Carbohydrates

A. Definition
B. Roles
C. Monosaccharides-Chemistry
   1. Chirality
      a. One or more asymmetric carbons
      b. Linear and ring forms
   2. Derivatives: the chemistry of carbohydrates
      a. Oxidation
         i. C1
         ii. C6
      b. Reduction
         i. C1/C2
         ii. Other carbons
      c. Ester formation
      d. Amino sugars
   3. Polymerization
      a. The Glycosidic Bond
      b. Non-covalent bonds in macro-molecular structure
      c. Disaccharides
D. Oligosaccharides
   1. Glycoproteins & glycolipids
   2. O-linked
   3. N-linked
   4. Sequence determination-ABO
E. Polysaccharides
   1. Polymers of glucose
   2. Polymers of disaccharides

Nucleic Acids

A. Nucleotides
   1. Parts-structure
   2. Nomenclature
   3. Numbering
   4. Properties
B. Nucleic Acids
   1. Polymer: phospho-diester bond
   2. H-bonds
   3. Roles
      a. Nucleotides
      b. Nucleic acids
C. The 4 S's
   1. Size
   2. Solubility
   3. Shape
      a. B-DNA
      b. A-DNA
      c. Z-DNA
      d. Topology
         i. Packaging
         ii. Supercoiling
         iii. Topoisomerases
   4. Stability
      a. Nucleotides: mutagenesis
         i. Tautomers
         ii. Acid/base
      b. Nucleic Acids
         i. Chemistry
         ii. Denaturation
         iii. Stability
         iv. Nucleases
D. Structure of the Information
   1. Exceptions to flow
   2. Structure
   3. Levels of Control
E. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
   3. Transformation of hosts
   4. Selection of transformants
   5. Expression
   6. Site-directed mutagenesis
F. Replication
   1. Polymerases
   2. Fidelity
   3. Sequence determination

Lecture 27 (11/19/25)

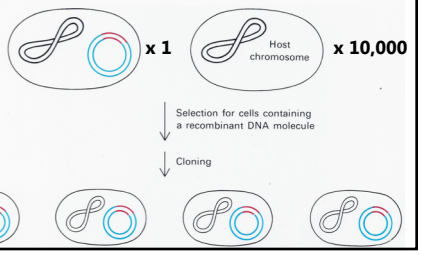
TODAY
- Reading: Ch25; 916-921
- Homework: #27

NEXT
Exam 4
After Break: Ch26, 960-964; Ch27, 1006-1013, 1018-1024, 1015-1018, 1028-1036
Homework: #28, #29, #30

Recombinant DNA and Biotechnology

- Biochemical Basis of Biotechnology
  - Restriction enzymes, DNA ligase
  - Vectors and Inserts to make recombinant DNA (rDNA)
  - Transformation of hosts
  - Selection of transformants

- Use of antibiotic resistance gene (e.g., ampicilin resistance) on a plasmid
- For viral vectors, use of "infected" phenotype.
- Use of "selectable markers" to detect either insertion into the vector or incorporation into the host. Some of these are a type of **reporter gene**—a gene whose expression is easily observed.
- Many plasmids contain the *lacZ* gene with a multiple cloning site within its sequence. *lacZ* codes for an enzyme that can convert the substrate X-Gal into a bright blue product



How can you distinguish between recDNA vectors and "empty" vectors?

