Virginia Tech
Invent the Future

Institute for Critical Technology and Applied Science

Introduction to Gene Cloning

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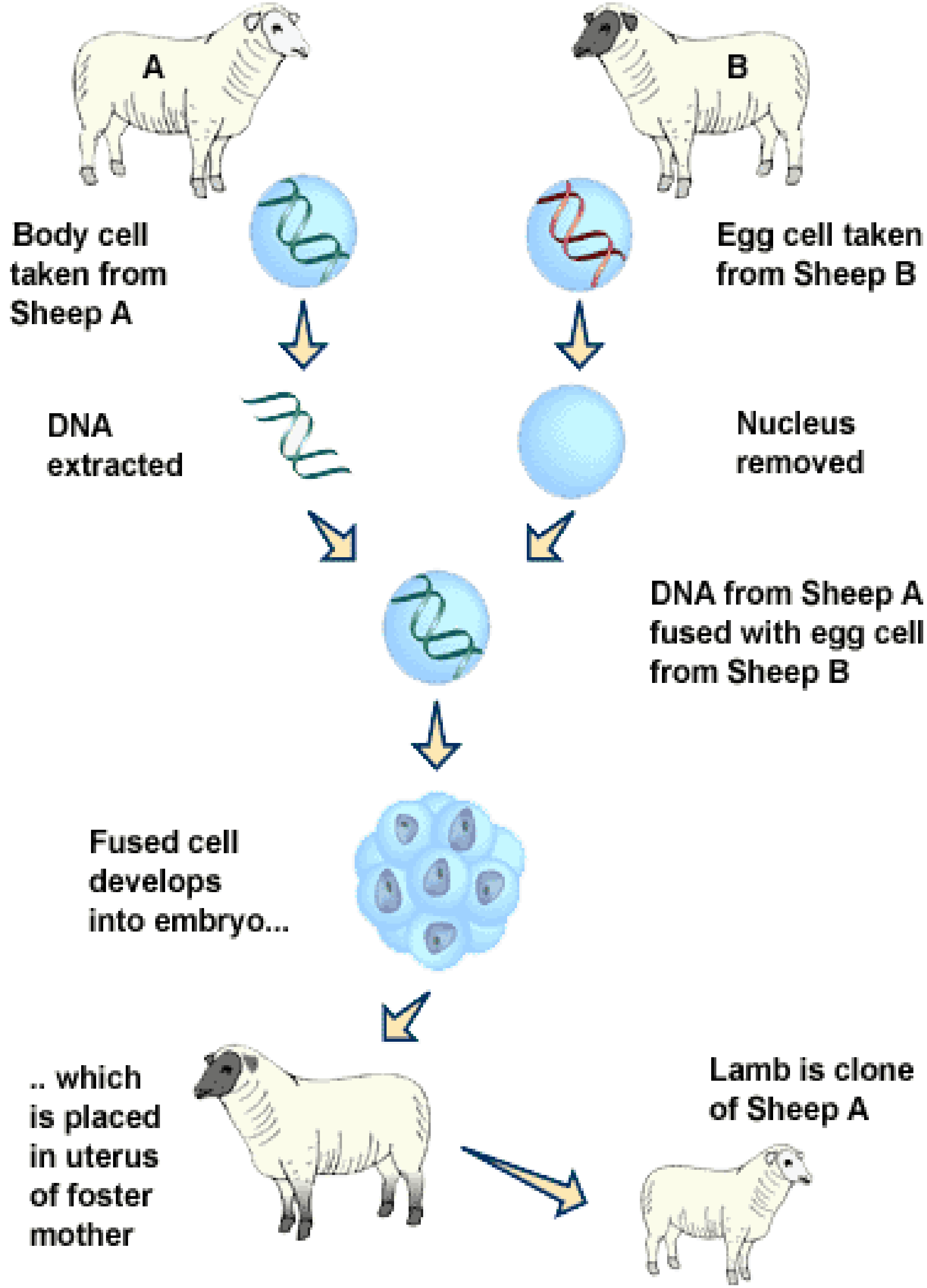
Gene Cloning

- Making multiple copies of a target gene
- Generally use bacteria as the "factory"

Cloning


A
B
Body cell taken from Sheep A
Egg cell taken from Sheep B
DNA extracted
Nucleus removed
DNA from Sheep A fused with egg cell from Sheep B
Fused cell develops into embryo...
.. which is placed in uterus of foster mother
Lamb is clone of Sheep A

