Analyzing & Engineering Genes NOTES

The beginning: Solving the problem of Pituitary Dwarfism

treatment = hGH (human growth hormone) produced from GH1

Genetically modified E. coli used to synthesize hGH in bacteria

E. coli cells had a modified gene composed of the coding sequence for ___________ to synthetic bacterial ___________ to produce large quantities

I. The field of biotechnology

✓ recombinant DNA technology
✓ genetic engineering
✓ gene splicing
✓ cloning

A. What is a recombinant?

Recombinant DNA refers to a combination of DNA molecules that are __________, produced by joining DNA obtained from ___________ biological sources

• ___________ = carrier DNA molecules that transfer and replicate inserted DNA fragments

Permit entry into host cell, where it can be replicated ___________ into many copies

__________ enzymes cut DNA at a specific sequence of nucleotides = __________ site

B. Making recombinants = Cutting DNA and pasting it in new combinations

1) DNA is isolated & purified
2) Restriction Enzymes
3) Fragments inserted
4) Vector transferred to host cell (transformation / transduction)
5) As host cell replicates, recombinant molecules are passed on to progeny

• ___________ holds all of the DNA fragments of the genome in different host (bacterial or yeast) cells

• ___________ = DNA produced from RNA

6) Cloned DNA can be recovered & analyzed – can be transcribed & translated in the
C. Selecting for recombinants:
How do you know if transformation or transduction worked?
Did the vector insert the foreign DNA?
Insert a ____________________ & then screen for presence of that = Gene inserted that can be selected for, e.g. antibiotic resistance; ________ production.

- selecting recombinant vectors using X gal - identified by white colonies. White cells lack ______________ because they have plasmids with foreign DNA inserted into the polylinker site; blue cells contain plasmids with ______________

II. Commonly used Molecular Techniques:
A. The Polymerase Chain Reaction Makes DNA Copies ______________
Host Cells

- Directed Replication of a target sequence: PCR requires two oligonucleotide primers, one complementary to the 3’ end of one strand of the DNA to be amplified and one complementary to the 3’ end of the other strand.
- primers anneal to denatured DNA, and the complementary strands are synthesized by a heat-stable __________________________
- __________________________ way of replicating specific target sequences
Visualizing PCR products to see if PCR worked

- gel electrophoresis: allows for separation of nucleic acids (fragments) and proteins

B. DNA sequencing

- process for sequencing PCR products (specific regions of DNA)
- 4 sequencing reactions made for each base
- sequencing is automated and uses fluorescent dye-labeled dideoxynucleotides

- an argon laser excites the fluorescent dye attached to the ddNTP and it fluoresces at a specific wavelength

III. Applications of biotechnology

pharmaceuticals

Example:

Insulin - cDNA made from mRNA for human insulin protein is put into E. coli chromosome next to the lac operon promoter → bacteria synthesizes human insulin

agriculture: genetically modified organisms

- temperature-resistance
- pest-resistance
- improve shipping
- improved flavor
- vaccines
- growth hormones
**Case study: golden rice**

Genes for \__________ synthesis  
cloned into Ti plasmids and recombinant plasmids  
introduced into rice plants  
Transformed plants produce

3. diagnosis of genetic disorders

if gene sequence or genetic markers identified, can use  
PCR and DNA hybridization genetic testing: detection can  
be made before birth  
example:

4. gene therapy  
current best vector: retrovirus - has RNA genome and reverse transcriptase  
but, viruses are pathogens (e.g., HIV is a retrovirus); so disease genes need to be deactivated

5. forensics

6. environmental cleanup – bioremediation

**The Future**
- Genomics, Proteomics, Metabolomics  
Many applications of DNA technology  
But, there is much public controversy about genetically modified organisms (GMOs)  
Many questions about potential effects on the environment and human health  
Government policies and regulations need to be developed and refined

**IV. Ethical considerations**
1. controversial issues  
   - amplification of fossil DNA - e.g., scientists have analyzed mtDNA from wooly mammoth that died 40,000 years ago - should we try to clone?

2. ethical dilemmas  
   - Do you want to know  
   - Should insurance companies be allowed to set insurance premiums based on your genotype?  
   - Should parents be able to  
   - Should genetic screening be a