

Figure 9.1 A Typical Genetic Modification Procedure.

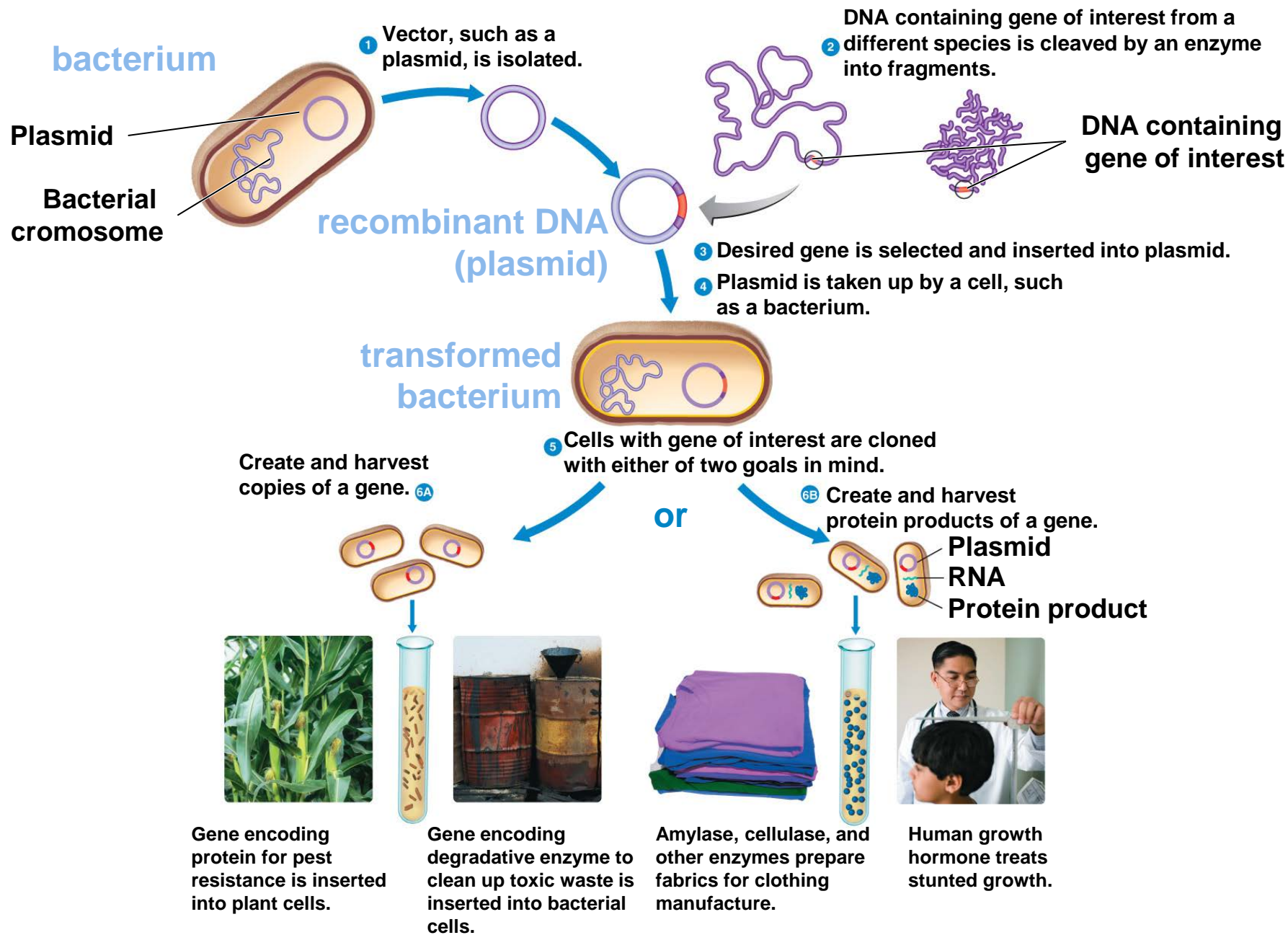


Table 9.2 Some Pharmaceutical Products of rDNA

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Product	Comments
α-Glucosidase	Produced by genetically modified mammalian cells to treat Pompe disease
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep
Bone Morphogenic Proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture
Cervical Cancer Vaccine	Consists of viral proteins; produced by <i>S. cerevisiae</i> or by insect cells
Colony-Stimulating Factor	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; treatment of leukemia; produced by <i>E. coli</i> and <i>S. cerevisiae</i>
Epidermal Growth Factor (EGF)	Heals wounds, burns, ulcers; produced by <i>E. coli</i>
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture
Factor VII	Treatment of hemorrhagic strokes; produced by mammalian cell culture
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture
Interferon	
IFN-α	Therapy for leukemia, melanoma, and hepatitis; produced by <i>E. coli</i> and <i>S. cerevisiae</i> (yeast)
IFN-β	Treatment for multiple sclerosis; produced by mammalian cell culture
IFN-γ	Treatment of chronic granulomatous disease; produced by <i>E. coli</i>
Hepatitis B Vaccine	Produced by <i>S. cerevisiae</i> that carries hepatitis-virus gene on a plasmid
Human Growth Hormone (hGH)	Corrects growth deficiencies in children; produced by <i>E. coli</i>
Human Insulin	Therapy for diabetes; better tolerated than insulin extracted from animals; produced by <i>E. coli</i>
Influenza Vaccine	Vaccine made from <i>E. coli</i> or <i>S. cerevisiae</i> carrying virus genes
Interleukins	Regulate the immune system; possible treatment for cancer; produced by <i>E. coli</i>
Monoclonal Antibodies	Possible therapy for cancer and transplant rejection; used in diagnostic tests; produced by mammalian cell culture (from fusion of cancer cell and antibody-producing cell)
Orthoclone OKT3 Muromonab-CD3	Monoclonal antibody used in transplant patients to help suppress the immune system, reducing the chance of tissue rejection; produced by mouse cells
Prourokinase	Anticoagulant; therapy for heart attacks; produced by <i>E. coli</i> and yeast
Pulmozyme (rhDNase)	Enzyme used to break down mucous secretions in cystic fibrosis patients; produced by mammalian cell culture
Relaxin	Used to ease childbirth; produced by <i>E. coli</i>
Superoxide Dismutase (SOD)	Minimizes damage caused by oxygen free radicals when blood is resupplied to oxygen-deprived tissues; produced by <i>S. cerevisiae</i> and <i>Komagataella pastoris</i> (yeast)
Taxol	Plant product used for treatment for ovarian cancer; produced in <i>E. coli</i>
Tissue Plasminogen Activator	Dissolves the fibrin of blood clots; therapy for heart attacks; produced by mammalian cell culture
Tumor Necrosis Factor (TNF)	Causes disintegration of tumor cells; produced by <i>E. coli</i>
Veterinary Use	
Canine Distemper Vaccine	Canarypox virus carrying canine distemper virus genes
Feline Leukemia Vaccine	Canarypox virus carrying feline leukemia virus genes