Ethical Issue of the Day #1

Reproductive Cloning

Arguments for a ban on human reproductive* cloning

- hundreds of cloned embryos must be created and placed into a mother to produce a single live birth
- dying, stillborn and deformed babies frequent
- Even those who have lived a long time (such as Dolly) have a large number of serious health problems

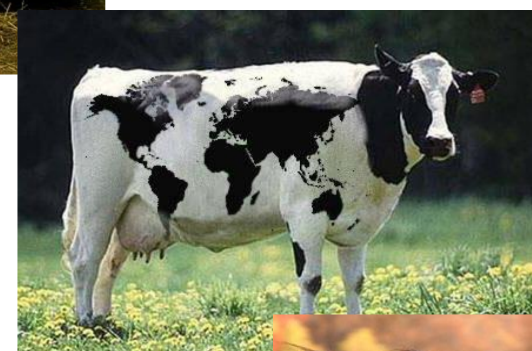
*cloning to make a baby

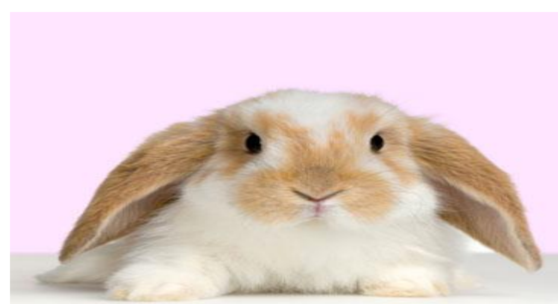
Animal cloned so far

- Frog (1952)
- Sheep (1996)
- Mouse (1997)
- Cow (1998)
- Goat (2000)
- Cat (2001)
- Rabbit (2002)
- Horse, Rat (2003)









ANDi Monkey

Snuppy the Afghan Hound

April 2009 Injaz, or 'Achievement' Dubai

Cloning a gene

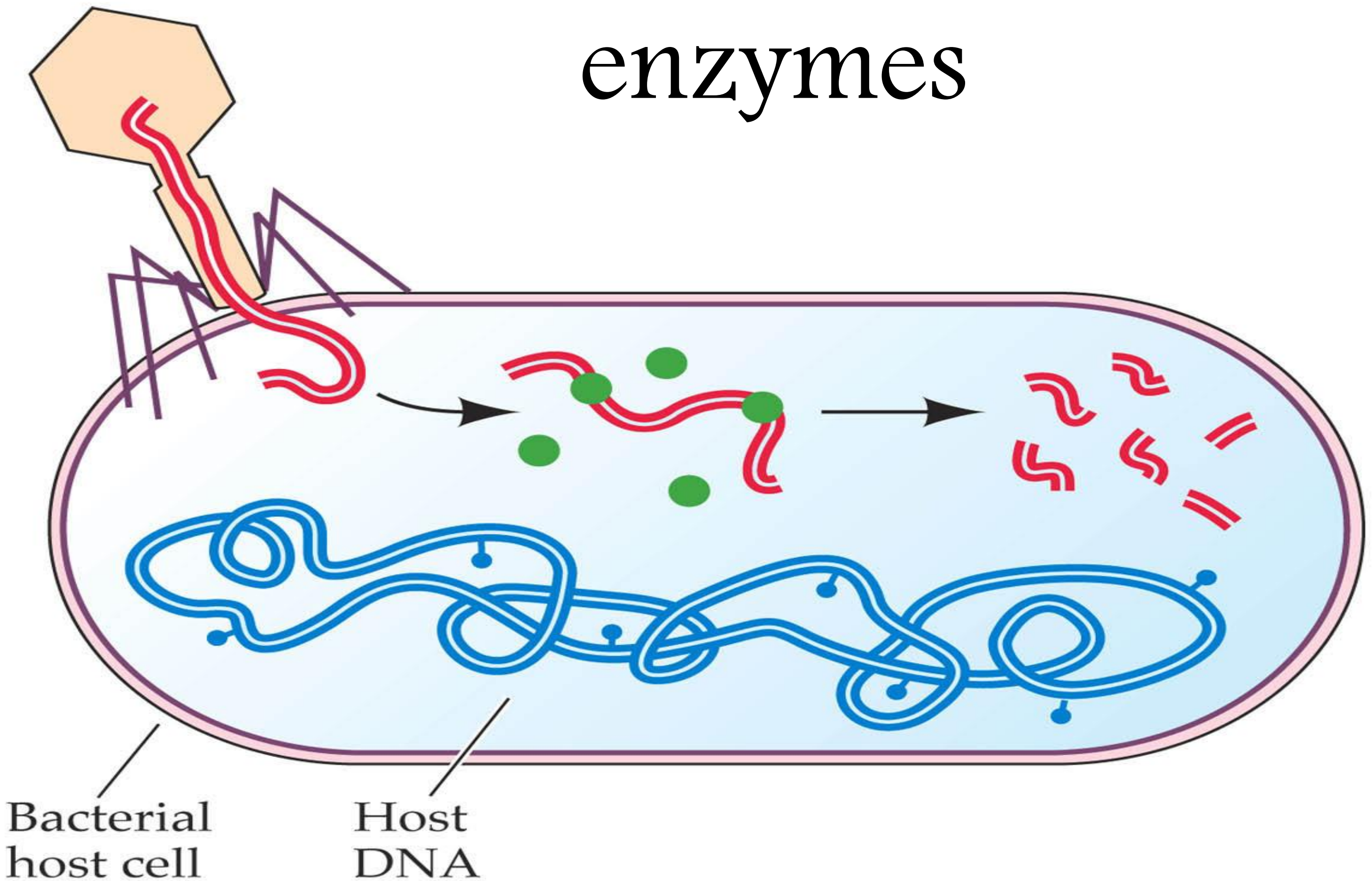
- Insert a particular fragment of DNA (a particular gene) into a vector
- A vector is another DNA molecule that can be put into a host
- This creates a new DNA molecule

