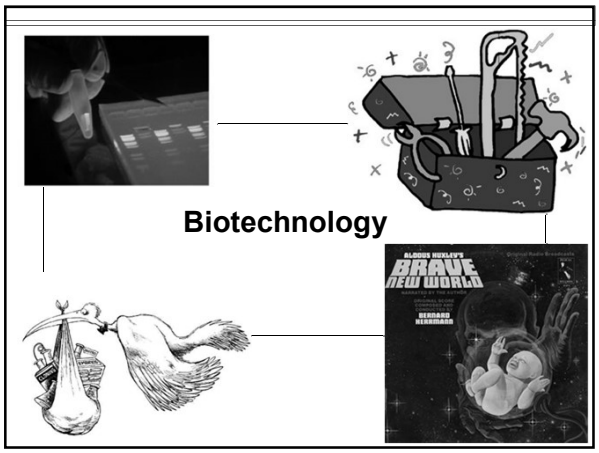
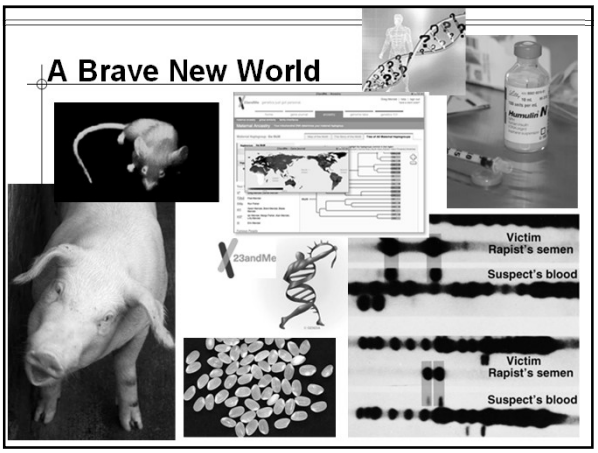
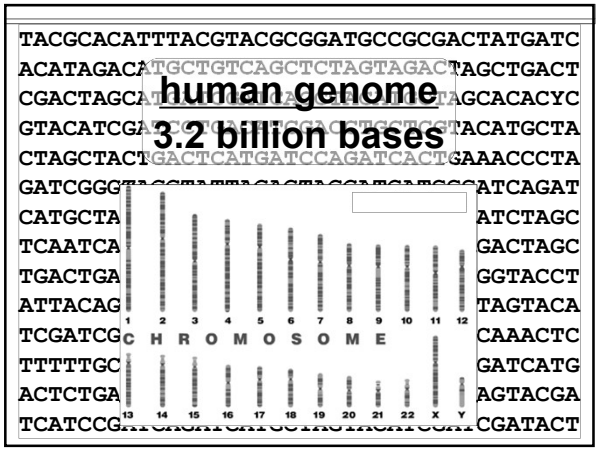
AP Biology







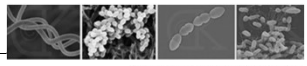
Biotechnology today

- 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…

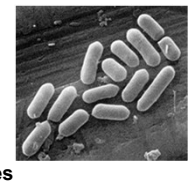
AP Biology

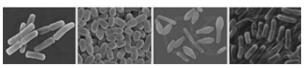
Our tool kit…

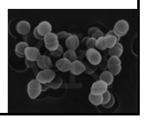
Bacteria



- Bacteria review
  - one-celled prokaryotes
  - reproduce by mitosis
    - binary fission
  - rapid growth
    - generation every ~20 minutes
    - 10^8 (100 million) colony overnight!
  - dominant form of life on Earth
  - incredibly diverse

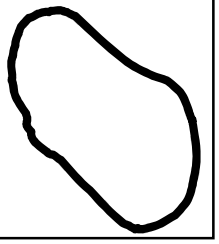






Bacterial genome

- Single circular chromosome
  - haploid
  - naked DNA
    - no histone proteins
  - ~4 million base pairs
    - ~4300 genes
    - 1/1000 DNA in eukaryote



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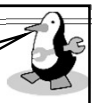
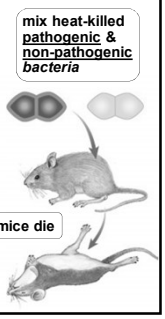
Transformation