Table 9.3 Some Agriculturally Important Products of rDNA Technology

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Product	Comments
AGRICULTURAL PRODUCTS	
Bt cotton and Bt corn	Plants have toxin-producing gene from <i>Bacillus thuringiensis</i> ; toxin kills insects that eat plants.
Genetically modified tomatoes , raspberries	Antisense gene blocks pectin degradation, so fruits have longer shelf life.
<i>Pseudomonas fluorescens</i> bacterium	Has toxin-producing gene from insect pathogen <i>B. thuringiensis</i> ; toxin kills root-eating insects that ingest bacteria.
<i>Pseudomonas syringae</i> , ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
<i>Rhizobium meliloti</i> bacterium	Modified for enhanced nitrogen fixation.
Round up (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.
ANIMAL HUSBANDRY PRODUCTS	
Bovine growth hormone (bGH)	Improves weight gain and milk production in cattle; produced by <i>E. coli</i> .
Porcine growth hormone (pGH)	Improves weight gain in swine; produced by <i>E. coli</i> .
Transgenic animals	Genetic modification of animals to produce medically useful products in their milk.
OTHER FOOD PRODUCTION PRODUCTS	
Cellulase	Enzyme that degrades cellulose to make animal feedstocks; produced by <i>E. coli</i> .
Chymogen	Causes formation of milk curds in cheese-making; produced by <i>Aspergillus niger</i> .

Table 9.1 Selected Restriction Enzymes Used in rDNA Technology

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Enzyme	Bacterial Source	Recognition Sequence
<i>Bam</i> HI	<i>Bacillus amyloliquefaciens</i>	G↓G A T C C G C T A G↑G
<i>Eco</i> RI	<i>Escherichia coli</i>	G↓A A T T C C T T A A↑G
<i>Hae</i> III	<i>Haemophilus aegyptius</i>	G G↓C C C C↑G G
<i>Hind</i> III	<i>Haemophilus influenzae</i>	A↓A G C T T T T C G A↑A

Figure 9.2 The role of a restriction enzyme in making recombinant DNA.

1 Restriction enzyme cuts (red arrows) double-stranded DNA at its particular recognition sites, shown in blue.

2 These cuts produce a DNA fragment with two sticky ends.

DNA from another source, perhaps a plasmid, cut with the same restriction enzyme.

3 When two such fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.

4 The joined fragments will usually form either a linear molecule or a circular one, as shown here for a plasmid. Other combinations of fragments can also occur.

5 The enzyme DNA ligase is used to unite the backbones of the two DNA fragments, producing a molecule of recombinant DNA.