  --> Recombinant DNA
- Greatly aids further study of that gene, and its use in diagnosis or treatment

Cloning Tools

- Restriction endonucleases
- Ligase
- Vectors
- Host
- Methods for introducing DNA into a host cell

Restriction endonucleases enzymes

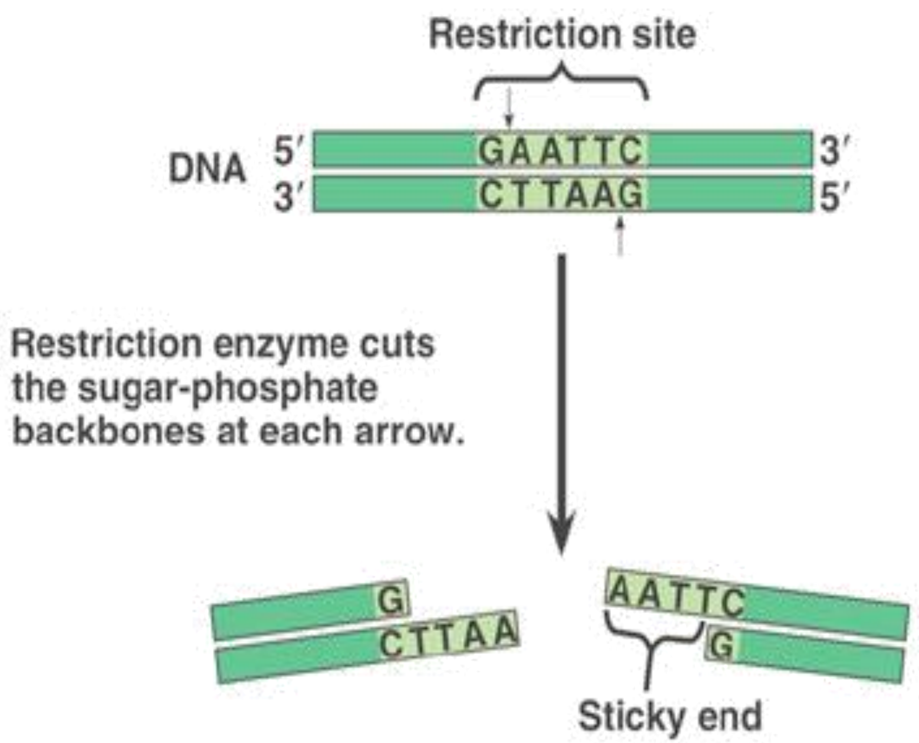


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.1 Bacteria Fight Invading Viruses with Restriction Enzymes

