


What do you notice about these phrases?

radar
racecar
Madam I'm Adam
Able was I ere I saw Elba
a man, a plan, a canal,
Panama
Was it a bar or a bat I saw?

MCC BP Based on work by K. Foglia
www.kimunity.com

Chapter 20.



**Biotechnology:
DNA Technology & Genomics**

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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

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Biotechnology

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



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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

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Evolution & breeding of food plants

- “Descendants” of the wild mustard
 - ◆ Brassica spp.



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Animal husbandry / breeding



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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...

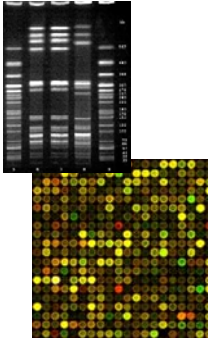


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Our tool kit...

Bioengineering Tool kit


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



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Cut, Paste, Copy, Find...


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



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Cut DNA

- **Restriction enzymes**
 - ◆ **restriction endonucleases**
 - ◆ discovered in 1960s
 - ◆ evolved in bacteria to cut up foreign DNA ("restriction")
 - 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

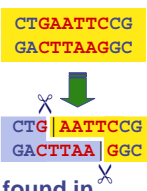


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Restriction enzymes Madam I'm Adam

- **Action of enzyme**
 - ◆ cut DNA at specific sequences
 - **restriction site**
 - ◆ symmetrical "palindrome"


CTGAATTCG
GACTTAAGGC
 - ◆ produces protruding ends
 - **sticky ends**


- **Many different enzymes**
 - ◆ named after organism they are found in
 - EcoRI, HindIII, BamHI, SmaI

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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



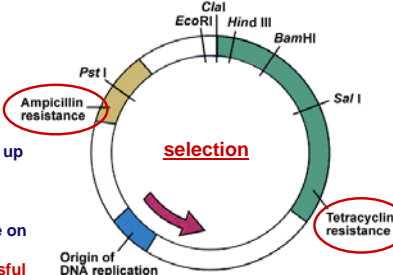
MCC BP **DNA → RNA → protein → trait** Based on work by K. Foglia www.kimunity.com

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




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Selection for plasmid uptake

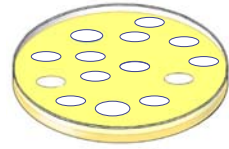
- Ampicillin becomes a selecting agent
 - only bacteria with the plasmid will grow on amp plate