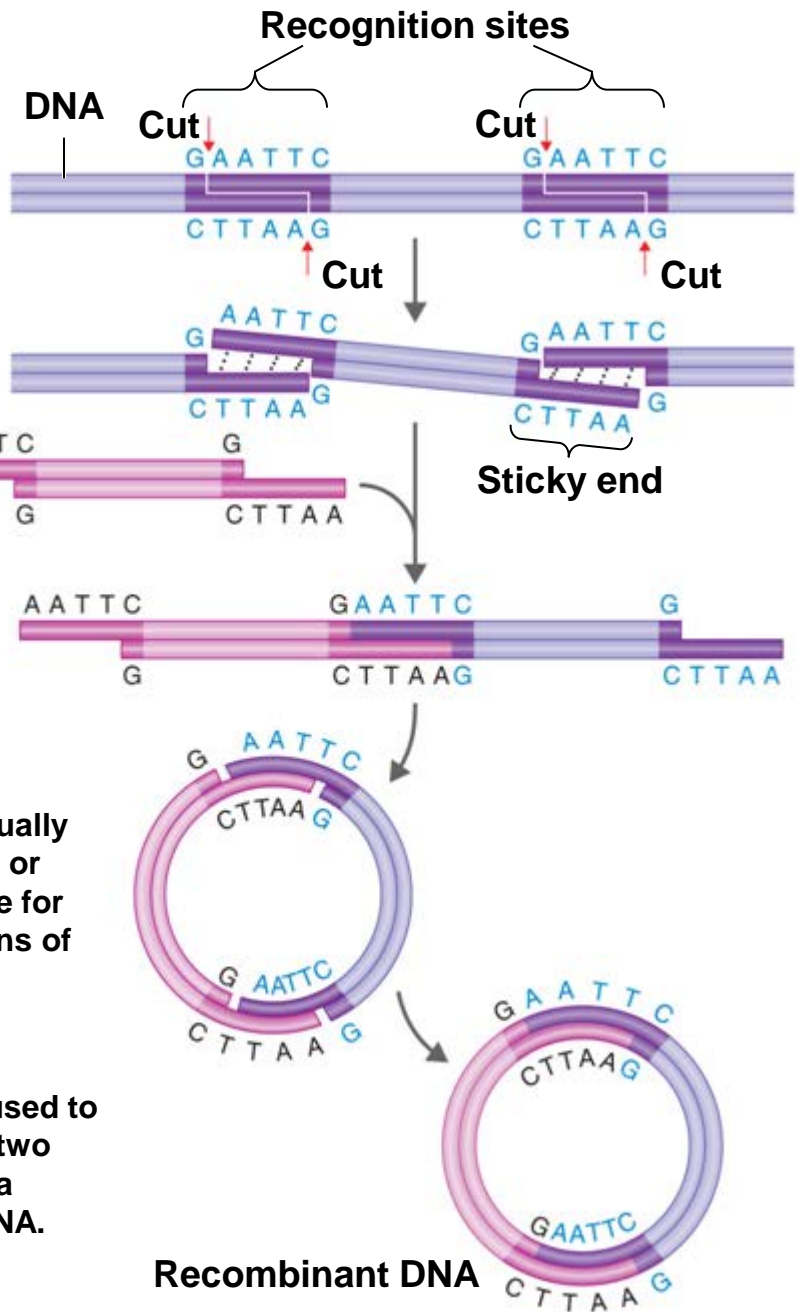
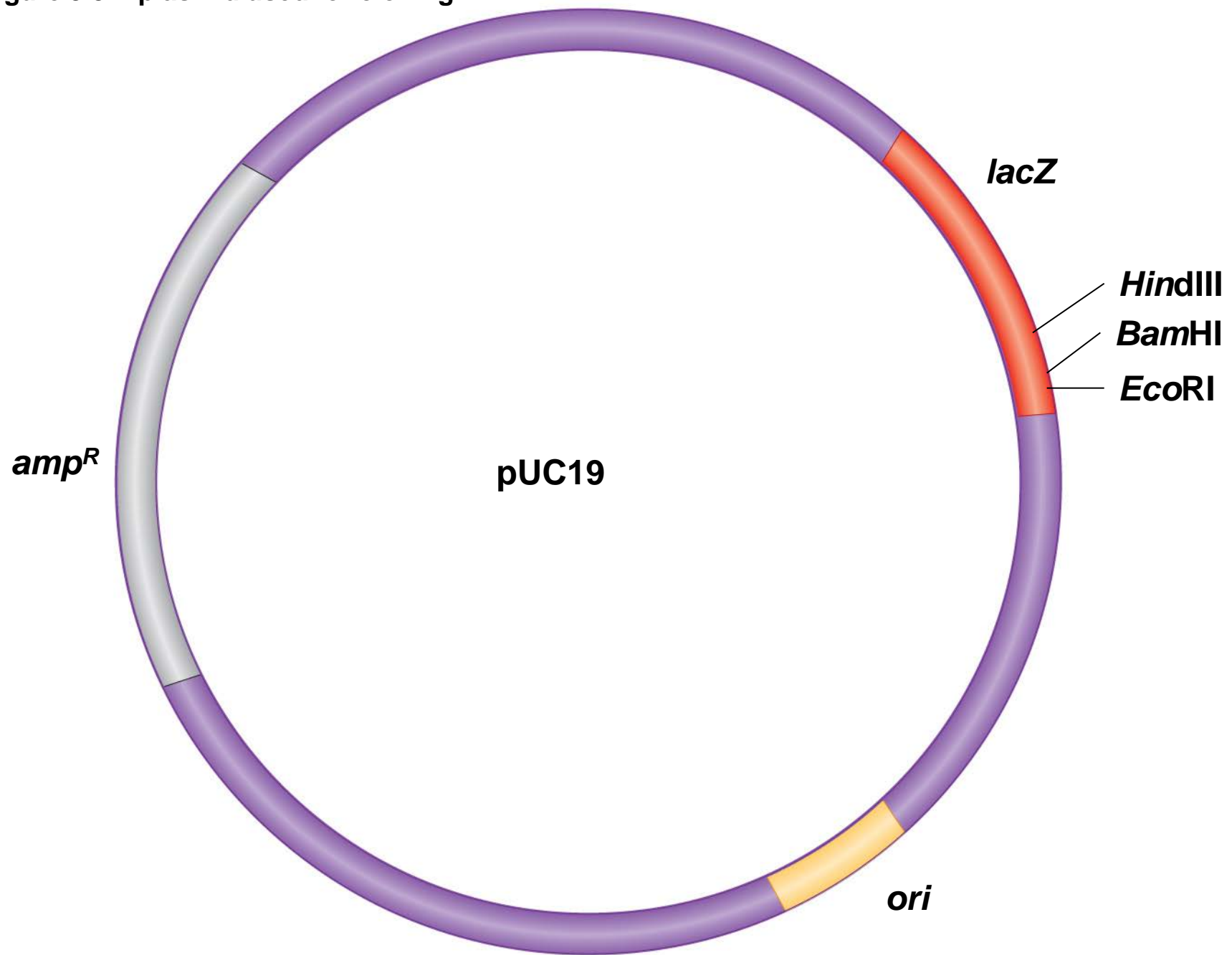
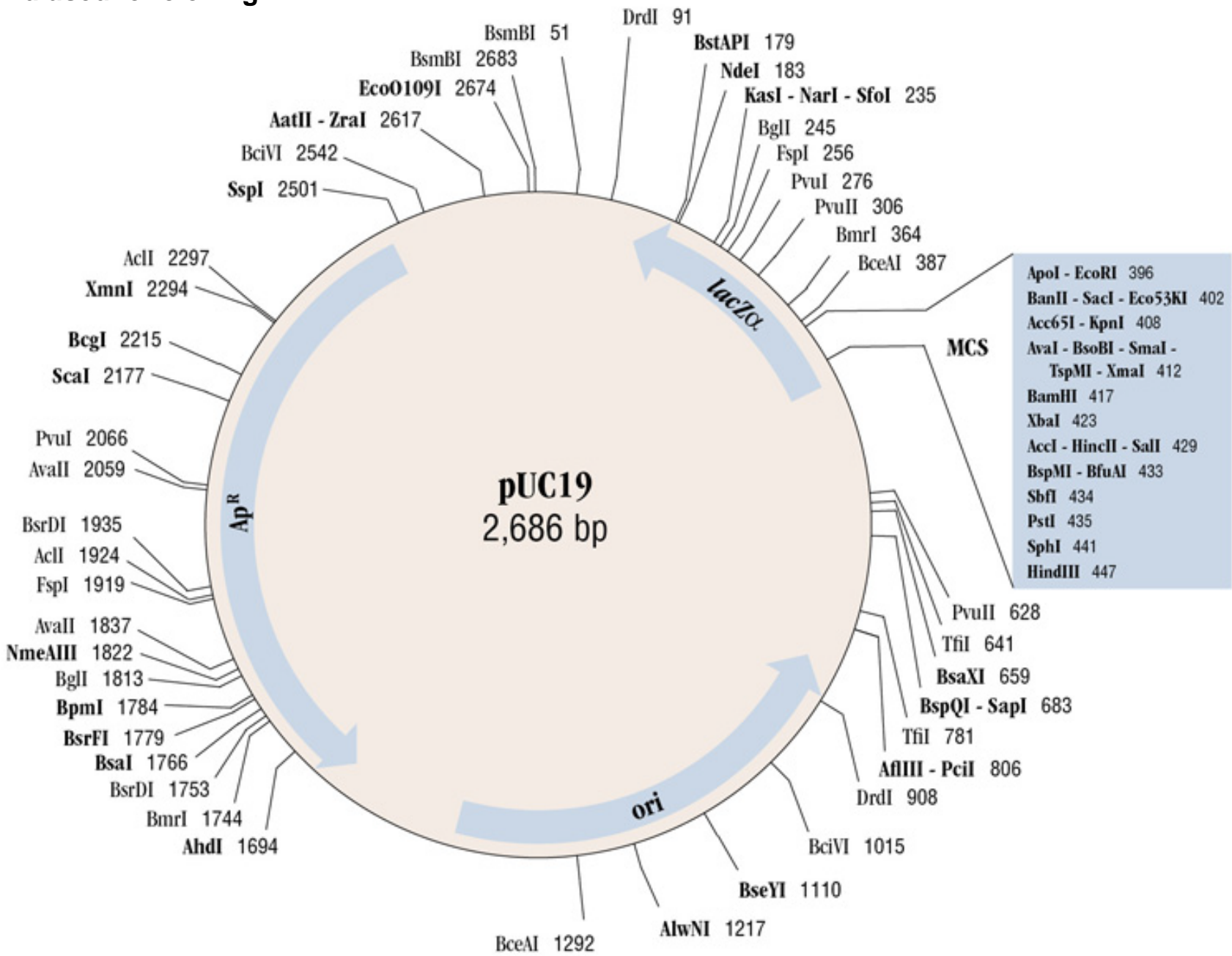