Cutting DNA

- Restriction endonucleases (restriction enzymes)
  - sticky ends
  - blunt ends

- Nomenclature
  - *Eco*RI
  - *E* = genus (*Escherichia*)
  - *co* = species (*coli*)
  - R = strain
  - I = # of enzyme



Some restriction enzymes

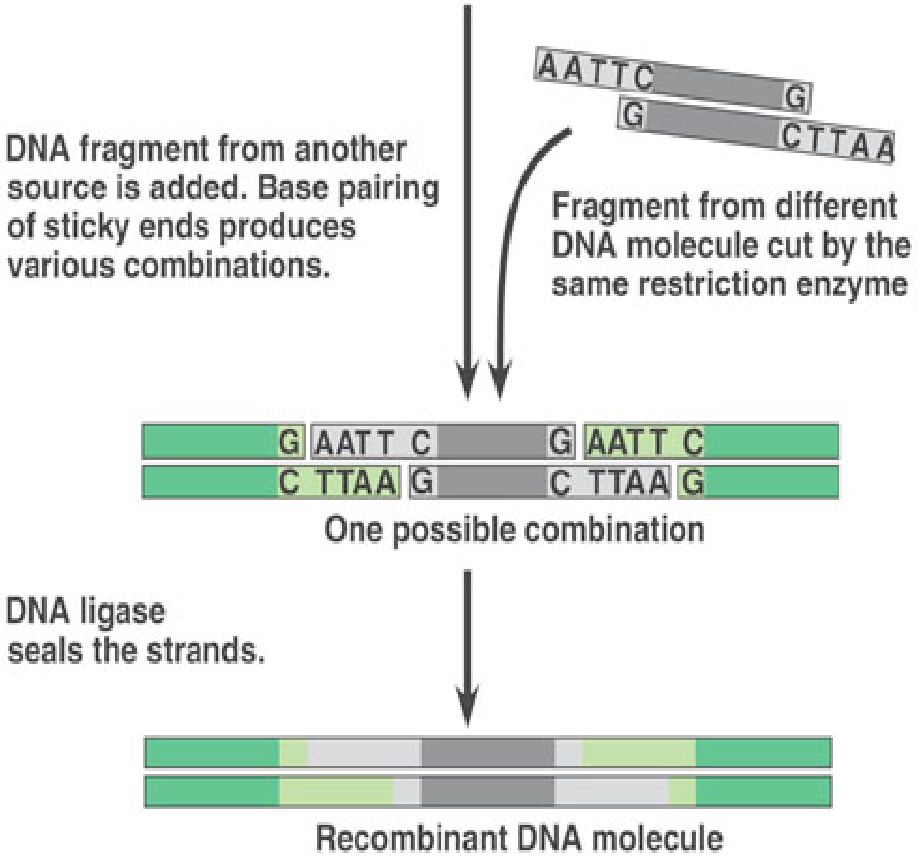
Table 8.1 Properties of Some Restriction Enzymes

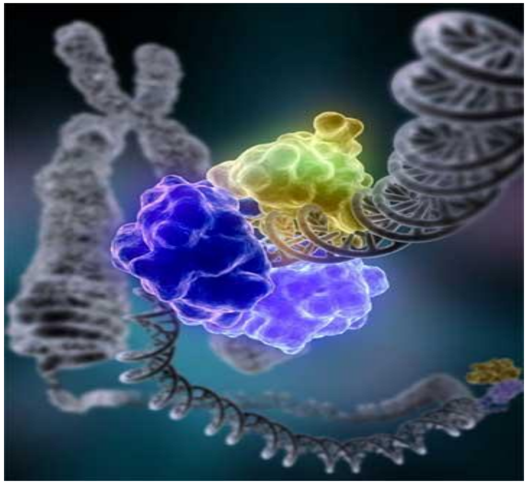
Enzyme	Bacterial Source	Restriction Site*
*Bam*HI	*Bacillus amyloliquefaciens* H	G↓GATCC CCTAG↑G
*Eco*RI	*Escherichia coli* RY13	G↓AATTC CTTAA↑G
*Eco*RII	*E. coli* R245	CC↓GG GG↑CC
*Hin*dII	*Haemophilus influenzae* Rd	GTPy↓PuAC CAPu↑PyTG
*Hin*dIII	*H. influenzae* Rd	A↓AGCTT TTCGA↑A
*Hin*fI	*H. influenzae* Rf	G↓ANTC CTNA↑G
*Hpa*I	*H. parainfluenzae*	GTT↓AAC CAA↑TTG
*Msp*I	*Moraxella* sp.	CC↓GG GG↑CC
*Sma*I	*Serratia marcescens*	CCC↓GGG GGG↑CCC

