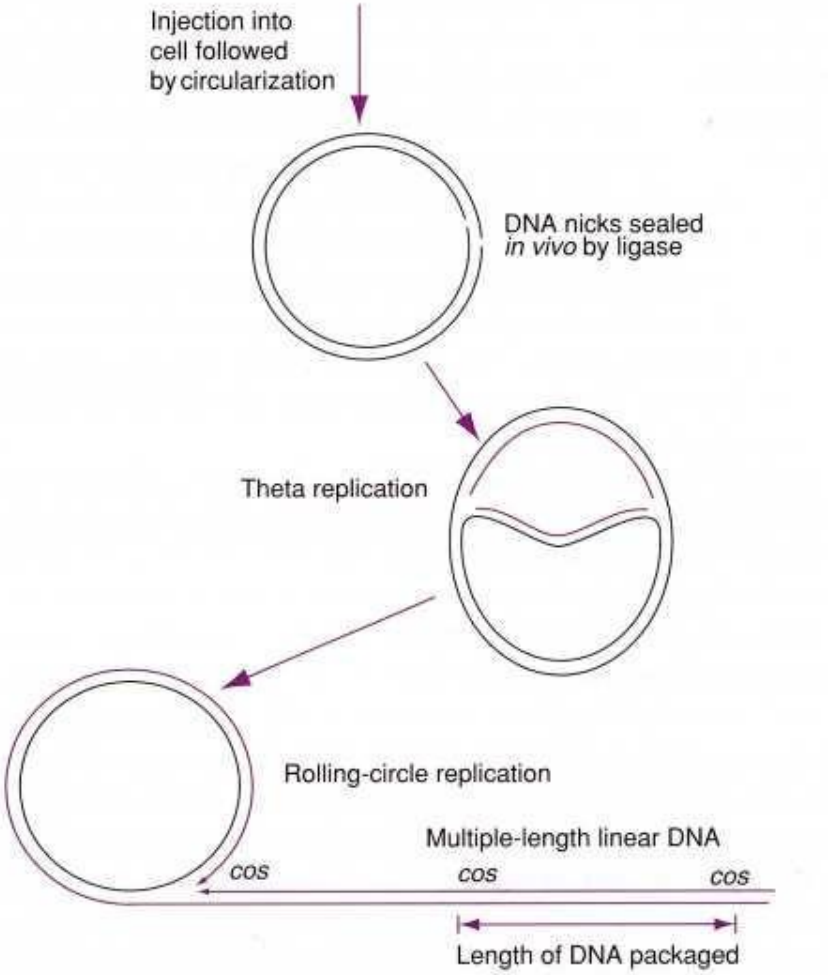
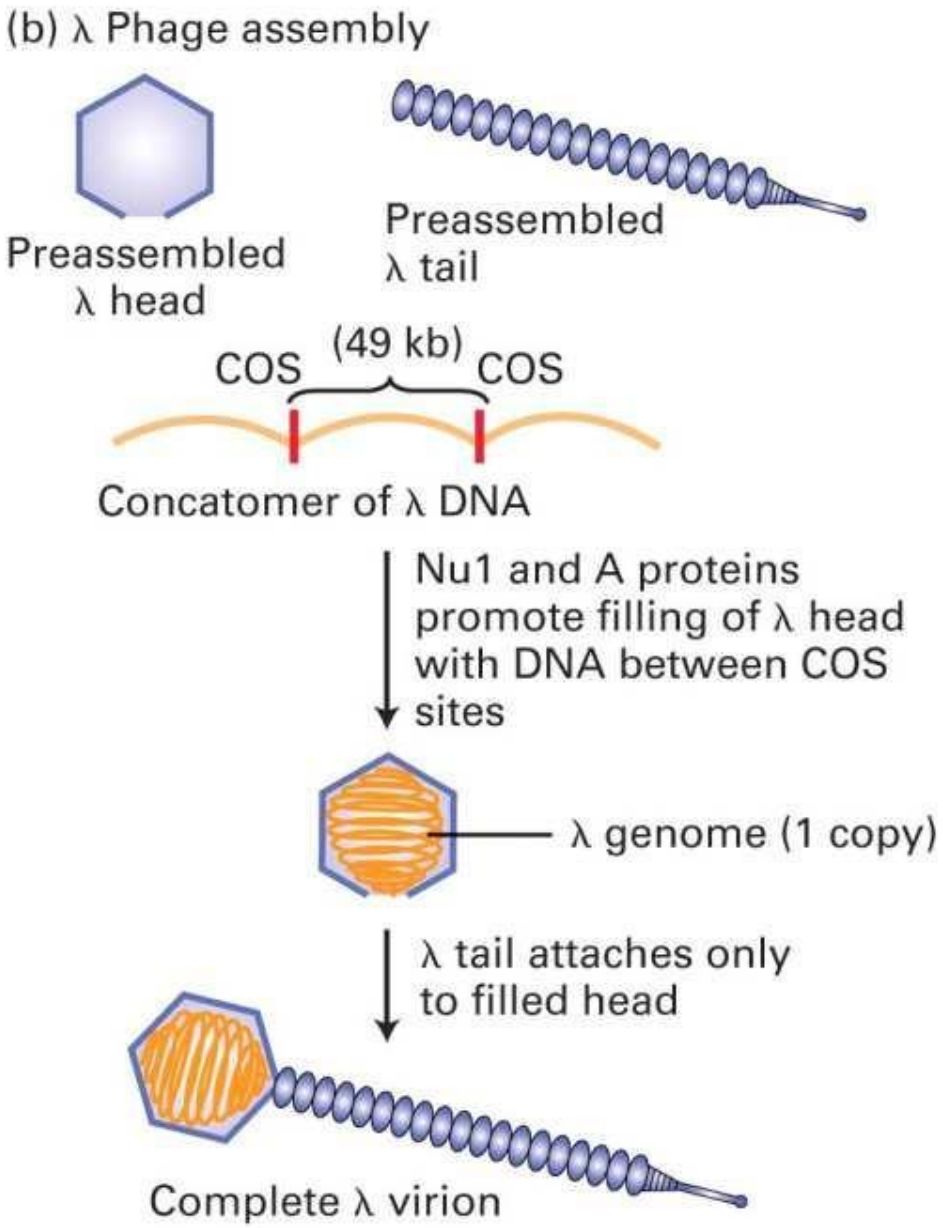
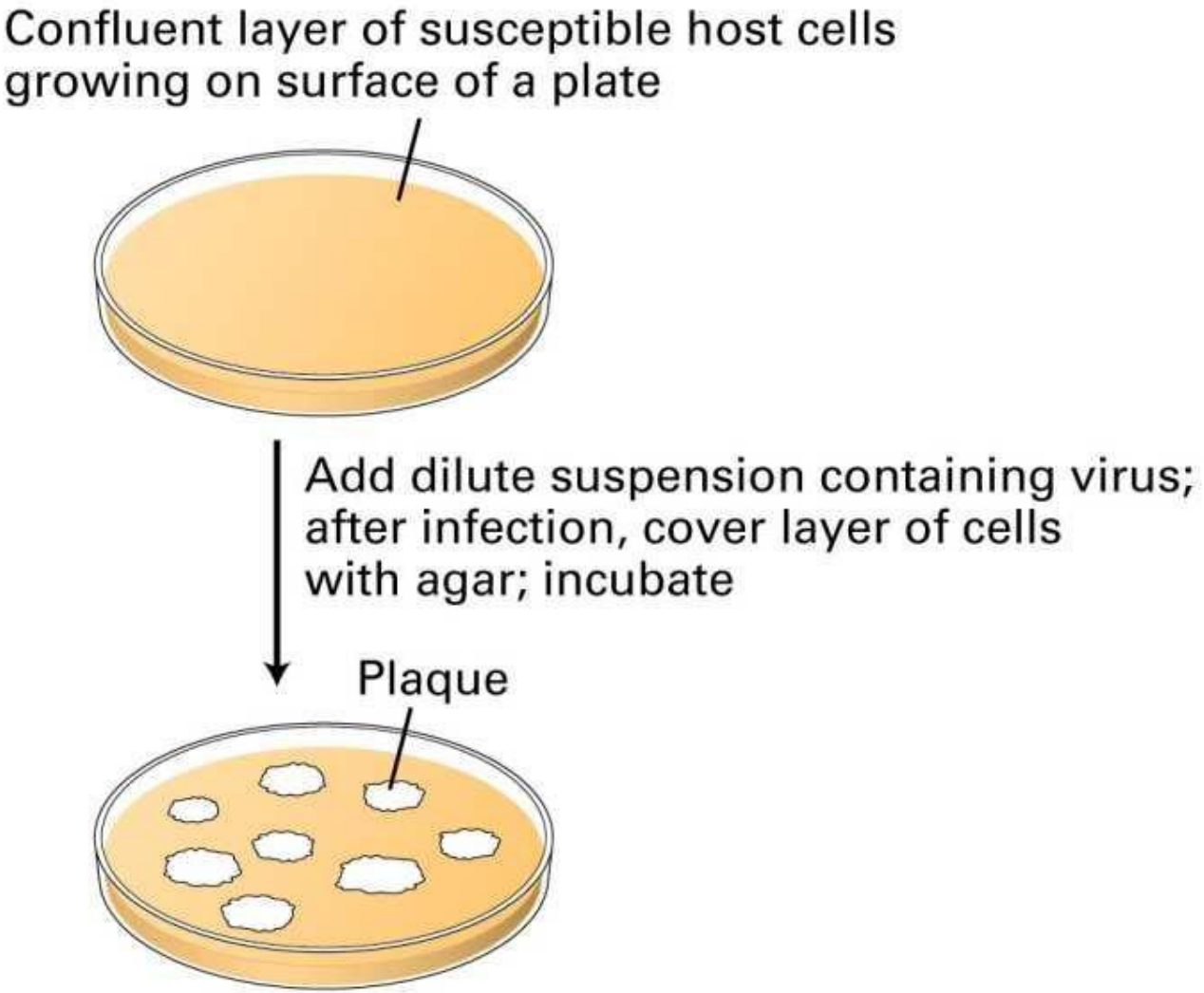


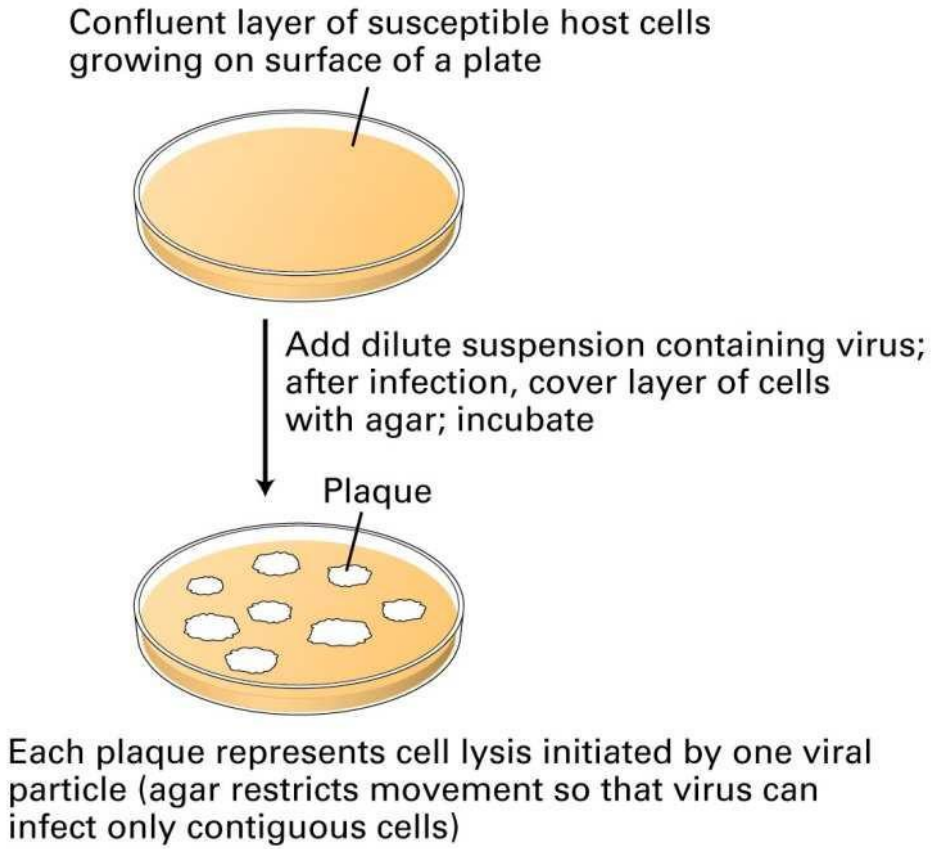
Figure 6.6 Replication of bacteriophage lambda DNA