Recombinant DNA and Biotechnology

DNA Cloning

Introduce DNA into Organism

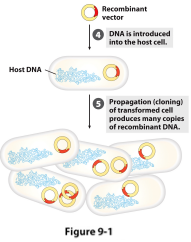
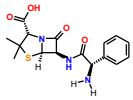


Figure 9-1

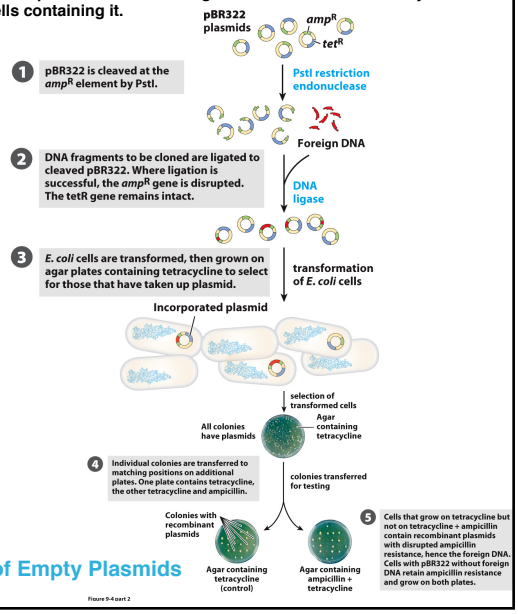
Antibiotic Selection

- Antibiotics, such as penicillin and ampicillin, kill bacteria.
- Plasmids can carry genes that give a host bacterium a resistance against antibiotics.
- Allows growth (selection) of bacteria that have taken up the plasmid



Ampicillin

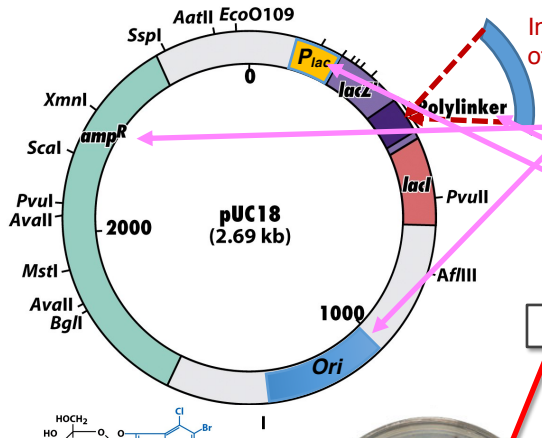
Use of pBR322 to clone foreign DNA in *E. coli* and identify cells containing it.



Identification of Empty Plasmids

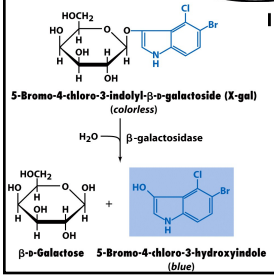
Figure 9-4 part 2

Selection using Reporter Gene

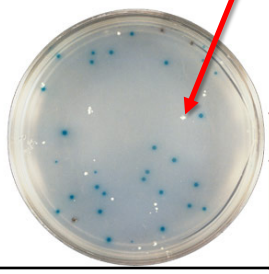


Insert ORF of interest

- Origin of replication
- Antibiotic resistance gene
- Multiple cloning site
- Promoter for transcription, translation (host specific)



Recombinant DNA Clone



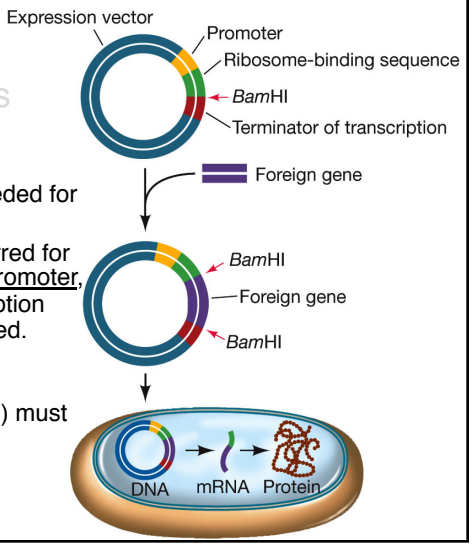
(–) Insert – Makes β-Galactosidase

(+) Insert – Doesn't make β-Galactosidase

DNA taken up by amp^s, *lacZ*⁻ *E. coli*	Phenotype for ampicillin	Phenotype for *lacZ*
None	Sensitive	~~White~~ N/A
Foreign DNA	Sensitive	~~White~~ N/A
Plasmid	Resistant	Blue
Recombinant DNA	Resistant	White