*Arrows indicate sites of cleavage; Py = pyrimidine (either T or C); Pu = purine (either A or G); N = any nucleotide (A, T, G, or C).

- 100's of restriction enzymes (RE's) are commercially available
- Artificial RE sites can be inserted at ends of any gene
- RE's and ligase allow precise cutting and pasting of any DNA sequences

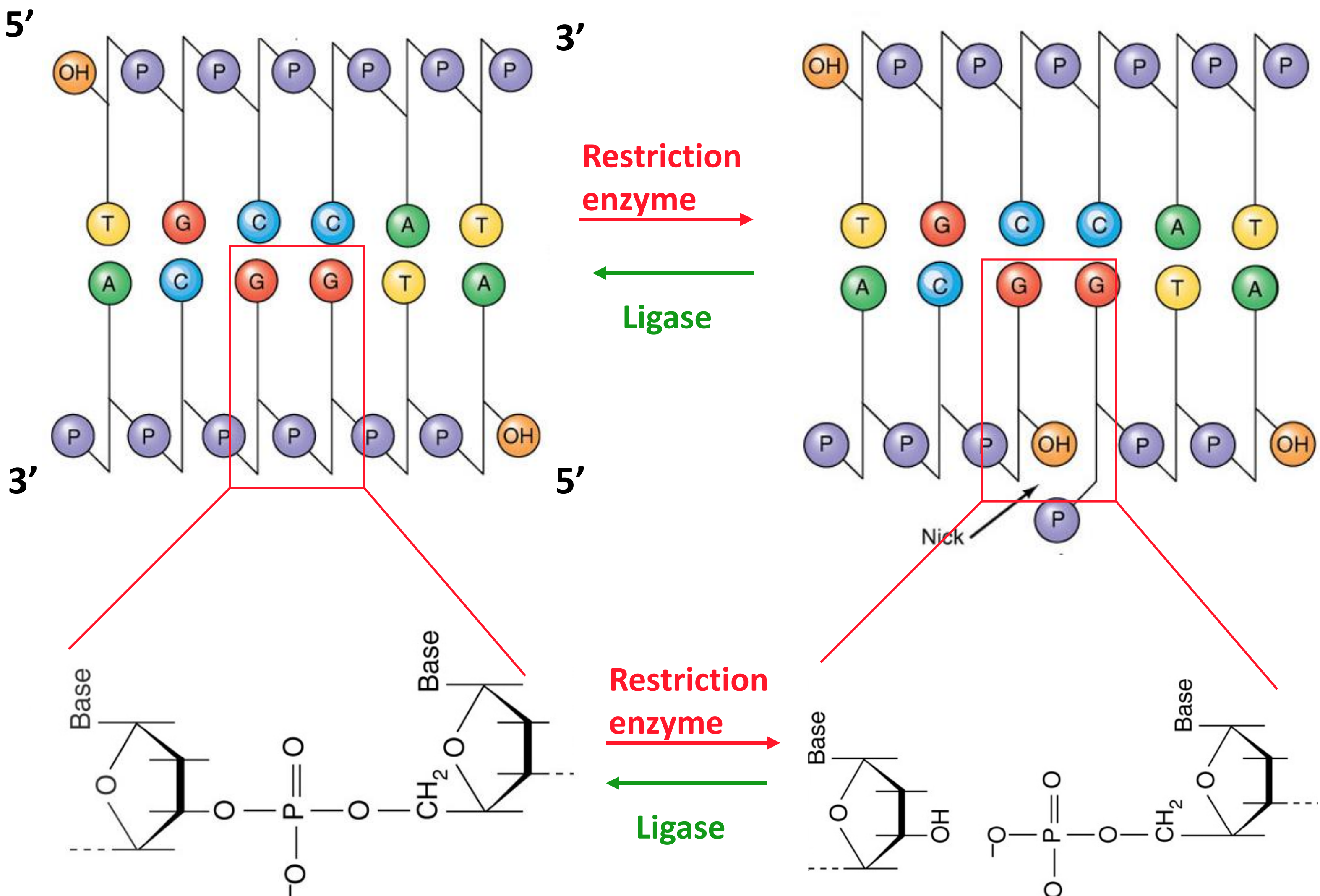
Pasting DNA



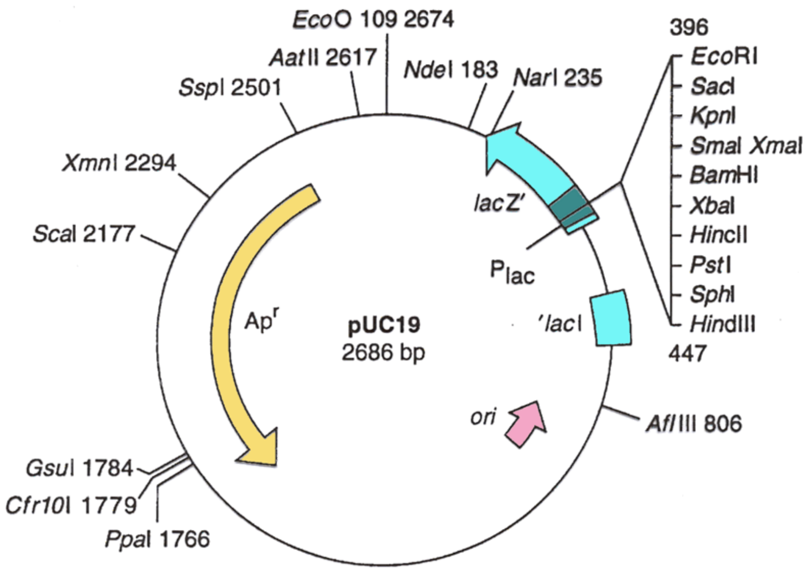


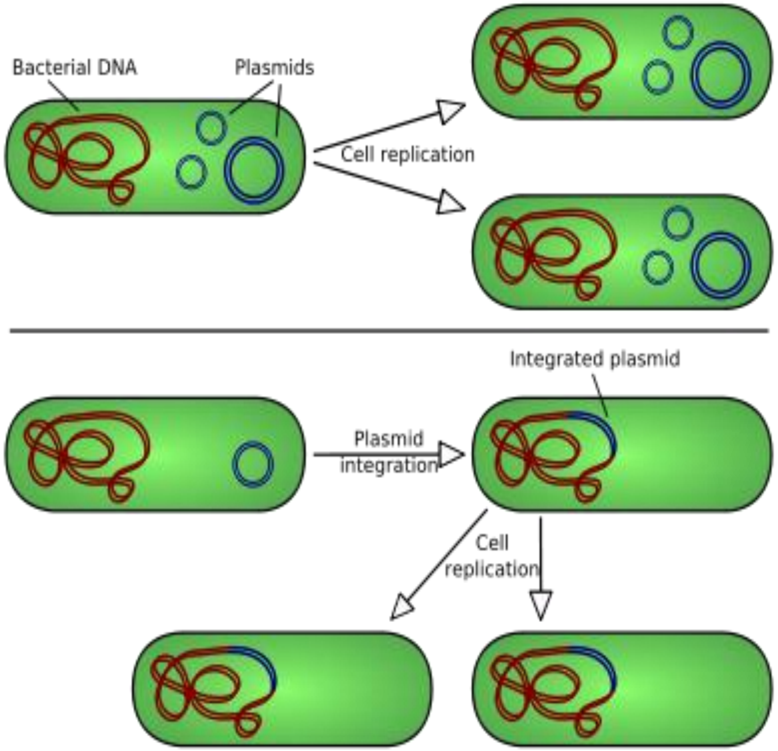
- Complementary ends (sticky ends) H-bond
- Ligase forms phosphodiester bond to seal strands together.

