

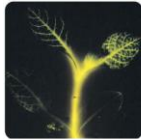
"Okay—is there anybody ELSE whose homework ate their dog?"

Chapter 16

Biotechnology: DNA Technology & Genomics

The BIG Questions...

- How can we use our knowledge of DNA to:
 - ◆ diagnose disease or defect?
 - ◆ cure disease or defect?
 - ◆ change/improve organisms?
- What are the techniques & applications of biotechnology?
 - ◆ direct manipulation of genes for practical purposes



Because all organisms use the same genetic code, scientists can make a plant glow like a firefly.

Biotechnology

- Genetic manipulation of organisms is **not** new
 - ◆ humans have been doing this for thousands of years
 - plant & animal breeding






Evolution & Breeding of Food Plants





Evolution of *Zea mays* from ancestral *teosinte* (left) to modern corn (right). The middle figure shows possible hybrids of teosinte & early corn varieties

Evolution & Breeding of Food Plants

- “Descendants” of the wild mustard
 - ◆ *Brassica* genus

Animal Husbandry / Breeding

Biotechnology Today

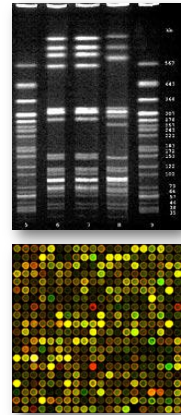
- Genetic Engineering
 - ◆ manipulation of DNA
 - ◆ if you are going to engineer DNA & genes & organisms, then you need a **set of tools** to work with
 - ◆ this unit is a survey of those tools...



Our tool kit...

Bioengineering Tool Kit

- Basic Tools
 - ◆ restriction enzymes
 - ◆ ligase
 - ◆ plasmids / cloning
 - ◆ DNA libraries / probes
- Advanced Tools
 - ◆ PCR
 - ◆ DNA sequencing
 - ◆ gel electrophoresis
 - ◆ Southern blotting
 - ◆ microarrays



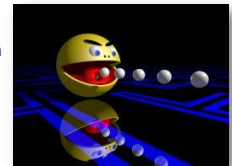
Cut, Paste, Copy, Find...

- Word processing metaphor...
 - ◆ cut
 - restriction enzymes
 - ◆ paste
 - ligase
 - ◆ copy
 - plasmids
 - ◆ bacteria
 - ◆ transformation
 - PCR
 - ◆ find
 - Southern blotting
 - probes



Cutting DNA

- Restriction enzymes
 - ◆ **restriction endonucleases**
 - ◆ discovered in 1960s
 - ◆ evolved in bacteria to cut up foreign DNA (“action **restricted** to foreign DNA”)
 - protection against viruses & other bacteria
 - ◆ bacteria protect their own DNA by methylation & by not using the base sequences recognized by the enzymes in their own DNA

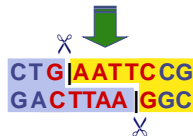


Restriction Enzymes

- Action of enzyme
 - ◆ cut DNA at specific sequences
 - **restriction site**
 - ◆ symmetrical “palindrome”
 - ◆ produces protruding ends
 - **sticky ends**
- Many different enzymes
 - ◆ named after organism they are found in
 - EcoRI, HindIII, BamHI, SmaI

Madam I'm Adam

CTGAATTCCG
GACTTAAGGC



Discovery of Restriction Enzymes

1960s | 1978



Werner Arber

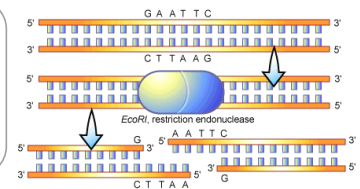


Daniel Nathans



Hamilton O. Smith

Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in E. coli



Paste DNA

- Sticky ends allow:
 - H bonds between complementary bases to anneal
- Ligase
 - enzyme "seals" strands
 - bonds sugar-phosphate bonds
 - covalent bond of DNA backbone

Biotech Use of Restriction Enzymes

Gel Electrophoresis

- Separation of DNA fragments by size
 - DNA is negatively charged
 - moves toward + charge in electrical field
 - agarose gel
 - "swimming through Jello"
 - smaller fragments move faster

Gel Electrophoresis

Gel Electrophoresis

Gel Electrophoresis

Southern Blot

Want to locate a sequence on a gel?