Recombinant DNA and Biotechnology

- Biochemical Basis of Biotechnology
  - Restriction enzymes, DNA ligase
  - Vectors and Inserts to make recombinant DNA (rDNA)
  - Transformation of hosts
  - Selection of transformants
  - Expression

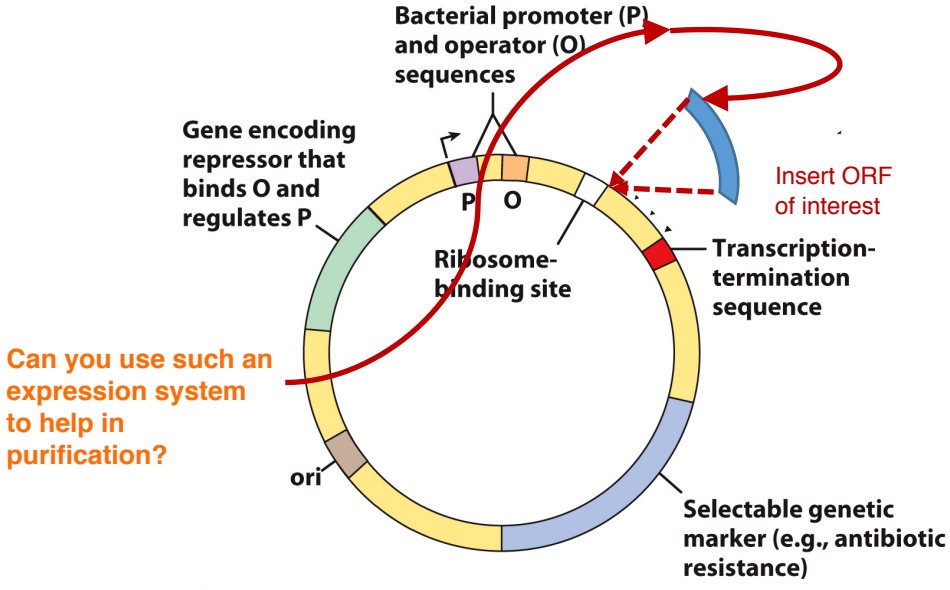


Expression vectors include sequences needed for expression of a *transgene* in a host cell.

- For prokaryote host, which are preferred for making large amounts: A **bacterial** promoter, ribosome binding site, and a transcription termination signal, must all be included.
- For eukaryote (mostly yeast): The **eukaryotic** promoter/enhancer and terminator (poly-A addition signal/site) must be included.

Recombinant DNA and Biotechnology

Typical Expression Vector



Can you use such an expression system to help in purification?

Figure 9-7

Recombinant DNA and Biotechnology

Purification of Recombinant Proteins

- Purification of natural proteins is difficult.
- Recombinant proteins can be tagged for purification.
- The tag binds to the affinity resin, binding the protein of interest to a purification column.

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

Recall:

Affinity Chromatography

Basis = function

Biotechnology (recombinant DNA technology) has revolutionized protein purification.

At the level of the DNA sequence, the DNA sequence encoding such binding proteins or "tags" can be "fused" to the sequence encoding YFP. In this way, a chimeric protein is produced that has the binding function, which allows the use of affinity chromatography.

Common "tags" are:

- Maltose-binding protein
- Chitin-binding protein
- Glutathione-S-transferase (GST)
- His-His-His-His-His-His

Column beads have attached:

- Maltose
- Chitin
- Glutathione (γ-Glu-Cys-Gly)
- Ni-chelate

Recombinant DNA and Biotechnology

Purification of Recombinant Proteins

Can you use such an expression system to help visualize proteins INSIDE of cells?