DNA ligase covalently links two DNA strands

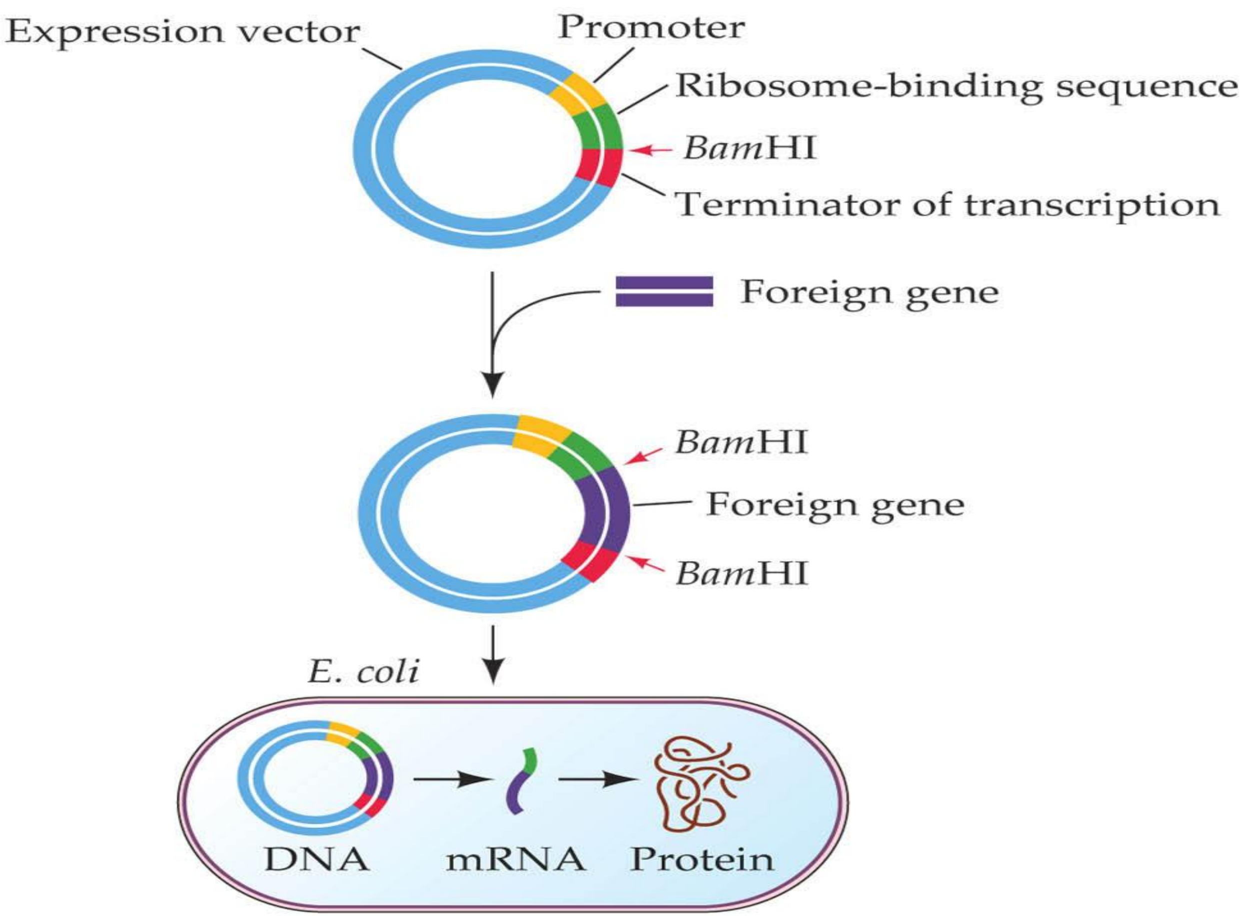


Plasmid vectors





An Expression Vector Allows a Foreign Gene to Be Expressed in a Host Cell



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.13 An Expression Vector Allows a Foreign Gene to Be Expressed in a Host Cell

Cloning vectors

allowing the exogenous DNA to be inserted, stored, and manipulated mainly at DNA level.

