BIOL 300 – Foundations of Biology
Summer 2017 – Telleen
Lecture 14 Outline

Biotechnology

I. What is Biotechnology?
- A. Biotechnology is the use of cells and/or biological molecules to solve problems or make useful products
- B. In a broad sense, many things are actually the products of biotechnology:
  1. Bread, Beer/wine, cheese, yogurt
- C. Current examples include antibiotics, many pharmaceuticals, genetically modified organisms
- D. Modern biotechnology uses modern tools including computers, informatics, and recombinant DNA technology

II. Recombinant DNA

Recombinant DNA is combining DNA from two or more different sources into a single molecule

- A. **Gel Electrophoresis** is a method of separating DNA molecules based on their size
  1. DNA has a negative charge from the phosphates so it will migrate toward the positive pole if an electric current is applied
  2. Agarose is a polysaccharide that dissolves in water when heated and forms a solid matrix when cooled; it is basically highly refined agar
  3. If we put DNA in an agarose gel and apply an electric current, DNA moves toward the positive pole at a rate inversely proportional to its size
  4. DNA can be visualized with chemical staining and UV light
- B. DNA modifying enzymes
  1. **Restriction enzymes** recognize specific DNA sequences and cut DNA within that sequence only
     a. **CRISPR-**based techniques are basically programmable **Restriction Enzymes**
  2. **DNA Ligase** joins the ends of linear DNA molecules
  3. **DNA Polymerases** make new DNA molecules based on existing template (e.g. **PCR**)
- C. Making recombinant molecules combines the above enzymes and techniques
  1. Recall that **plasmids** are small circular DNA molecules that replicate in bacteria and often contain antibiotic resistance genes
  2. **Plasmids** can be used as **vectors** to carry foreign DNA sequences:
     1. **Plasmid** and DNA of interest are digested with restriction enzymes
     2. Both are run on an agarose gel to separate different fragments
     3. Desired fragments (plasmid and insert) are excised from gel and purified
     4. Fragments are mixed together with DNA ligase, which joins the fragments
     5. The resulting recombinant DNA molecule (plasmid+insert) are transformed into host bacteria (usually *E. coli*)
     6. Bacteria containing the plasmid can be selected using antibiotic resistance
     7. Once identified, colonies containing the recombinant plasmid can be grown to make large amounts of the plasmid (along with the insert DNA)
  3. When we **clone** a gene, this is what we mean
  4. Collections of cloned DNA fragments from a particular source are called libraries

III. DNA profiling can be used to distinguish between individuals (including strains of microbes)

A. DNA profiling examines the DNA of an individual (or clonal strain) so that it can be distinguished from other such entities

B. These techniques are used in forensic DNA analysis

C. Most are based on PCR (Polymerase Chain Reaction)

D. Specific DNA sequences can be amplified by PCR

1. PCR makes many copies of specific DNA sequences in a test tube
2. Involves DNA Polymerase, short DNA primers, and dNTPs
3. Here's how it works:
    a. Template DNA is heated to 95^{o}C to denature (separate strands by breaking H-bonds)
    b. Temperature is reduced so that primers can bind to denatured DNA
    c. Temperature is raised to 72^{o}C and DNA Polymerase extends the primers, copying the template sequence
    d. Return to step a (and repeat the whole process 30 times)
4. Each cycle of PCR doubles the number of molecules of the target sequence
5. After 30 cycles, there are more than 2^{30} copies (that's a lot!)

E. See examples used in class

IV. Genetic Engineering

A. Genetic engineering refers to directed alterations of the genome of an organism

B. Technically, selective breeding/domestication is genetic engineering in this sense

C. Often used to refer to the insertion of one or more genes from one organism into another

D. Modern genetic engineering uses recombinant DNA technology

E. First commercial genetically engineered product was human insulin from Genentech (1982)

1. Insulin gene cloned into a plasmid and expressed in *E. coli*
2. Large cultures could then be grown and the protein extracted

F. Now engineering of plants and animals is routine

IV. Genomics and Bioinformatics

A. Genomics is the study of the entire DNA sequence of an organism

B. Bioinformatics is the use of computer and information technology to analyze biological information (sequence data)

C. The National Center for Biotechnology Information (NCBI) provides many useful tools and databases to store biological sequence information

D. Genomics includes Physical mapping of chromosomes and Whole genome sequencing

E. Applications of Genomics include:

1. Predicting disease risk for individuals
2. GWAS
3. Pharmacogenomics
4. Metagenomics
5. Engineered biofuels
6. Forensic analyses
7. Agriculture

F. Proteomics studies the complete sets of proteins in cells (proteomes)