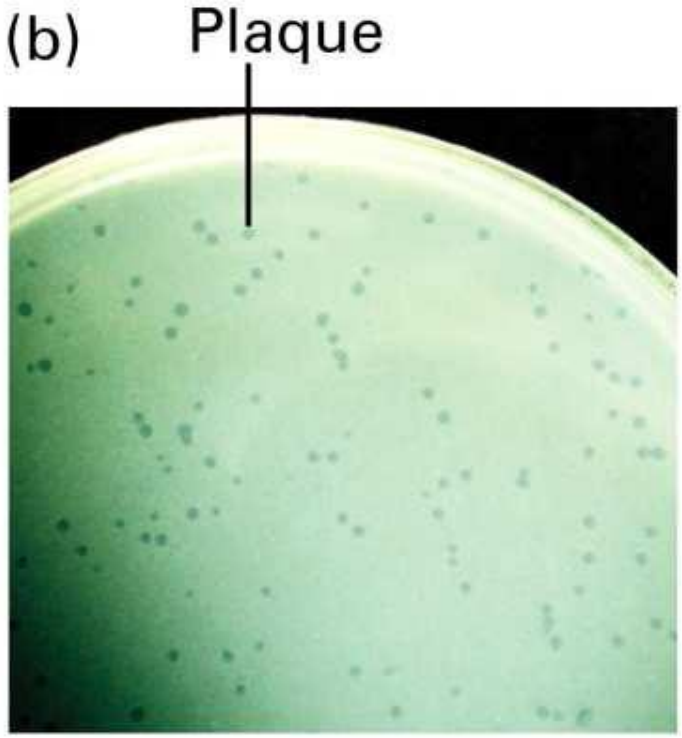

(b) λ Phage assembly
Preassembled λ head
Preassembled λ tail
COS
(49 kb)
COS
Concatomer of λ DNA
Nu1 and A proteins promote filling of λ head with DNA between COS sites
λ genome (1 copy)
λ tail attaches only to filled head
Complete λ virion



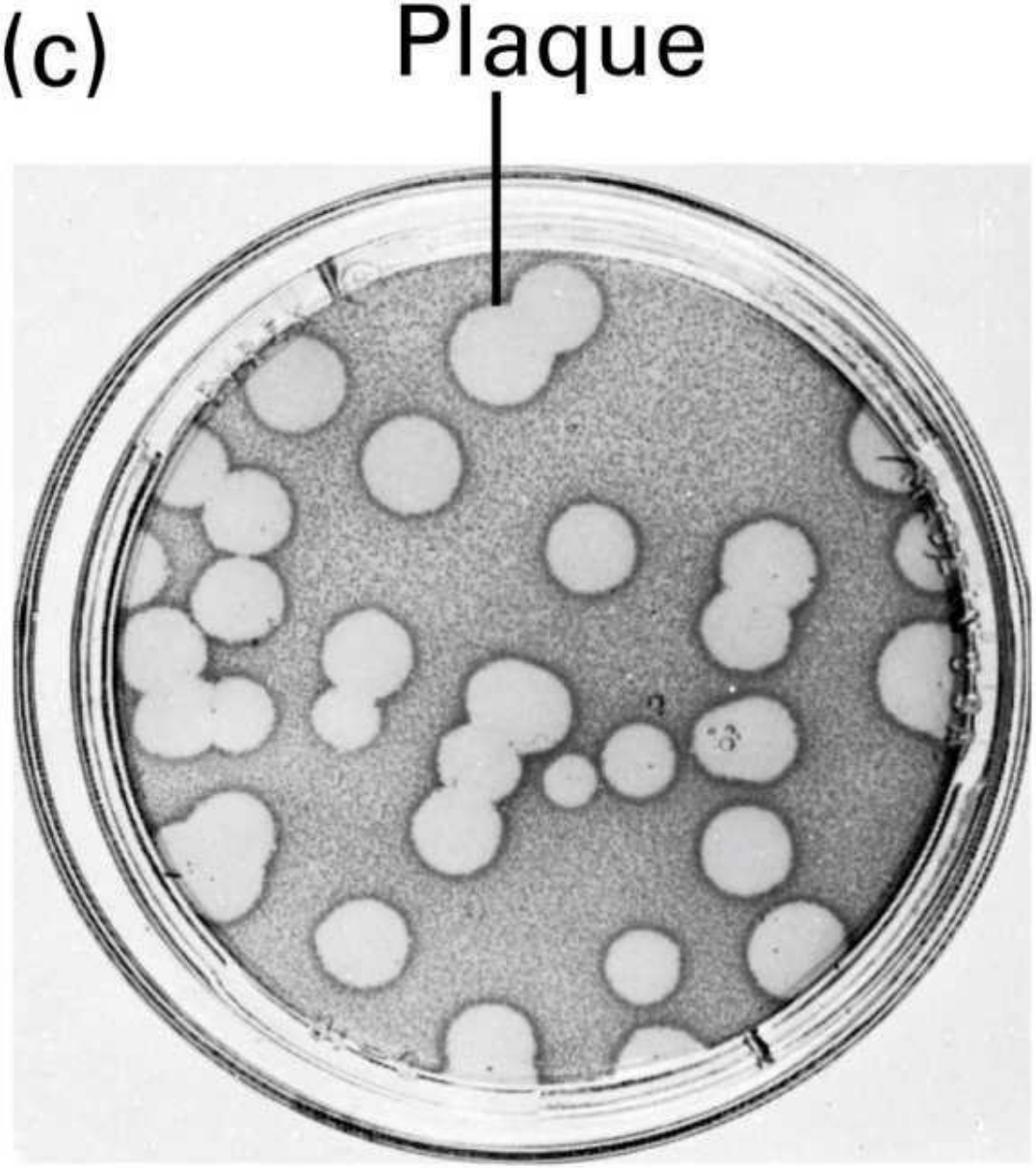
Each plaque represents cell lysis initiated by one viral particle (agar restricts movement so that virus can infect only contiguous cells)

Molecular genetics and bacteriophage

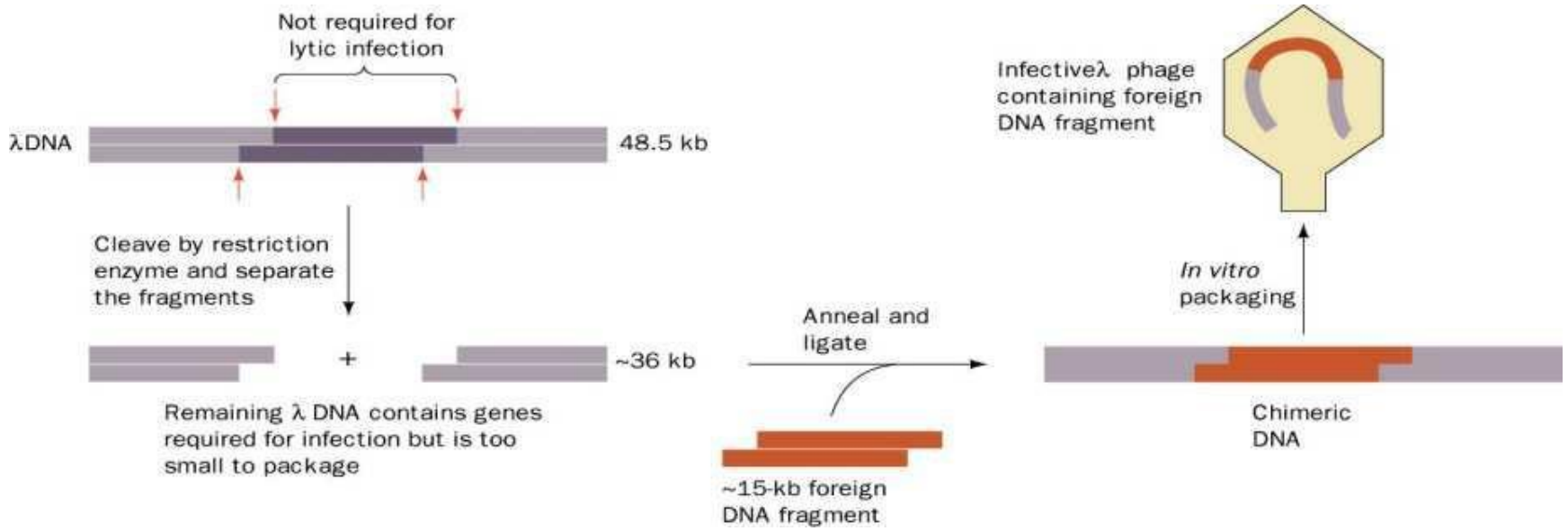




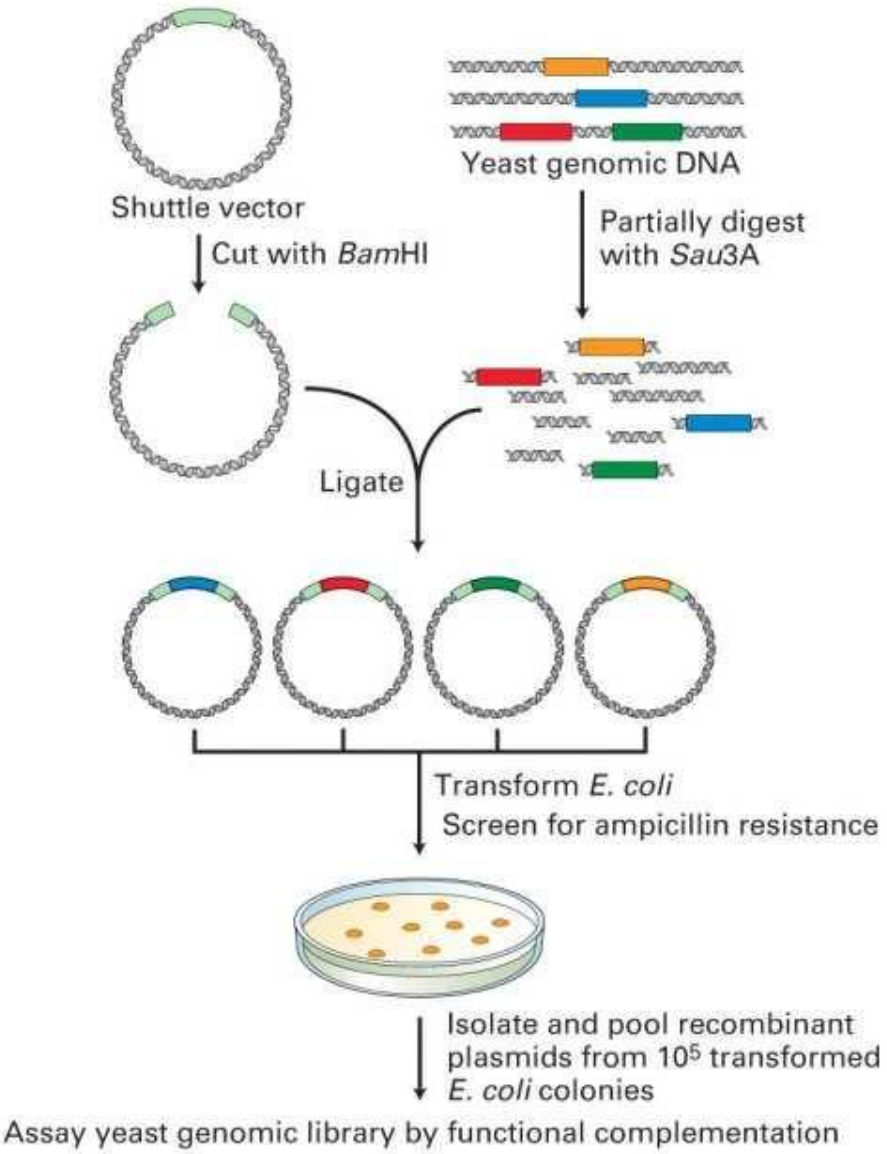
(c)



