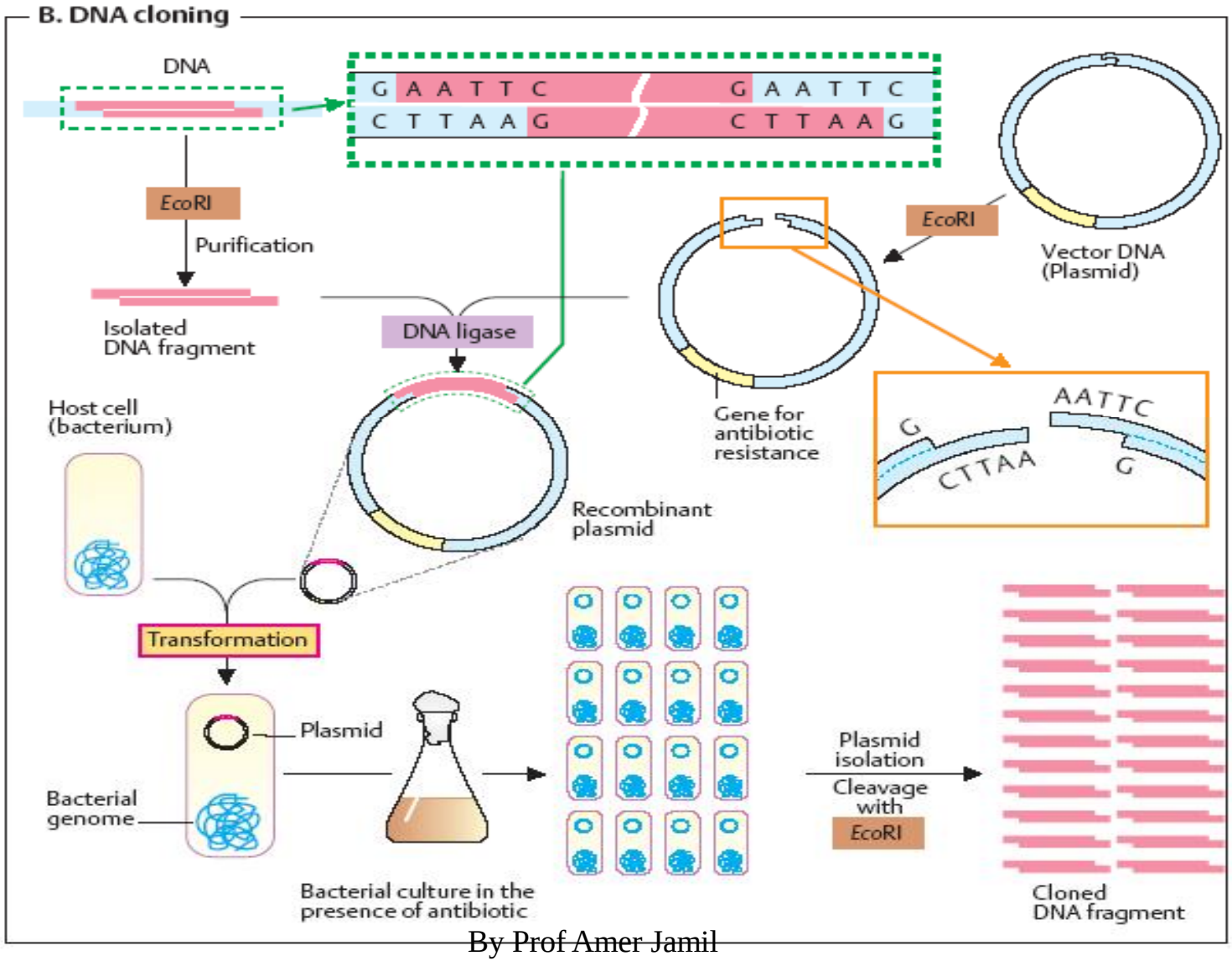
B. DNA cloning
DNA
G A A T T C
C T T A A G
G A A T T C
C T T A A G
EcoRI
Purification
Isolated DNA fragment
DNA ligase
EcoRI
Vector DNA (Plasmid)
Gene for antibiotic resistance
G
AATTC
CTTAA
G
Host cell (bacterium)
Recombinant plasmid
Transformation
Plasmid
Bacterial genome
Bacterial culture in the presence of antibiotic
Plasmid isolation
Cleavage with EcoRI
Cloned DNA fragment

Schematic illustration of DNA cloning

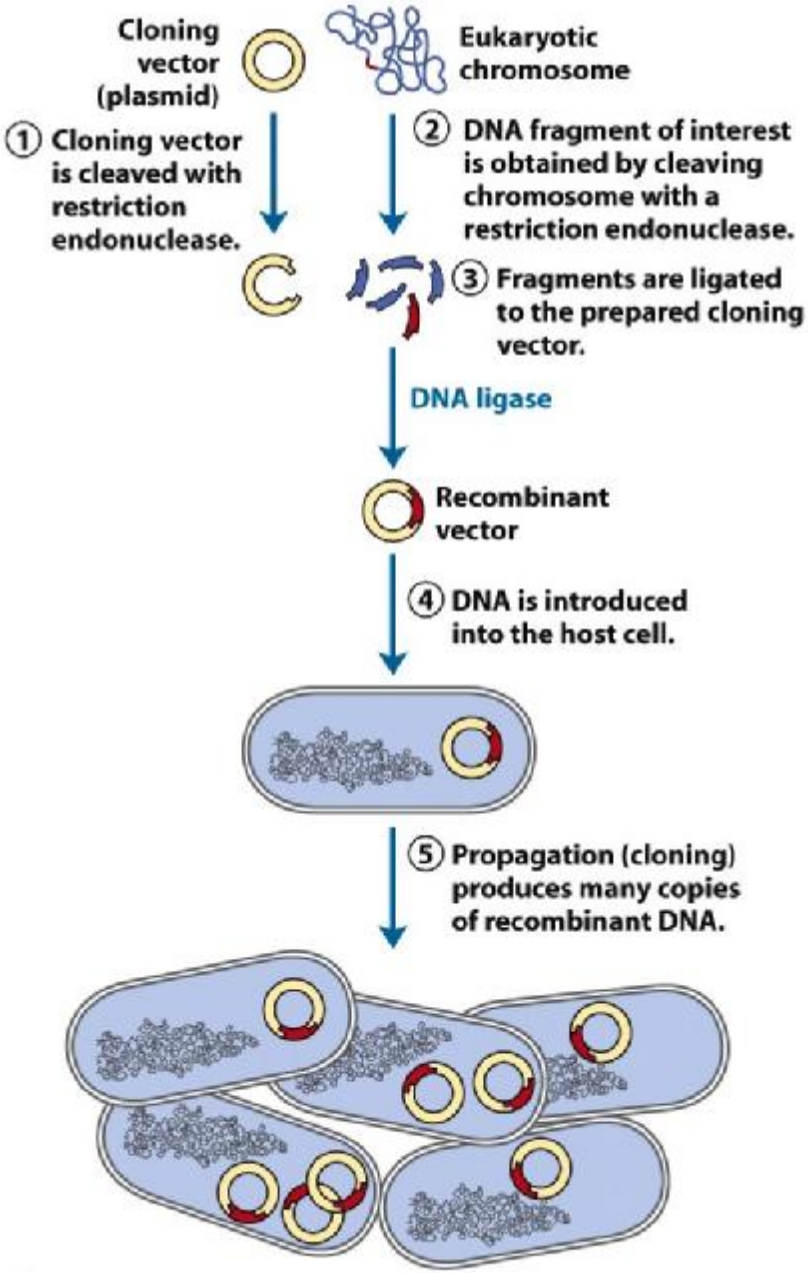

Figure 9-1
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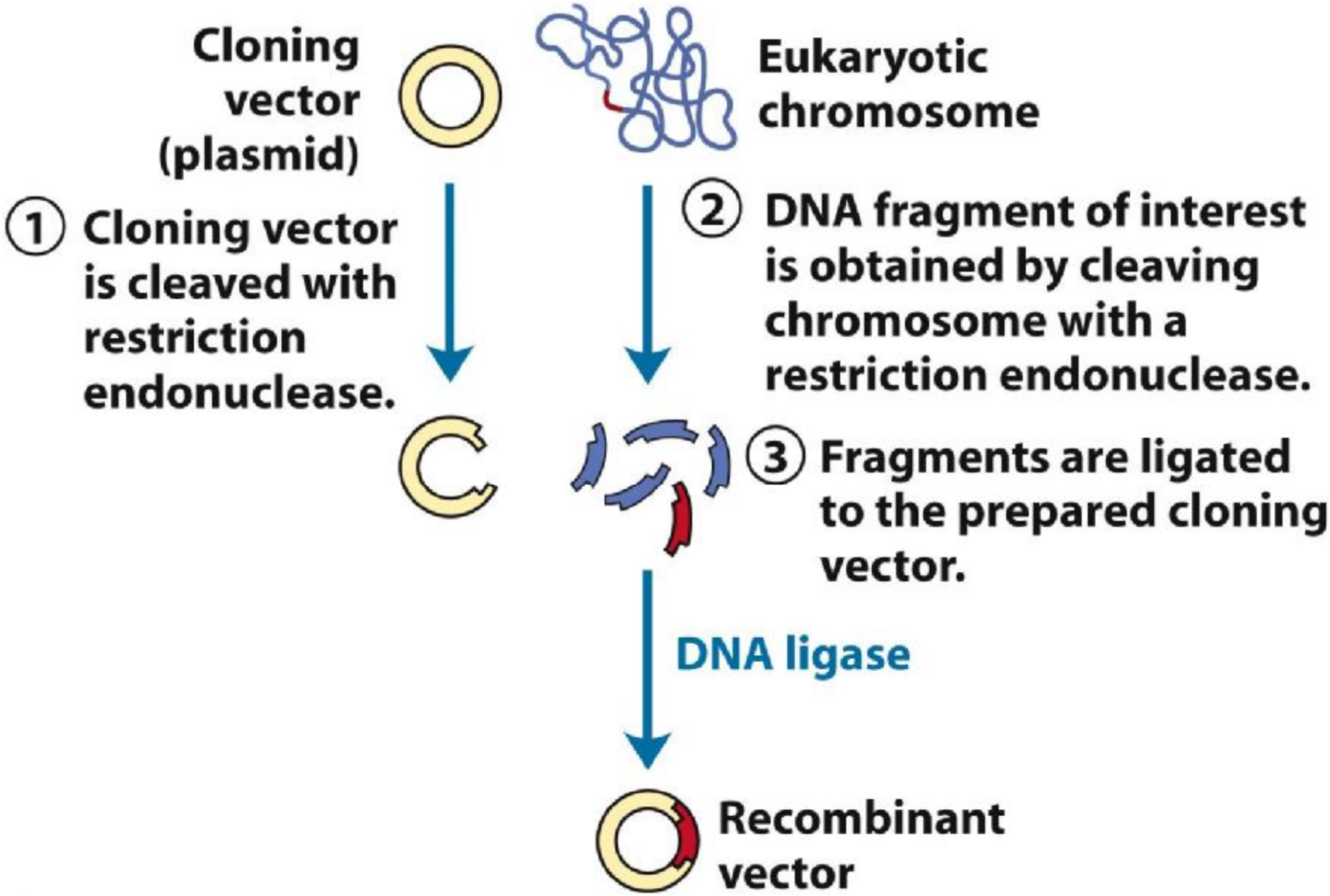

Figure 9-1 part 1
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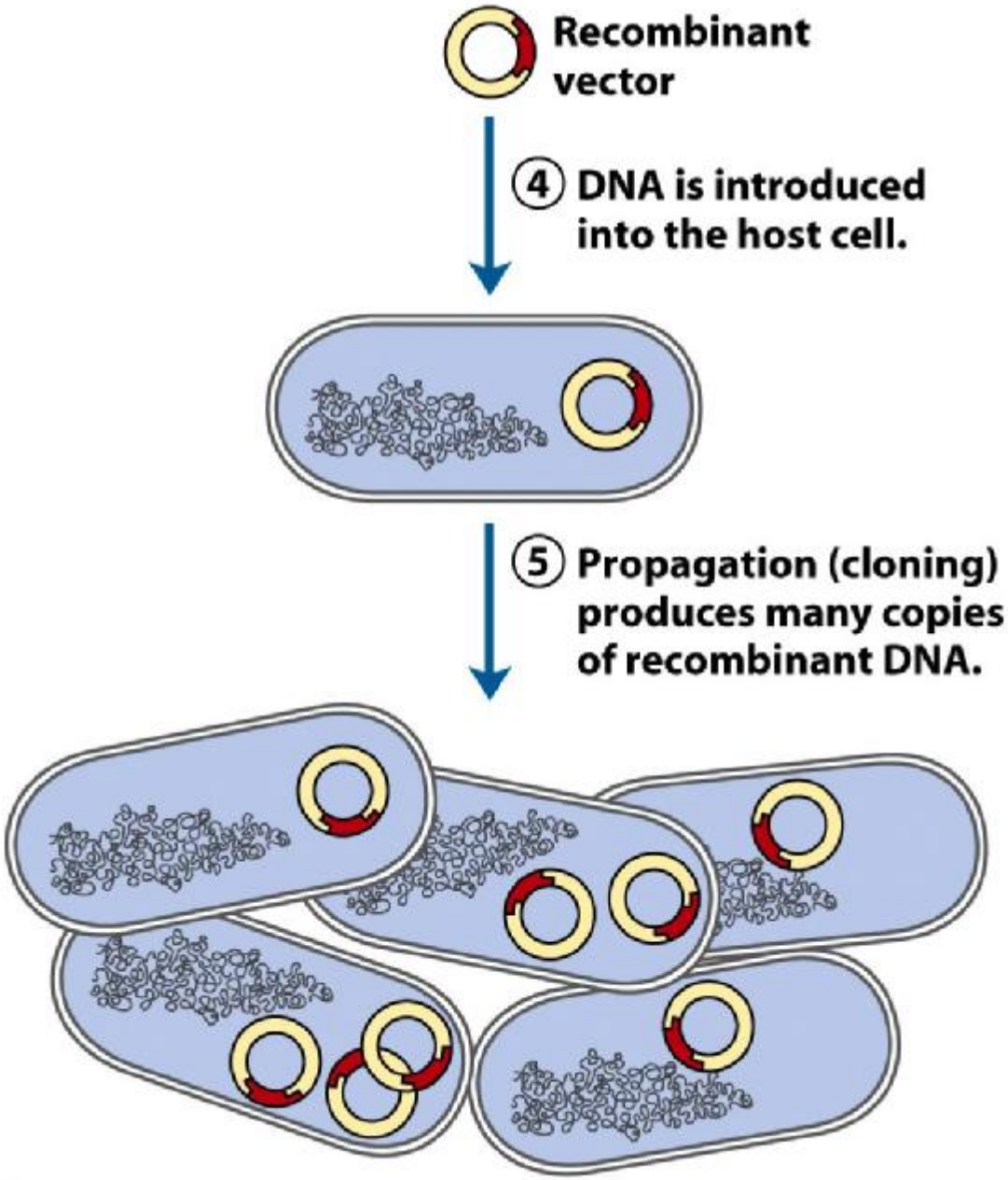

Figure 9-1 part 2
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TABLE 9–1 Some Enzymes Used in Recombinant DNA Technology

Enzyme(s)	Function
Type II restriction endonucleases	Cleave DNAs at specific base sequences
DNA ligase	Joins two DNA molecules or fragments
DNA polymerase I (*E. coli*)	Fills gaps in duplexes by stepwise addition of nucleotides to 3′ ends
Reverse transcriptase	Makes a DNA copy of an RNA molecule
Polynucleotide kinase	Adds a phosphate to the 5′-OH end of a polynucleotide to label it or permit ligation
Terminal transferase	Adds homopolymer tails to the 3′-OH ends of a linear duplex
Exonuclease III	Removes nucleotide residues from the 3′ ends of a DNA strand
Bacteriophage λ exonuclease	Removes nucleotides from the 5′ ends of a duplex to expose single-stranded 3′ ends
Alkaline phosphatase	Removes terminal phosphates from either the 5′ or 3′ end (or both)

Table 9-1
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Restriction endonucleases

- Also called restriction enzymes
- Occur naturally in bacteria
- Hundreds are purified and available commercially
- Named for bacterial genus, species, strain, and type

Example: *EcoR*I

Genus: *Escherichia*
Species: *coli*
Strain: R

TABLE 9–2 Recognition Sequences for Some Type II Restriction Endonucleases

***Bam*HI**	(5′) G G A T C C (3′) C C T A G G	***Hin*dIII**	(5′) A A G C T T (3′) T T C G A A
***Cla*I**	(5′) A T C G A T (3′) T A G C T A	***Not*I**	(5′) G C G G C C G C (3′) C G C C G G C G
***Eco*RI**	(5′) G A A T T C (3′) C T T A A G	***Pst*I**	(5′) C T G C A G (3′) G A C G T C
***Eco*RV**	(5′) G A T A T C (3′) C T A T A G	***Pvu*II**	(5′) C A G C T G (3′) G T C G A C
***Hae*III**	(5′) G G C C (3′) C C G G	***Tth*111I**	(5′) G A C N N N G T C (3′) C T G N N N C A G

Arrows indicate the phosphodiester bonds cleaved by each restriction endonuclease. Asterisks indicate bases that are methylated by the corresponding methylase (where known). N denotes any base. Note that the name of each enzyme consists of a three-letter abbreviation (in italics) of the bacterial species from which it is derived, sometimes followed by a strain designation and Roman numerals to distinguish different restriction endonucleases isolated from the same bacterial species. Thus *Bam*HI is the first (I) restriction endonuclease characterized from B*acillus* amy*loliquefaciens,* strain H.

Table 9-2
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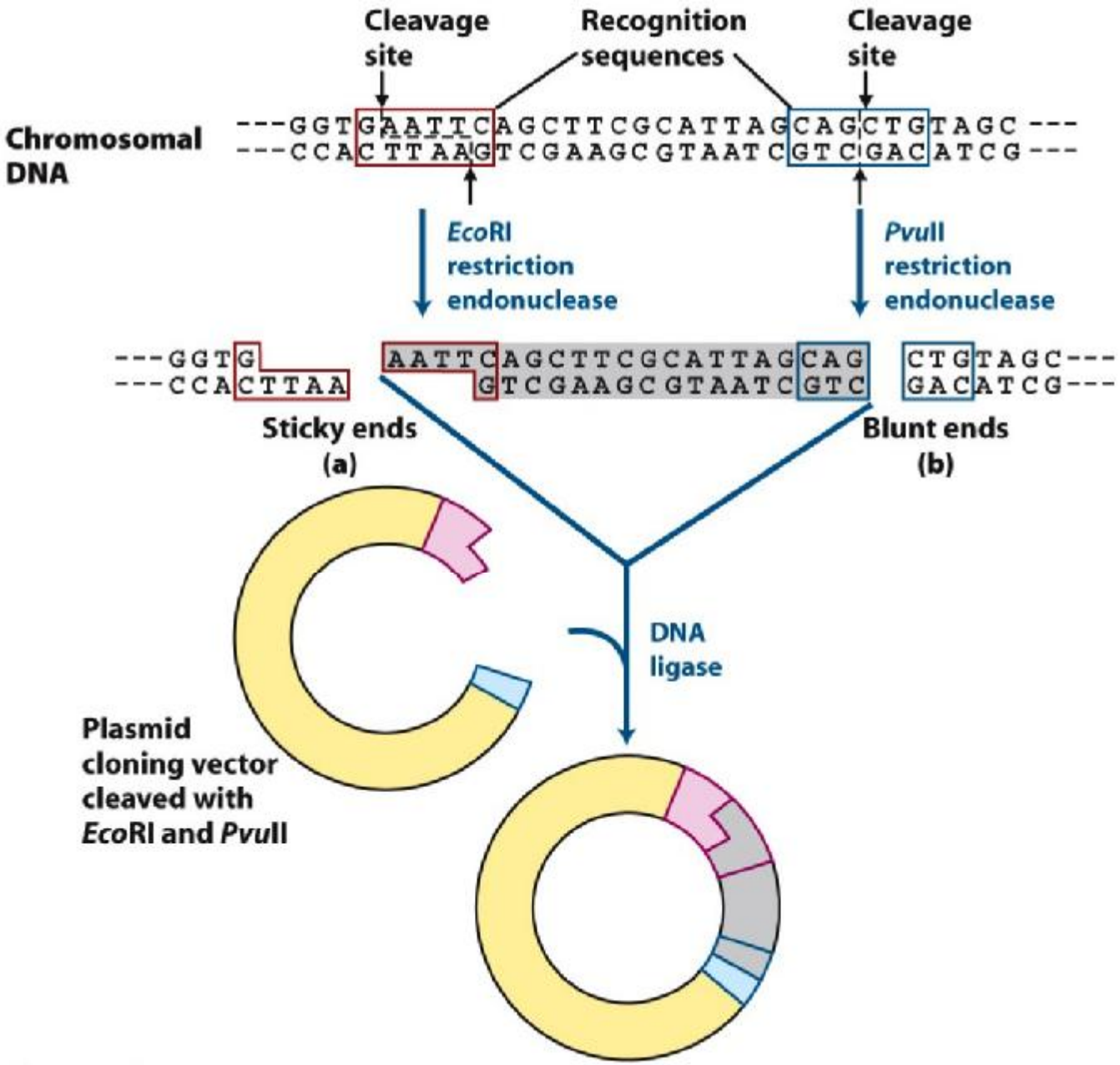