Figure 9.3 A plasmid used for cloning.



A plasmid used for cloning.



A plasmid used for cloning.

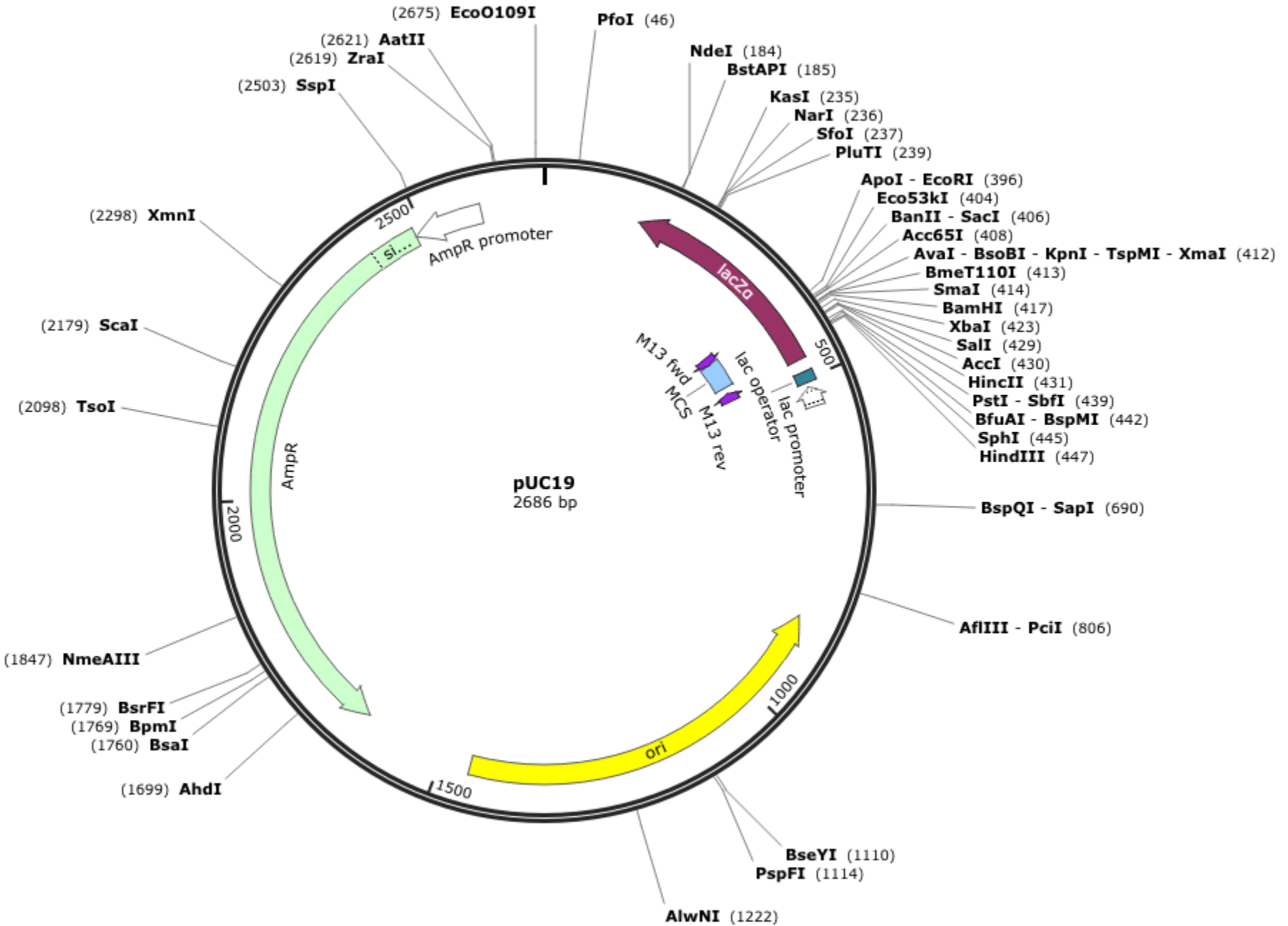


Figure 9.4 The polymerase chain reaction.

- First cycle
- 1 Incubate target DNA at 94° C for 1 minute to separate the strands.
 - 2 Add primers, nucleotides (deoxynucleotides, dNTP), and DNA polymerase.
 - 3 Primers attach to single-stranded DNA during incubation at 60° C for 1 minute.
 - 4 Incubate at 72° C for 1 minute; DNA polymerase copies the target DNA at this temperature.
- Second cycle
- 5 Repeat the cycle of heating and cooling to make two more copies of target DNA.