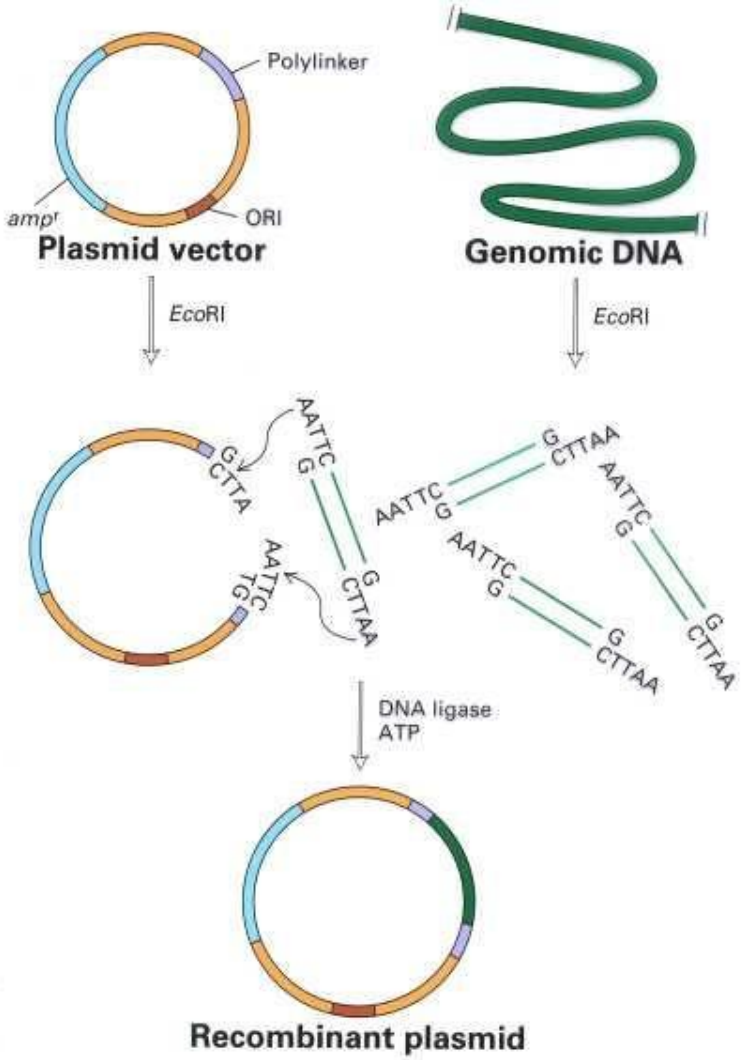
Cloning of foreign DNA in λ phages.



What is a gene library ?



Creation of Libraries



Creation of Libraries

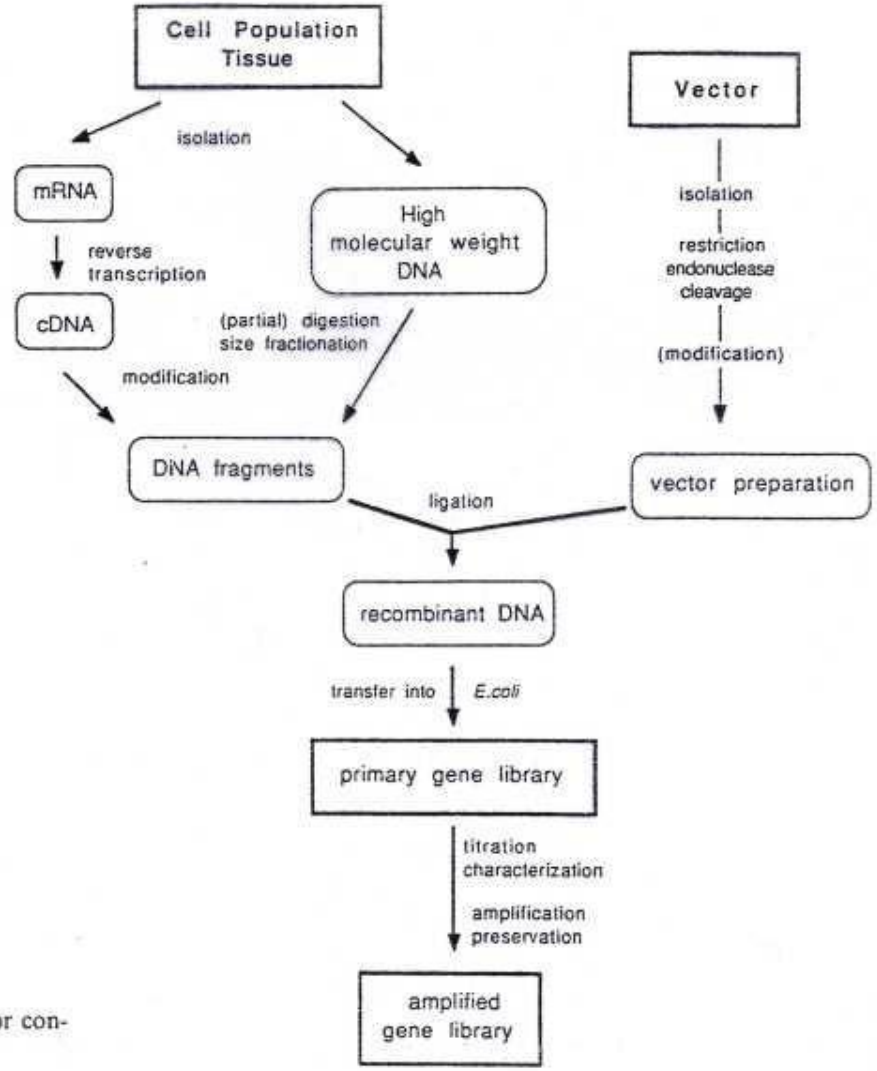


Fig. 6. General scheme for constructing gene libraries.

Sizes of Some DNA Molecules.

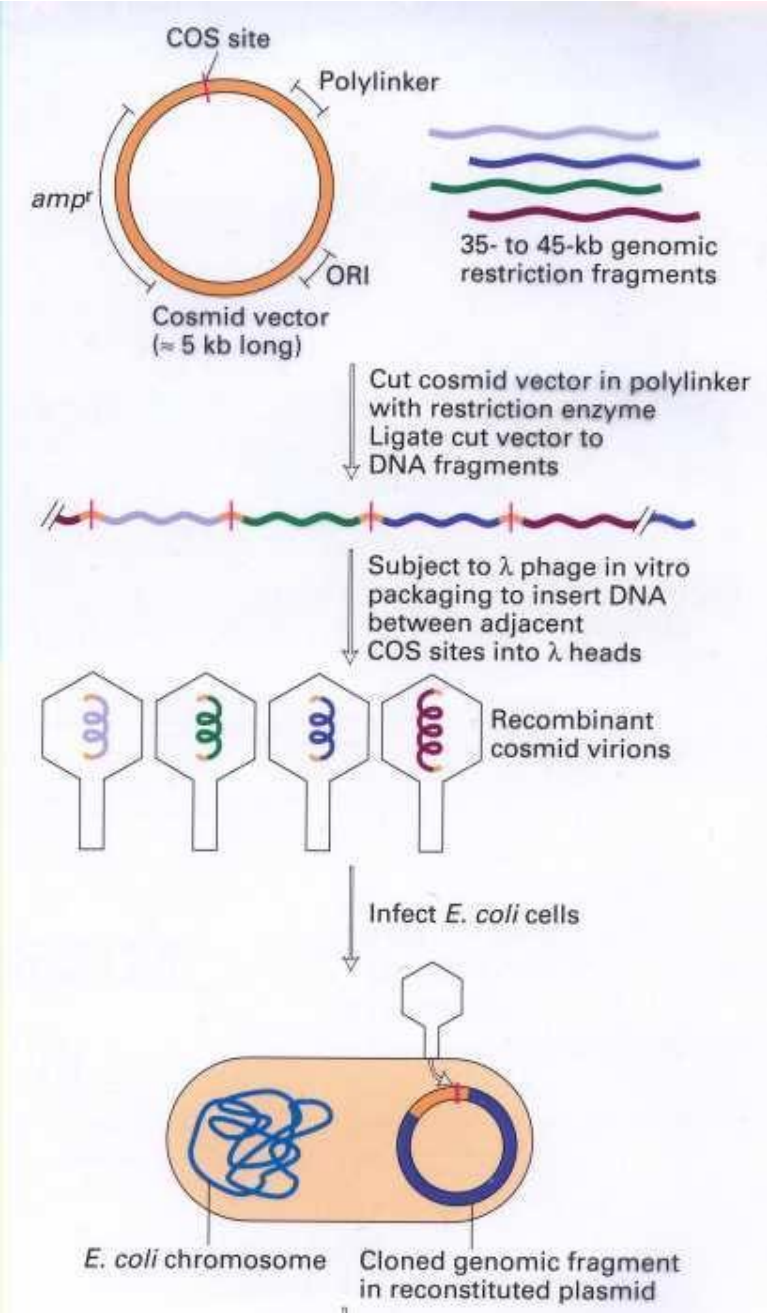
Organism	Number of base pairs (kb)[a]	Contour length (μm)
Viruses		
Polyoma, SV40	5.2	1.7
λ Bacteriophage	48.6	17
T2, T4, T6 bacteriophage	166	55
Fowlpox	280	193
Bacteria		
Mycoplasma hominis	760	260
Escherichia coli	4,600	1,600
Eukaryotes		
Yeast (in 17 haploid chromosomes)	12,000	4,100
Drosophila (in 4 haploid chromosomes)	180,000	61,000
Human (in 23 haploid chromosomes)	3,200,000	1,100,000
Lungfish (in 19 haploid chromosomes)	102,000,000	35,000,000

[a]kb = kilobase pair = 1000 base pairs (bp).