Figure 9-2ab
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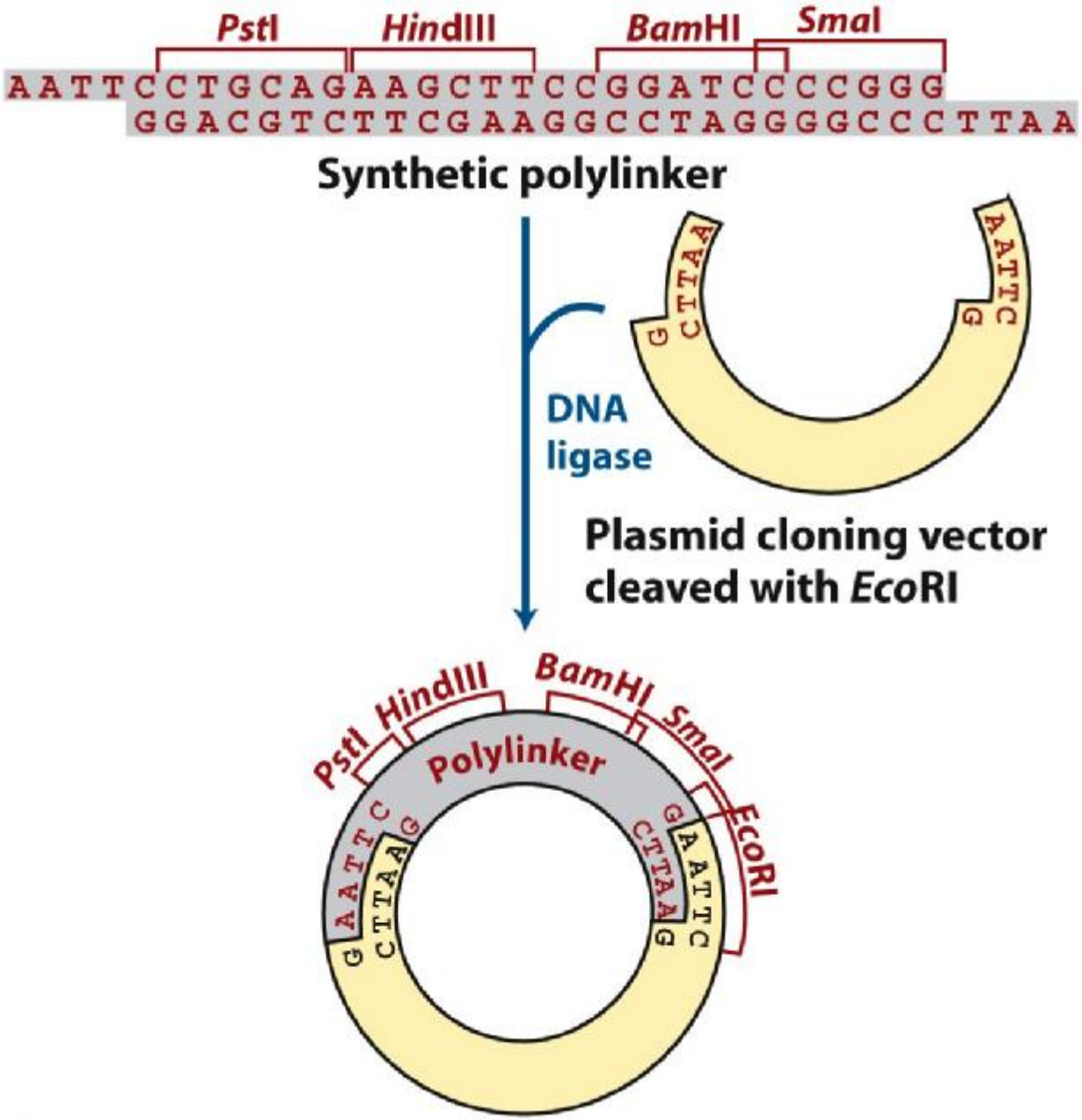

Figure 9-2c

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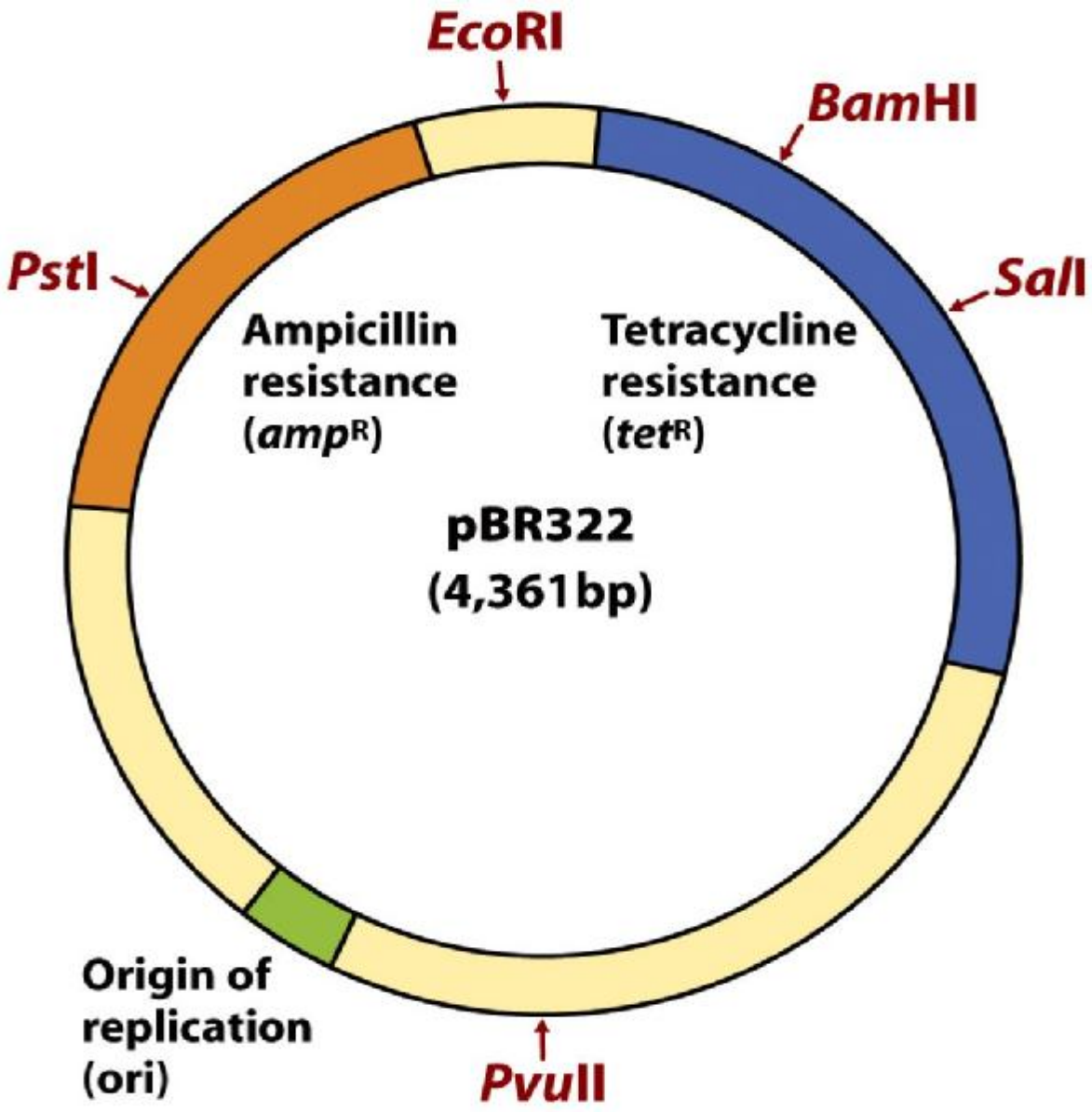

Figure 9-3
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The constructed *E. coli* plasmid pBR322

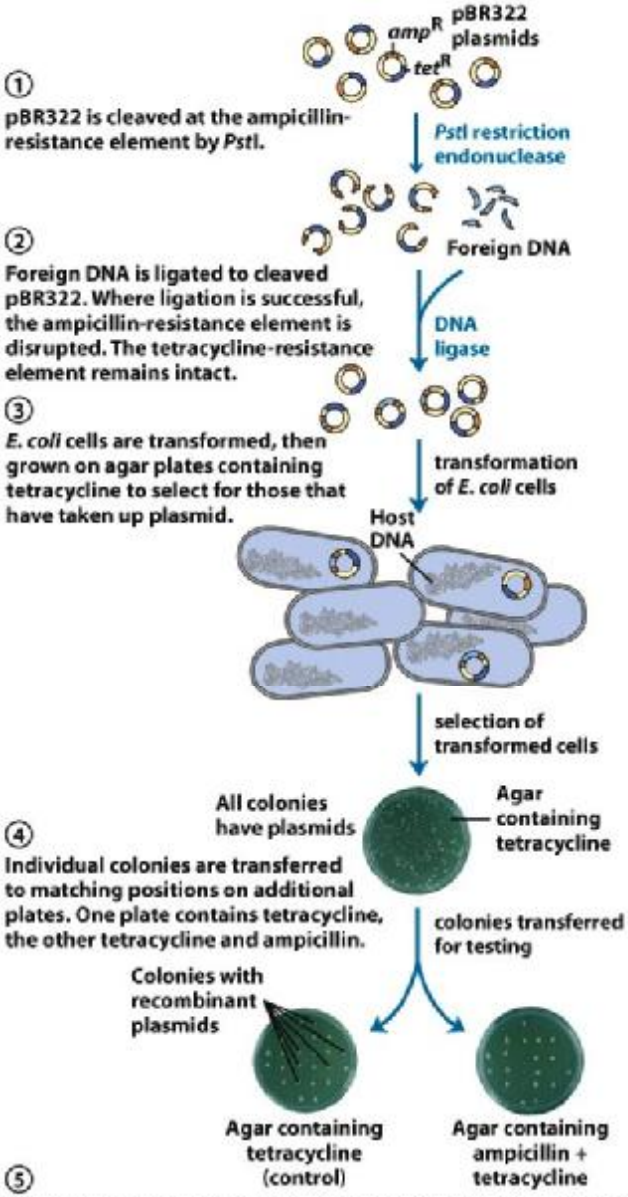

Figure 9-4

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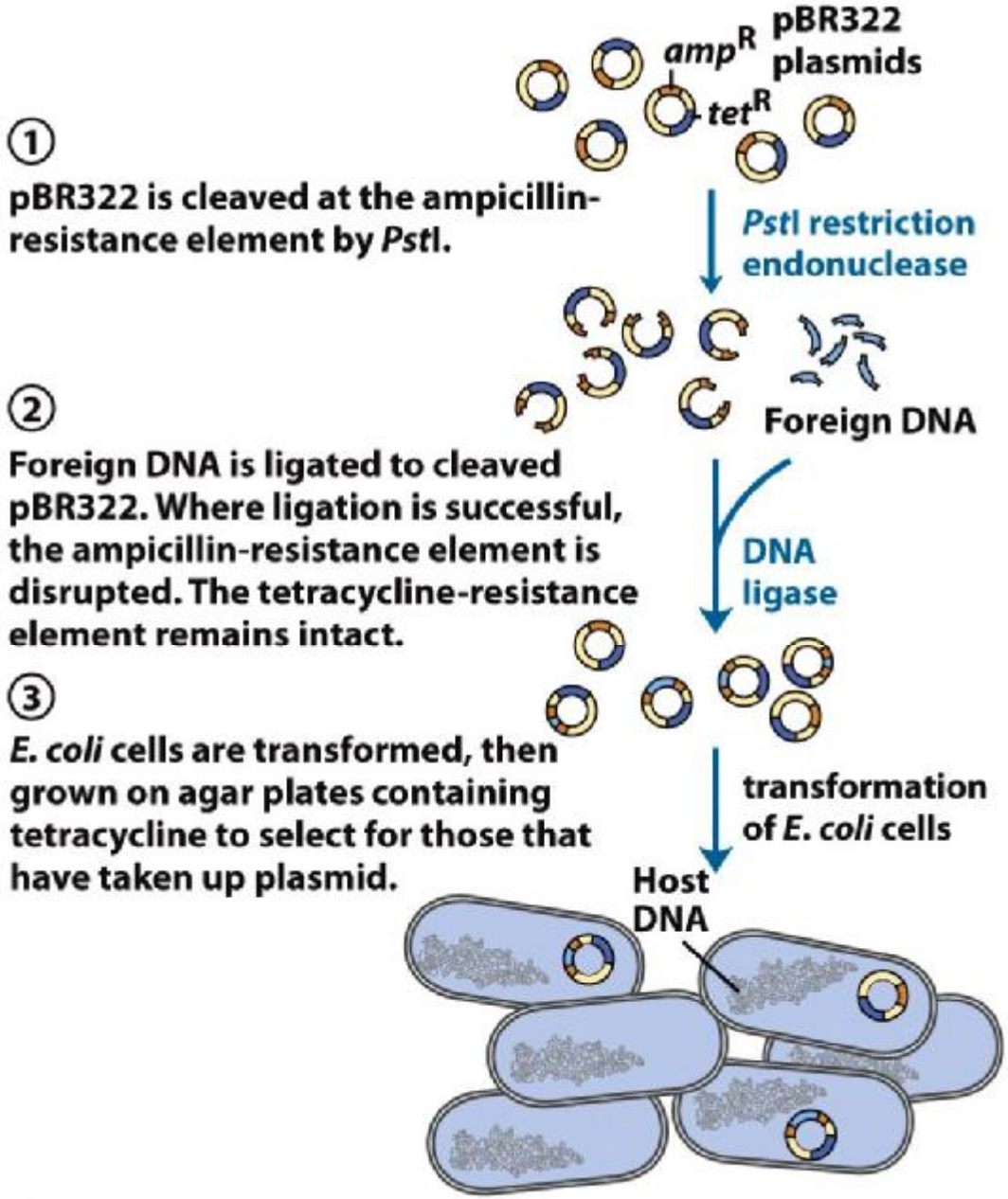

Figure 9-4 part 1
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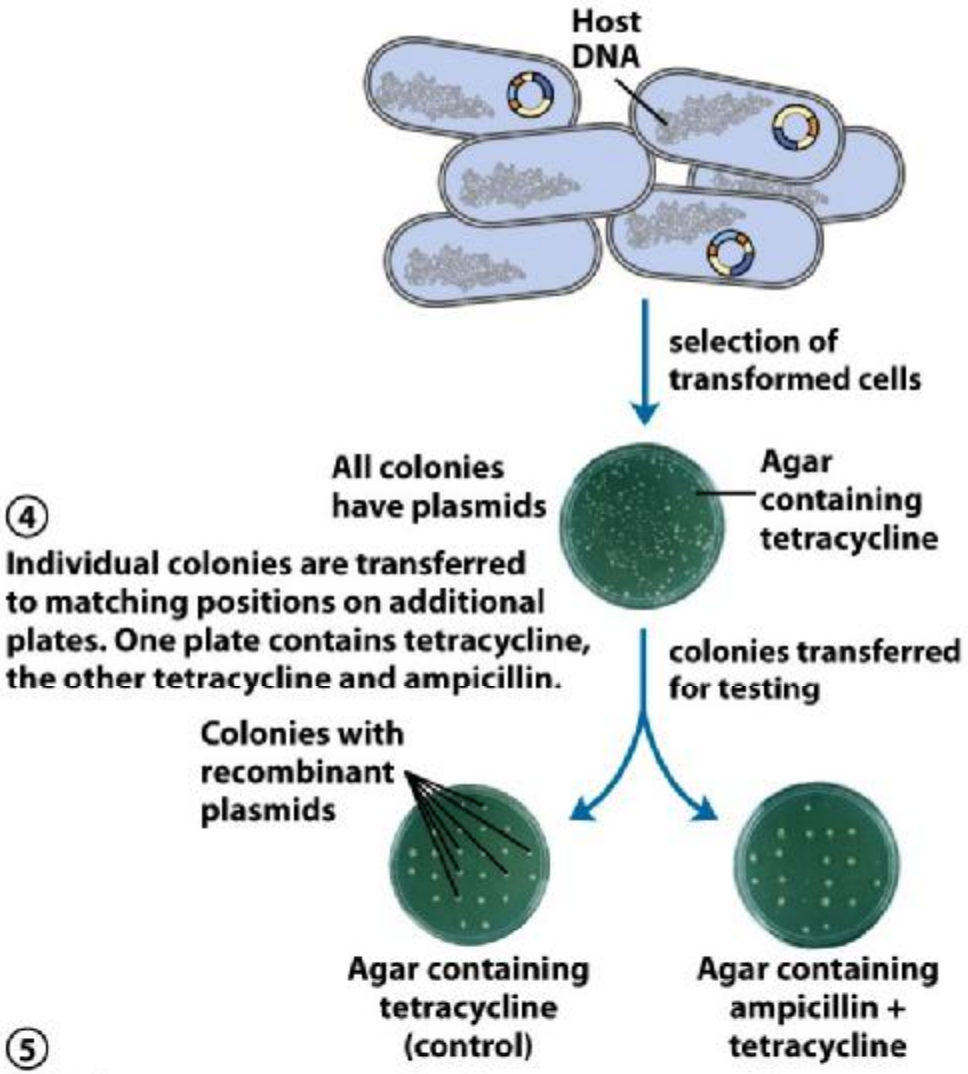

Figure 9-4 part 2

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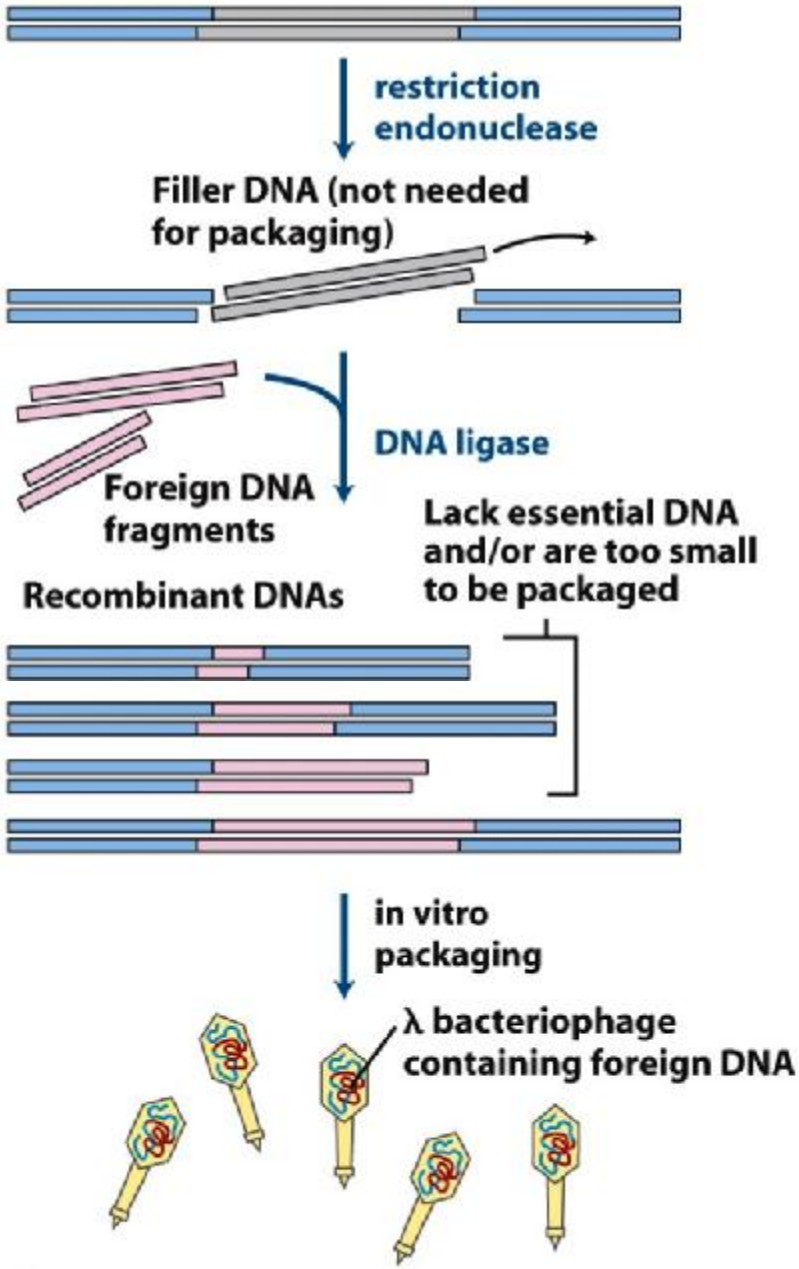

Figure 9-5
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Bacteriophage cloning vectors

