GENETIC ENGINEERING:
manipulating genes and genomes

RECOMBINANT DNA.
Combining DNA from different organisms

APPLICATIONS OF DNA TECHNOLOGY
• DIAGNOSIS OF DISEASE
  Virus detection;
  ID genetic carriers/disorders
• GENE THERAPY
  ID mutant genes; purify replacements
• PHARMACEUTICAL PRODUCTION
  Bacterial production of insulin,
  Human Growth hormone
• FORENSICS/PATERNITY
  Crime scene analysis
  Who's the daddy?
• GENETICALLY MODIFIED ORGANISMS
  "Golden" rice-gene for Vitamin A added
  Bt-corn -resists insect pests
  "frost resistant" strawberries
  toxin/pollution “eating” bacteria
• ENDANGERED SPECIES ZOO
  cloning extinct/endangered species

PLASMIDS = small self replicating DNA loops
• Can carry genes for ANTIBIOTIC RESISTANCE (used as genetic markers
• Used as VECTORS to carry recombinant DNA
• Can be cut with RESTRICTION ENZYMES
• Used to incorporate foreign DNA into bacteria
• Bacteria then reproduce, copying the inserted gene along with the plasmid

Ways to make bacteria able to “take up” foreign DNA = make them “COMPETENT”
1) ELECTROPORATION- zap with electricity
2) Use calcium chloride and “heat shock” to change their cell walls (We did this in LAB 8)
   - makes cells better able to pick up plasmids/DNA
   - rapidly growing cells are made competent more easily

GFP (Green Fluorescent Protein)
• Originally discovered in jellyfish
• Linked to plasmids carrying recombinant genes
• Used as a genetic tool to identify presence of recombinant genes
**Restriction Enzymes (Restriction Endonucleases)**

- Occur naturally in bacteria
- Function: protect bacteria from invasion by foreign DNA
- Each enzyme recognizes different specific code sequences ~often palindromes
- Cut DNA into short segments with staggered "sticky ends"
- Named for bacteria they come from: EX: EcoR1; HindIII; BamH1
- DNA ligase used to join DNA pieces cut with same enzymes
- Used to combine DNA from different organisms (recombinant DNA)

**Reverse Transcriptase**

- Enzyme from retroviruses (RNA containing viruses)
- Info flows backwards RNA → DNA
- Can be used to put eukaryotic genes into bacteria
- Bacteria don’t process DNA so eukaryotic genes with introns can’t be used directly
- Reverse transcriptase enzymes can take an "edited" message and change it into a gene

**Gene Cloning in Bacteria**

- Process used to produce multiple copies of specific segments of DNA
- Isolate bacterial plasmid and foreign DNA
- Treating with same restriction enzyme produces same "sticky ends"
- Mix DNA
- DNA ligase joins "sticky ends"
- Recombinant plasmid into taken up by bacteria (transformation)
- Bacteria reproduce resulting in multiple copies of the inserted gene

**Identifying Bacteria with Recombinant Plasmids**

- Ability to grow in presence of antibiotics
- Presence of GFP (glow under UV light)
GENE CLONING WITH POLYMERASE CHAIN REACTION (PCR)
Used to AMPLIFY DNA
Makes billions of copies of even small quantities of DNA
NEED:
- target DNA, primers, nucleotides, & DNA Polymerase
- Heat (94° C) - DNA helix unwinds/separates
- Cool (54° C) - DNA hybridizes with primers and builds chain
Both strands of DNA are copied; then copied strands are used as templates in next round
Heating/cooling process is repeated many times to get many copies

PROBLEM*
HEAT destroys DNA Polymerase
SOLUTION:
- Use Taq POLYMERASE from hot springs archeabacteria (Thermus aquaticus)
  ~THERMOSTABLE-withstands 90° heat needed for strand separation

RFLP’s (RESTRICTION FRAGMENT LENGTH POLYMORPHISM) ANALYSIS
- inherited differences found among the individuals in a population
- differences in DNA code result in different restriction sites in DNA
- produces fragments of different lengths
  = restriction fragment length polymorphisms (RFLP’s)
- treat DNA with restriction enzymes
- use gel electrophoresis to separate the restriction fragments

AGAROSE GEL ELECTROPHORESIS = “swimming through JELLO”
*** CAN BE USED TO SEPARATE ANY MOLECULE WITH A CHARGE
  ~ not just for DNA (Ex: proteins)
- DNA is negatively charged (due to phosphates) so moves in electric field
- Used to separate DNA fragments after cut with restriction enzymes
- Separates by size and electric charge
- Can identify DNA molecules by banding patterns
- Can isolate and purify genes
- DNA molecules can be identified by specific band patterns
  ~ separated by size and electric charge
  ~ DNA has - charge due to phosphates in backbone
  ~ Smaller fragments move farther
  ~ More voltage-move faster
DNA DETECTION

- **ETHIDIUM BROMIDE** -
  Glows under UV light but highly carcinogenic
- **METHYLENE BLUE DYE** -
  - see as blue bands
- **SOUTHERN BLOT** -
  - uses radioactively labeled probes to ID specific DNA segments
  - expose X-ray film