- Restriction fragment preparation
- Electrophoresis
- Blotting
- Hybridization with radioactive probe
- Autoradiography

Southern blot

- Transfer DNA from gel to filter paper
- hybridize filter paper with tagged probe
 - fragment with matching sequence "lights up"

Hybridization in Southern Blotting

- Use radioactive probe to locate gene on filter paper
 - go back to gel & cut out piece of DNA you want to collect

Copy DNA

- Plasmids
 - small, self-replicating circular DNA molecules
 - insert DNA sequence into plasmid
 - vector = "vehicle" into organism
 - transformation
 - insert recombinant plasmid into bacteria
 - bacteria make lots of copies of plasmid
 - grow recombinant bacteria on agar plate
 - clone of cells = lots of bacteria
 - production of many copies of inserted gene

DNA → RNA → protein → trait

Recombinant Plasmid

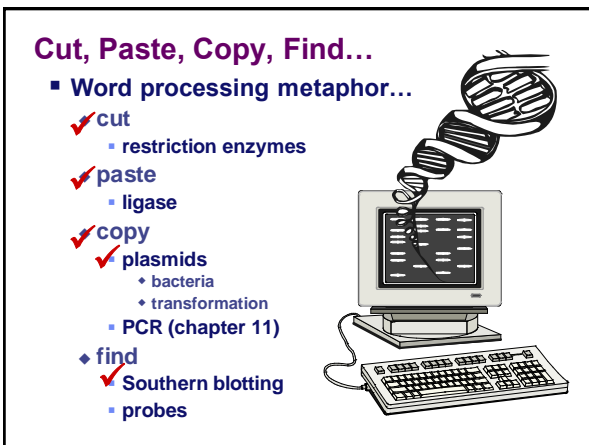
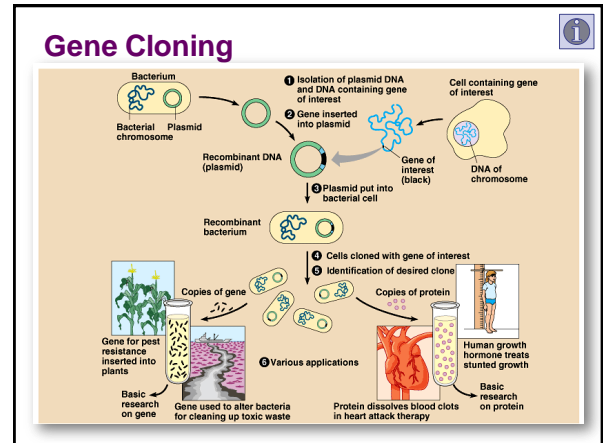
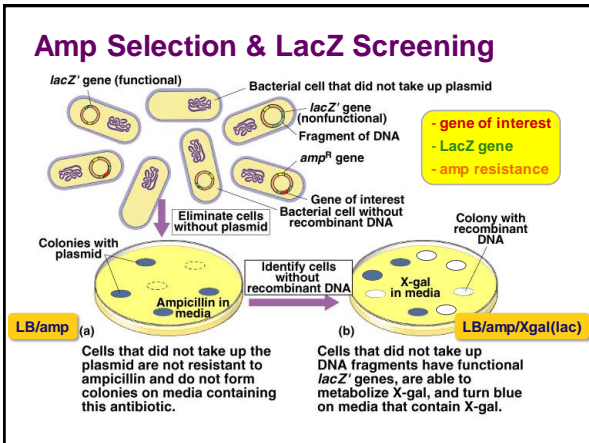
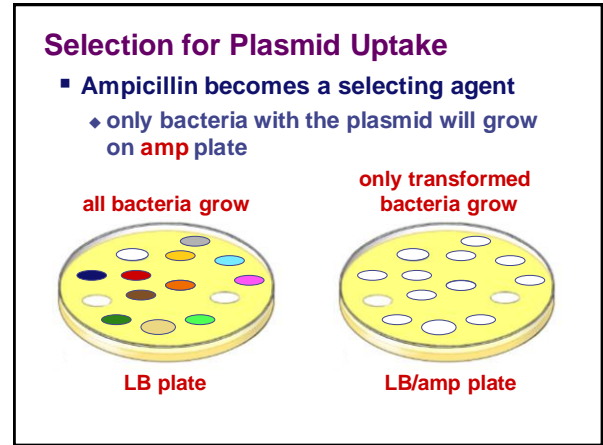
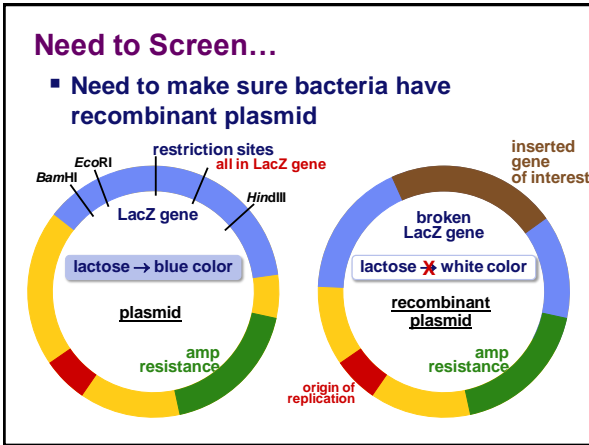
- Antibiotic resistance genes as a **selectable marker**
- Restriction sites for splicing in gene of interest