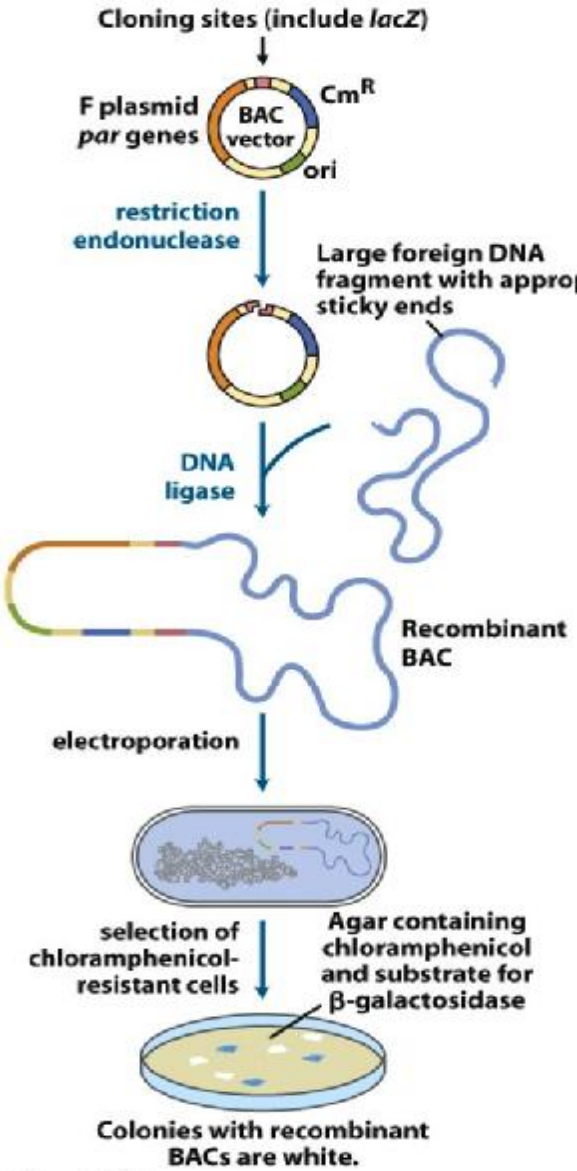

Figure 9-6
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Bacterial artificial chromosomes (BACs) as cloning vectors

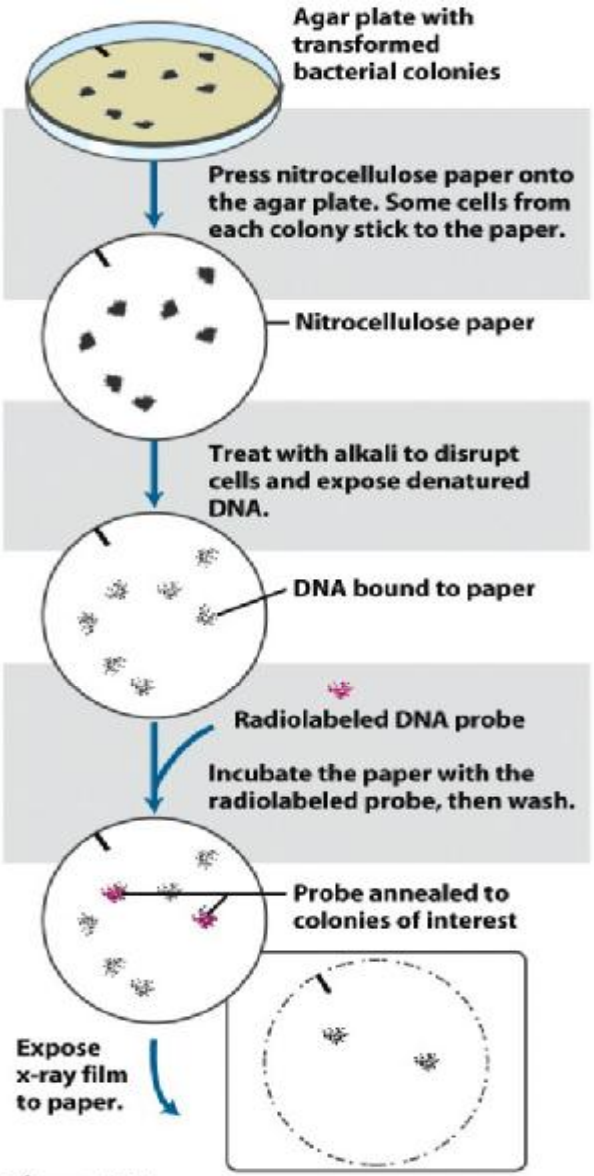

Figure 9-8
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Use of hybridization to identify a clone with a particular DNA segment

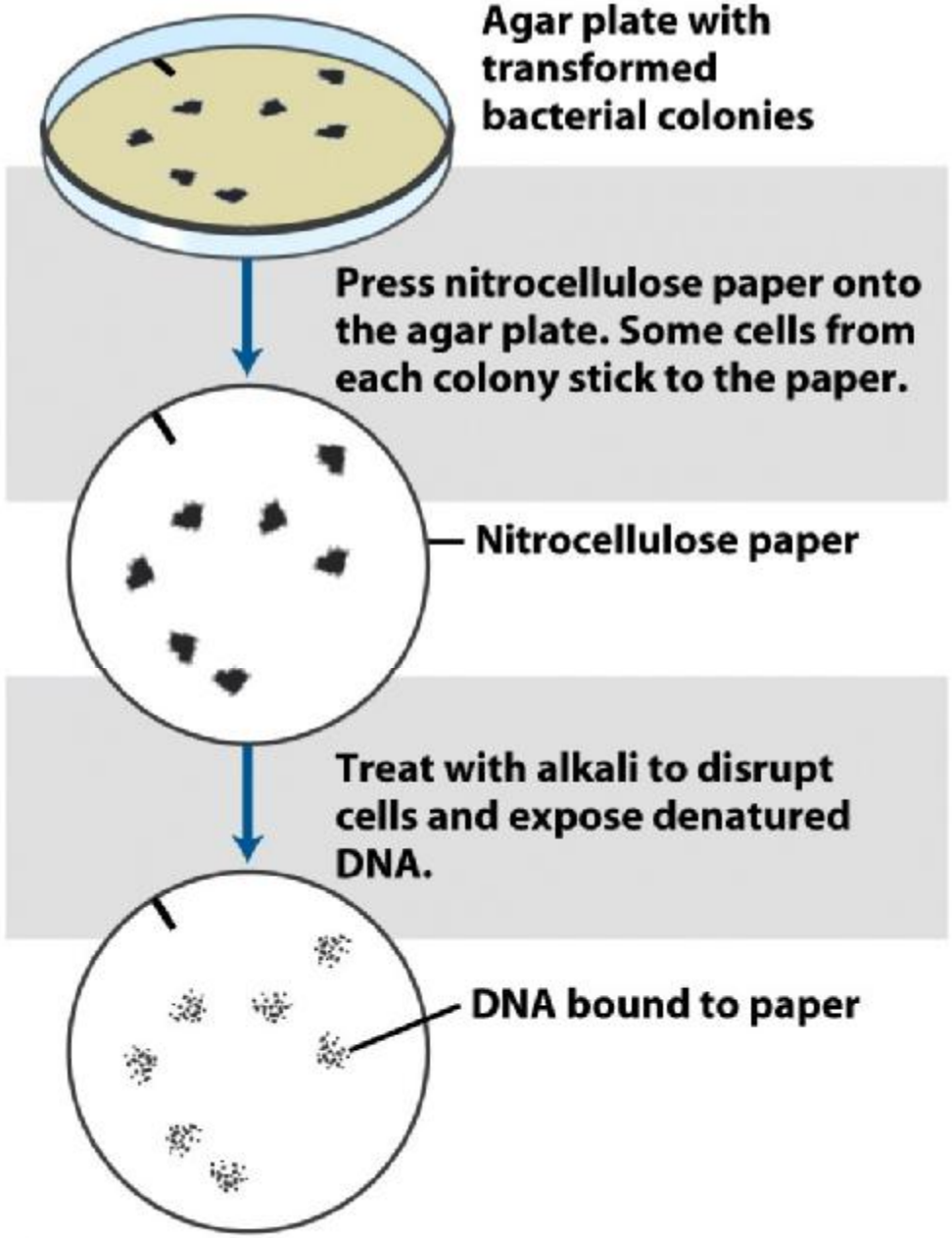

Figure 9-8 part 1
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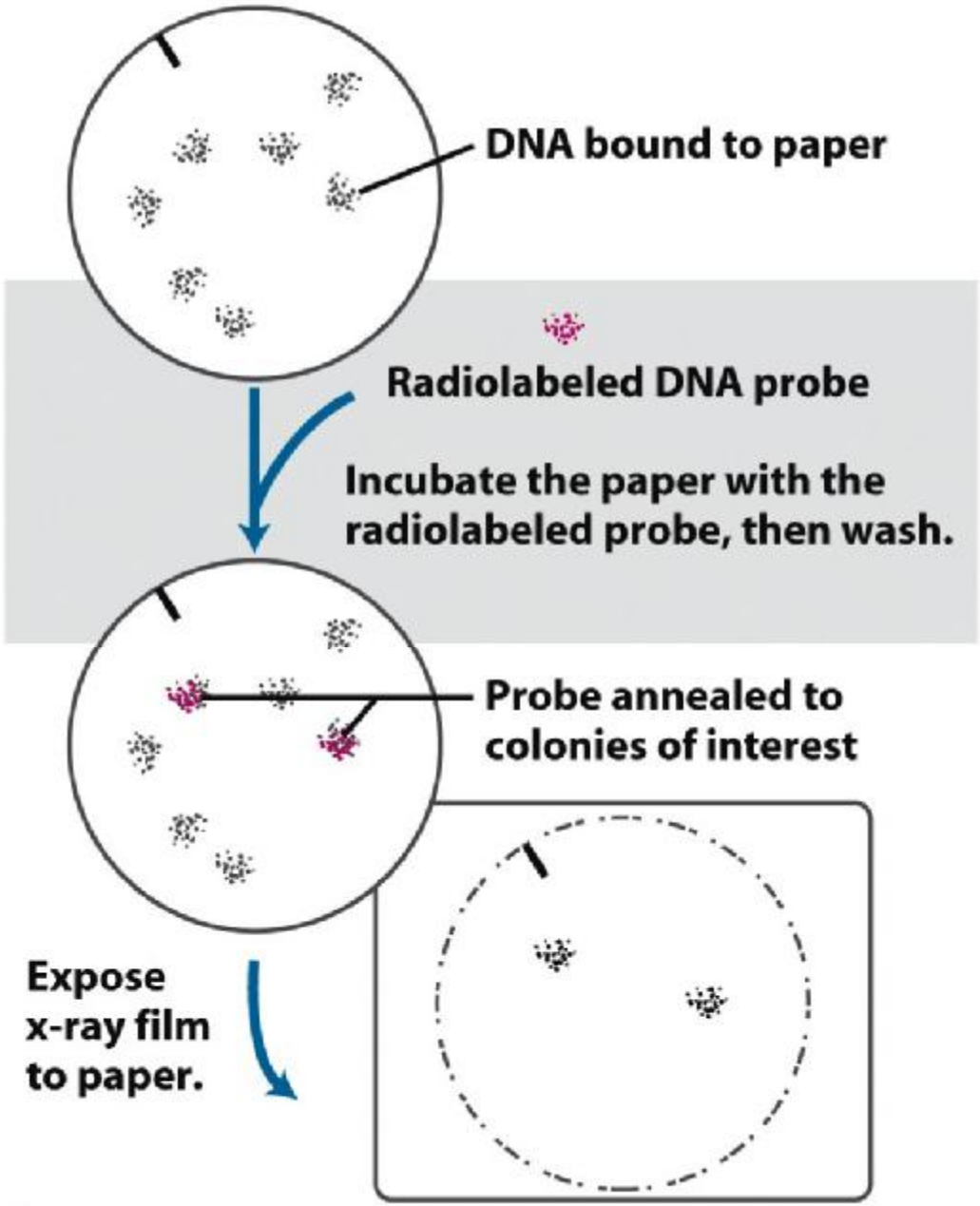

Figure 9-8 part 2
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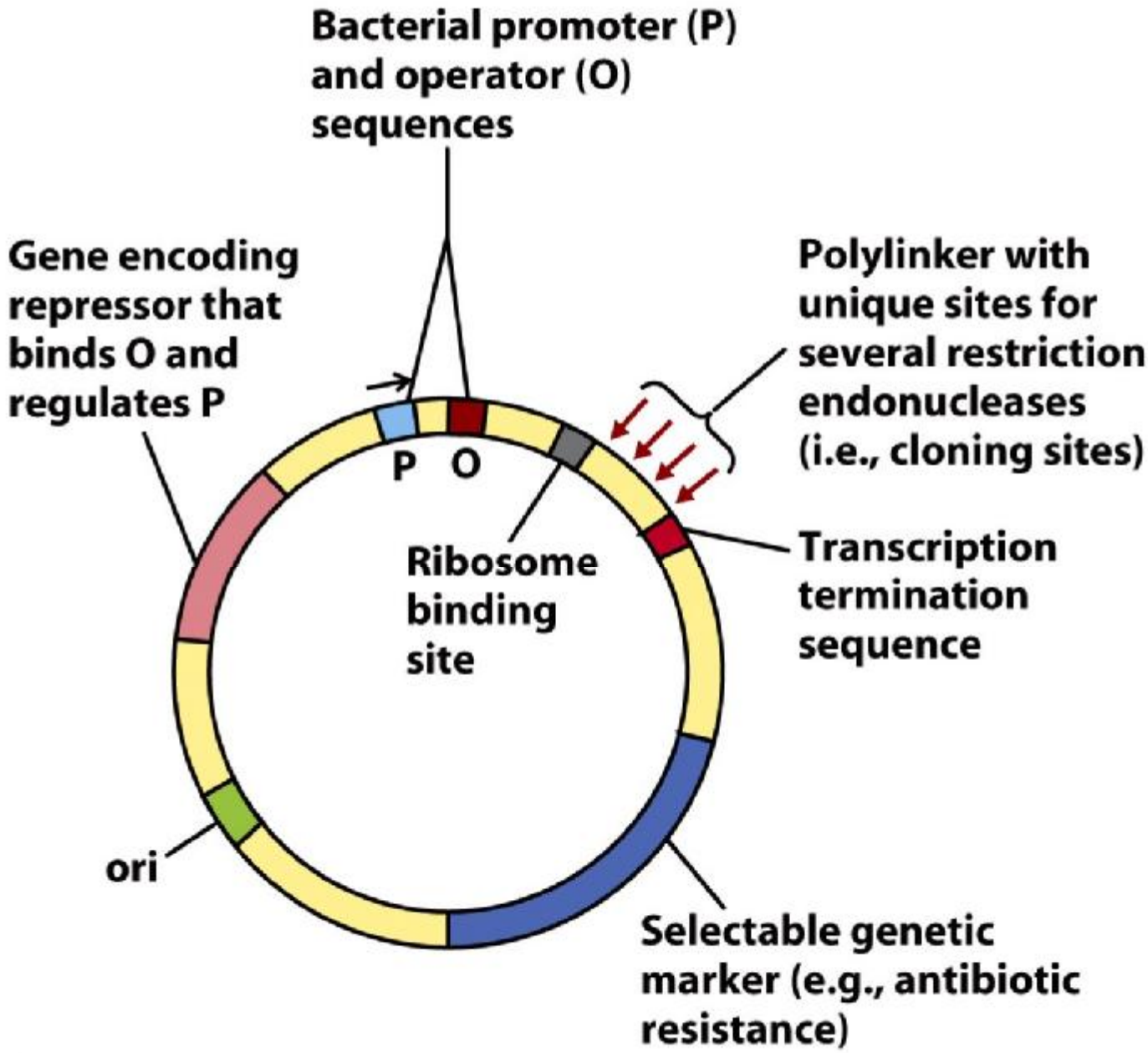

Figure 9-10
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DNA sequences in a typical *E. coli* expression vector

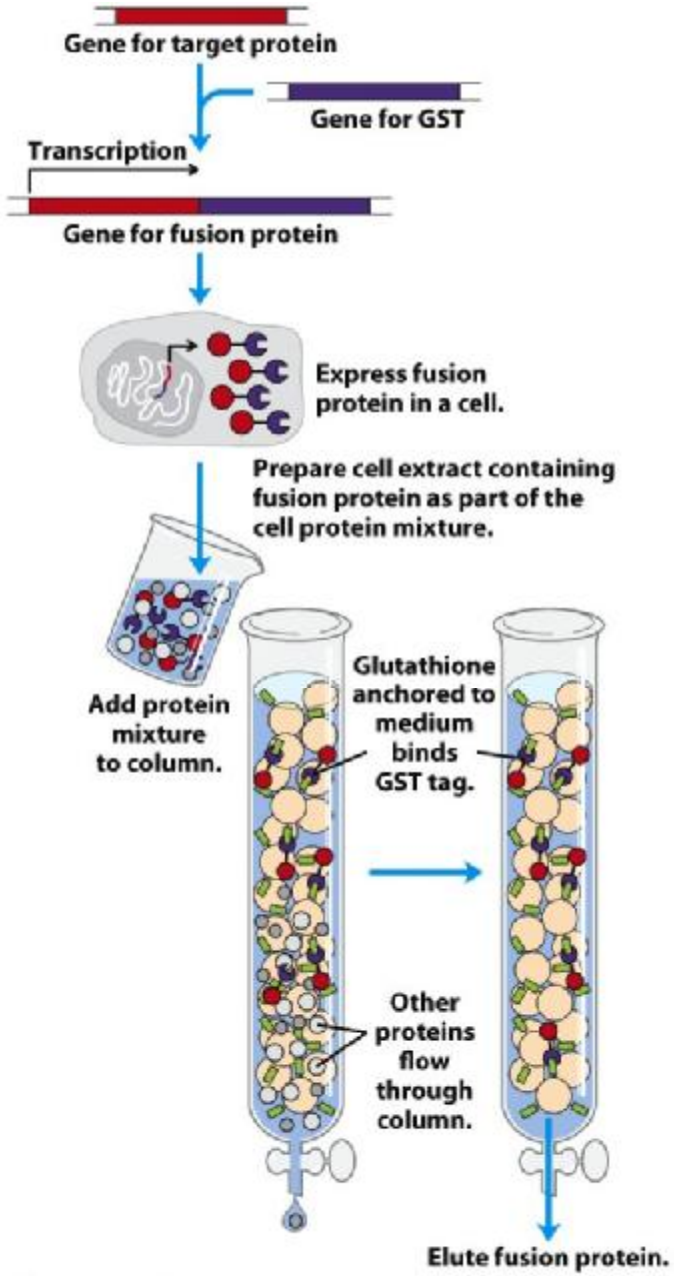

Figure 9-12b
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The use of tagged proteins in protein purification