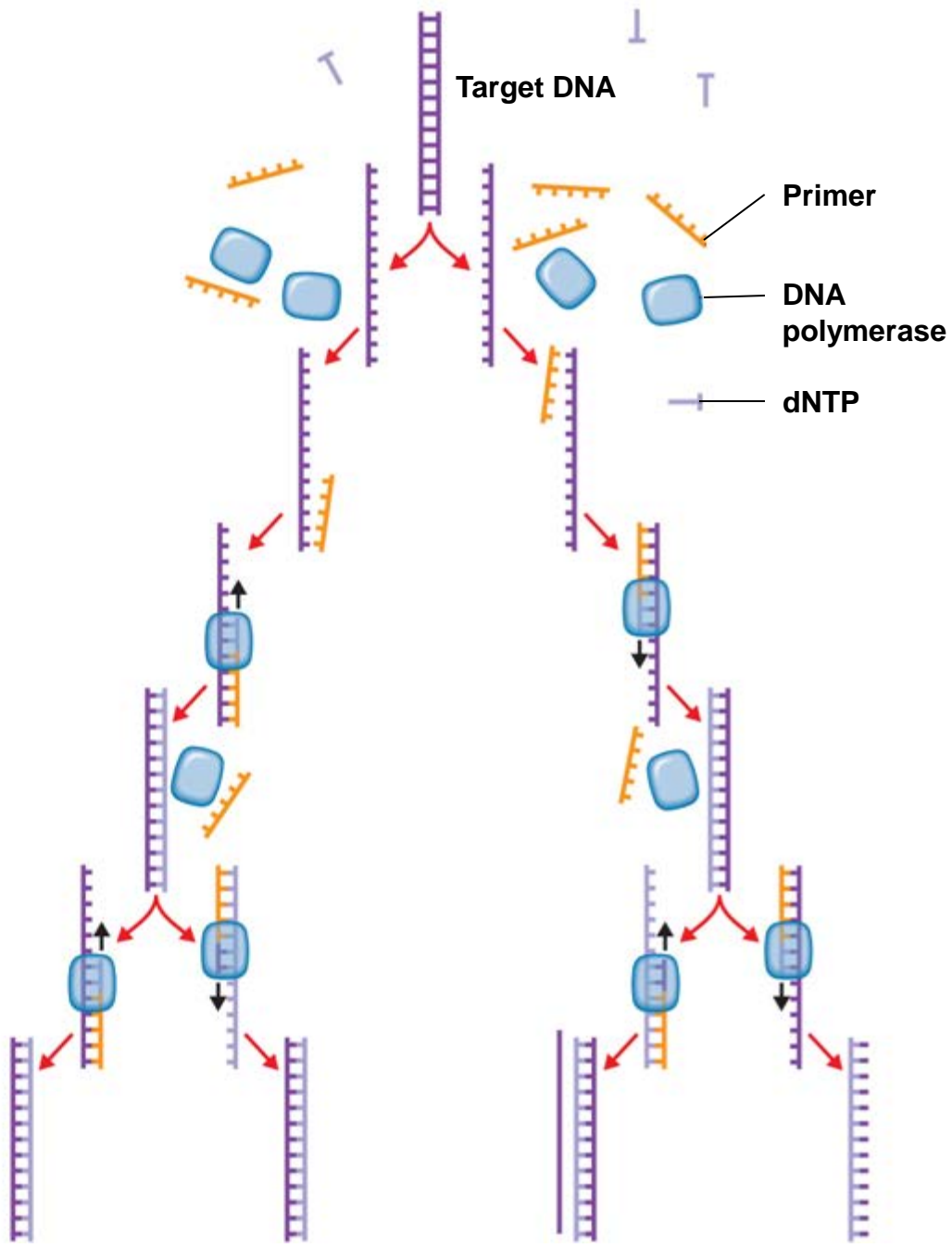
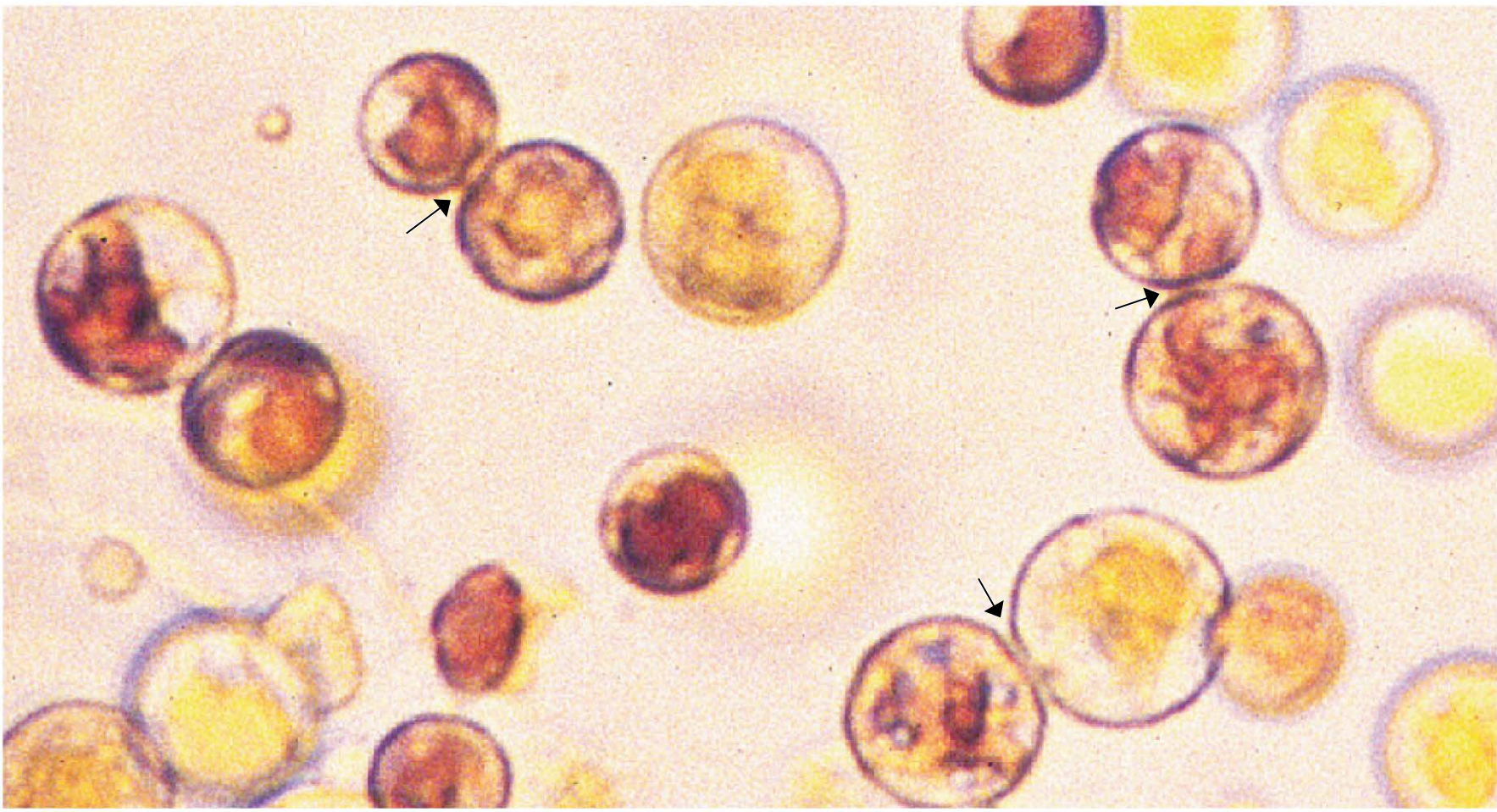