all bacteria grow



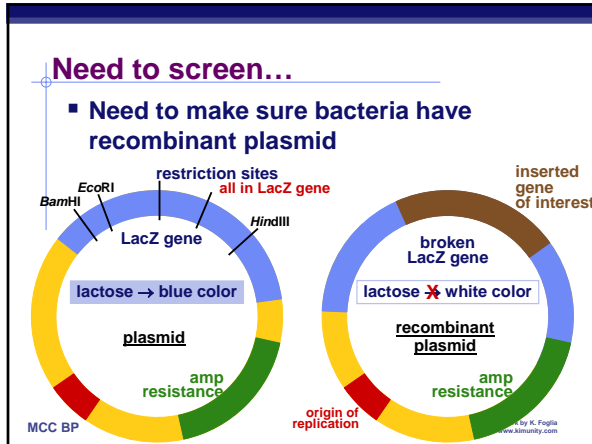
LB plate

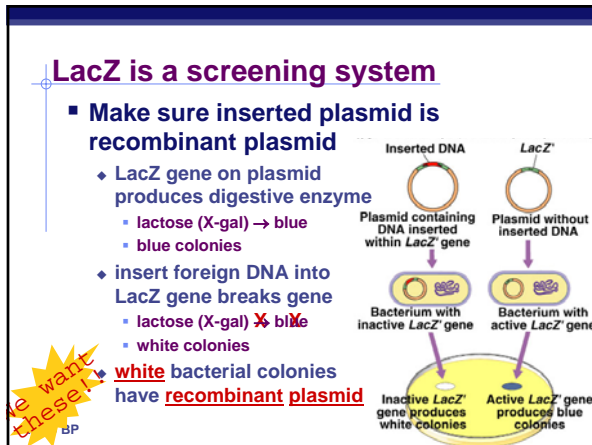
only transformed bacteria grow

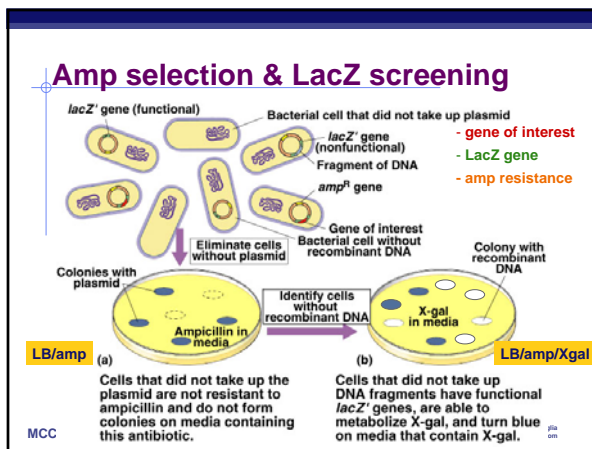


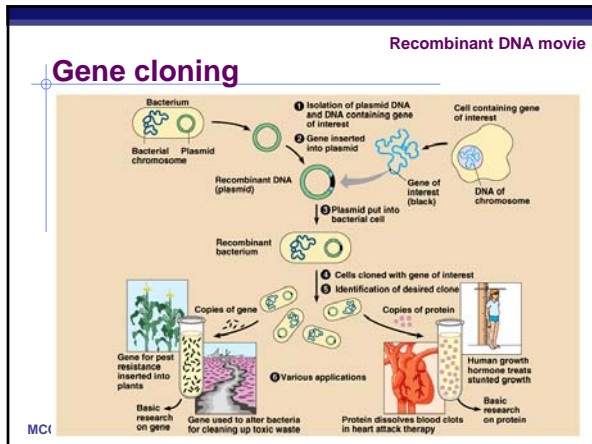
LB/amp plate

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Cut, Paste, Copy, Find...

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

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Any Questions??

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**Biotechnology:
DNA Technology & Genomics
Part 2**



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

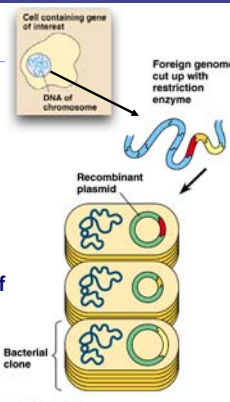
Have to find your “**gene of interest**” out of the entire genome of the organism...

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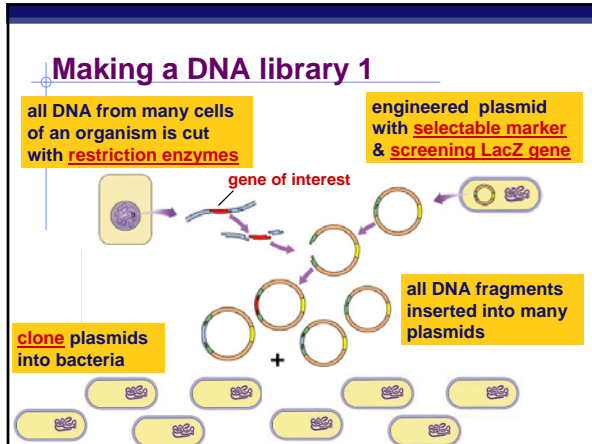
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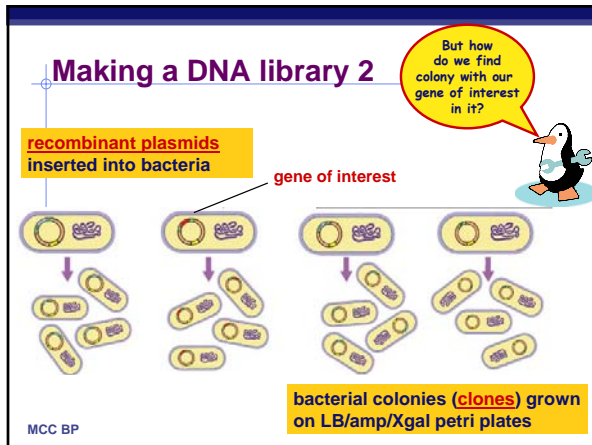
DNA libraries

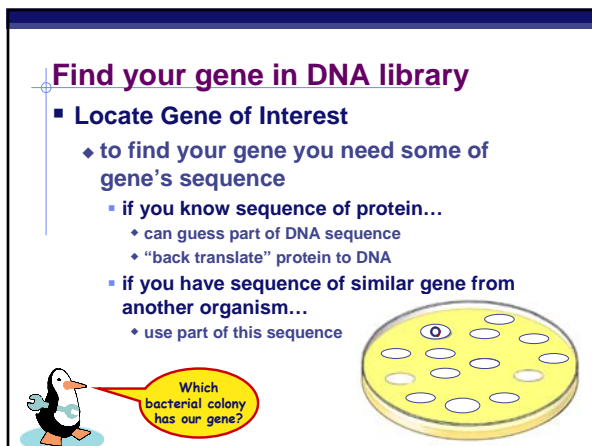
- Cut up all of nuclear DNA from many cells of an organism
 - ♦ restriction enzyme
- Clone all fragments into plasmids at same time
 - ♦ “shotgun” cloning
- Create a stored collection of DNA fragments
 - ♦ petri dish has a collection of all DNA fragments from the organism



(a) Plasmid library







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


- Cloning**
 - plate with bacterial colonies carrying recombinant plasmids
- Replicate plate**
 - press filter paper onto plate to take sample of cells from every colony
- Hybridization**
 - heat filter paper to denature DNA
 - wash filter paper with radioactive probe which will only attach to gene of interest
- 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.... **introns**

Solution...

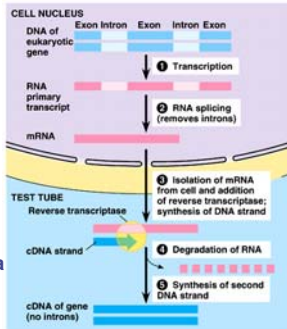
- Don't start with DNA...
- Use mRNA
 - ◆ copy of the gene without the junk!
- But in the end, you need DNA to clone into plasmid...
- How do you go from RNA → DNA?
 - ◆ **reverse transcriptase!**



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cDNA (copy 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



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Any Questions??

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