1 Plasmid vectors

2 Bacteriophage vectors

3 Cosmids

4 BACs & YACs

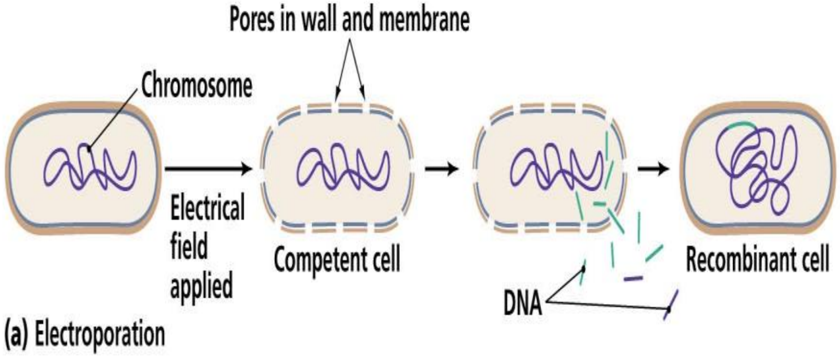
What determines the choice vector?

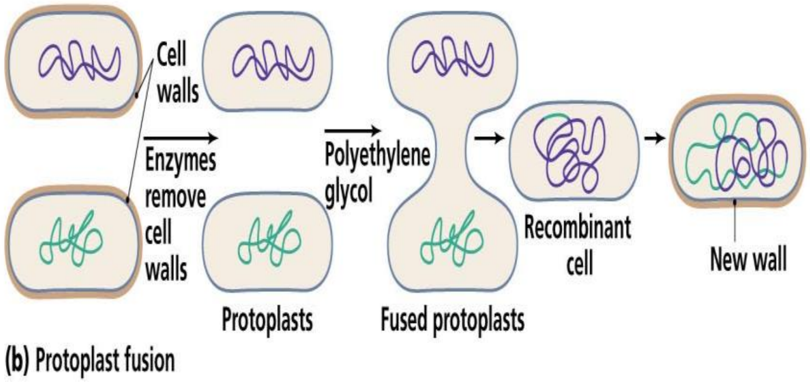
- insert size
- vector size
- restriction sites
- copy number
- cloning efficiency
- ability to screen for inserts
- **what down-stream experiments do you plan?**

Table 5.1 Maximum DNA insert possible with different cloning vectors. YACs are discussed on p. 159.

Vector	Host	Insert size
λ phage	*E. coli*	5–25 kb
λ cosmids	*E. coli*	35–45 kb
P1 phage	*E. coli*	70–100 kb
PACs	*E. coli*	100–300 kb
BACs	*E coli*	≤ 300 kb
YACs	*Saccharomyces cerevisiae*	200–2000 kb

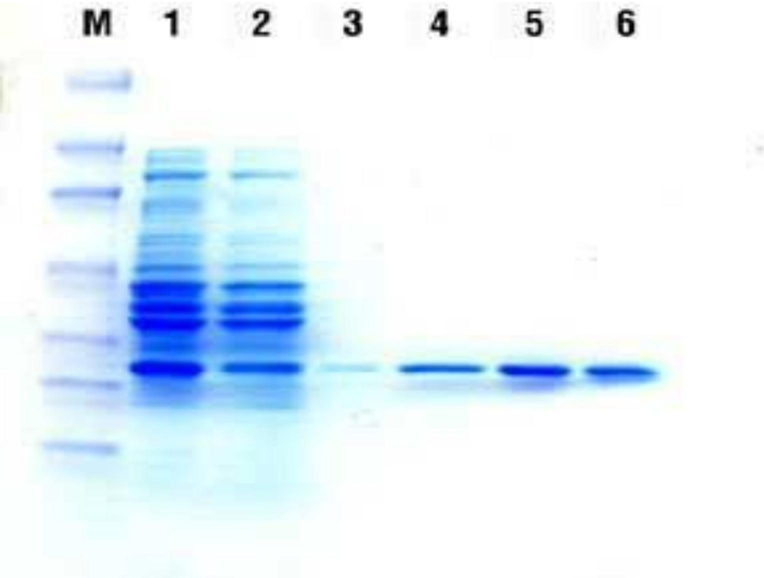
Getting DNA into cells--Transformation of bacteria





- Cells are made competent via electroporation or via pre-treatment with $CaCl_2$/cold
- DNA (plasmid) is added and cells that have taken up the plasmid are identified by plating on selective media

Protein Purification



Bacterial Expression System Shortcomings

- There are problems with expression of eukaryotic proteins in a bacterial system
  – Bacteria may recognize the proteins as foreign and destroy them
  – Posttranslational modifications are different in bacteria
  – Bacterial environment may not permit correct protein folding
- Very high levels of cloned eukaryotic proteins can be expressed in useless, insoluble form