Figure 9.5b Protoplast fusion.



(b) Algal protoplasts fusing

LM |-----|
10 μ m

Figure 9.6 A gene gun, which can be used to insert DNA-coated “bullets” into a cell.



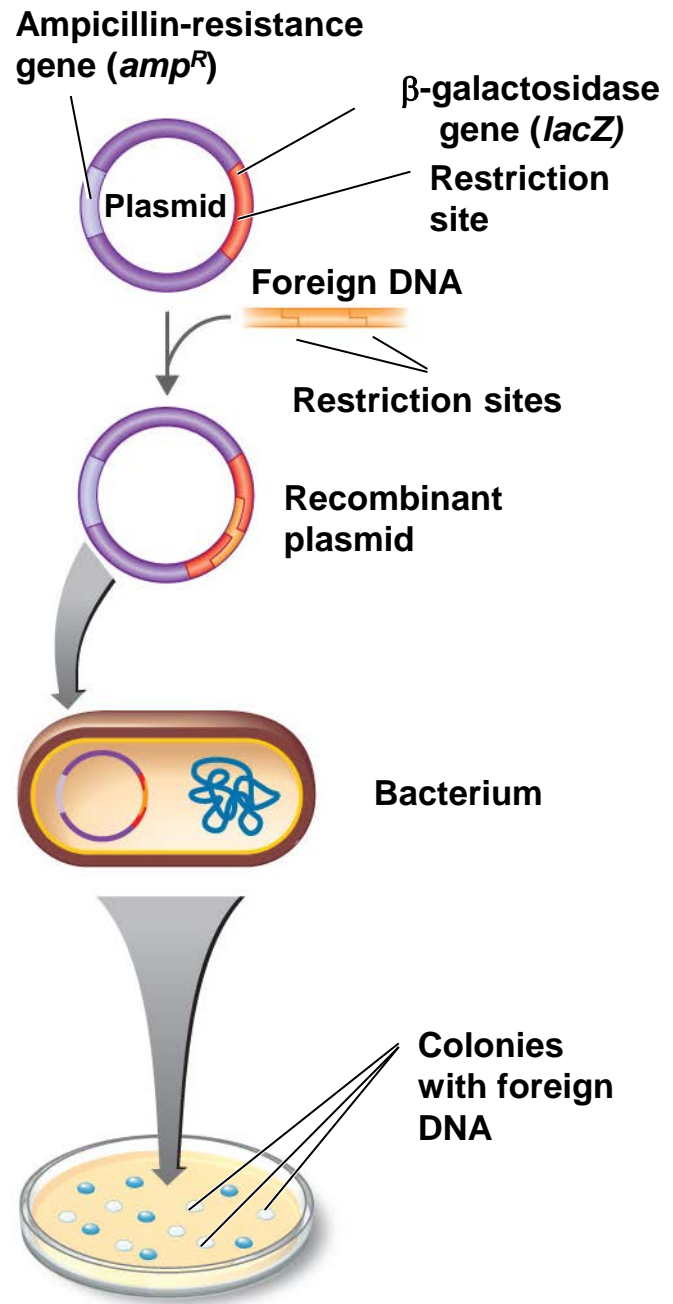
Figure 9.7 The microinjection of foreign DNA into an egg.



LM

80 μ m

Figure 9.11 Blue-white screening, one method of selecting recombinant bacteria.



Applications: Therapy



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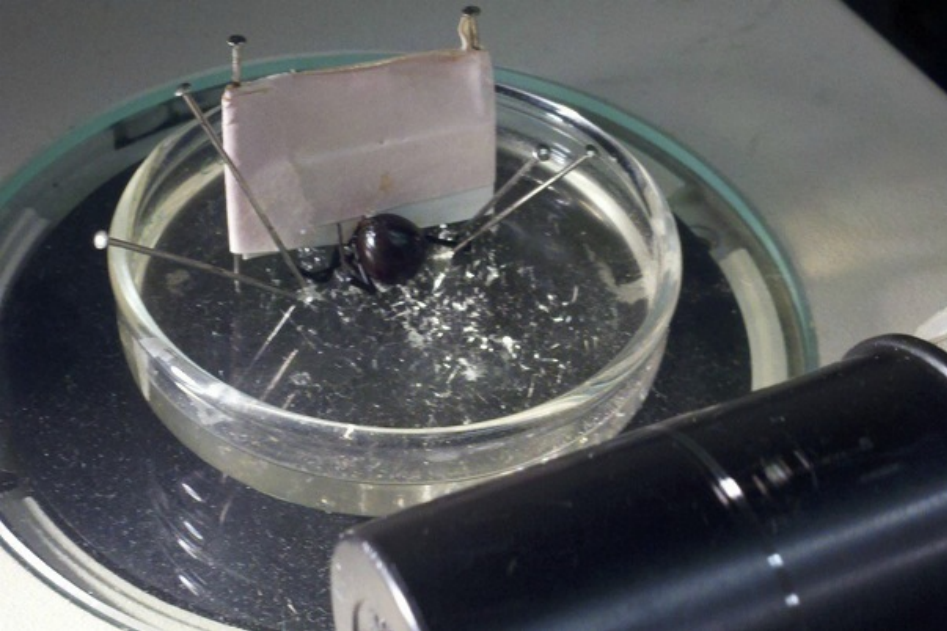
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Abstract ▾