Selectable marker

- Plasmid has both "added" gene & antibiotic resistance gene
- If bacteria **don't** pick up plasmid then **die** on antibiotic plates
- If bacteria pick up plasmid then survive on antibiotic plates
- selecting for successful transformation

LacZ is one Screening System

- Make sure inserted plasmid is recombinant plasmid
 - LacZ gene on plasmid produces digestive enzyme
 - lactose (X-gal) → blue
 - blue colonies
 - insert foreign DNA into LacZ gene breaks gene
 - lactose (X-gal) **not** blue
 - white colonies
 - white bacterial colonies have recombinant plasmid



What if you don't have your gene conveniently on a chunk of DNA ready to insert into a plasmid?

Have to find your "gene of interest" out of the entire genome of the organism...

DNA Libraries

- Cut up all of nuclear DNA from many cells of an organism
 - ♦ restriction enzyme
- Clone **all** fragments into plasmids at same time
 - ♦ “shotgun” cloning
- Create a stored collection of DNA fragments
 - ♦ petri dish has a collection of all DNA fragments from the organism

(a) Plasmid library

Making a DNA Library

all DNA from many cells of an organism is cut with **restriction enzymes**

engineered plasmid with **selectable marker & screening LacZ gene**

gene of interest

all DNA fragments inserted into many plasmids

clone plasmids into bacteria

Making a DNA Library

recombinant plasmids inserted into bacteria

gene of interest

bacterial colonies (clones) grown on LB/amp/Xgal petri plates

Finding Your Gene in DNA Library

- **Locate Gene of Interest**
 - ♦ to find your gene you need some of gene’s sequence
 - if you know sequence of protein...
 - ♦ can guess part of DNA sequence
 - ♦ “back translate” protein to DNA
 - if you have sequence of similar gene from another organism...
 - ♦ use part of this sequence

Locating Your Gene of Interest

- **DNA hybridization**
 - ♦ find gene in bacterial colony using a **probe**
 - short, single stranded DNA molecule
 - complementary to part of gene of interest
 - tagged with radioactive P³² or fluorescence
 - ♦ heat treat genomic DNA
 - unwinds (**denatures**) strands
 - ♦ DNA **hybridization** between probe & denatured DNA

Hybridization

- 1 Cloning** - plate with bacterial colonies carrying recombinant plasmids
- 2 Replicate plate** - press filter paper onto plate to take sample of cells from every colony
- 3 Hybridization** - heat filter paper to denature DNA - wash filter paper with radioactive probe which will only attach to gene of interest
- 4 Locate** - expose film - locate colony on plate from film

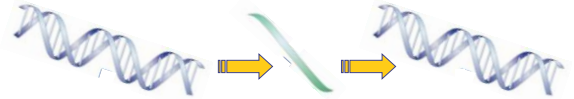
Problems...

- A lot of junk!
 - ◆ human genomic library has more “junk” than genes in it
- Introns, introns, introns!
 - ◆ if you want to clone a human gene into bacteria, you can't have 'em....



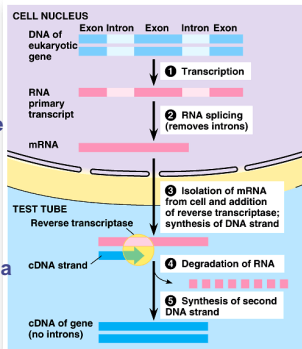
Solution...