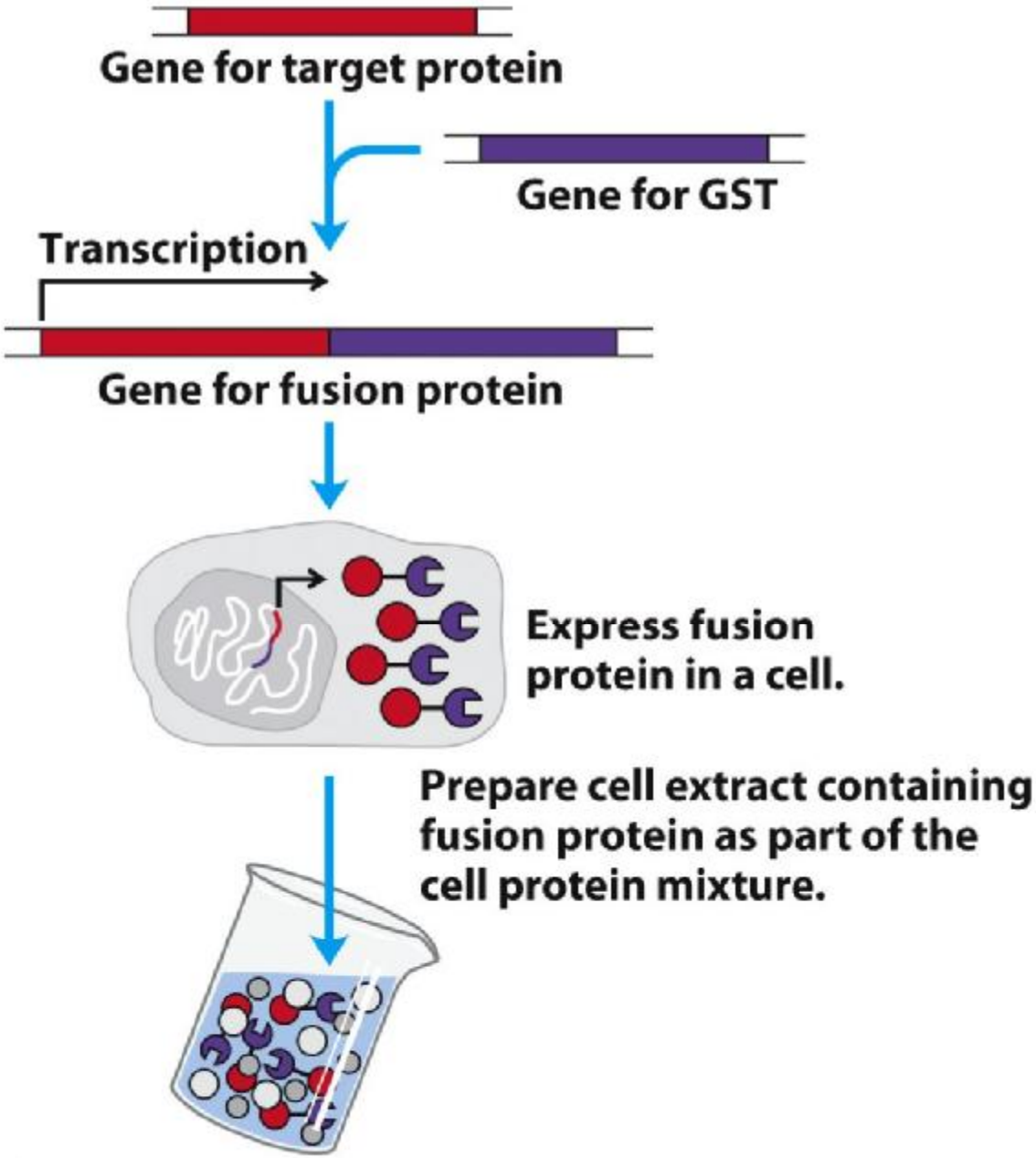

Figure 9-12b part 1
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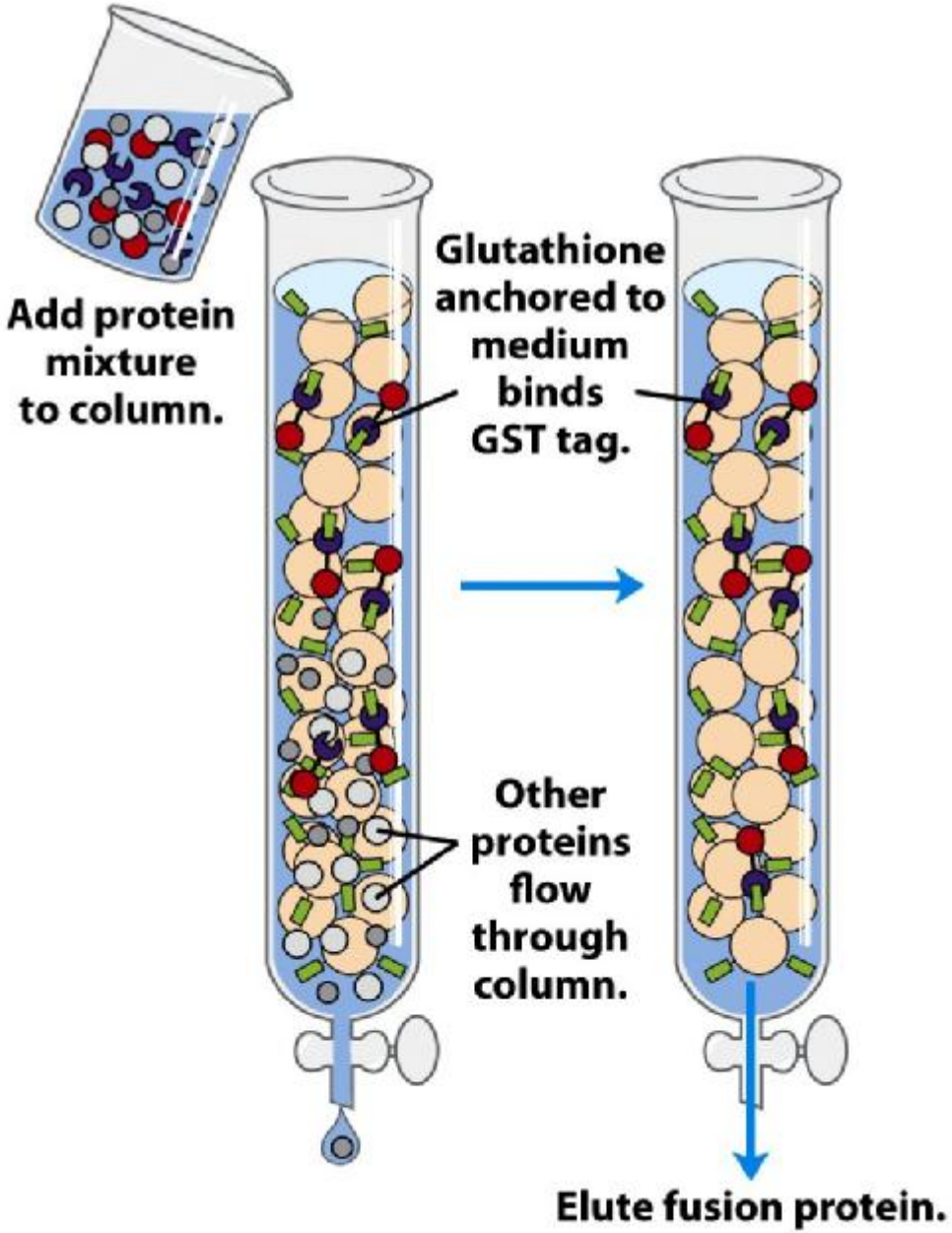

Figure 9-12b part 2
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TABLE 9–3 Commonly Used Protein Tags

Tag protein/ peptide	Molecular mass (kDa)	Immobilized ligand
Protein A	59	Fc portion of IgG
$(His)_6$	0.8	Ni^{2+}
Glutathione-*S*-transferase (GST)	26	Glutathione
Maltose-binding protein	41	Maltose
β-Galactosidase	116	*p*-Aminophenyl-β-D-thiogalactoside (TPEG)
Chitin-binding domain	5.7	Chitin

Table 9-3
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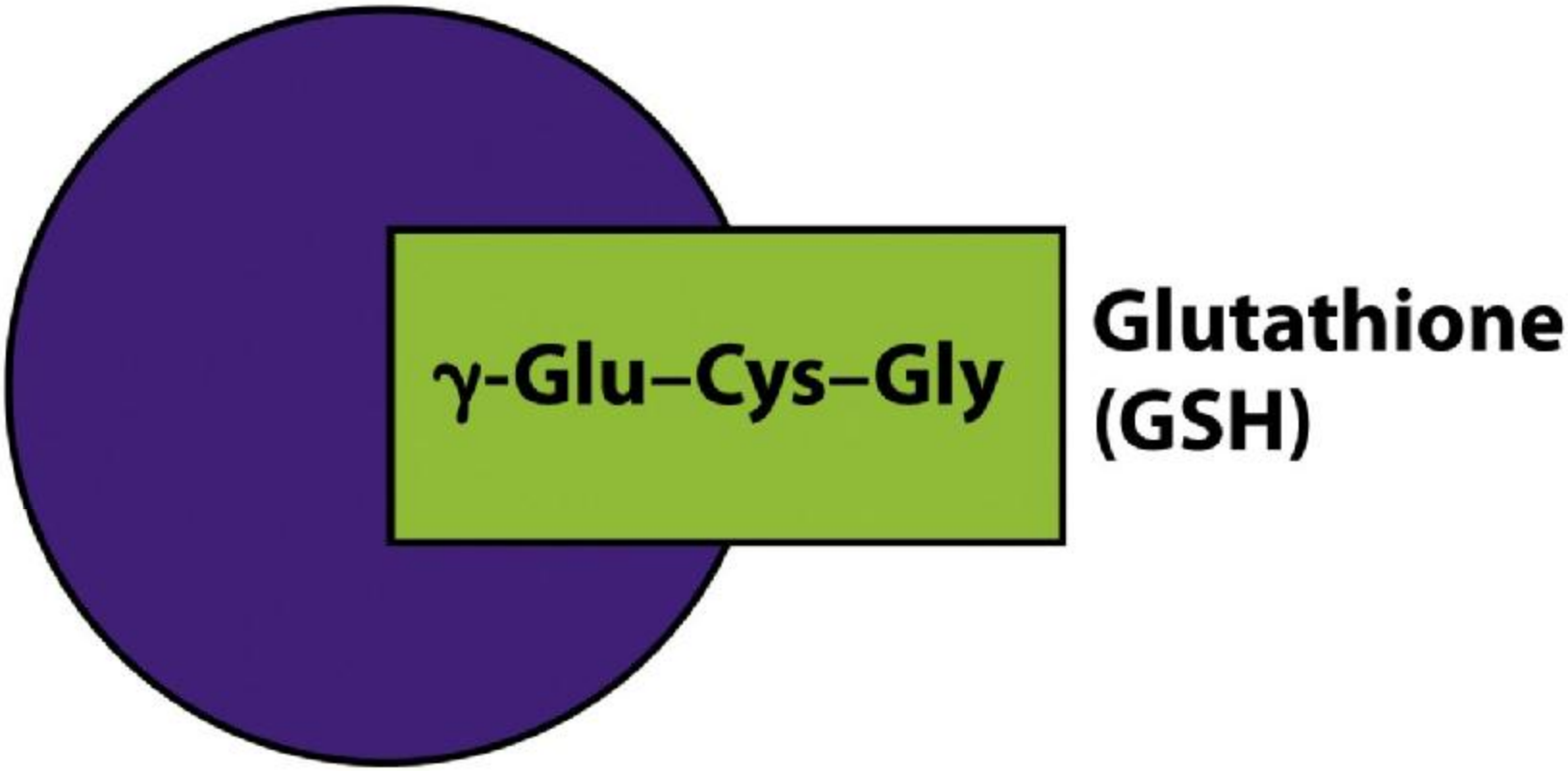

Figure 9-12a
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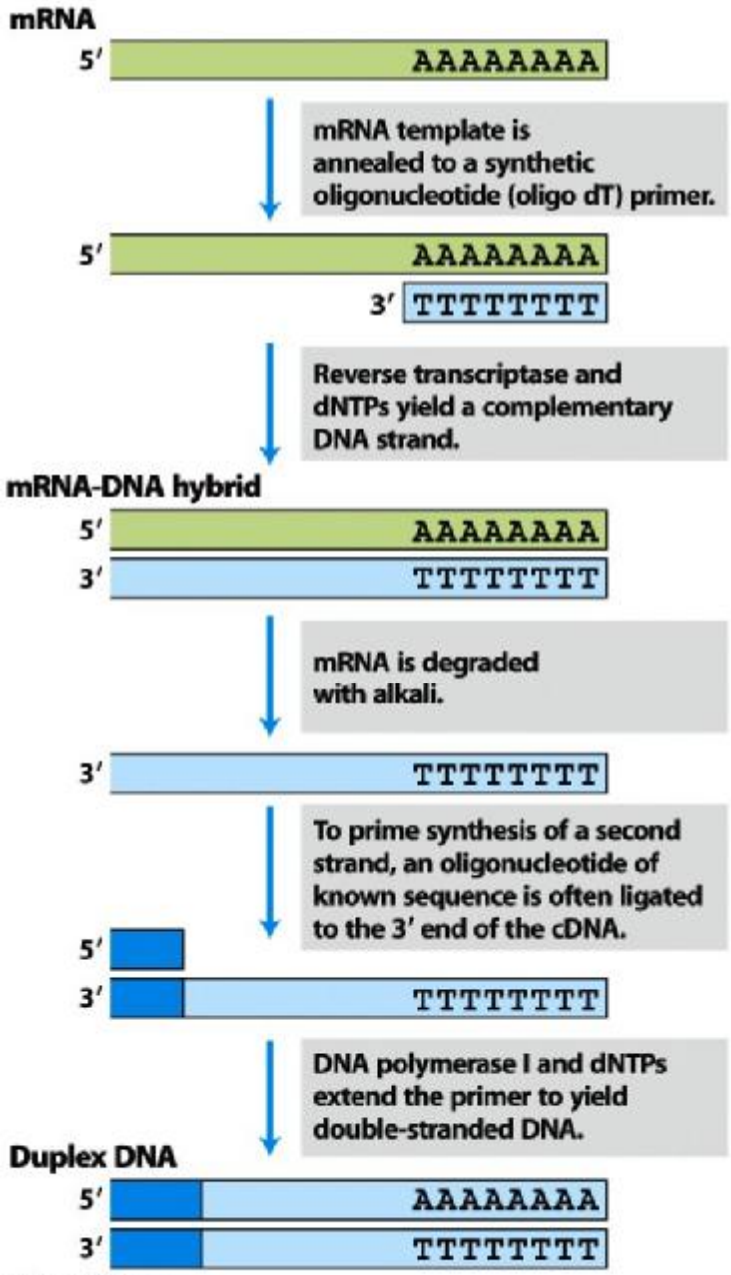

Figure 9-14

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Construction of a cDNA library from mRNA

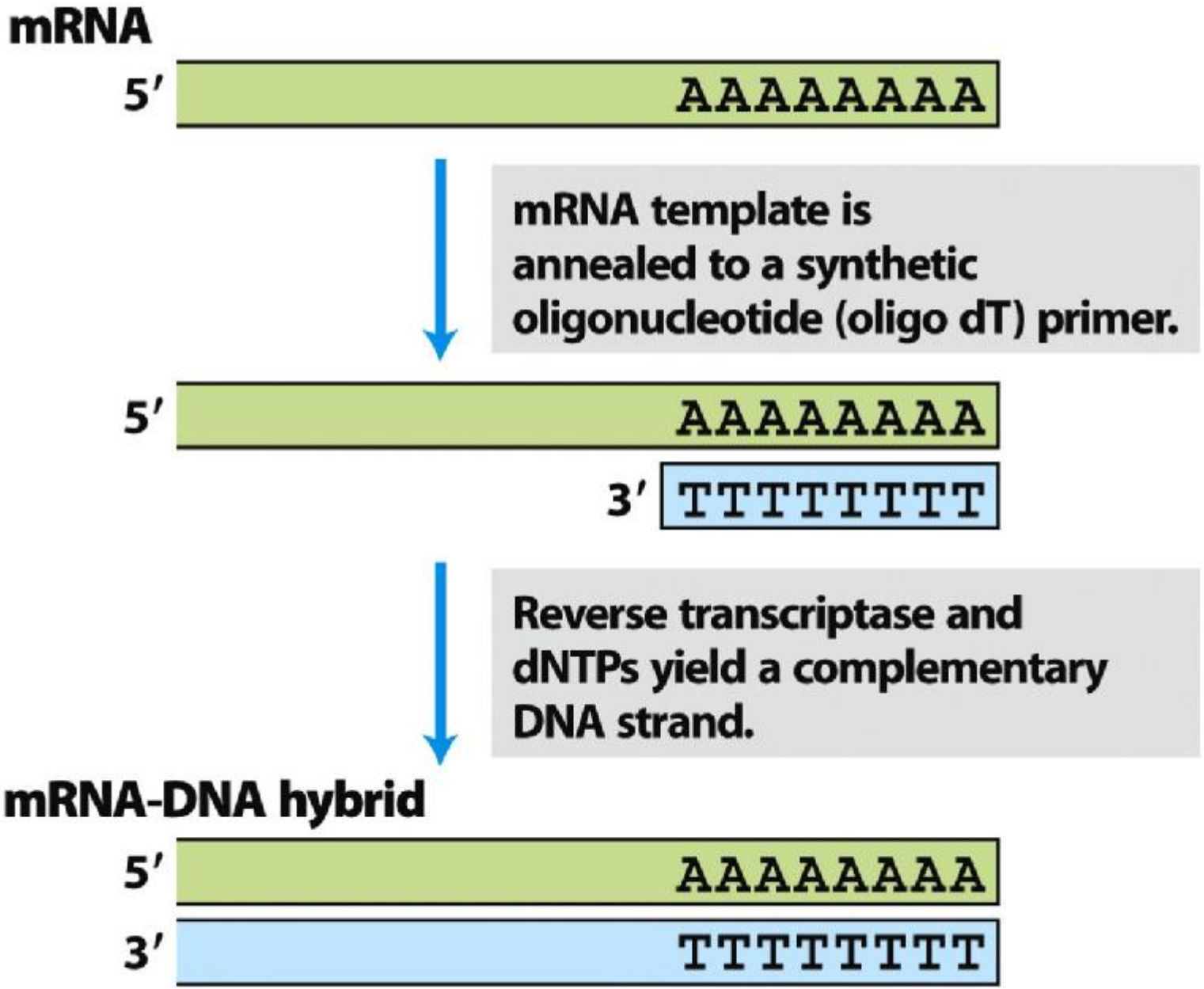

Figure 9-14 part 1
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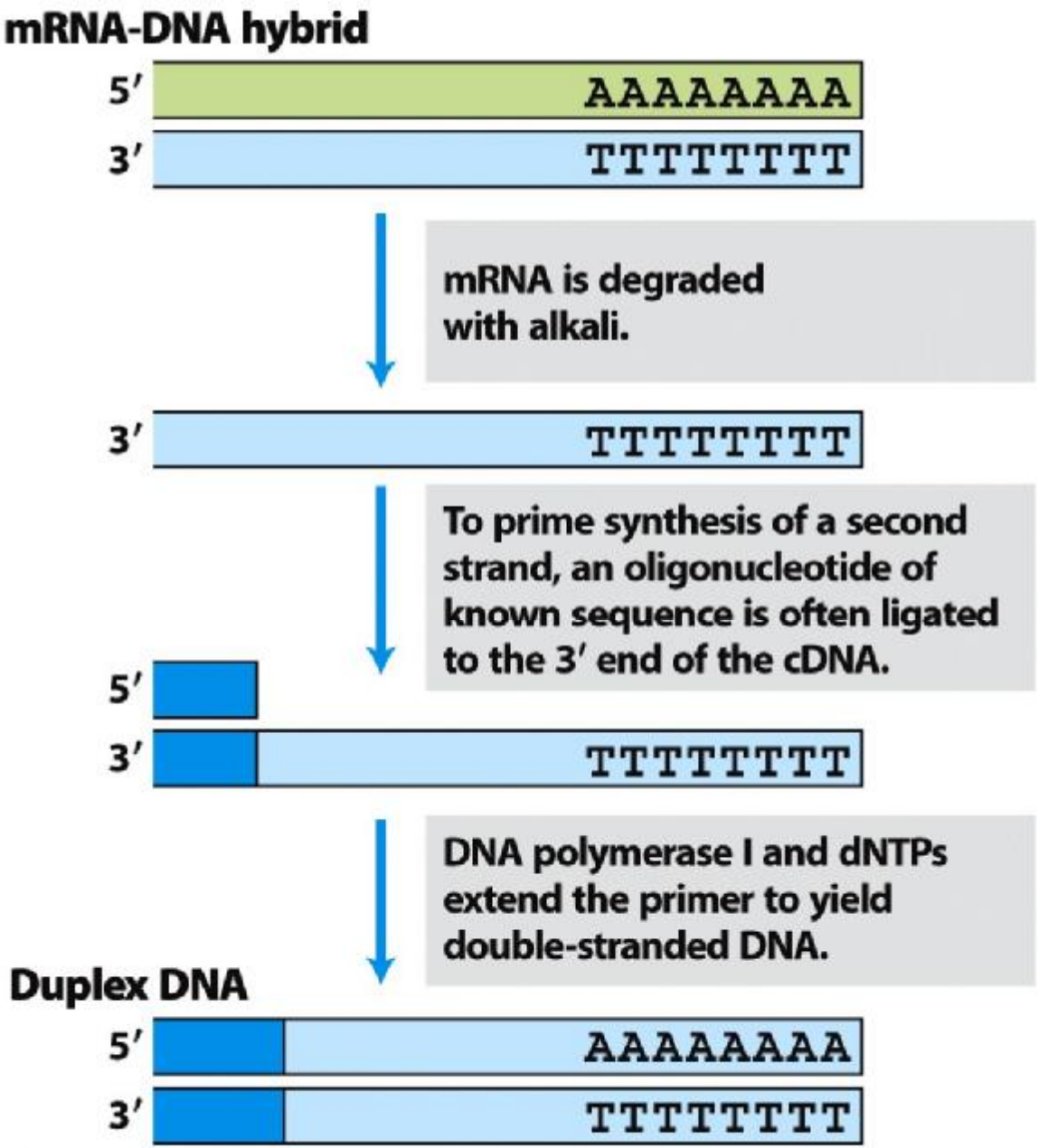

Figure 9-14 part 2

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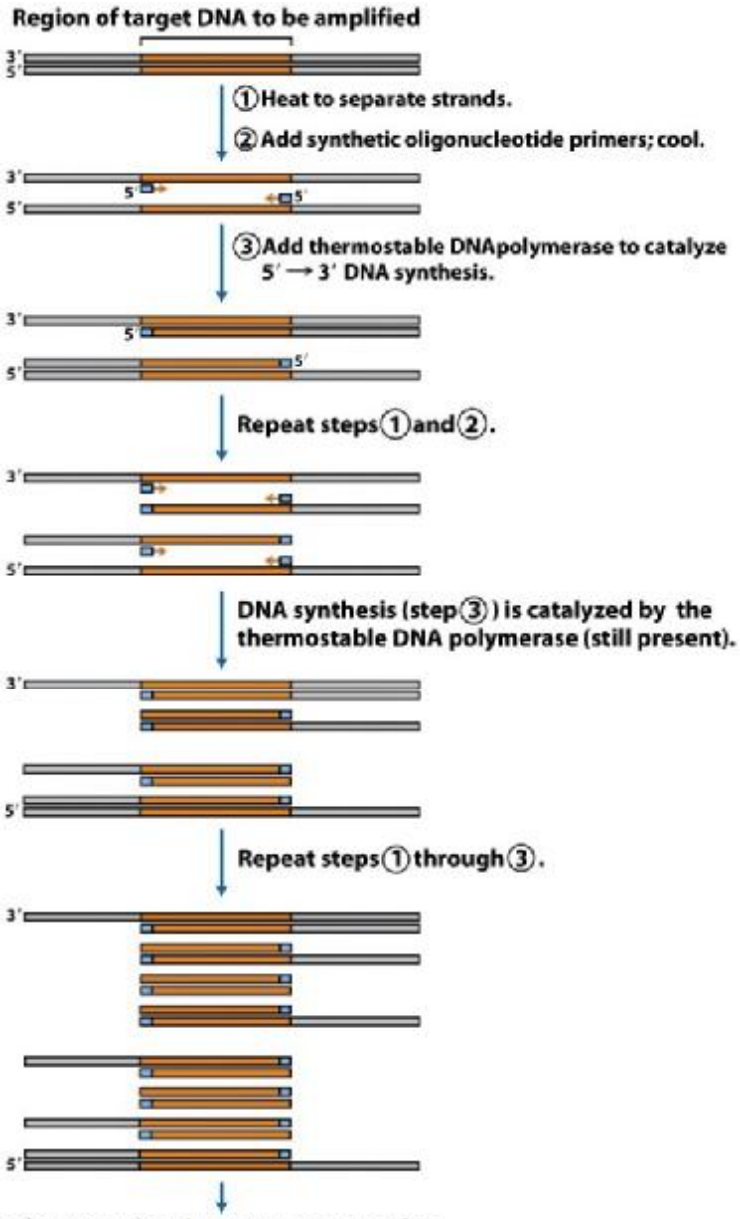