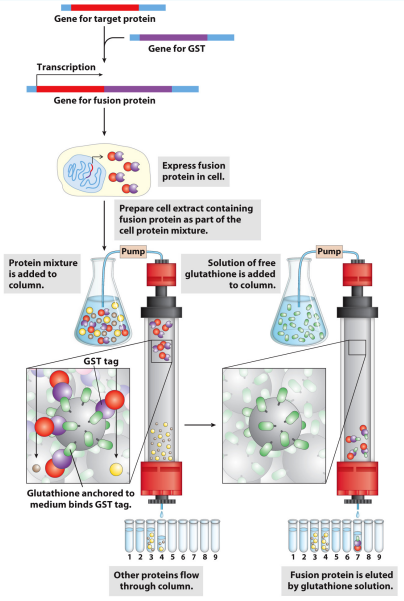
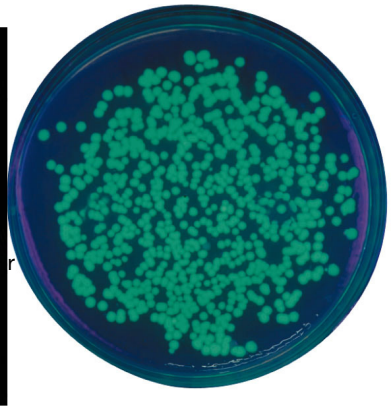


Figure 9-11b

Recombinant DNA and Biotechnology

Expression

- *EXAMPLE*: Green fluorescent protein, which normally occurs in a jellyfish, emits visible light when exposed to UV light. The gene for this protein has been isolated and incorporated into vectors as a reporter gene



Recombinant DNA and Biotechnology

GFP–Tagged Protein Localization

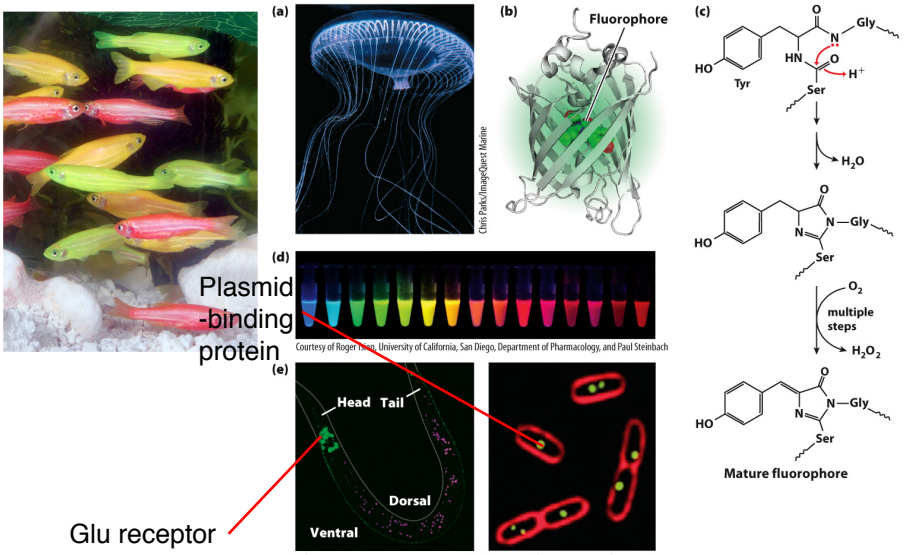


Figure 9-16

http://olympus.magnet.fsu.edu/primer/techniques/confocal/applications/fpcolorpalette.html