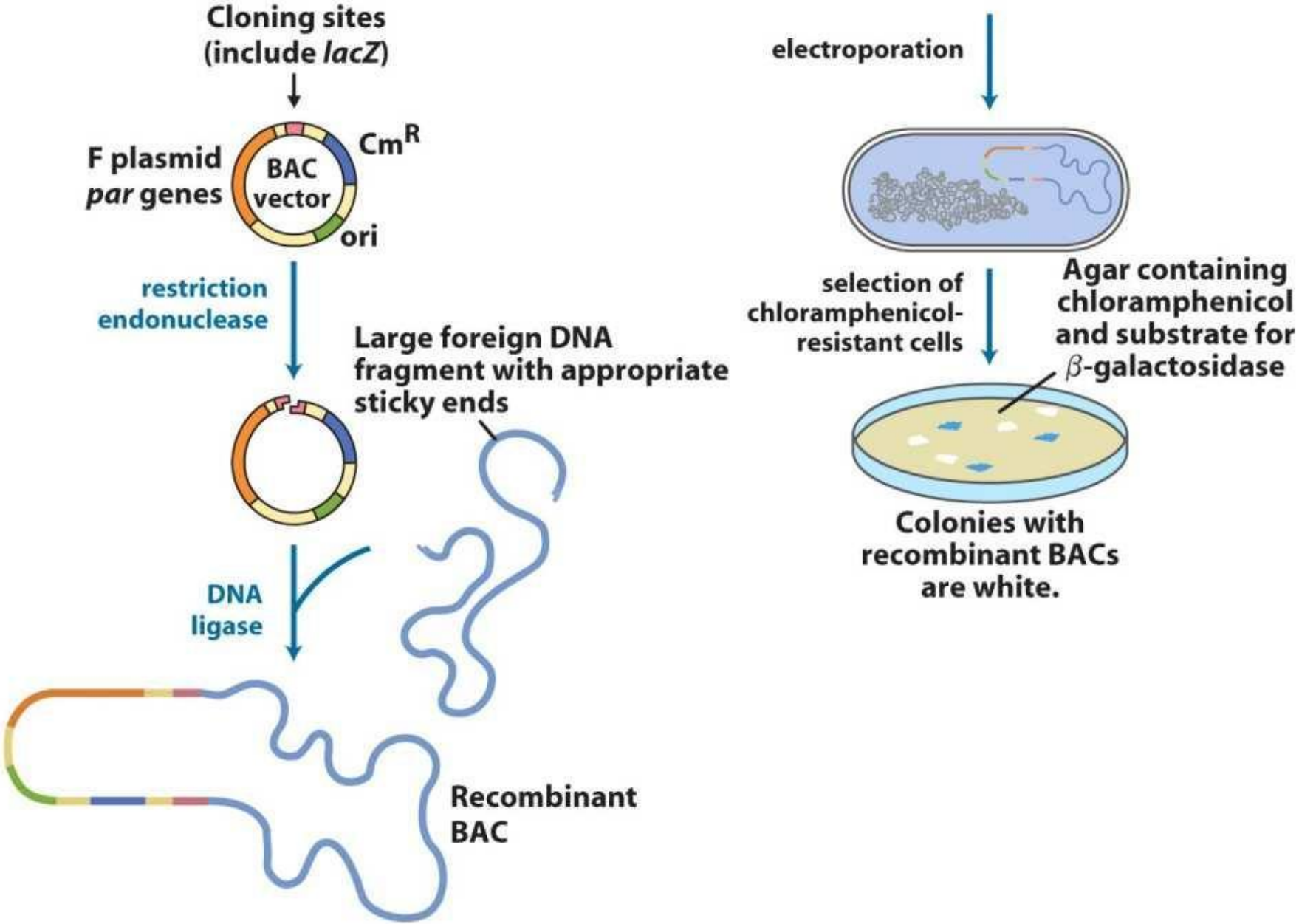
Source: Mainly Kornberg, A. and Baker, T.A., *DNA Replication* (2nd ed.), *p.* 20, Freeman (1992).

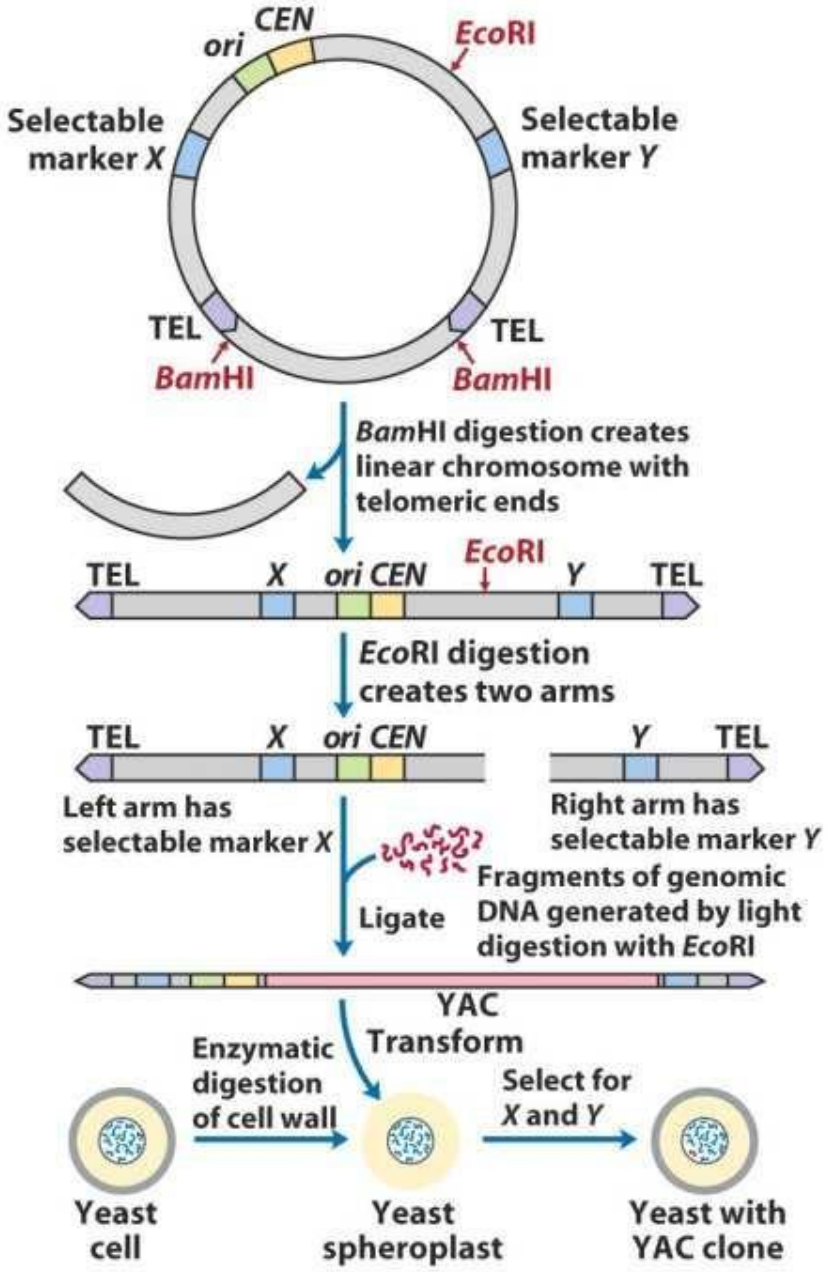
Table 4.6 Insert capacities of some commonly used vector systems

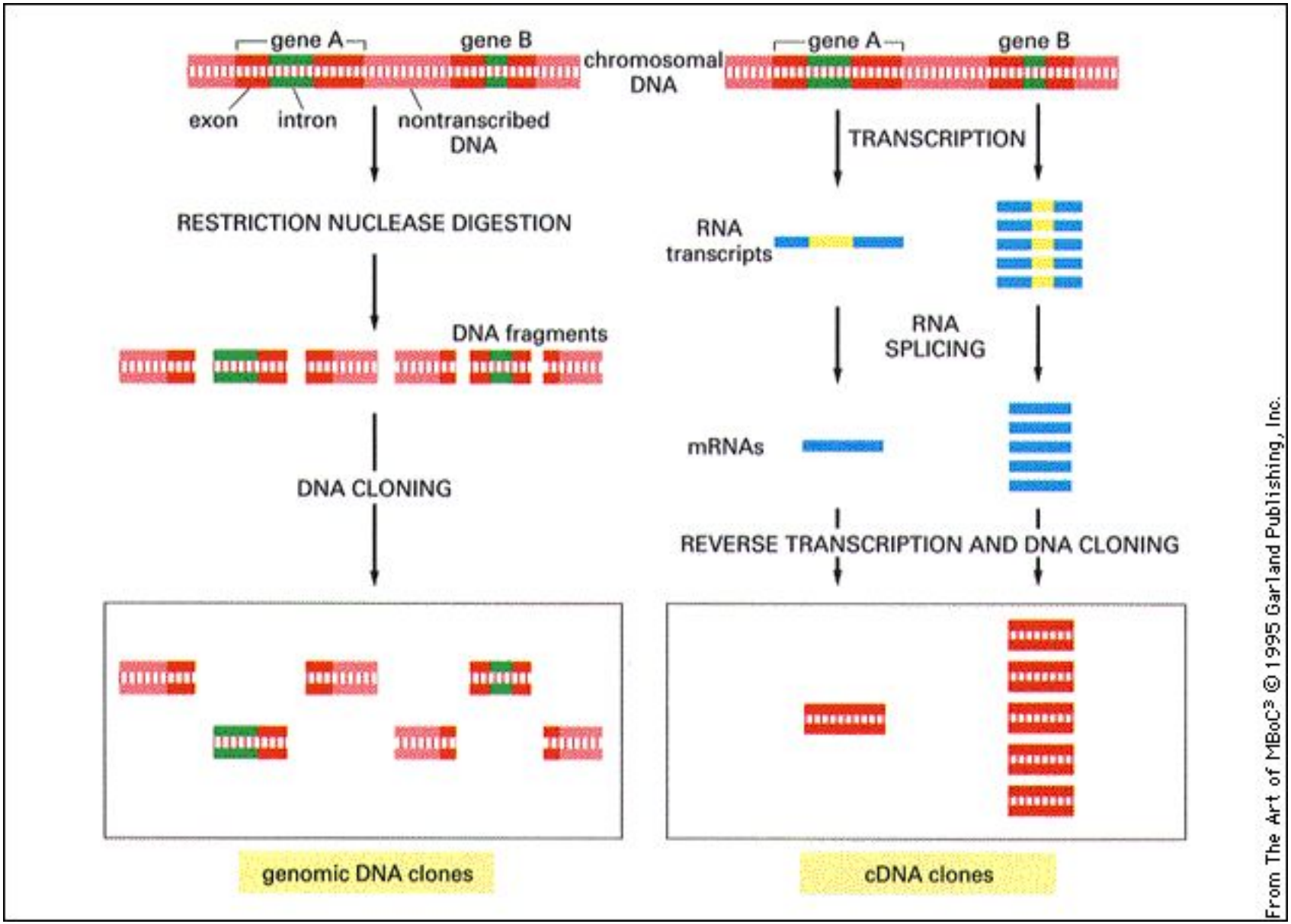
Vector system	Host cell	Insert capacity (kb)
Plasmid	*E. coli*	0.1–10
Bacteriophage λ	λ/*E. coli*	10–20
Cosmid	*E. coli*	35–45
Bacteriophage P1	*E. coli*	80–100
BAC	*E. coli*	50–300
P1 bacteriophage-derived artificial chromosome	*E. coli*	100–300
Yeast artificial chromosome	Yeast	100–2,000
Human artificial chromosome	Cultured human cells	>2,000