Figure 9-16a

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polymerase chain reaction

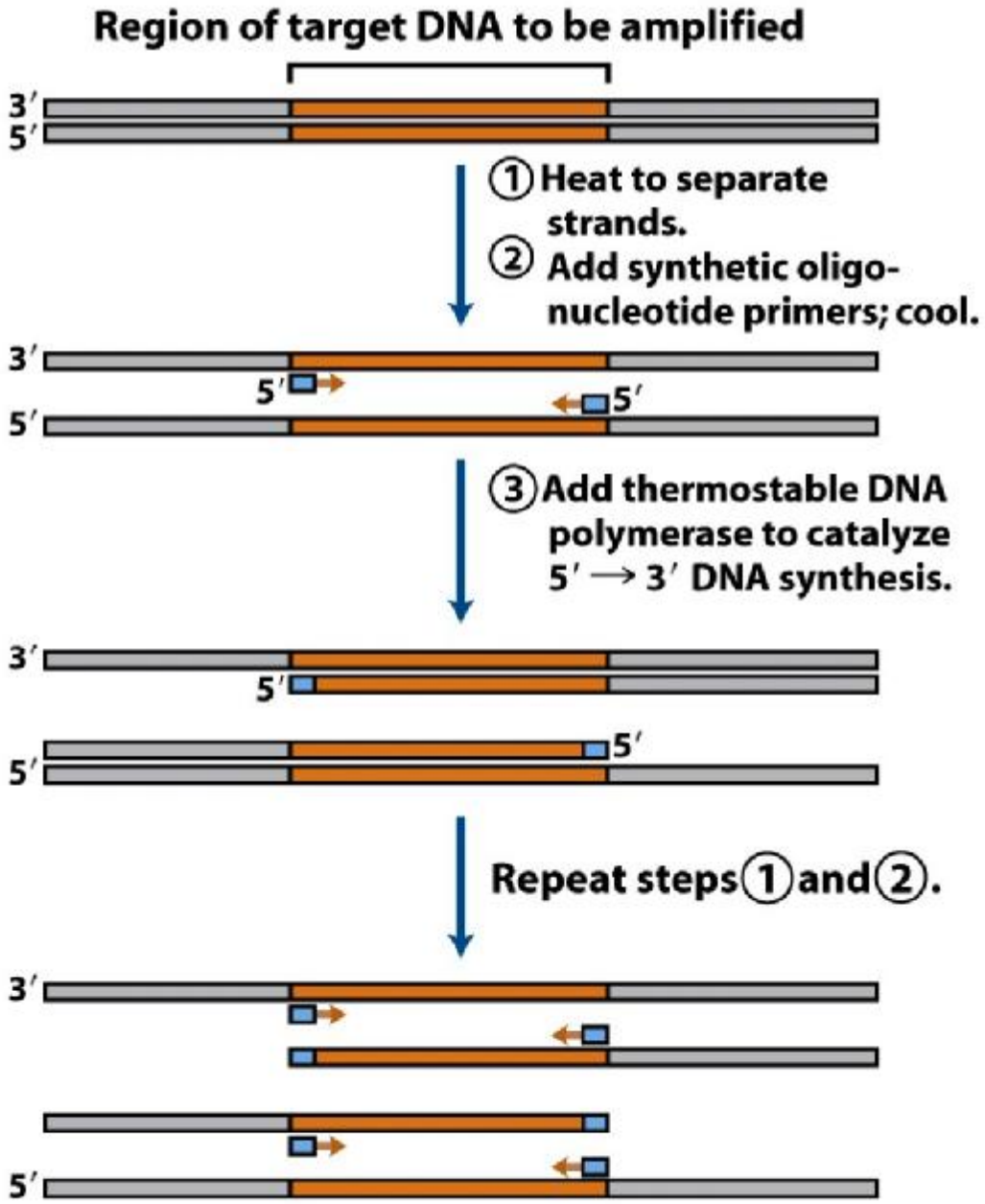

Figure 9-16a part 1

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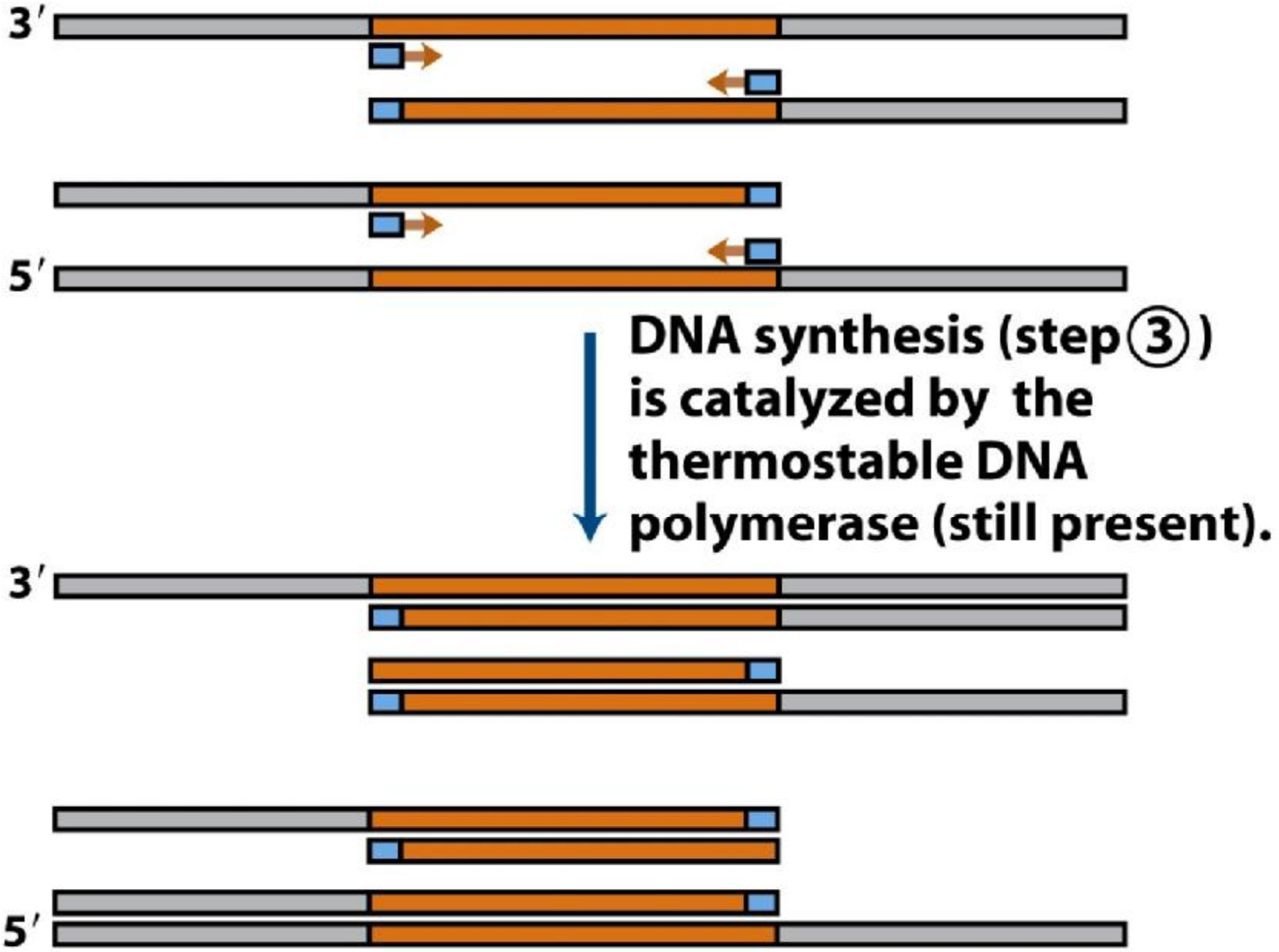

Figure 9-16a part 2
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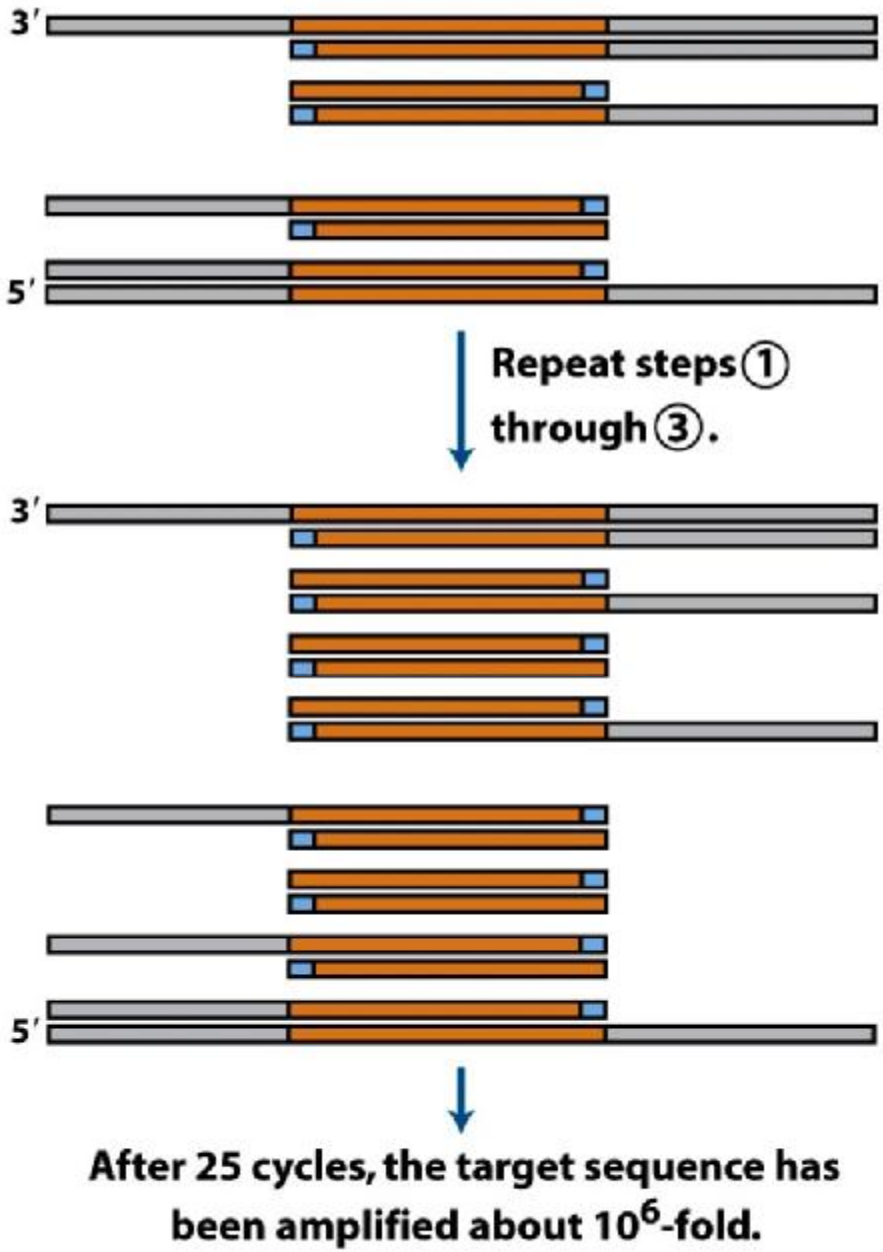

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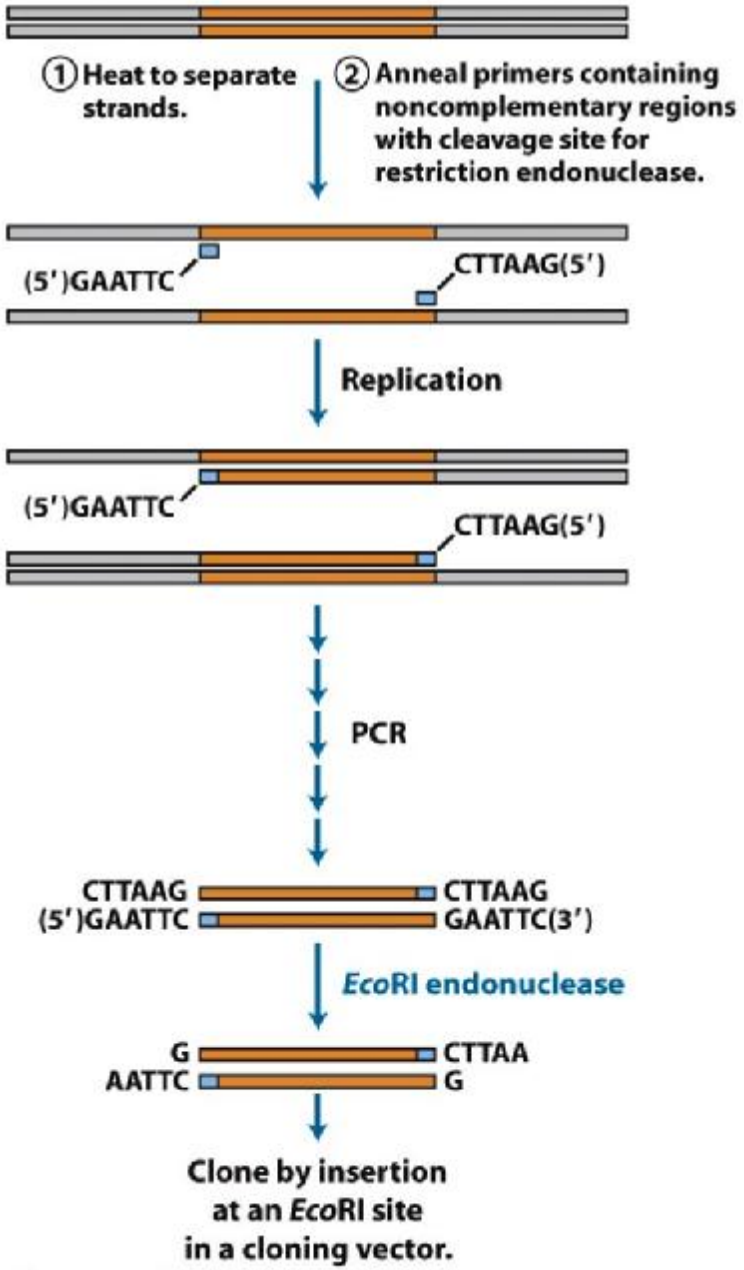

Figure 9-16b

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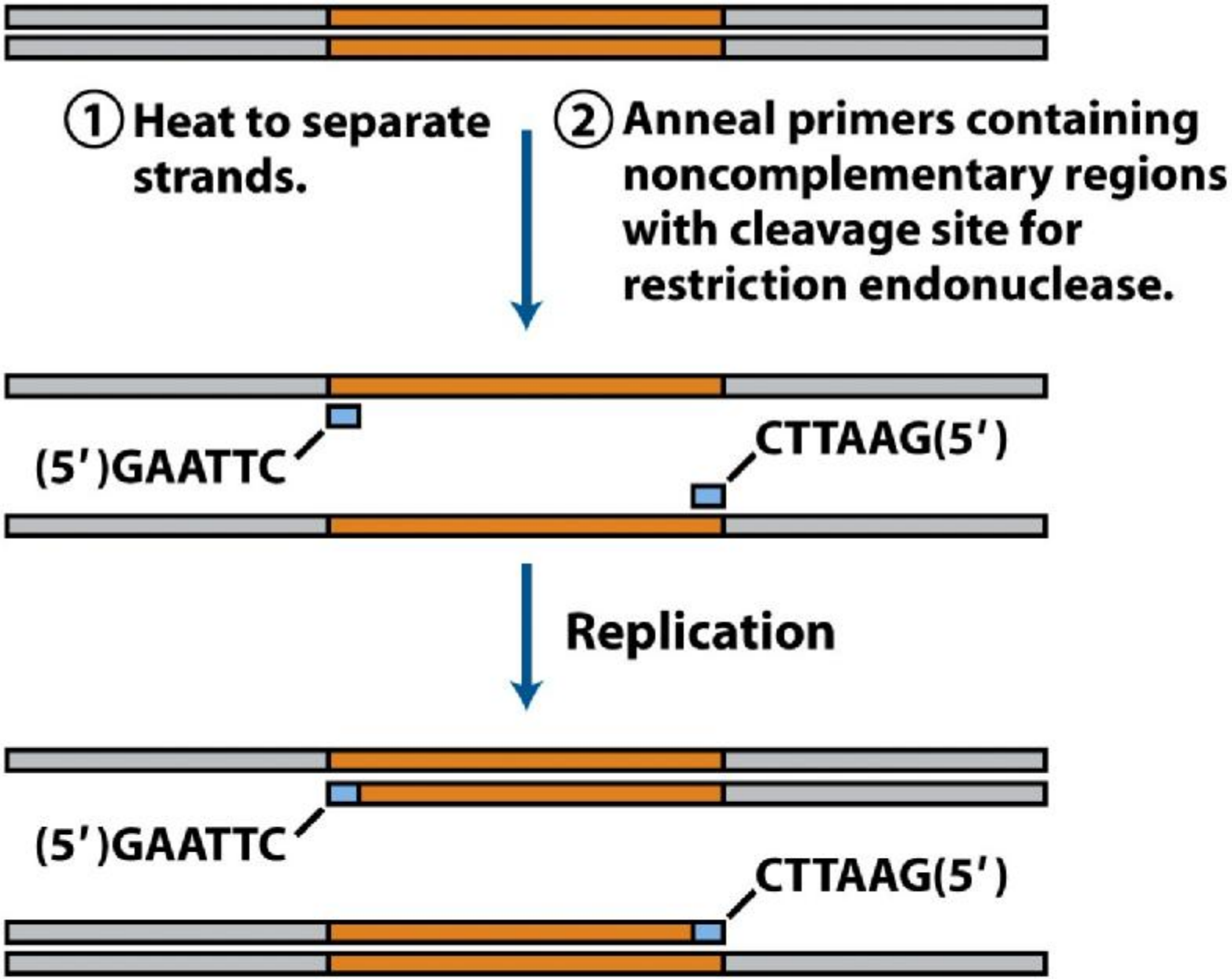

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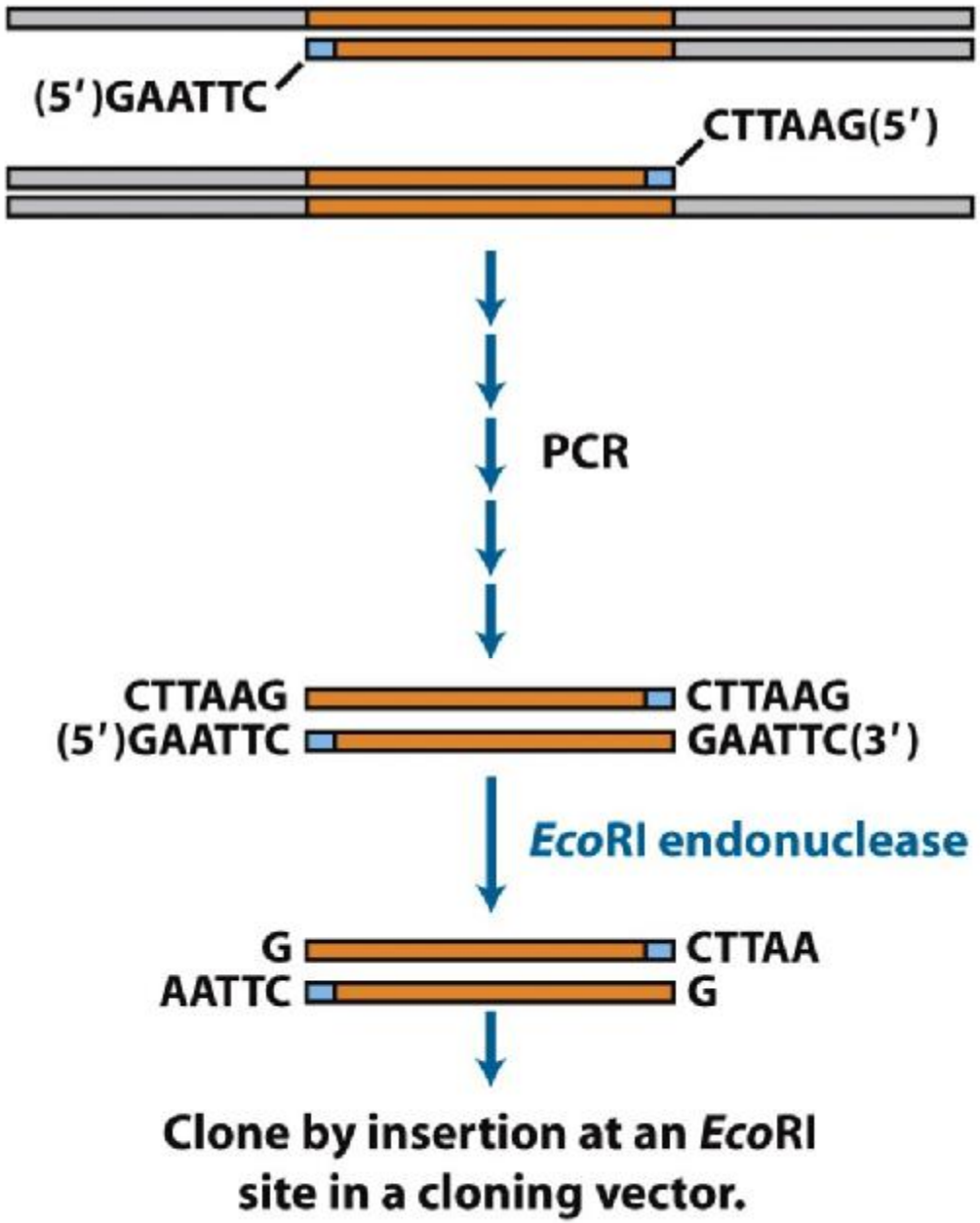