- Don't start with DNA...
- Use mRNA
 - ◆ copy of the gene without the junk!
- But in the end, you need DNA to clone into plasmid...
- How do you go from RNA → DNA?
 - ◆ **reverse transcriptase!**

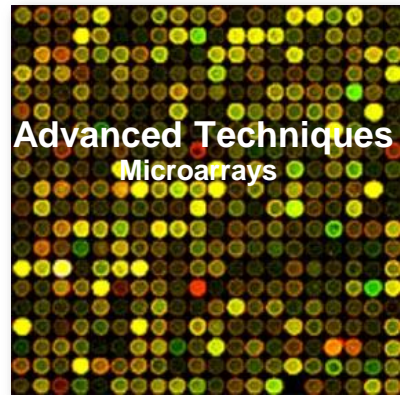


cDNA (complementary DNA) Libraries

- Collection of only the coding sequences of expressed genes
 - ◆ extract mRNA from cells
 - ◆ reverse transcriptase
 - RNA → DNA
 - from retroviruses
 - ◆ clone into plasmid
- Applications
 - ◆ need edited DNA for expression in bacteria
 - human insulin



Advanced Techniques Microarrays



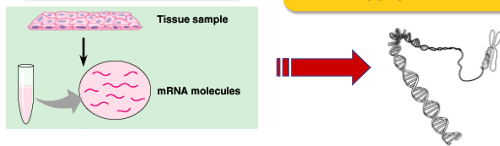
Where do we go next?



- When a gene is turned on, it creates a trait
 - ◆ want to know what gene is being expressed

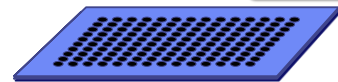
extract mRNA from cells
mRNA = active genes

How do you match
mRNA back to DNA in
cells???

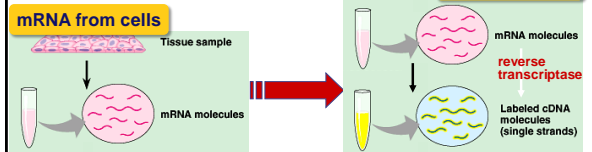


Microarrays

slide with spots of DNA
each spot = 1 gene

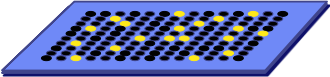


- Create a slide with a sample of each gene from the organism
 - ◆ each spot is one gene
- Convert mRNA → labeled cDNA



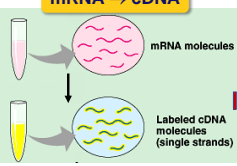
Microarrays

slide with spots of DNA
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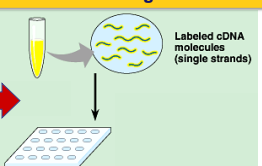


- Labeled cDNA hybridizes with DNA on slide
 - each yellow spot = gene matched to mRNA
 - each yellow spot = expressed gene

mRNA → cDNA




cDNA matched to genomic DNA



Application of Microarrays

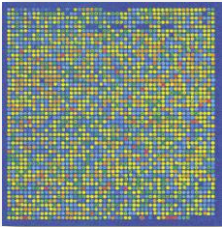
2-color fluorescent tagging



- Comparing treatments or conditions = Measuring change in gene expression
 - sick vs. healthy; cancer vs. normal cells
 - before vs. after treatment with drug
 - different stages in development
- Color coding: label each condition with different color
 - red = gene expression in one sample
 - green = gene expression in other sample
 - yellow = gene expression in both samples
 - black = no or low expression in both

“DNA Chip”

- Patented microarray technology from Affymetrix
 - automated DNA synthesis of genes of interest on chip
 - chips are more consistent
 - smaller spots/more spots per chip
 - can buy specific chips
 - human chip
 - mouse chip
 - etc.




Biotechnology Today: Applications

- Application of DNA technologies
 - basic biological research
 - medical diagnostics
 - medical treatment (gene therapy)
 - pharmaceutical production
 - forensics
 - environmental cleanup
 - agricultural applications

...and then there's the ethics issues!

Application of Recombinant DNA

- Combining sequences of DNA from 2 different sources into 1 DNA molecule
 - often from different species
 - human insulin gene in E. coli (humulin)
 - frost resistant gene from Arctic fish in strawberries
 - “Roundup-ready” bacterial gene in soybeans
 - BT bacterial gene in corn
 - jellyfish glow gene in Zebra “Glofish”



What's next?

- After you have cloned & amplified DNA (genes), you can then tackle more interesting questions
 - how does gene differ from person to person?
 - ...or species to species
 - is a certain allele associated with a hereditary disorder
 - in which cells is gene expressed?
 - where is gene in genome?