Recombinant DNA and Biotechnology

- Biochemical Basis of Biotechnology
  - Restriction enzymes, DNA ligase
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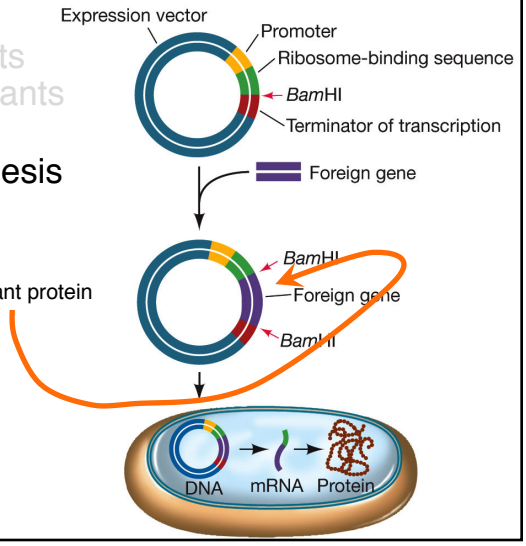
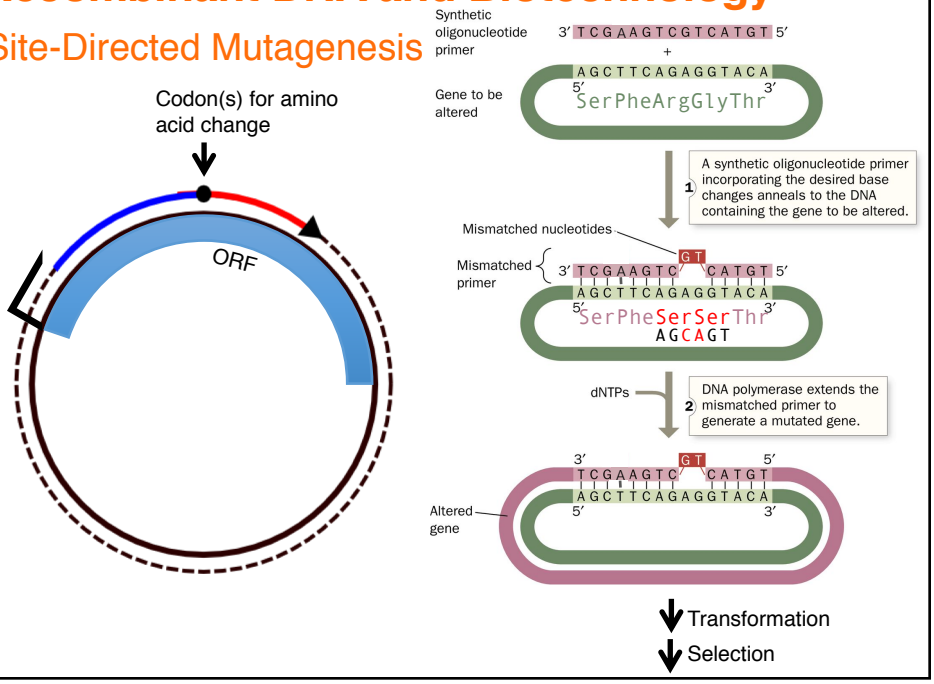
  - Selection of transformants
  - Expression
  - Site-directed mutagenesis

Make a specific mutation to make a variant protein

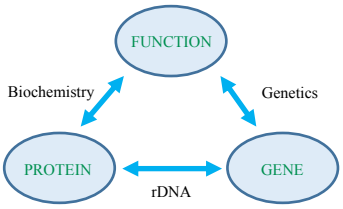


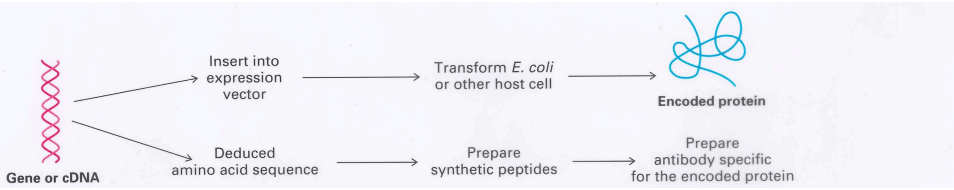
Recombinant DNA and Biotechnology

Site-Directed Mutagenesis



Recombinant DNA and Biotechnology





Recombinant DNA and Biotechnology