Figure 9-16b part 2
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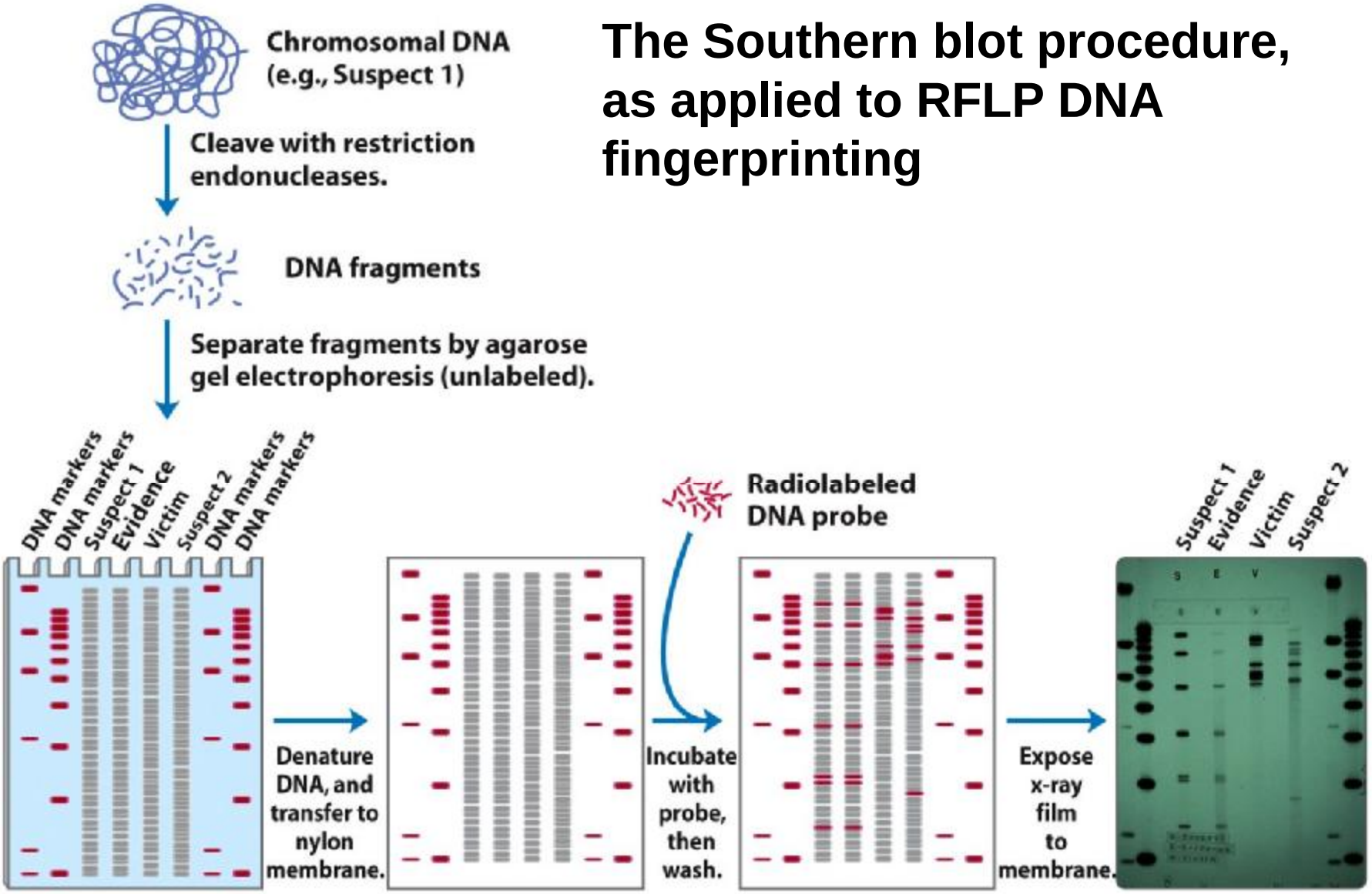
The Southern blot procedure, as applied to RFLP DNA fingerprinting



Box 9-1 figure 1

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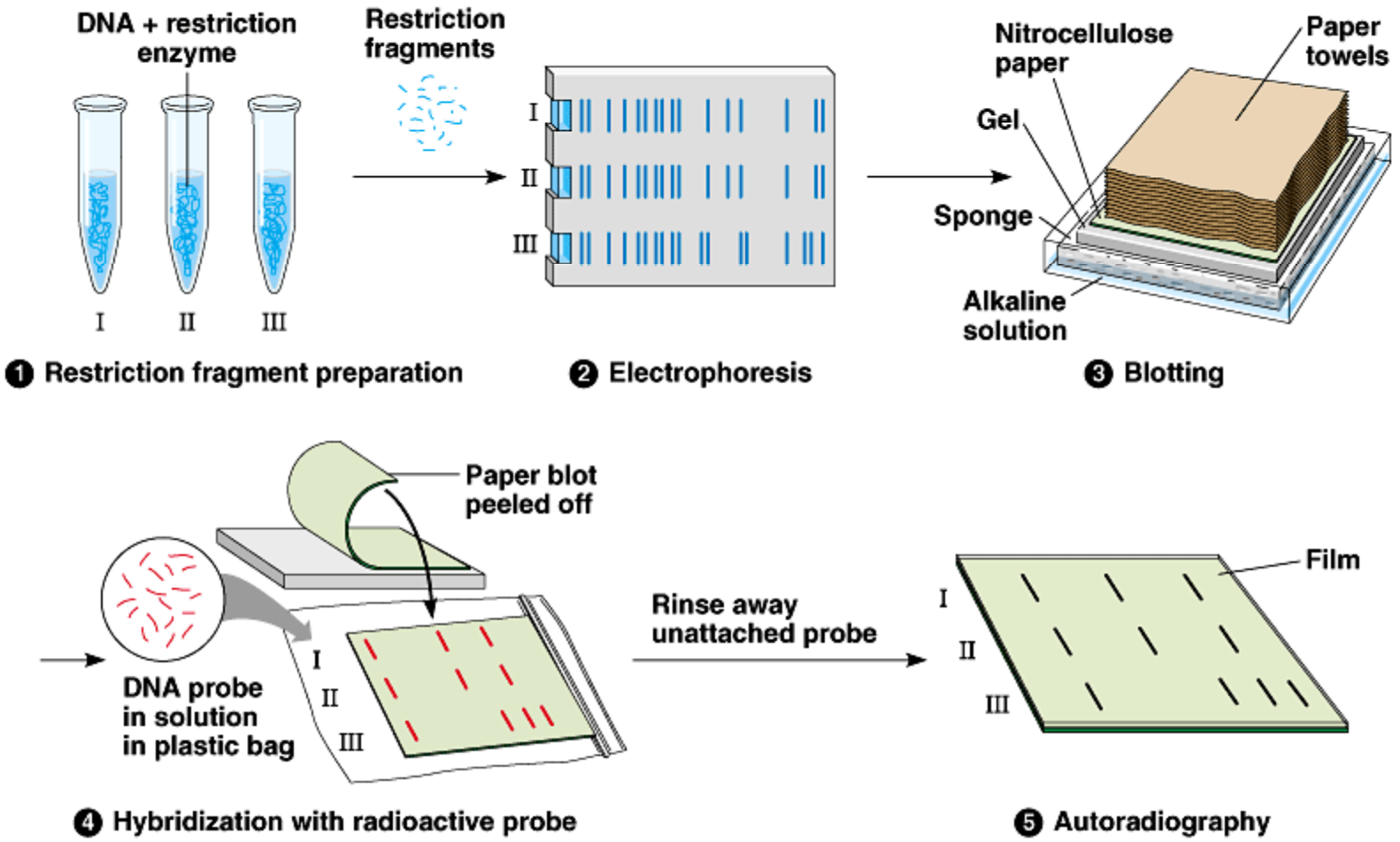
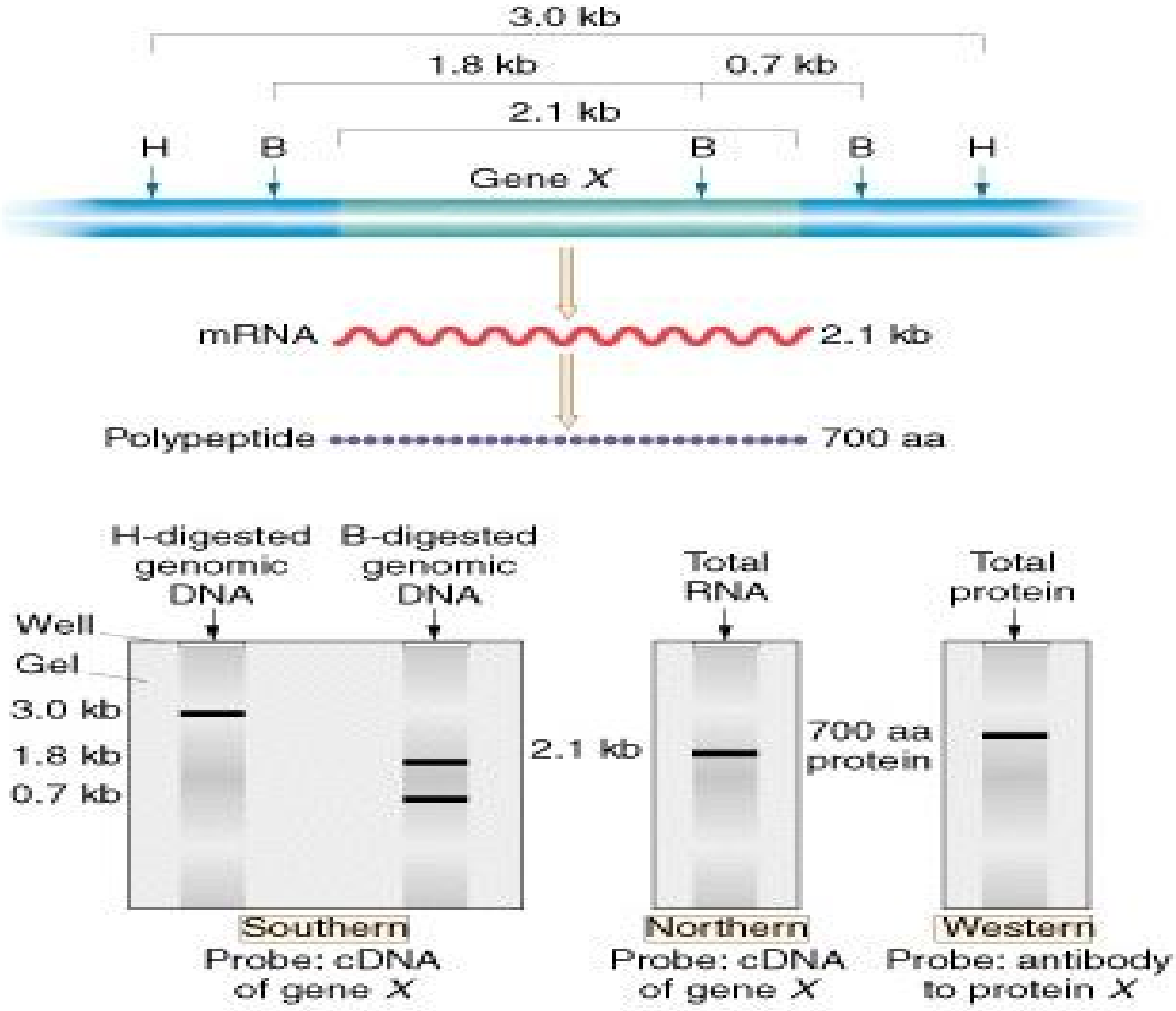
Southern Blotting

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1.8 kb
0.7 kb
2.1 kb
H
B
B
B
H
Gene X
mRNA
2.1 kb
Polypeptide
700 aa
H-digested genomic DNA
B-digested genomic DNA
Well
Gel
3.0 kb
1.8 kb
0.7 kb
2.1 kb
Southern
Probe: cDNA of gene X
Total RNA
700 aa protein
Northern
Probe: cDNA of gene X
Total protein
Western
Probe: antibody to protein X

TABLE 1 Properties of the Loci Used for the CODIS Database

Locus	Chromosome	Repeat motif	Repeat length (range)*	Number of alleles seen†
CSF1PO	5	TAGA	5–16	20
FGA	4	CTTT	12.2–51.2	80
TH01	11	TCAT	3–14	20
TPOX	2	GAAT	4–16	15
VWA	12	[TCTG][TCTA]	10–25	28
D3S1358	3	[TCTG][TCTA]	8–21	24
D5S818	5	AGAT	7–18	15
D7S820	7	GATA	5–16	30
D8S1179	8	[TCTA][TCTG]	7–20	17
D13S317	13	TATC	5–16	17
D16S539	16	GATA	5–16	19
D18S51	18	AGAA	7–39.2	51
D21S11	21	[TCTA][TCTG]	12–41.2	82
Amelogenin	X,Y	Not applicable		

Source: Adapted from Butler, J.M. (2005) *Forensic DNA Typing,* 2nd edn, Academic Press, San Diego, p. 96.

*Repeat lengths observed in the human population. Partial or imperfect repeats can be included in some alleles.

†Number of different alleles observed to date in the human population. Careful analysis of a locus in many individuals is a prerequisite to its use in forensic DNA typing.

Box 9-1 table 1

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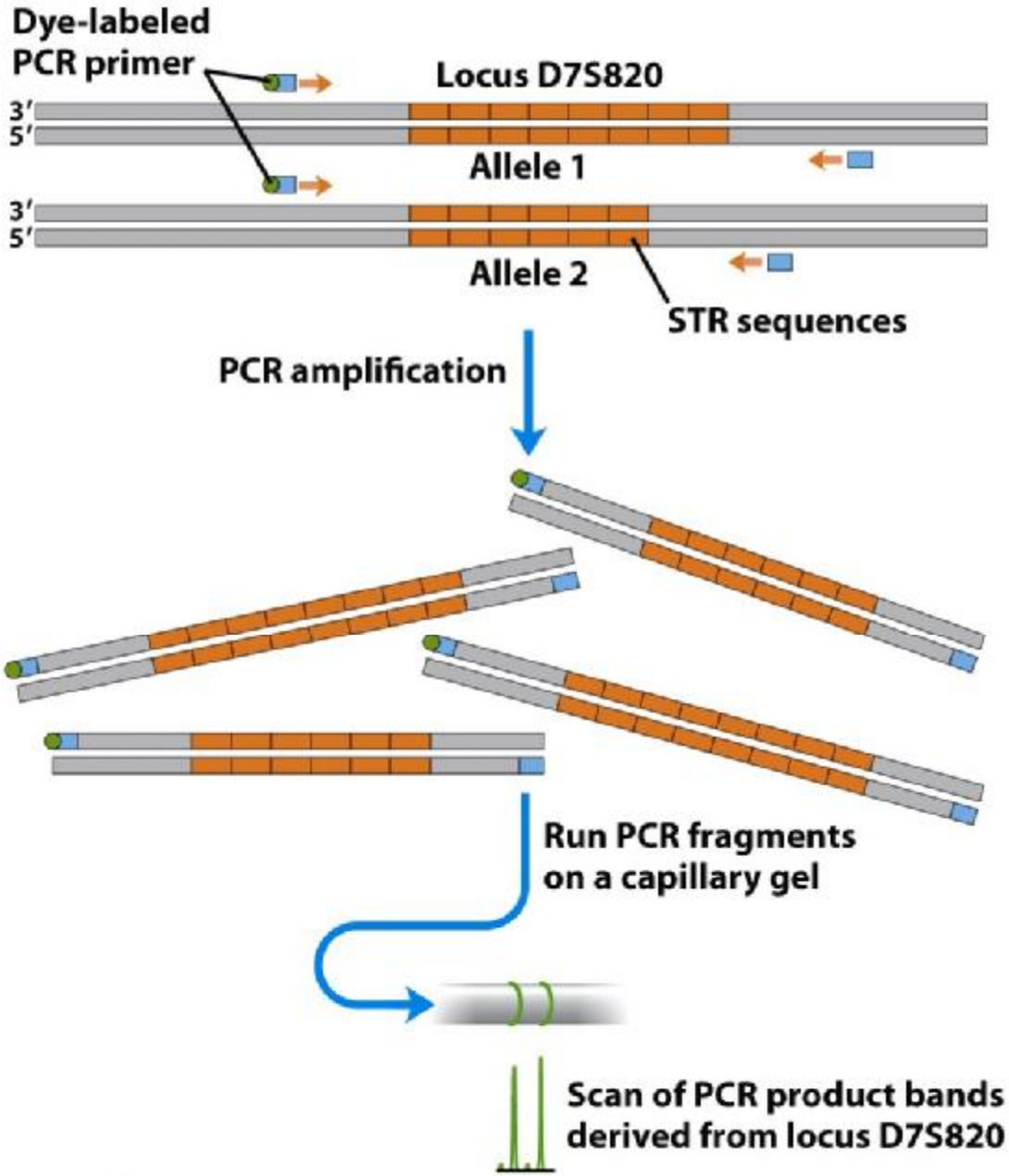

Box 9-1 figure 2a
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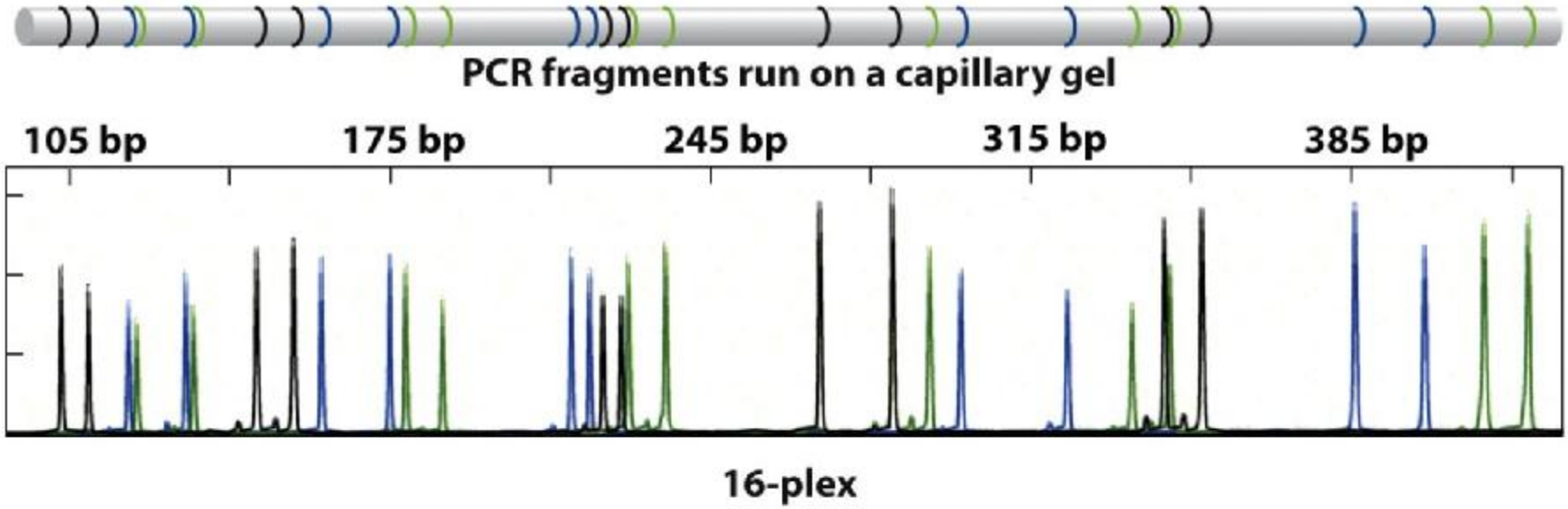
PCR analysis of an STR locus

PCR analysis of an STR locus



Box 9-1 figure 2b
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The Human Genome Project strategy