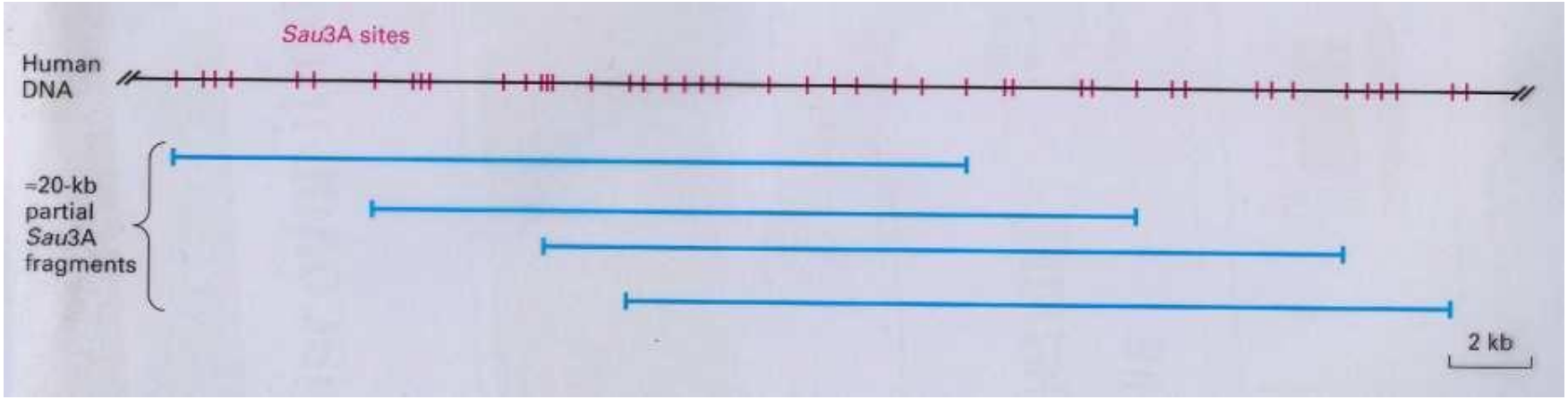
Cosmid = Cos - Plasmid

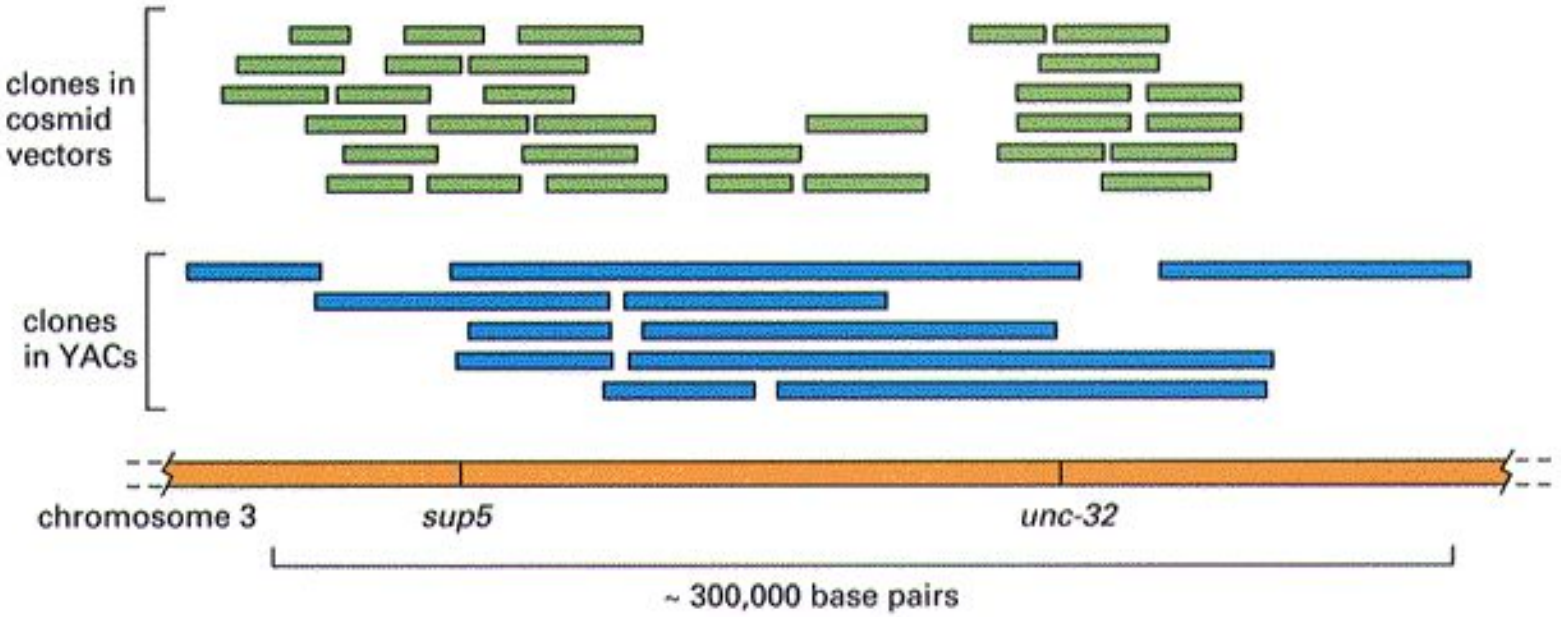

Cloning sites
(include lacZ)
F plasmid
par genes
BAC
vector
Cm^R
ori
restriction
endonuclease
Large foreign DNA
fragment with appropriate
sticky ends
DNA
ligase
Recombinant
BAC
electroporation
selection of
chloramphenicol-
resistant cells
Agar containing
chloramphenicol
and substrate for
β-galactosidase
Colonies with
recombinant BACs
are white.


CEN
ori
EcoRI
Selectable marker X
Selectable marker Y
TEL
TEL
BamHI
BamHI
BamHI digestion creates linear chromosome with telomeric ends
TEL X ori CEN EcoRI Y TEL
EcoRI digestion creates two arms
TEL X ori CEN
Y TEL
Left arm has selectable marker X
Right arm has selectable marker Y
Fragments of genomic DNA generated by light digestion with EcoRI
Ligate
YAC
Transform
Enzymatic digestion of cell wall
Select for X and Y
Yeast cell
Yeast spheroplast
Yeast with YAC clone

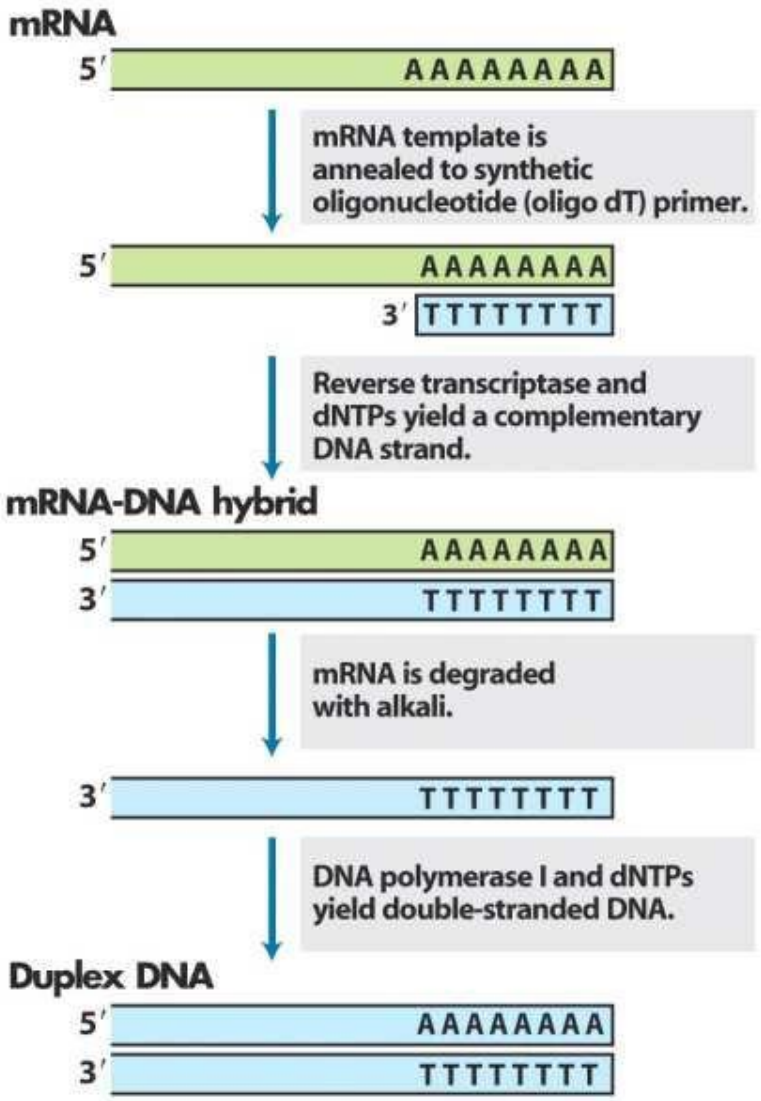

gene A
gene B
chromosomal DNA
exon
intron
nontranscribed DNA
TRANSCRIPTION
RESTRICTION NUCLEASE DIGESTION
RNA transcripts
DNA fragments
RNA SPLICING
mRNAs
DNA CLONING
REVERSE TRANSCRIPTION AND DNA CLONING
genomic DNA clones
cDNA clones
From The Art of MBoC3 © 1995 Garland Publishing, Inc.