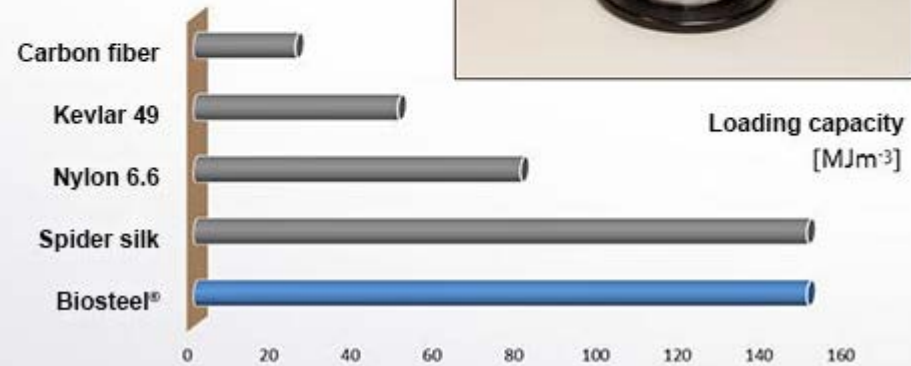
[Clin Lab Med](#). 2010 Jun;30(2):365-80. doi: 10.1016/j.cll.2010.02.001. Epub 2010 May 6.

Genetically engineered pigs as a source for clinical red blood cell transfusion.

[Cooper DK](#)¹, [Hara H](#), [Yazer M](#).



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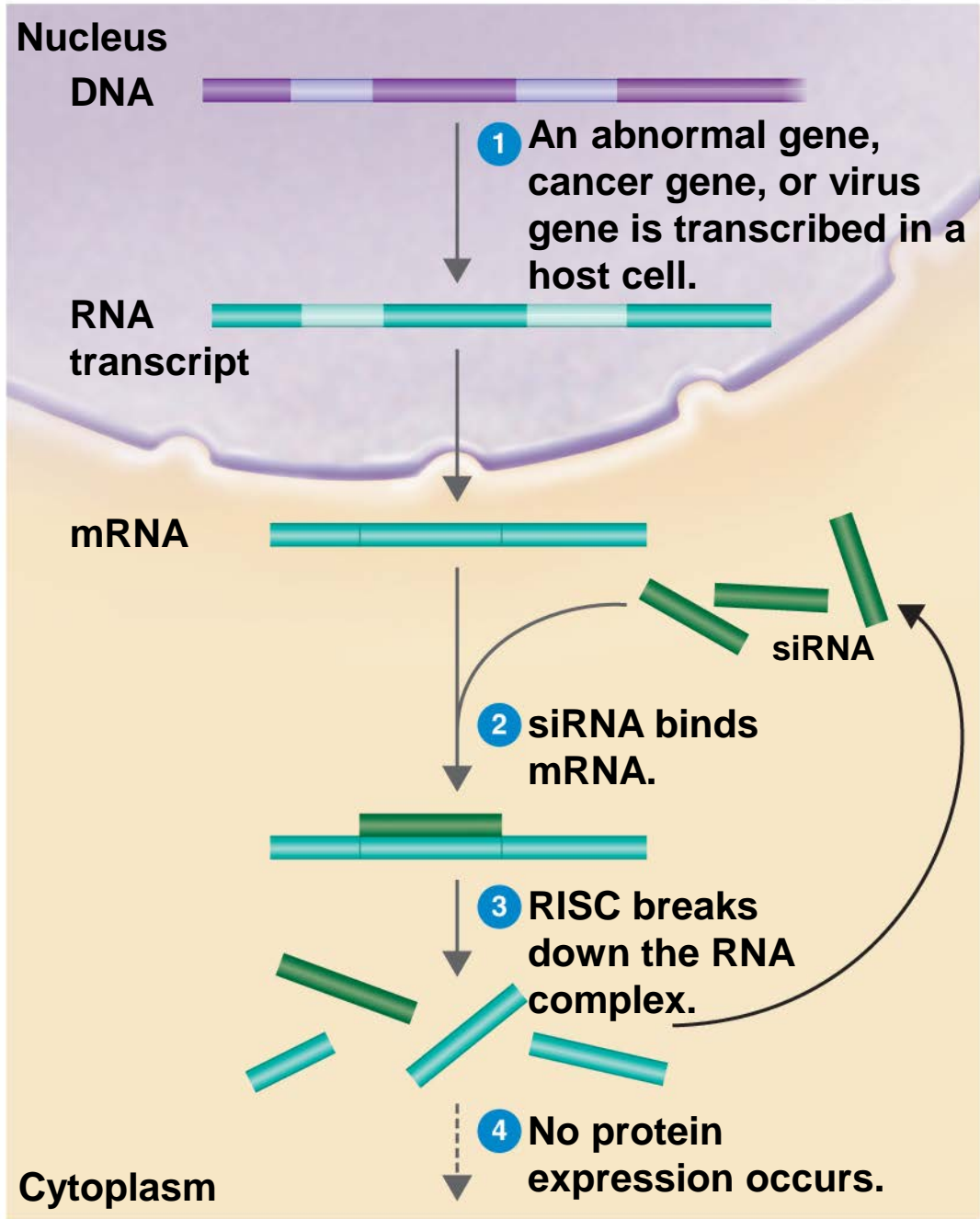


