Chapter 8: Recombinant DNA Technology

1. Tools of Recombinant DNA Technology

2. PCR & DNA Sequencing

1. Tools of Recombinant DNA Technology

Restriction enzyme recognition sequence

- Restriction enzyme cuts the DNA into fragments
- Addition of a DNA fragment from another source
- Two or more fragments stick together by base-pairing
- DNA ligase pastes the strand
- Recombinant DNA molecule

Restriction Enzymes

The cutting and splicing of DNA in vitro involves the use of restriction enzymes (RE’s):

- EcoR I cuts at: ..GAATTC.. ..CTTAAG..
- Hind III cuts at: ..AAGCTT.. ..TTCGAA..

There are many different RE’s, each cutting a different sequence
“Cloning” a Gene

DNA of interest is inserted into a plasmid & carried inside bacterial “clone”:

1) cut plasmid and DNA to be cloned with same RE
2) ligate fragments together using DNA ligase enzyme
3) transform bacteria, select for “clones” with plasmid

**Allows DNA of interest to be easily “grown” and purified**

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**Diagram**

1. Isolate plasmid.
2. Enzymatically cleave DNA into fragments.
3. Isolate fragment with the gene of interest.
4. Insert gene into plasmid.
5. Insert plasmid and gene into bacterium.

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- Harvest copies of gene to insert into plants or animals
- Produce vaccines, antibiotics, hormones, or enzymes
- Create beneficial combination of traits
- Eliminate undesirable phenotypic traits
Cloning Vectors
DNA designed to carry “DNA of interest” within another organism (or virus):
• plasmids (bacteria), viral DNA, artificial chromosomes (yeast)

Plasmid cloning vectors contain:
• convenient restriction sites
• origin of replic.
• antibiotic resistance genes for selection

2. PCR & DNA Sequencing

PCR & DNA sequencing are techniques that involve manipulating DNA replication in vitro...
Each technique requires the following:

1) Source of DNA of Interest
   • tissue sample that may contain pathogenic DNA (PCR)
   • “cloned” DNA from species of interest inserted into a vector (plasmid, virus, artificial chromosome)

2) Artificial (man-made) Primer(s)
   • ~20 nt DNA fragment that targets DNA synthesis to region of interest

3) Raw Materials for DNA Synthesis
   • nucleotides (dNTP’s), DNA polymerase

a. PCR

Overview of PCR

Every PCR (Polymerase Chain Reaction) reaction requires the following:

1) source of target DNA template
2) artificial primers “flanking” DNA of interest
3) heat-stable DNA polymerase (from hyperthermophile)
4) dNTP’s
5) automated thermocycler to facilitate repeated:
   • denaturation of DNA (separating the 2 strands)
   • hybridization of primers to template
   • DNA synthesis
Specificity of PCR

- Primers must be specific for DNA of interest
- Complementary to target DNA
- If target DNA is present, the sequence flanked by the primers will be selectively amplified

Illustration of PCR

- Gene of interest
- Original DNA molecule with gene of interest
- Heat to 94°C
- Denaturation
- DNA polymerase (heat stable)
- Cool to 60°C
- Extension at 72°C
- DNA primer
- dNTPs
- Heat-stable DNA polymerase must remain active after heating to 94°C

Isolation of PCR DNA Fragments

The resulting PCR products can be separated by size via gel electrophoresis:

- Once purified from the gel, PCR fragments can be cloned into vectors (plasmids) and sequenced to verify!
b. DNA Sequencing

DNA Sequencing uses Chain Terminators

Normal nucleotide (dNTP)  Dideoxynucleotide (ddNTP)

4 separate DNA synthesis reactions are carried out, each containing:

- DNA template to be sequenced
- dNTP's
- DNA primer (fluorescently labeled)
- DNA polymerase

Each of the 4 reactions is spiked with a different labeled ddNTP:

- ddATP, ddCTP, ddGTP or ddTTP

  - primers used for each reaction labeled with a different color fluorescent marker
**“A” reaction**
- termination of DNA synthesis from the primer will occur at random “A’s” due to ddATP

**“C” reaction**
- termination of DNA synthesis from the primer will occur at random “C’s” due to ddCTP

**“G” reaction**
- termination of DNA synthesis from the primer will occur at random “G’s” due to ddGTP

**“T” reaction**
- termination of DNA synthesis from the primer will occur at random “T’s” due to ddTTP

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**Sanger ddNTP Chain Termination Sequencing**

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**Resolving the DNA Fragments**
Separation of the resulting labeled DNA fragments from each reaction by gel electrophoresis reveals the sequence!
- each labeled DNA strand begins at the primer
- the length of each fragment depends on where the strand terminated
  - i.e., where the ddNTP was added, thus causing chain termination
  - shorter DNA fragments move faster through the gel, longer fragments move more slowly

**The relative lengths of the fragments from each reaction reveals the overall DNA sequence**
Key Terms for Chapter 8

• restriction enzyme
• “cloning” of a gene, cloning vector
• gel electrophoresis
• dideoxynucleotide triphosphates (ddNTPs)
• chain terminators

Relevant Chapter Questions
MC: 5, 8  T/F: 1-3