Fragmentation of genomic DNA

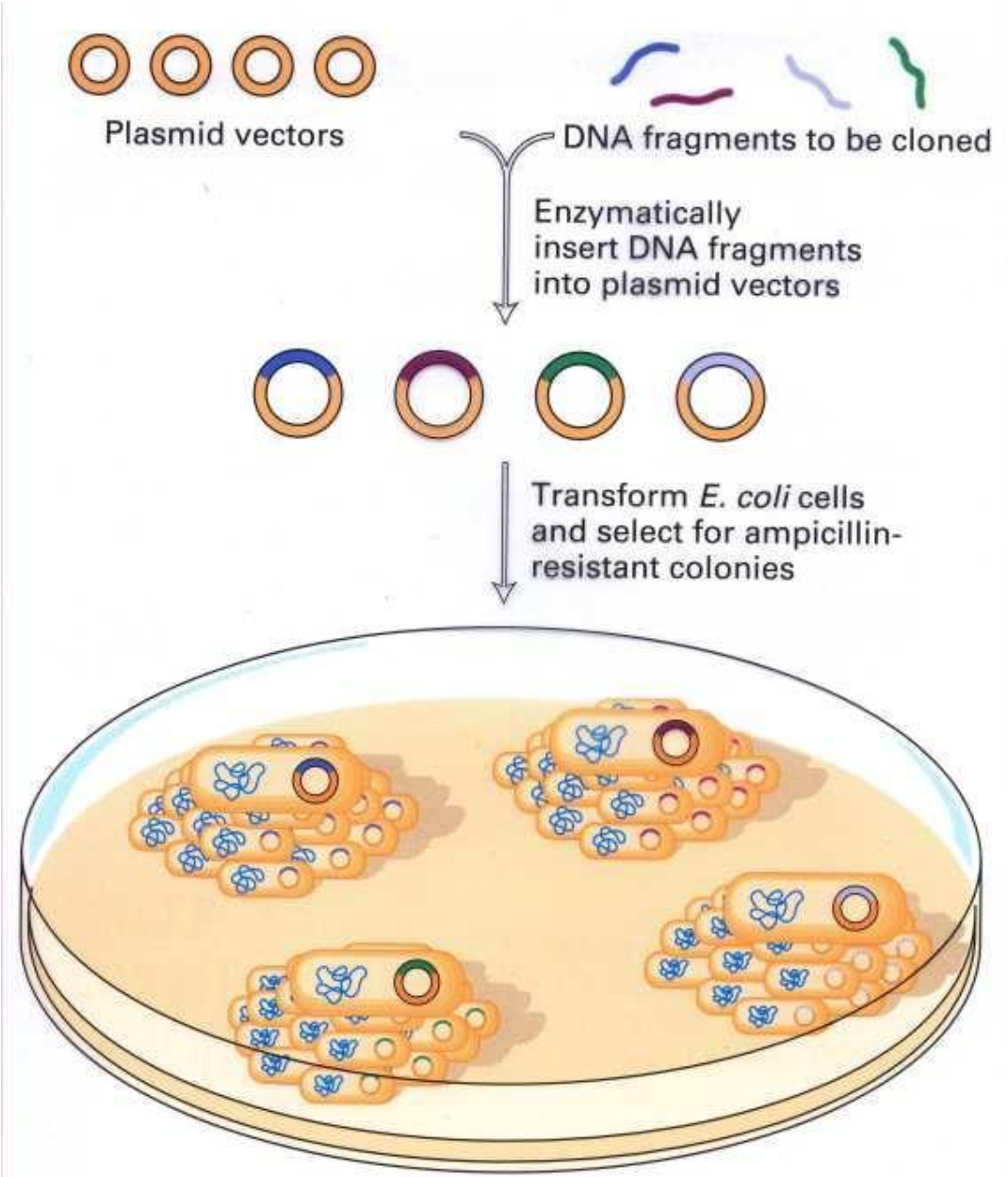




cDNA synthesis

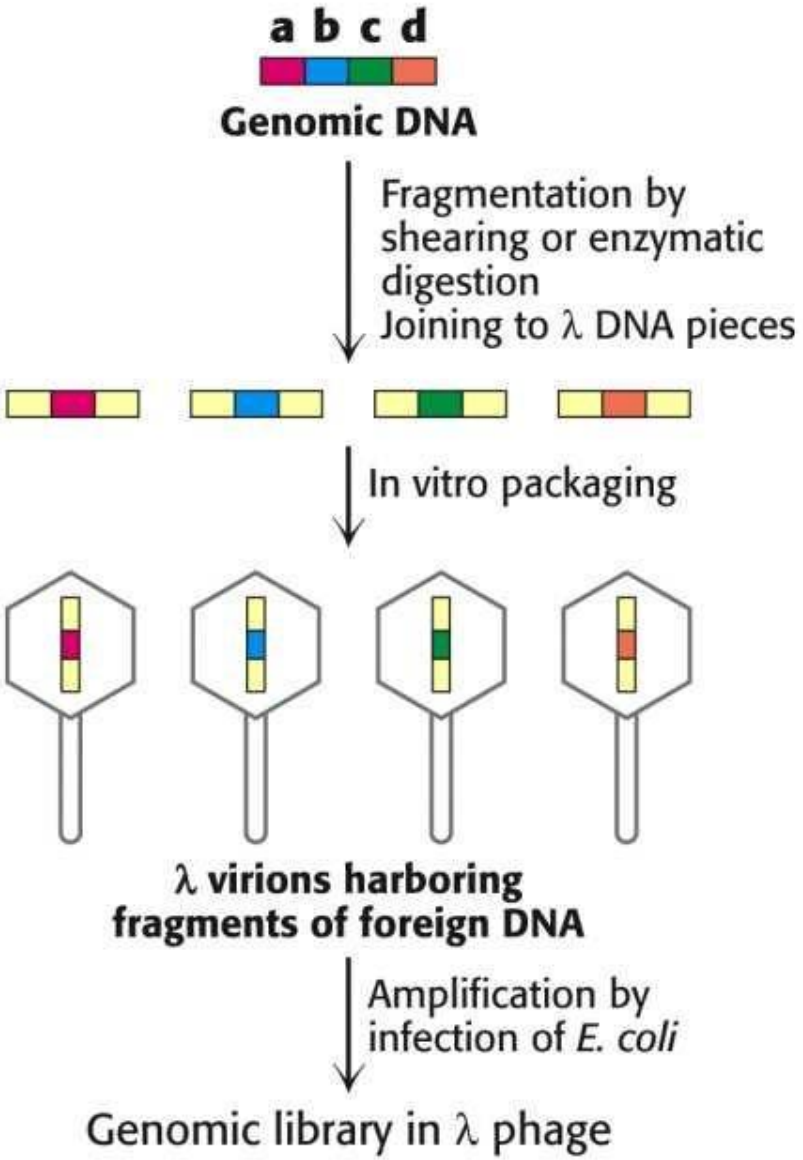


DNA Library



Clones -> genetically identical

Genomic phage library



Evaluation of library

Box 7.1 Estimates of the required size of genomic libraries

Organism	Genome size	Vector type	Insert size	*P*	Library size
Bacterium	4×10^6 bases	plasmid	4 kb	0.99	4.6×10^3
		lambda replacement	18 kb	0.99	1.0×10^3
		cosmid	40 kb	0.99	458
		BAC	300 kb	0.99	59
Mammal	3×10^9 bases	plasmid	4 kb	0.99	3.5×10^6
		lambda replacement	18 kb	0.99	7.7×10^5
		cosmid	40 kb	0.99	3.5×10^5
		BAC	300 kb	0.99	4.6×10^4

The values shown for the genome sizes of bacteria and mammals are examples for the purpose of this calculation. The actual genome sizes vary quite widely from one organism to another. The insert sizes for specific vectors will also vary.

Evaluation of library

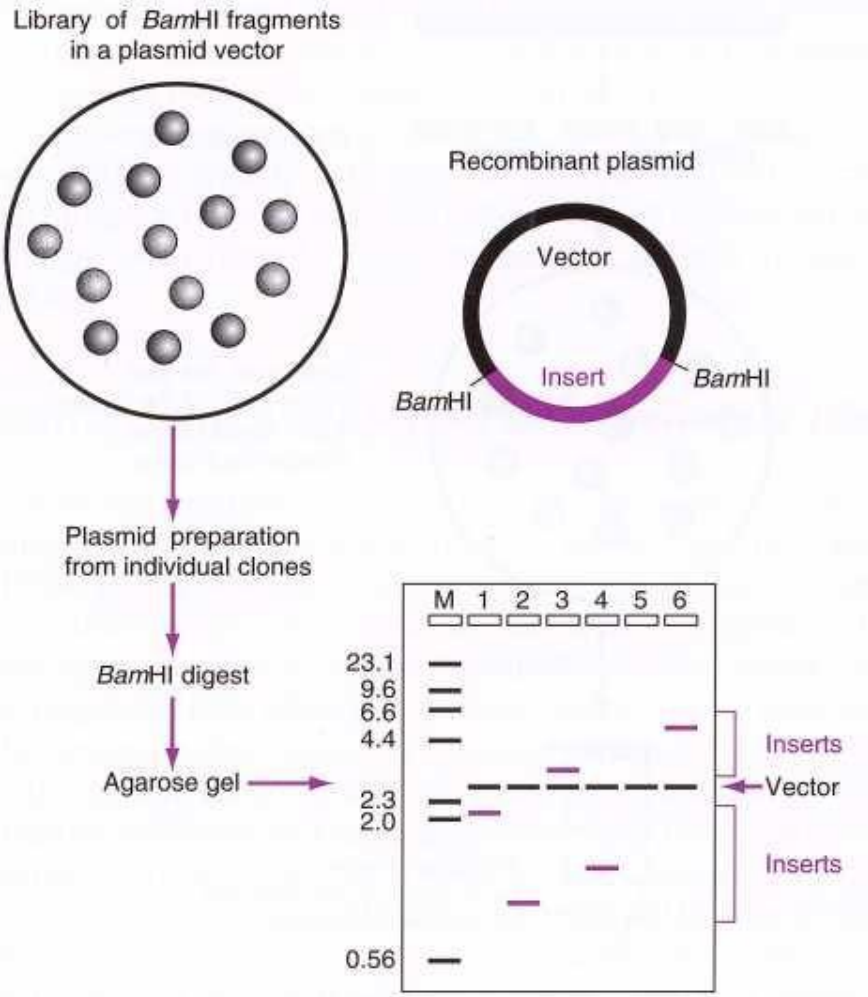


Figure 7.4 Assessing the quality of a gene library

Ordered library

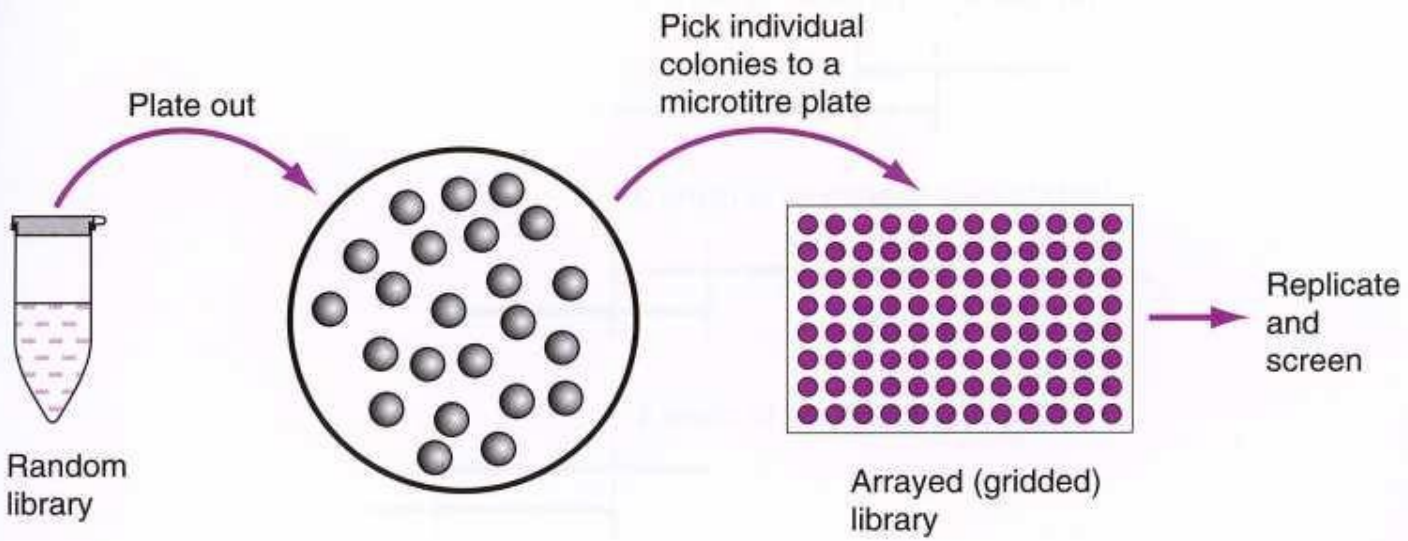


Figure 7.10 Production of an arrayed, or gridded, library

→ Microarrays

Ordered library

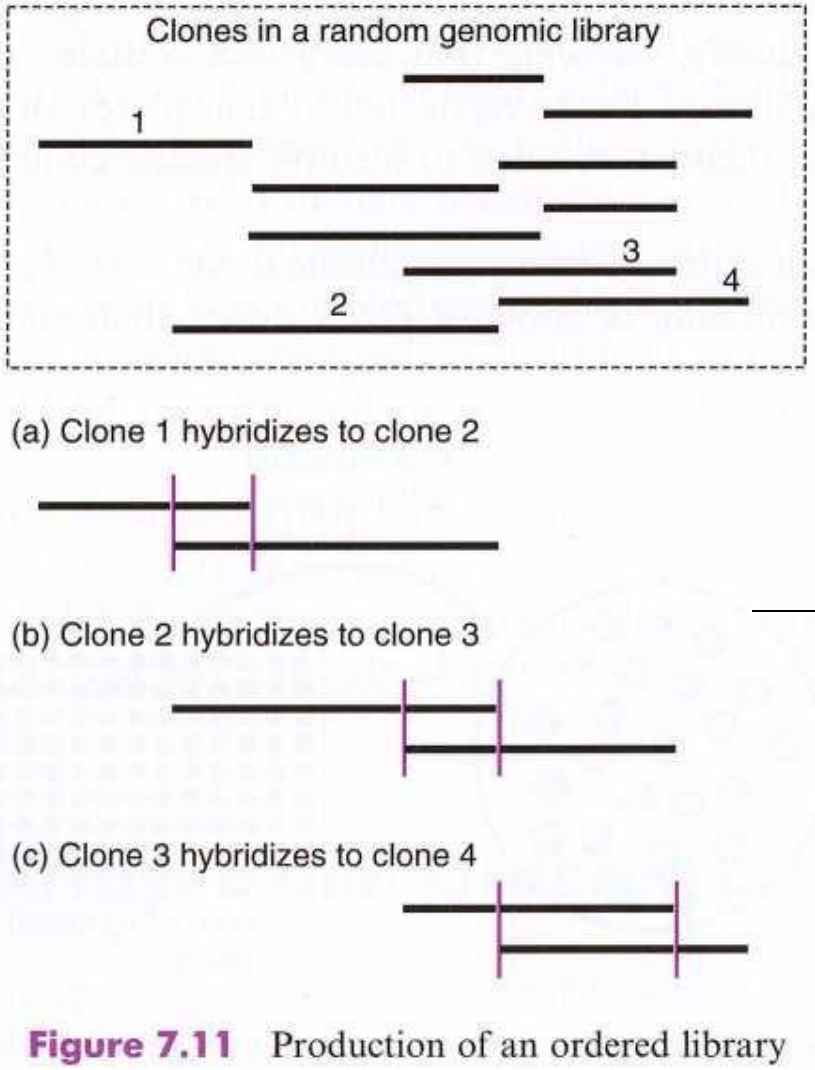
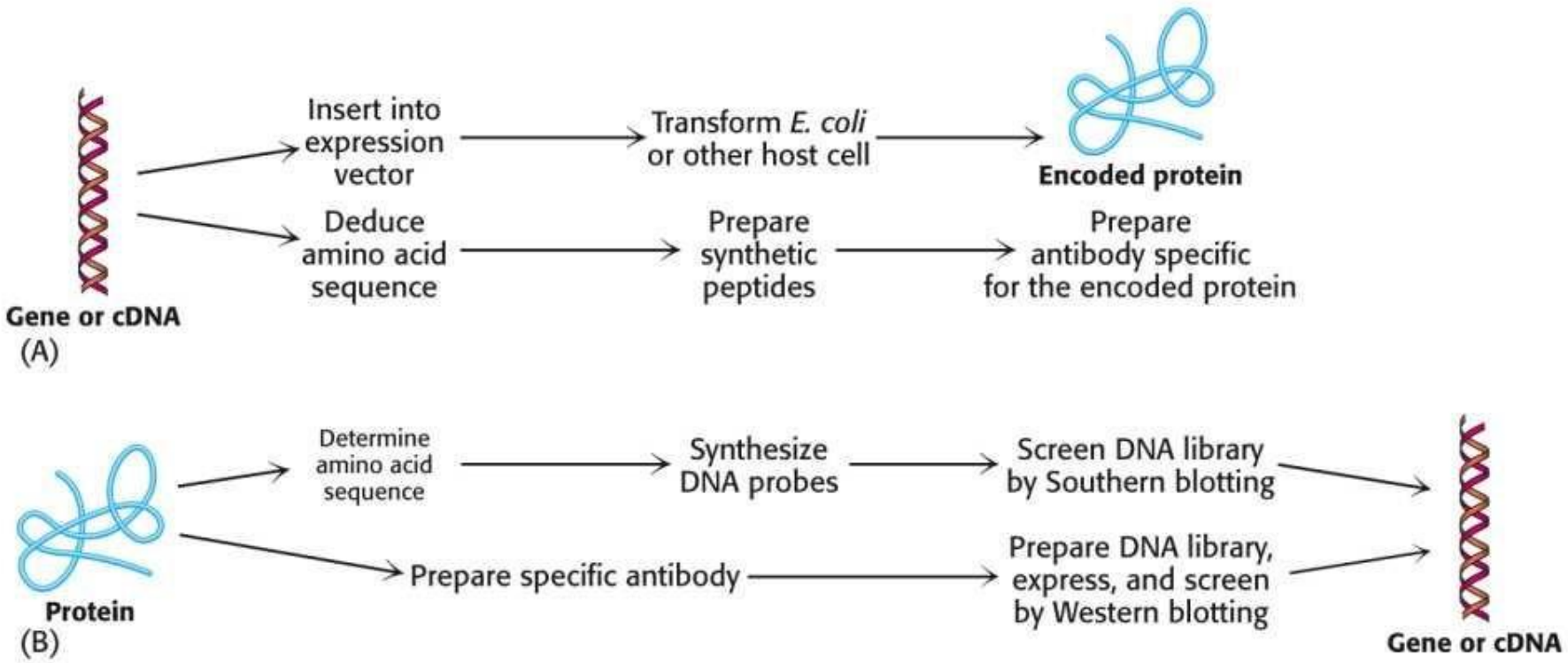