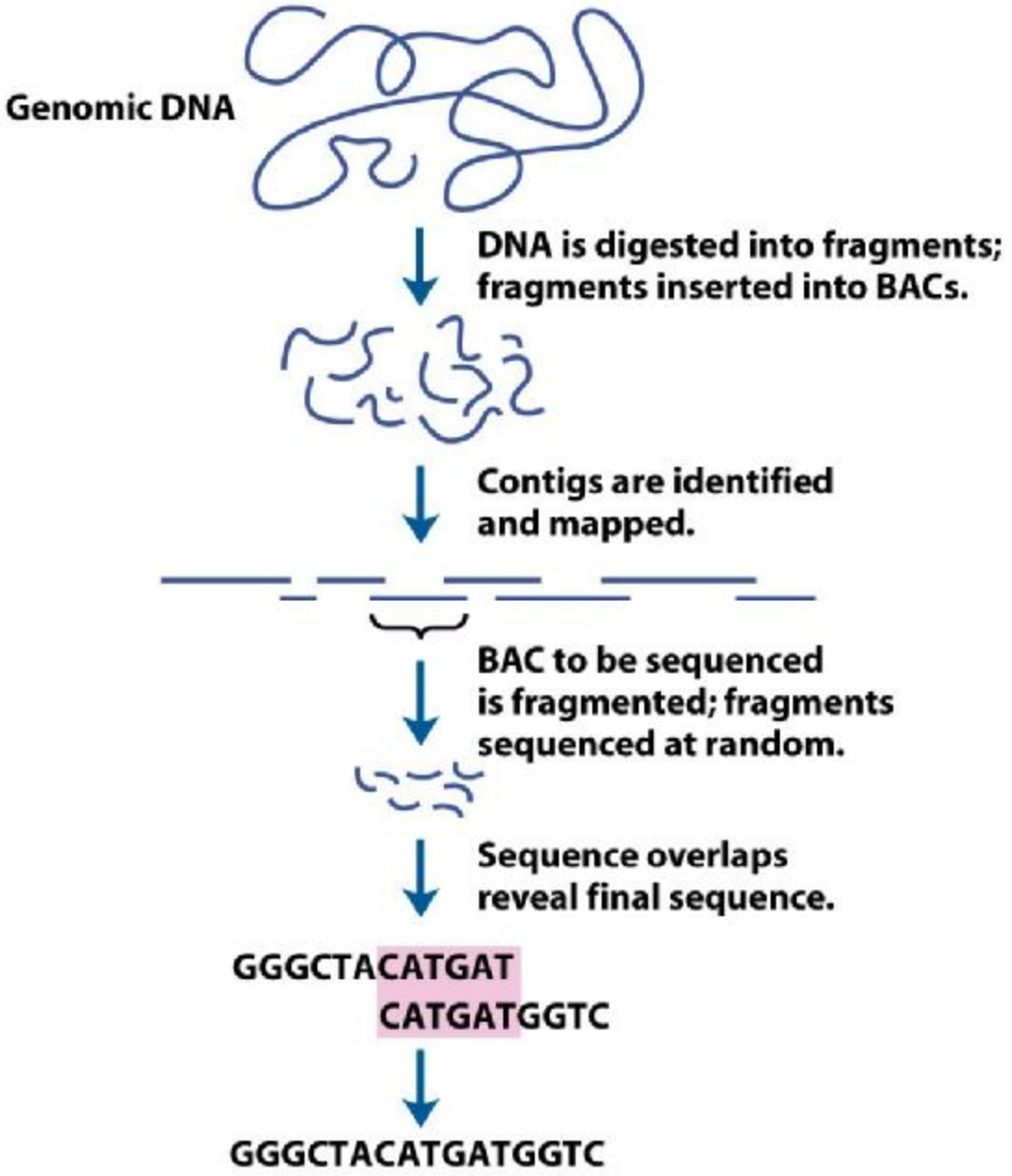

Figure 9-17
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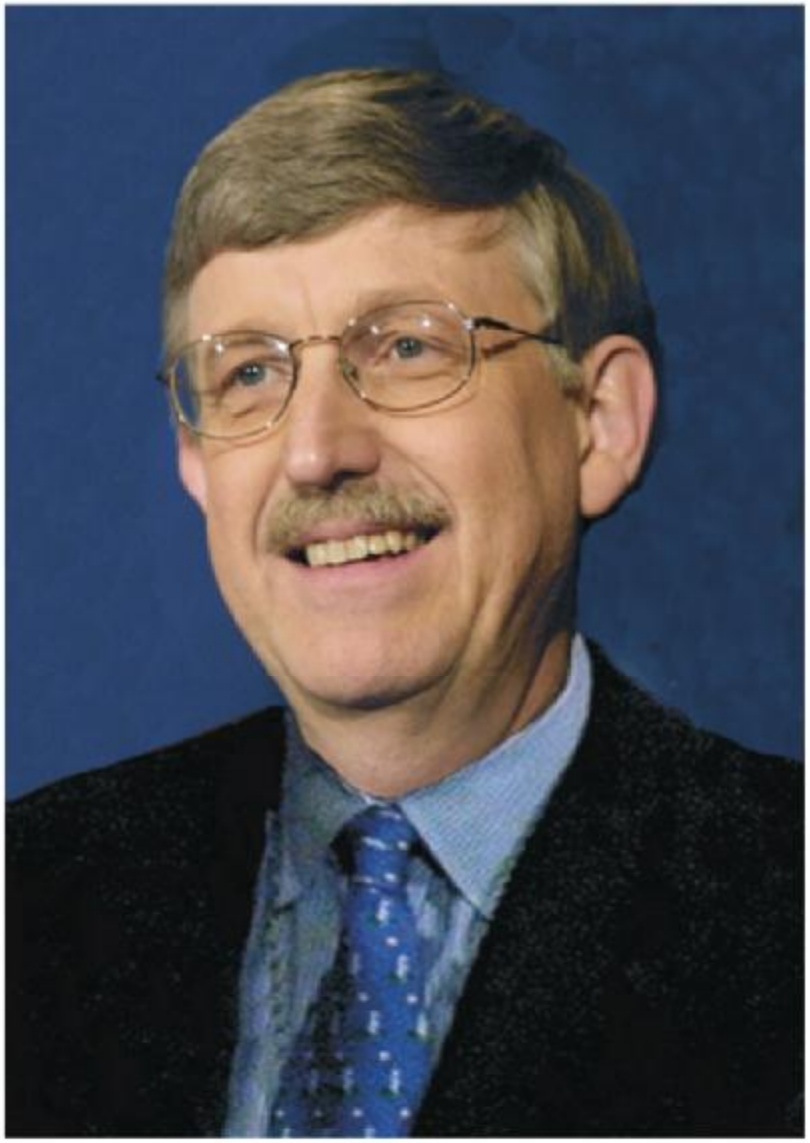
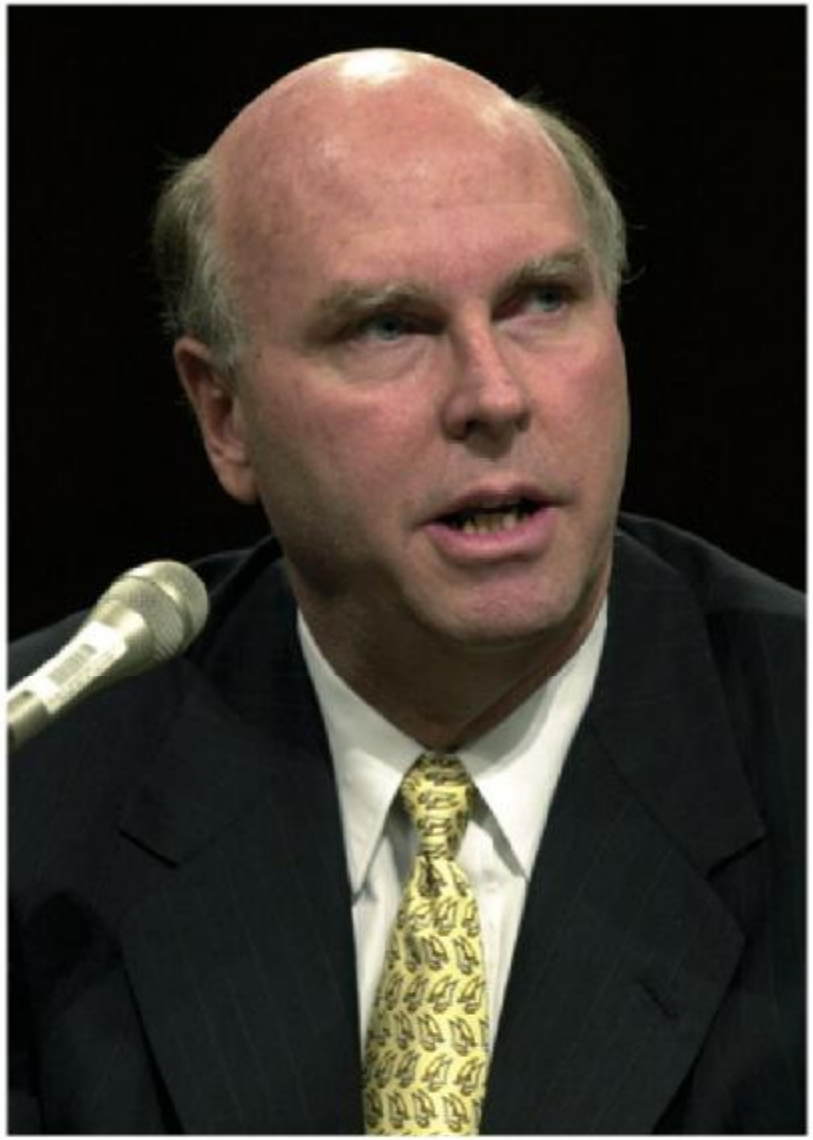


Francis S. Collins



J. Craig Venter

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Genomic sequencing timeline

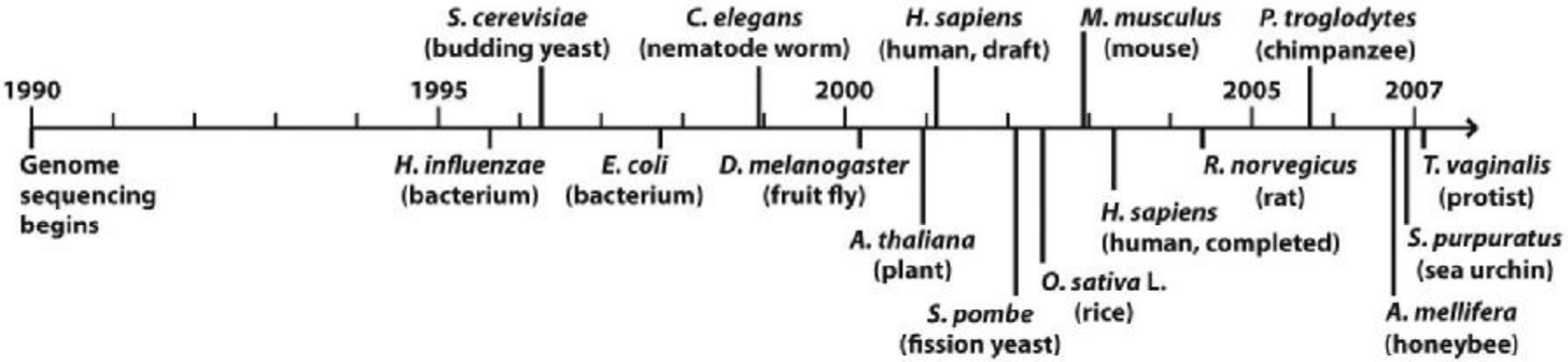

Figure 9-18
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DNA microarray

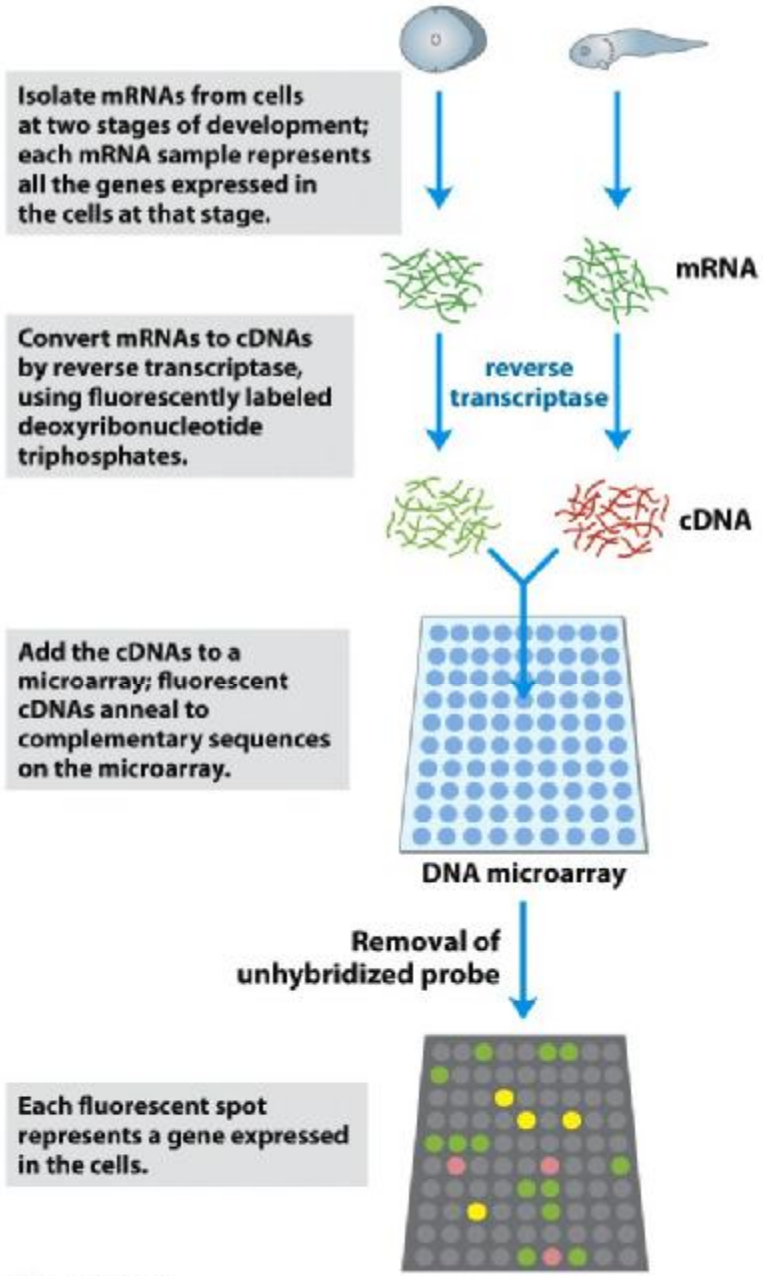

Figure 9-22
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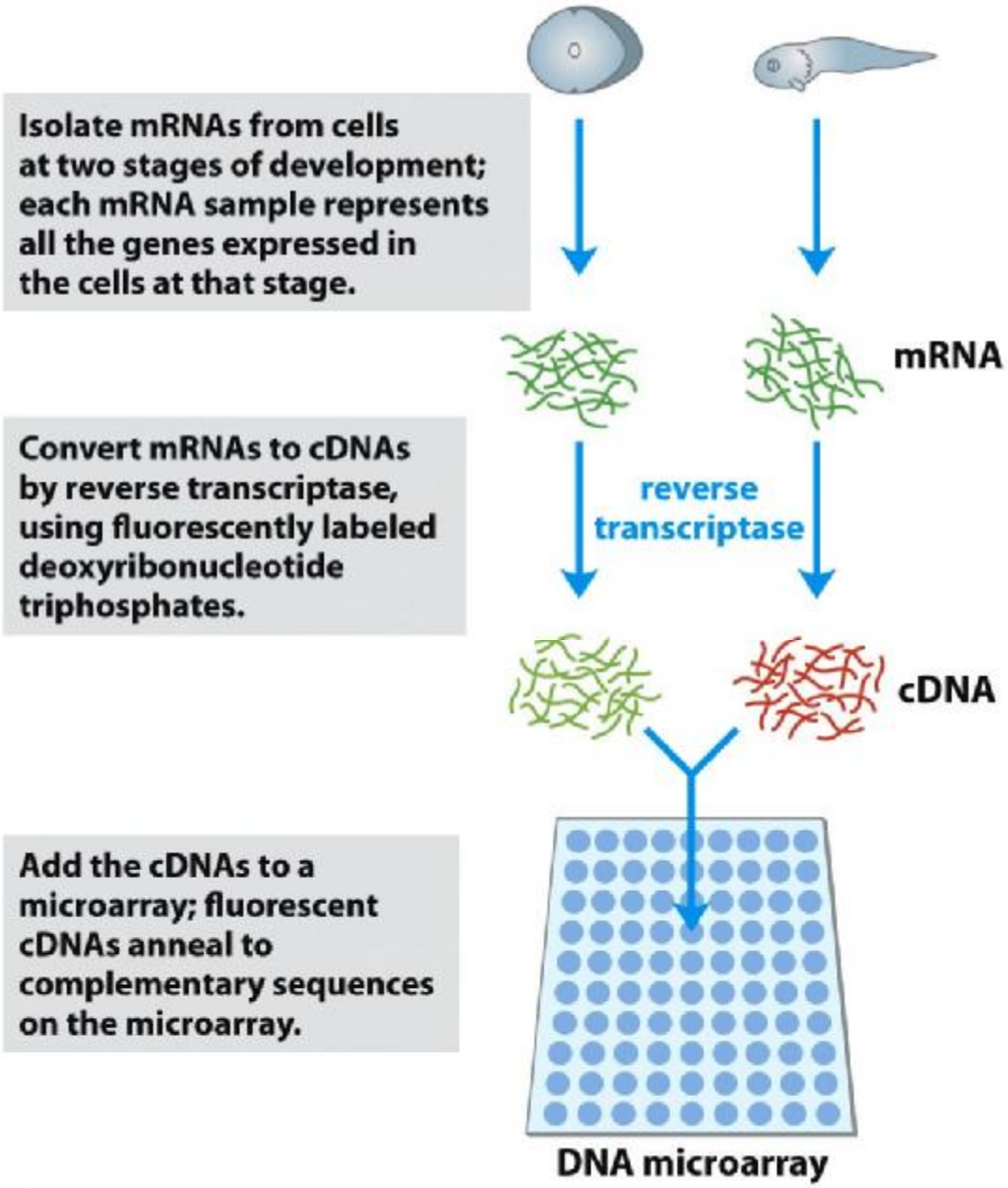

Figure 9-22 part 1
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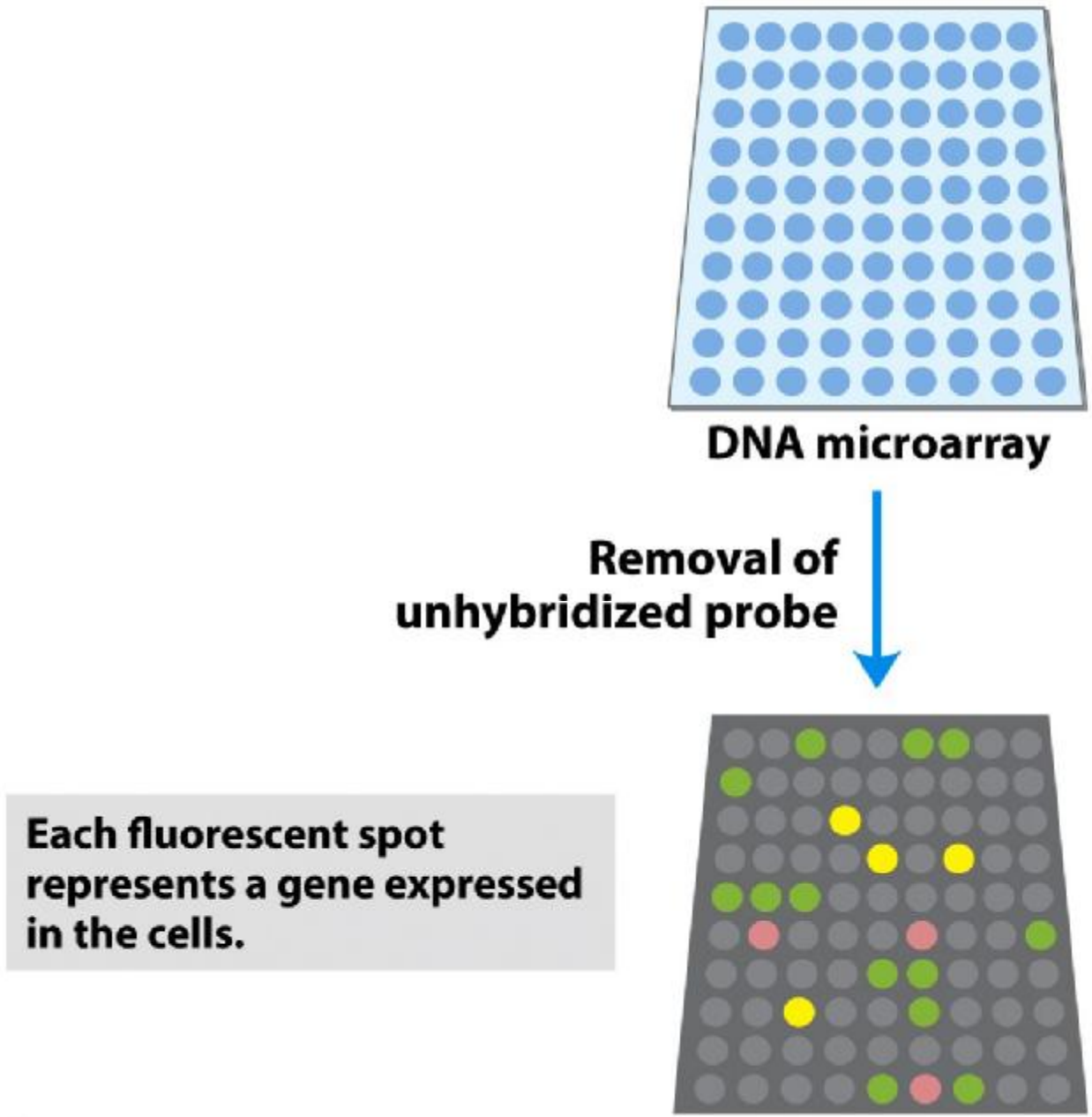