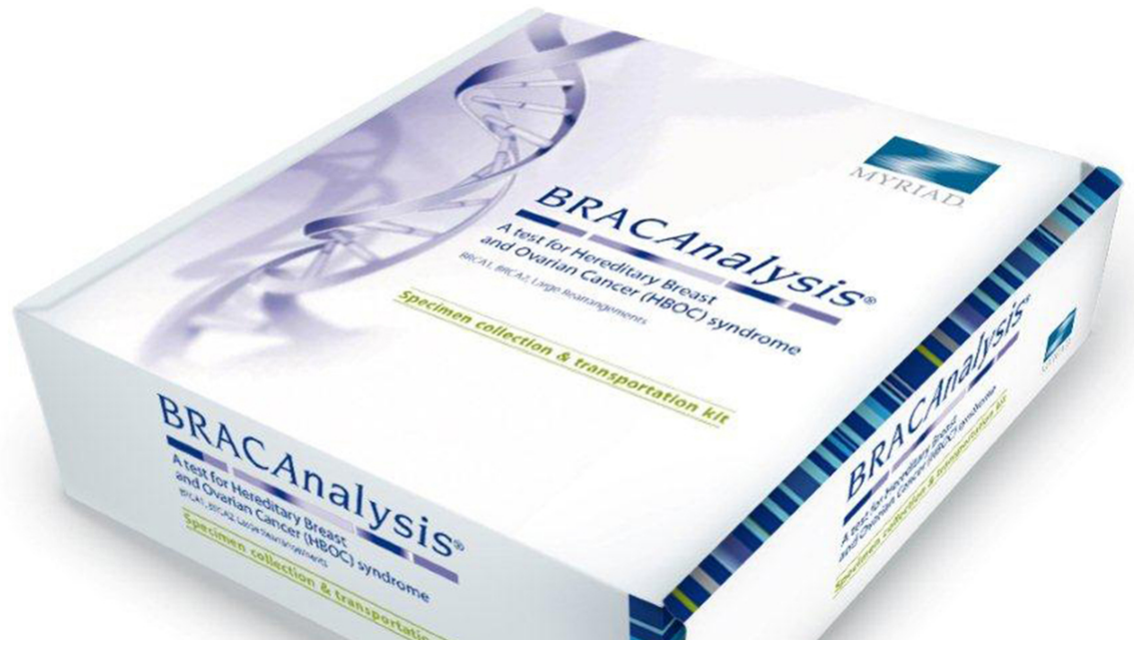
#UberFacts

Freckles, a goat in Utah, was implanted with spider genes as an embryo to produce spider silk protein in her milk. It is now being used to make "biosteel," a material stronger than kevlar.

Spidergoat?

Figure 9.14 Gene silencing could provide treatments for a wide range of diseases.





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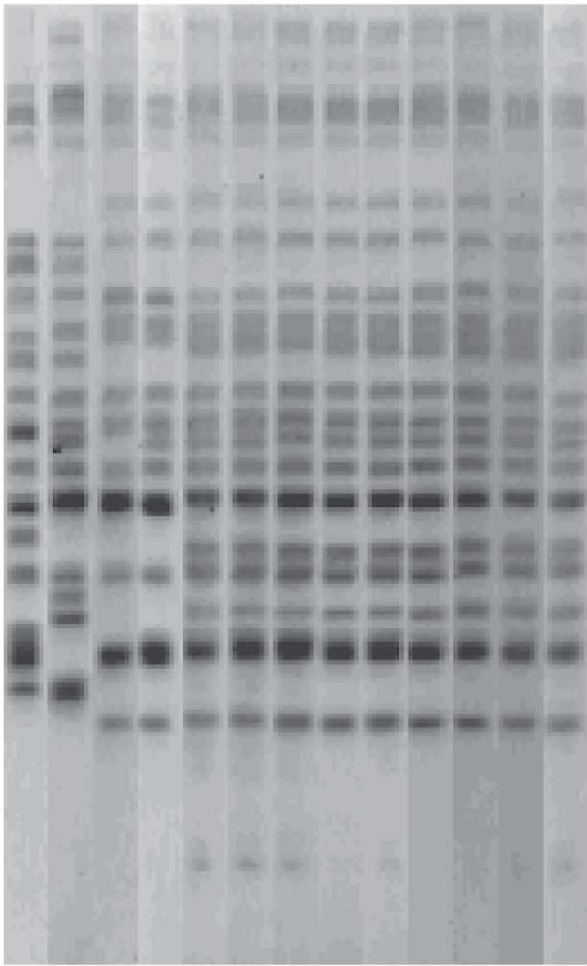
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Figure 9.17 DNA fingerprints used to track an infectious disease.

E. coli isolates from patients whose infections were not juice related

E. coli isolates from patients who drank contaminated juice


Apple juice isolates





GENUITY® SMARTSTAX® Products containing this technology contain Cry1A.105, Cry2Ab2, Cry1E, Cry3Bb1, Cry34Ab1 and Cry35Ab1 from *B.t.* that together control European corn borer, southwestern corn borer, southern cornstalk borer, corn earworm, fall armyworm, stalk borer, lesser corn stalk borer, sugarcane borer, western bean cutworm, black cutworm, western corn rootworm, northern corn rootworm, and Mexican corn rootworm. Routine applications of insecticides to control these insects are usually unnecessary when corn containing Genuity SmartStax is planted. Products containing this technology also contain Roundup Ready® 2 Technology and LibertyLink® technology that provide tolerance to in-crop applications of labeled Roundup® agricultural herbicides and Liberty® herbicides, respectively, when applied according to label directions.



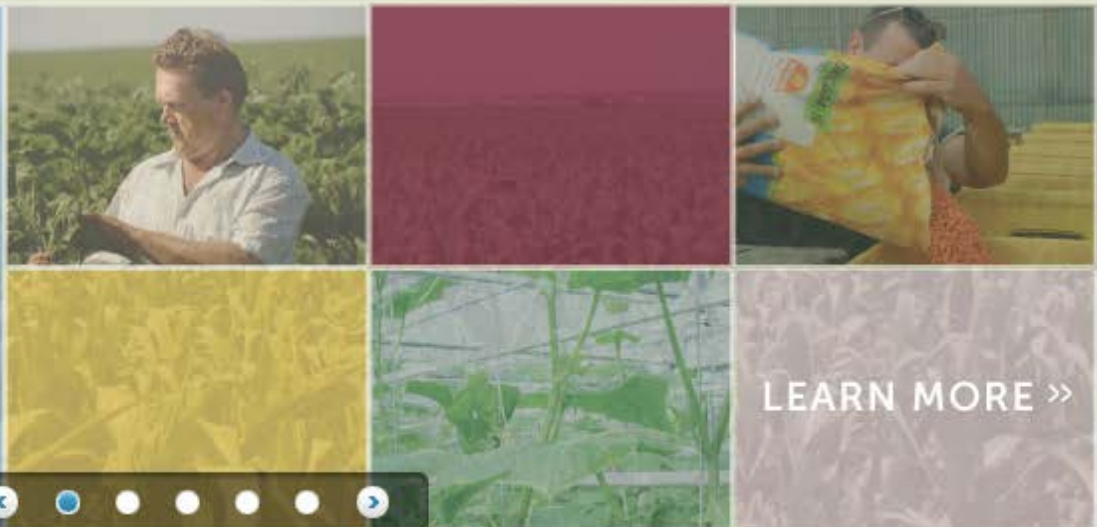
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