Figure 7.11 Production of an ordered library

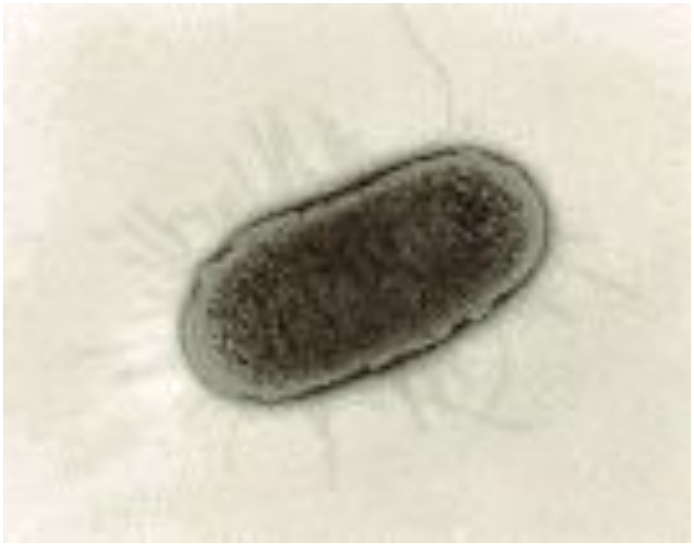
→ "Chromosome Walking"

-> also used in "Human Genome Project"

Different ways to clone a gene



Bacterial host engineering



Escherichia coli (E. coli) is a type of bacteria normally found in the intestines of people and animals.

Although most strains of E. coli are harmless, some can cause illness or even death. The most serious form is E. coli **0157:H7**.

E. coli leads to about 73,000 cases of infection and 61 deaths each year in the United States.

Genetic and physical maps of the *E. coli* chromosome

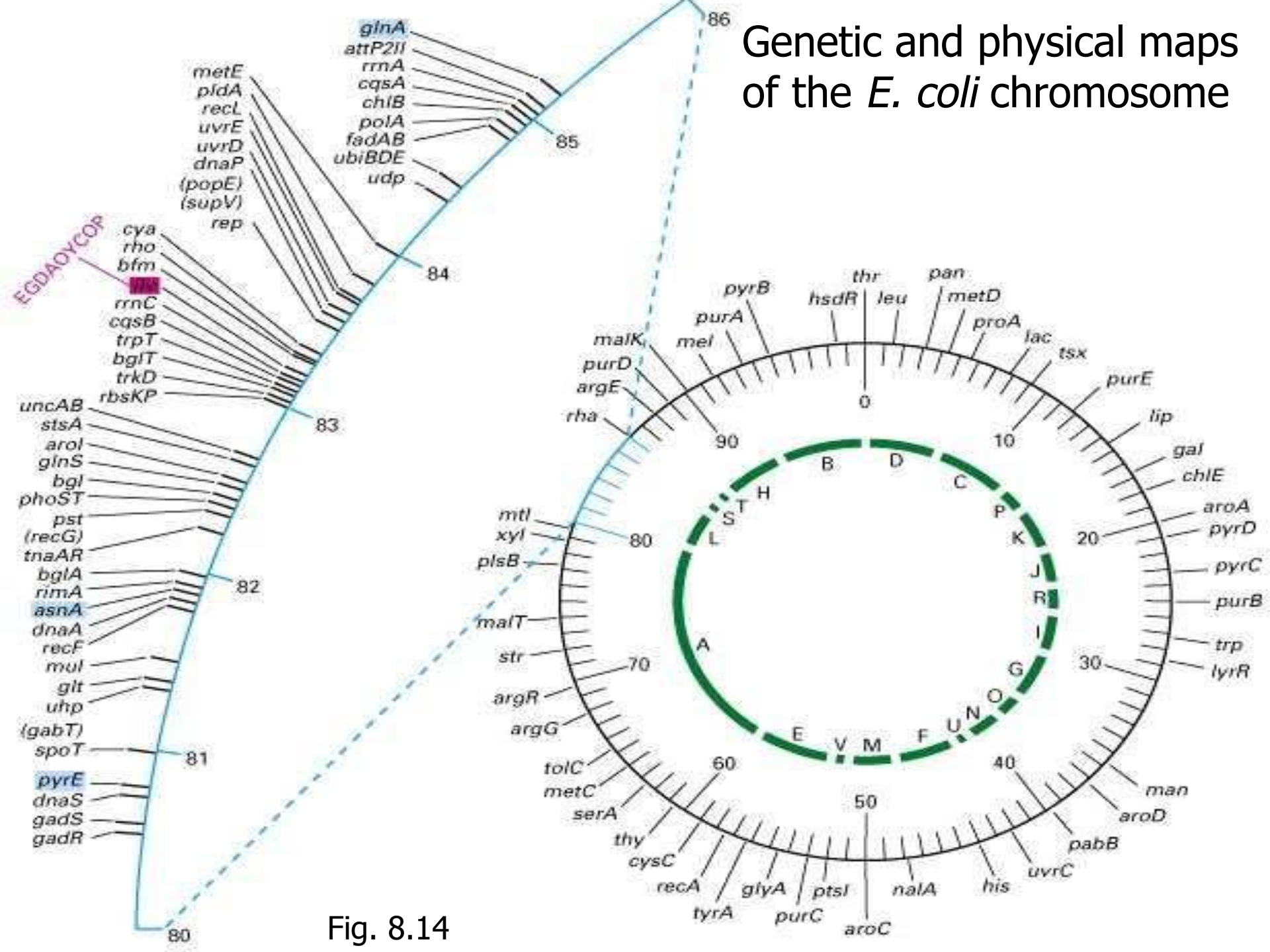


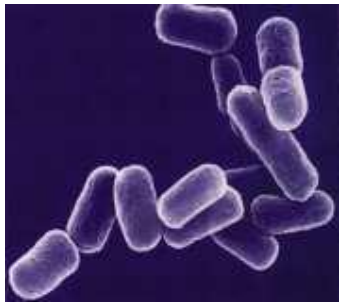
Fig. 8.14

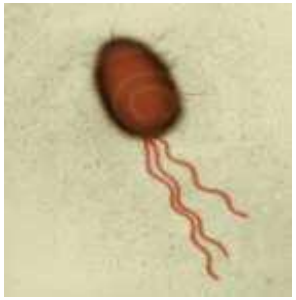
E.Coli K12 strain has been used for further engineering

The K12 strain was first isolated in 1921 from the stool of a malaria patient and it has been maintained in laboratory stocks as a pure strain for the last 75 years.

NIH Recombinant Advisory Committee (RAC) (1973)

Asilomar Conference on Recombinant DNA (February 1975)





Every strain comes with description of its genotype: DH5alpha (recA-; hsdR-; LacIq; uvrA-; mcrA-……)

Most strain in molecular biology are *recA- endA- hsdR-*

Additional changes in K12 E.coli for ease of the laboratory practice

1. Bacterial restriction modification systems have been removed.

(To prevent its interferention with the replication of foreign DNA in bacteria).

hsdR/hsdM/hsdS (EcoK) restriction system

Degrades DNA not methylated at the sequence 5'-AAC-(N)5-GTGC-3'

hsdM **recognises unmethylated DNA**
hsdM **is also involved in methylation of DNA**
hsdR **encodes an endonulease**
hsdS **encodes DNA sequence specific protein**

hsdR- **or** ***hsdS-*** ***mutants*** **facilitate propagation of any foreign DNA**

mcrA/mcrB/mrr complex

E.coli DNA is methylated by *dcm, dam* and *hsdM*

mcrA/mcrB/mrr cleaves DNA methylated by other systems

-mcrA-/mcrB-
strains are good for cloning eukaryotic DNA

Additional changes in K12 E.coli for ease of the laboratory practice

2. DNA recombination systems are modified to prevent rearrangements (RecA-)

(to prevent deletions and rearrangements)