Figure 9-22 part 2
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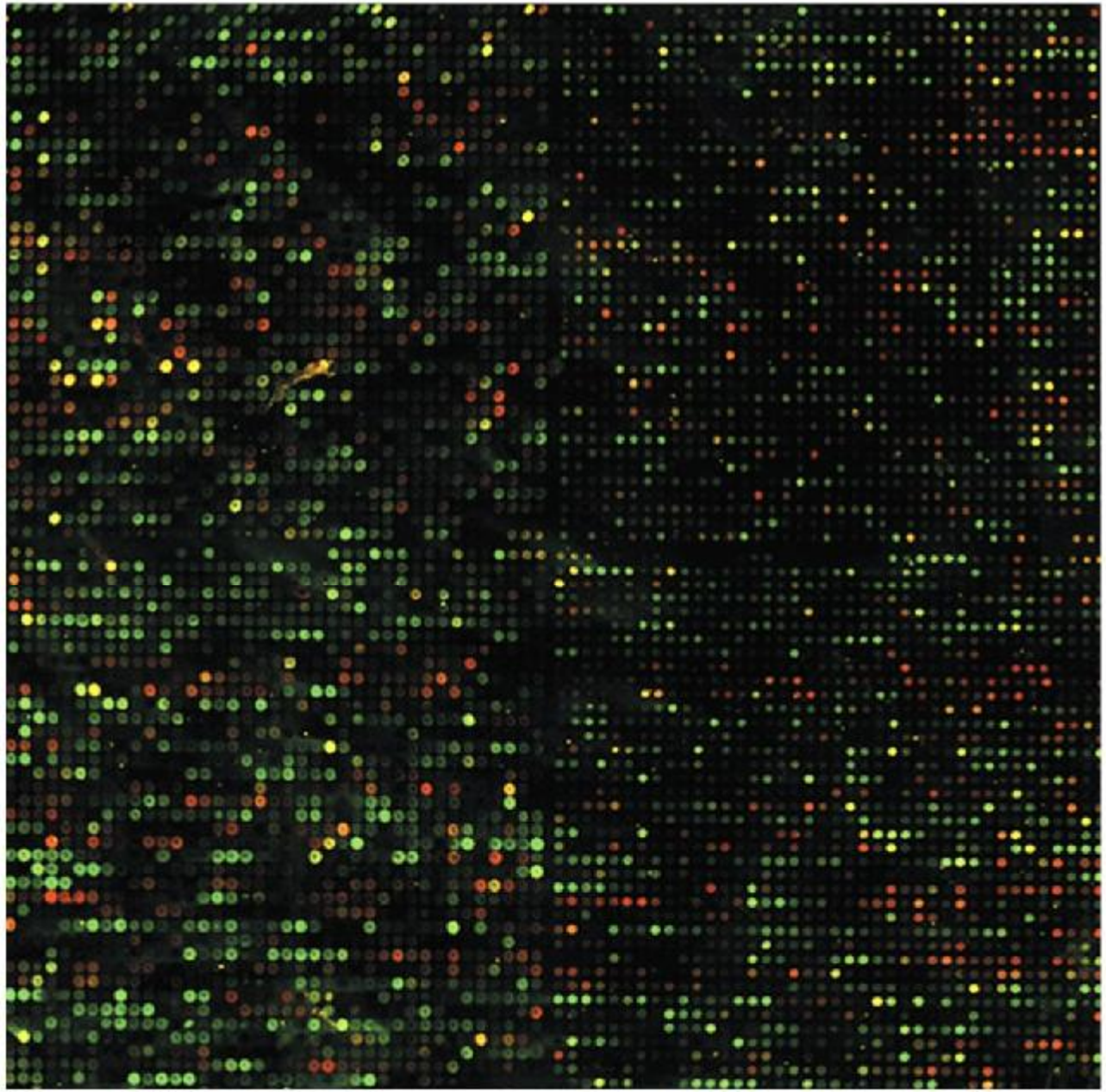

Figure 9-23

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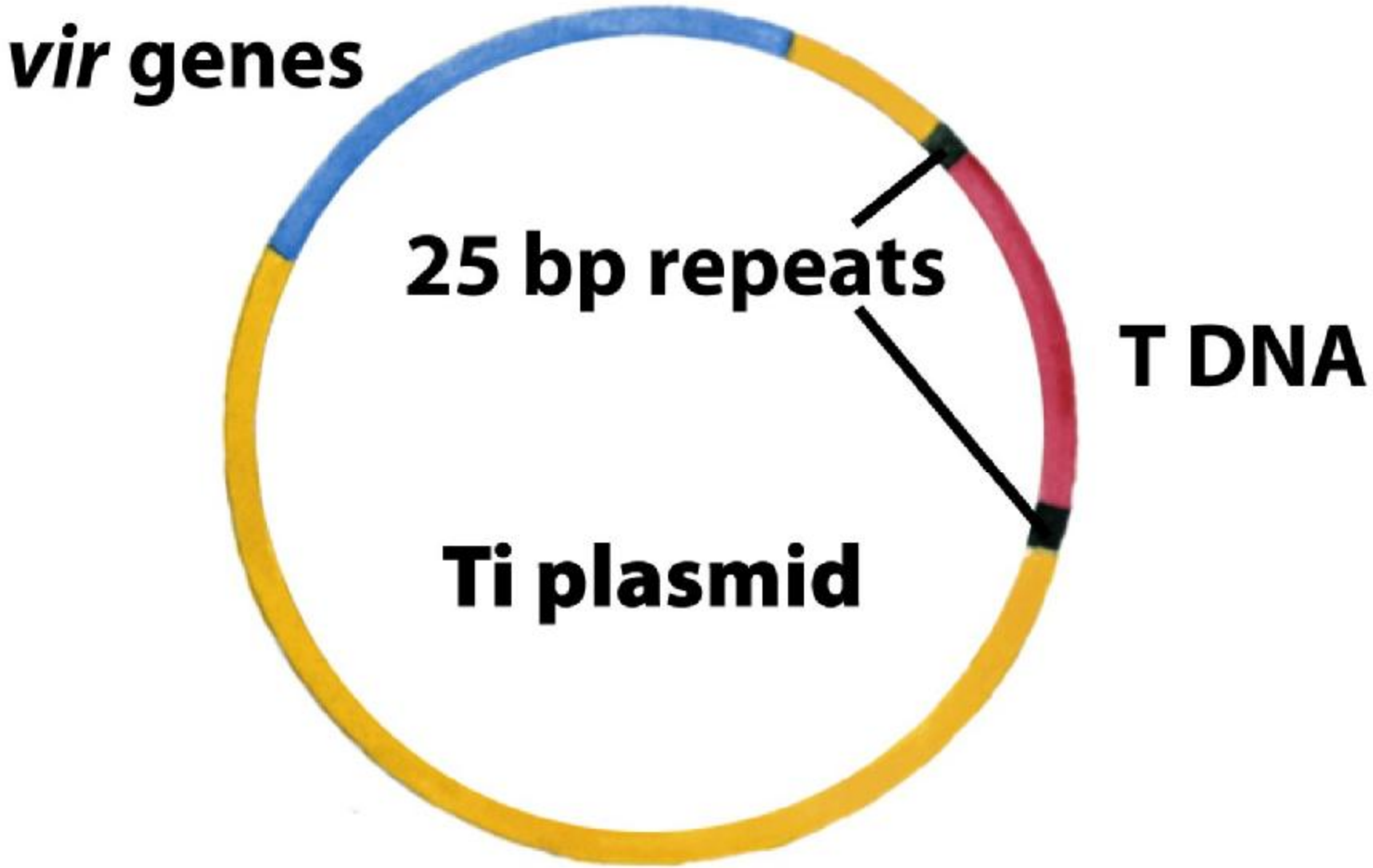

Figure 9-26a
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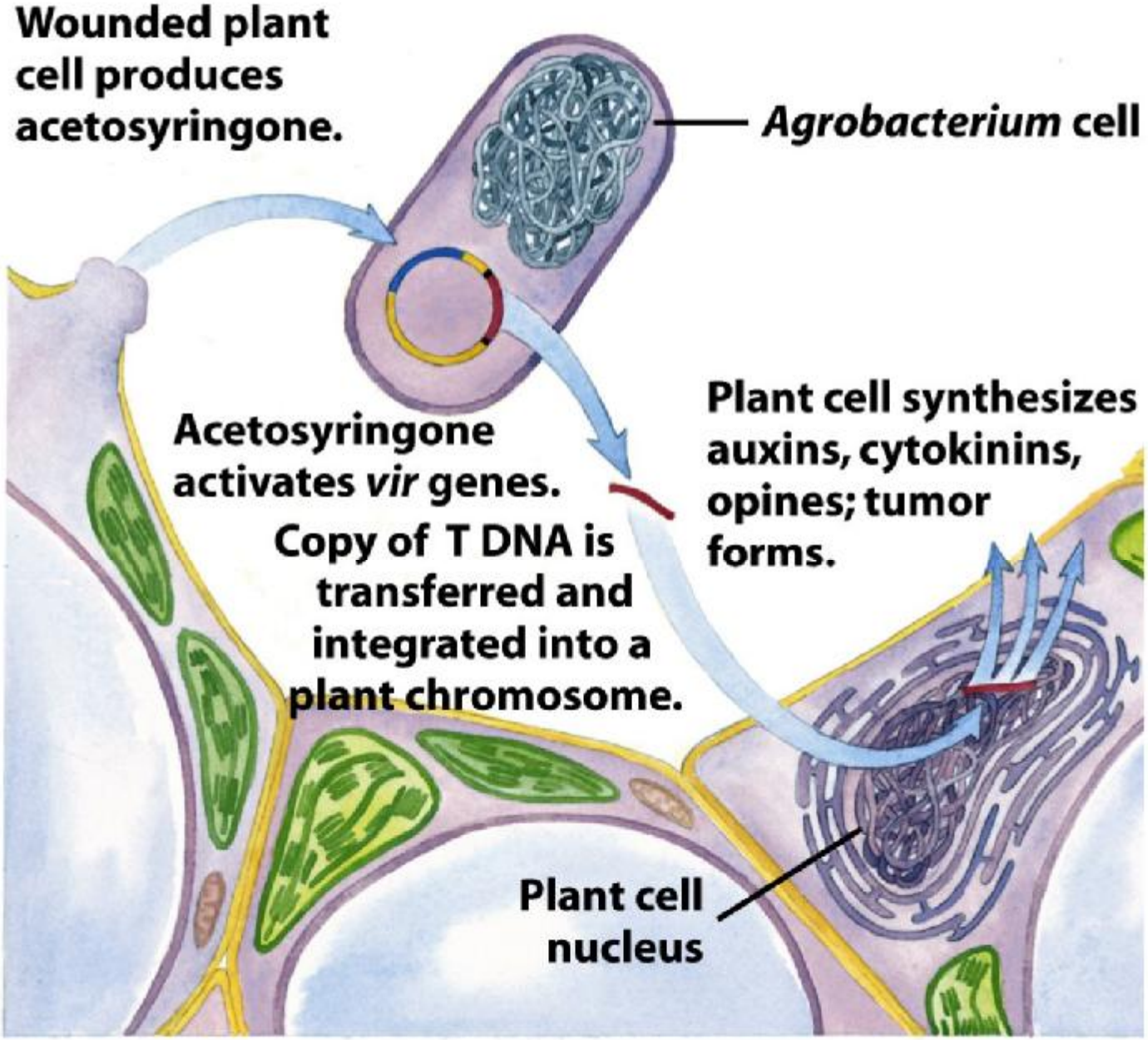

Figure 9-26b
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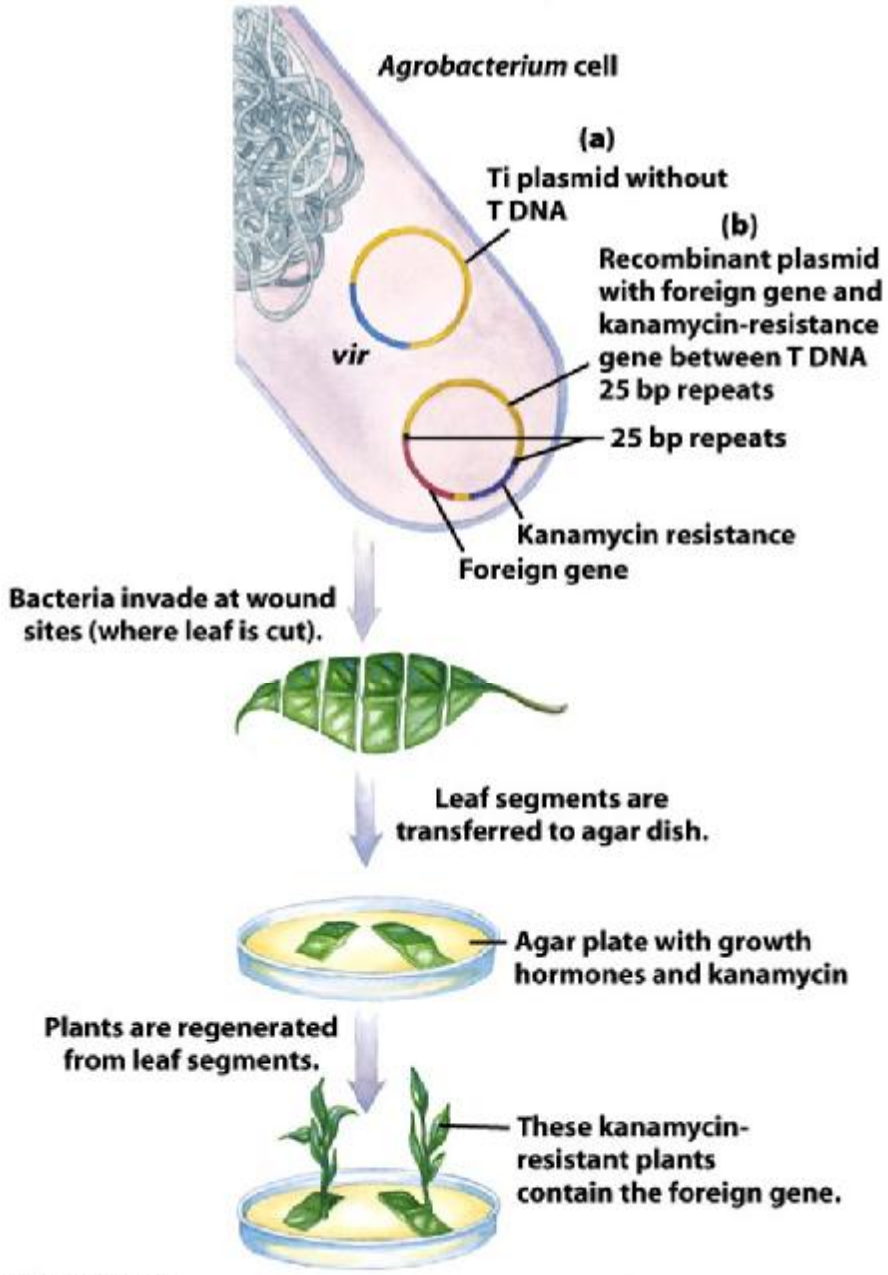

Figure 9-28

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A tobacco plant expressing the gene for firefly luciferase

Figure 9-29
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Figure 9-30
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Tomato plants engineered to be resistant to insect larvae

(a)

(b)

Figure 9-31
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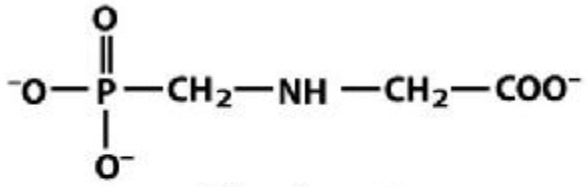
Glyphosate-resistant soybean plants



Glyphosate

Figure 9-33
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Genes for several variants of green fluorescent protein have been introduced into different strains of zebrafish

Transgenic Plants

Round Up Ready Soybeans are resistant to herbicide

Herbicide Tolerance, Insect Resistance, quality traits
Soybean, Corn, Cotton, Canola
Tomato

“Golden” rice with beta-carotene and extra iron

Bt Corn produces its own pesticide

A “clone” is a copy of something.

Computers that mimic IBMs are called “clones.”

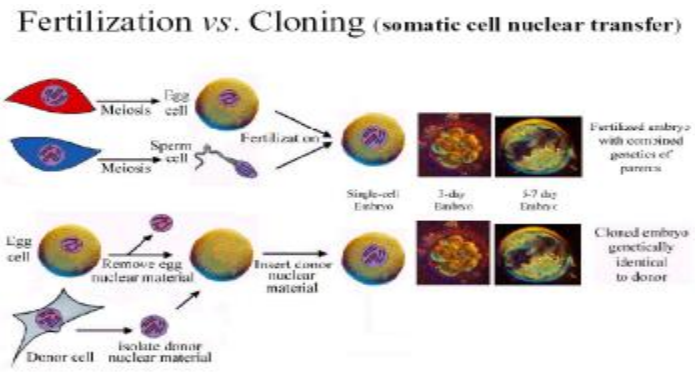
In genetics, a clone is a genetic copy of another organism.

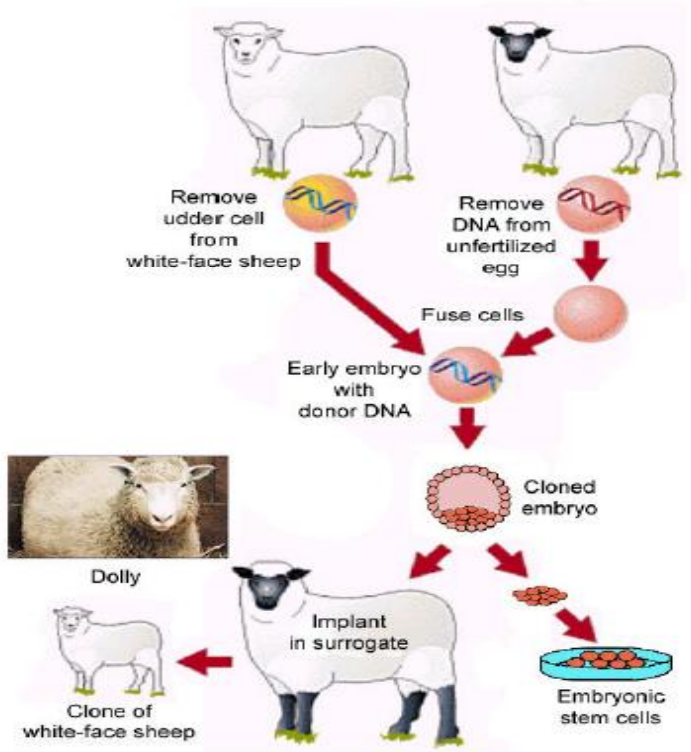
cloning

Clones occur naturally:

- Asexual breeding in plants & lower animals
- Identical twins (triplets) in higher animals

Dolly - 1996


Fertilization *vs.* Cloning (somatic cell nuclear transfer)
Meiosis
Egg cell
Sperm cell
Meiosis
Fertilization
Single-cell Embryo
3-day Embryo
5-7 day Embryo
Fertilized embryo with combined genetics of parents
Egg cell
Remove egg nuclear material
Insert donor nuclear material
Donor cell
Isolate donor nuclear material
Cloned embryo genetically identical to donor


Remove udder cell from white-face sheep
Remove DNA from unfertilized egg
Fuse cells
Early embryo with donor DNA
Cloned embryo
Dolly
Implant in surrogate
Clone of white-face sheep
Embryonic stem cells

Cloning since Dolly

Cloning of this sort has now been done on cattle, pigs and mice also.
The success rate has improved considerably.

Guar: First cloned endangered species dies 2 days after birth

Snuppy

TABLE 9–4 Some Recombinant DNA Products in Medicine

Product category	Examples/uses
Anticoagulants	Tissue plasminogen activator (TPA); activates plasmin, an enzyme involved in dissolving clots; effective in treating heart attack patients.
Blood factors	Factor VIII; promotes clotting; it is deficient in hemophiliacs; treatment with factor VIII produced by recombinant DNA technology eliminates infection risks associated with blood transfusions.
Colony-stimulating factors	Immune system growth factors that stimulate leukocyte production; treatment of immune deficiencies and infections.
Erythropoietin	Stimulates erythrocyte production; treatment of anemia in patients with kidney disease.
Growth factors	Stimulate differentiation and growth of various cell types; promote wound healing.
Human growth hormone	Treatment of dwarfism.
Human insulin	Treatment of diabetes.
Interferons	Interfere with viral reproduction; used to treat some cancers.
Interleukins	Activate and stimulate different classes of leukocytes; possible uses in treatment of wounds, HIV infection, cancer, and immune deficiencies.
Monoclonal antibodies	Extraordinary binding specificity is used in: diagnostic tests; targeted transport of drugs, toxins, or radioactive compounds to tumors as a cancer therapy; many other applications.
Superoxide dismutase	Prevents tissue damage from reactive oxygen species when tissues briefly deprived of O_2 during surgery suddenly have blood flow restored.
Vaccines	Proteins derived from viral coats are as effective in "priming" an immune system as is the killed virus more traditionally used for vaccines, and are safer; first developed was the vaccine for hepatitis B.

Table 9-4
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