***recA* is a core recombination protein**

***recA-* strains allow cloning of repetitive sequences**

***recA-/recB-/recC-* are enhanced strains with very low recombination efficiency**

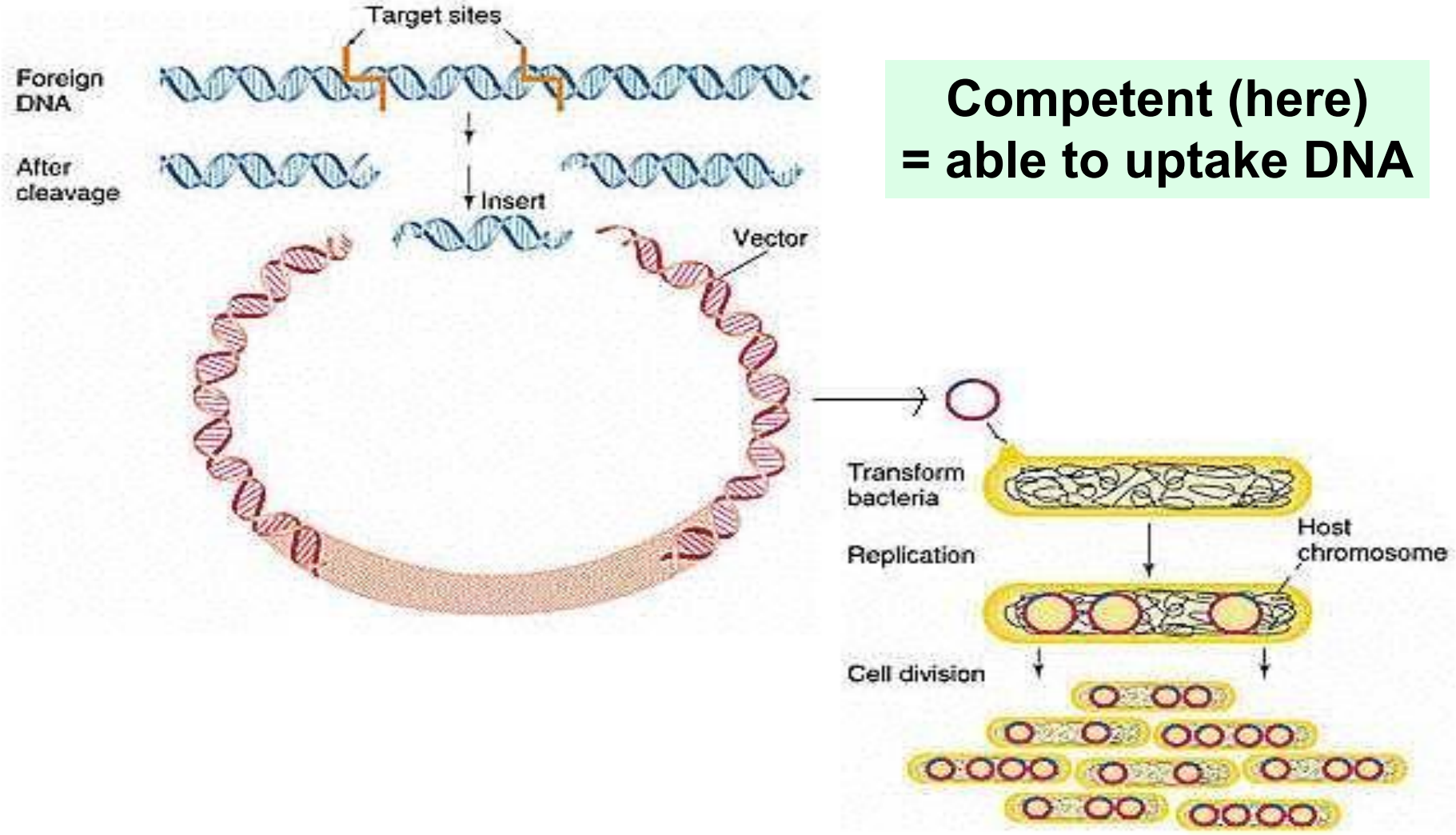
***uvrC/umuC* are involved in DNA repair**

***uvrC-/umuC-* are good for cloning of inverted repeats**

3. Endonuclease activity has been mutated (EndA-)

(to increase plasmid yields and improve the quality of DNA – no nicks)

Transformation of plasmid DNA in competent *E. coli* cells



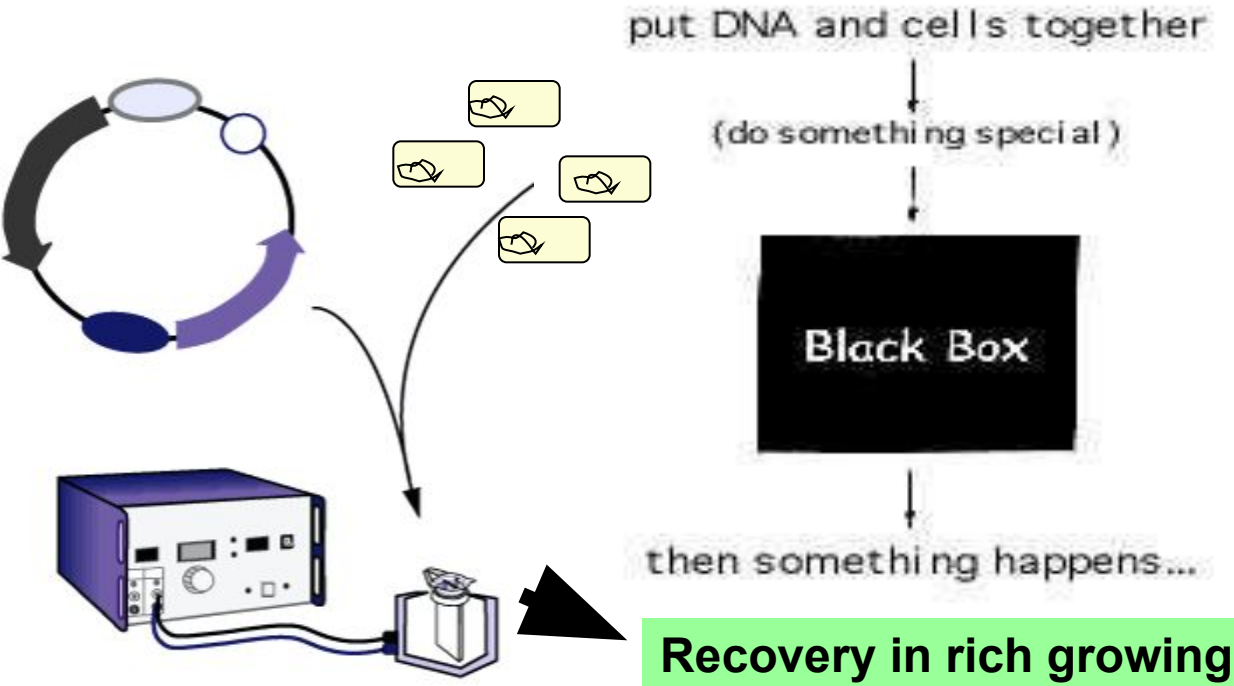
Competent (here) = able to uptake DNA

Transformation of plasmid DNA to competent *E. coli* cells

-- Electroporation and **electroporation-competent cells**

-- Heat shock transformation and **chemically competent cells**

Electroporation



Chemical transformation

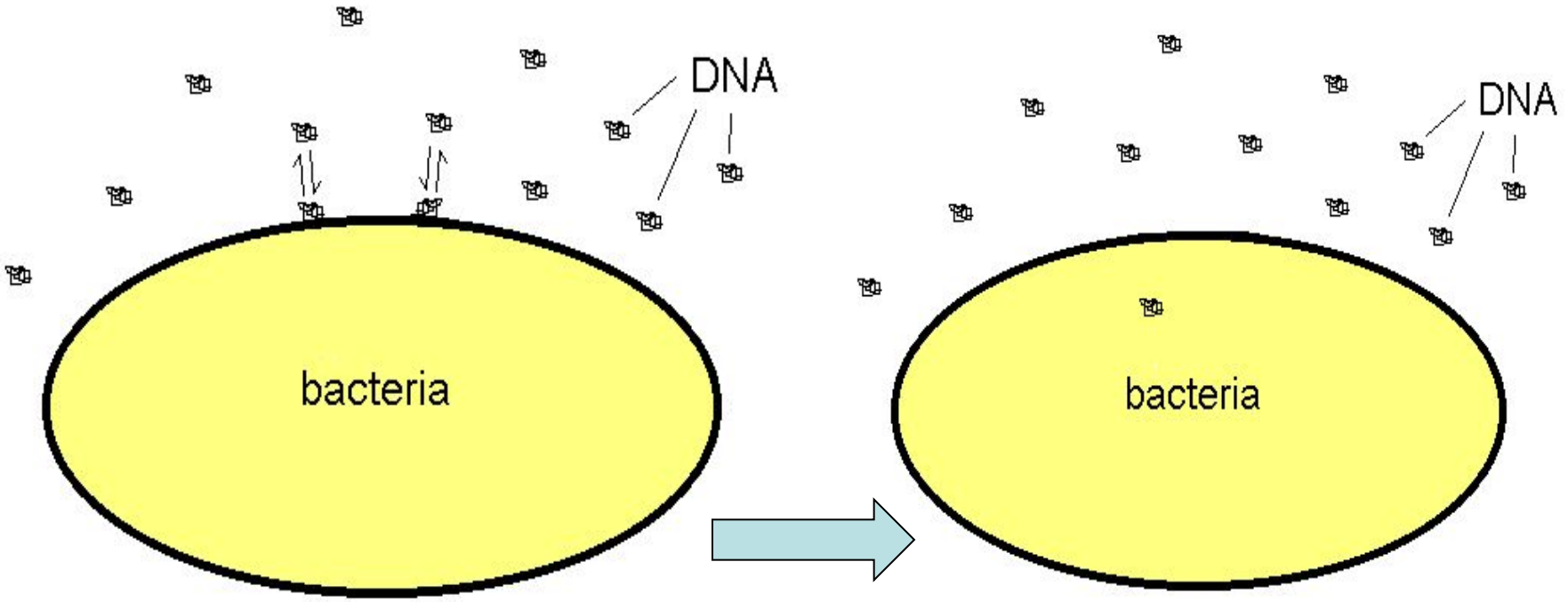
treating E. coli CaCl2 will batter the membranes and essentially make the bacteria very unhappy.

CaCl2 is gaping holes in the membrane

BRIEF HEAT SHOCK

Recovery in rich growing media

Calcium/phosphate (heat shock) method



During the incubation on ice, DNA binds to the surface of the bacterium as a calcium-phosphate-DNA complex

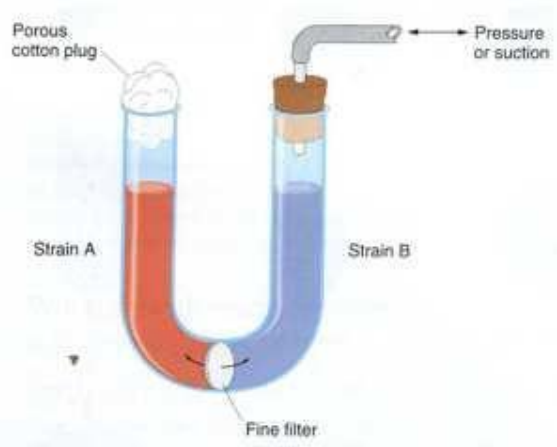
Following a sudden increase in temperature, one or more DNA molecules bound to the surface of the cell is taken up by the competent cell.

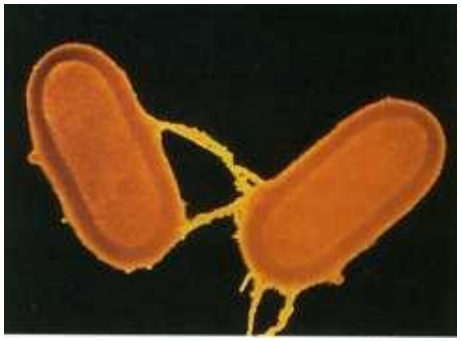
www.bch.msu.edu/bchug/web

www.bch.msu.edu/bchug/web/

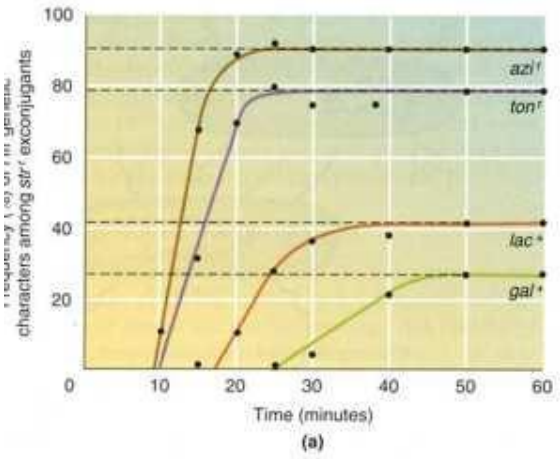
Conjugation

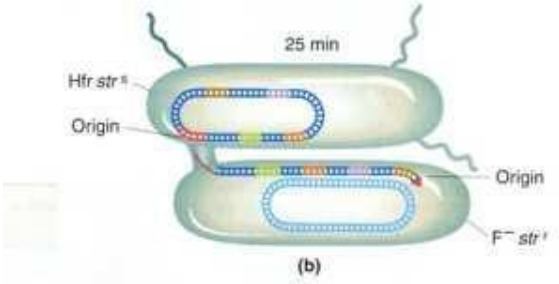
Lederberg





Monod





- F- to F+
- 100 minutes
- 4000 genes