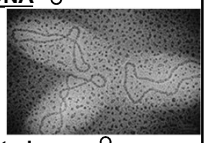


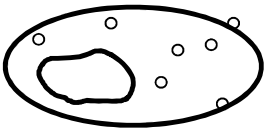
- Bacteria are opportunists
  - pick up naked foreign DNA wherever it may be hanging out
    - have surface transport proteins that are specialized for the uptake of naked DNA
  - import bits of chromosomes from other bacteria
  - incorporate the DNA bits into their own chromosome
    - express new genes
    - transformation
    - form of recombination



Plasmids

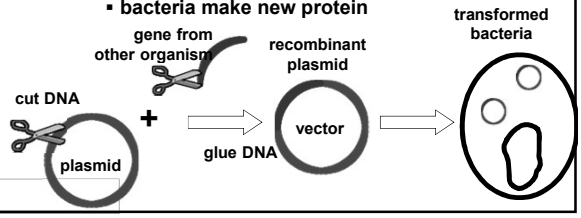
- Small supplemental circles of DNA
  - 5000 - 20,000 base pairs
  - self-replicating
  - carry extra genes
    - 2-30 genes
    - genes for antibiotic resistance
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
  - can be imported from environment





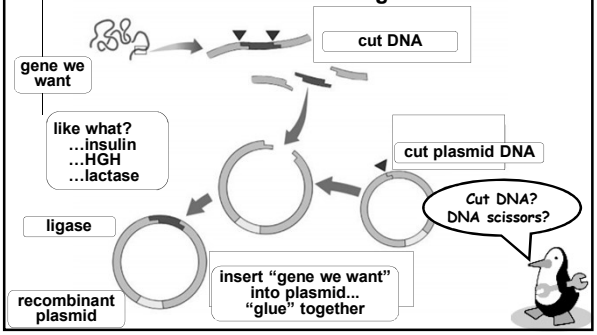
How can plasmids help us?

- A way to get genes into bacteria easily
  - insert new gene into plasmid
  - insert plasmid into bacteria = vector
  - bacteria now expresses new gene
    - bacteria make new protein

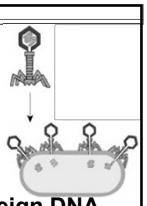


Biotechnology

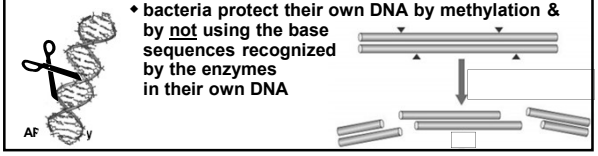
- Plasmids used to insert new genes into bacteria



How do we cut DNA?



- Restriction enzymes
  - restriction endonucleases
  - discovered in 1960s
  - evolved in bacteria to cut up foreign DNA
    - "restrict" the action of the attacking organism
    - 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



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?
go hang a salami I'm a lasagna hog

palindromes

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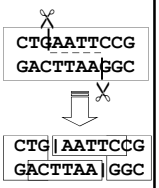
Madam I'm Adam

Restriction enzymes

- Action of enzyme
  - cut DNA at specific sequences
    - restriction site
  - symmetrical "palindrome"
  - produces protruding ends
    - sticky ends
    - will bind to any complementary DNA
- Many different enzymes
  - named after organism they are found in
    - EcoRI, HindIII, BamHI, SmaI



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1960s | 1978

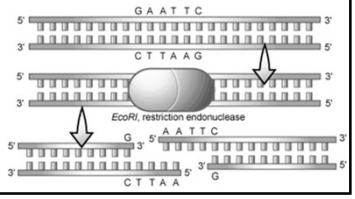
Discovery of restriction enzymes


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



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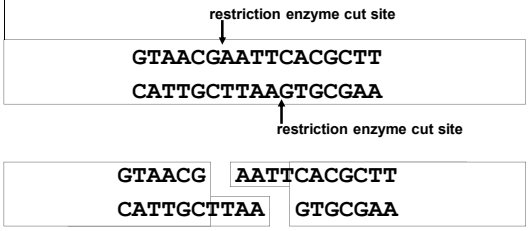
Restriction enzymes

- Cut DNA at specific sites
  - leave "sticky ends"



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Sticky ends

- Cut other DNA with same enzymes
  - leave "sticky ends" on both
  - can glue DNA together at "sticky ends"

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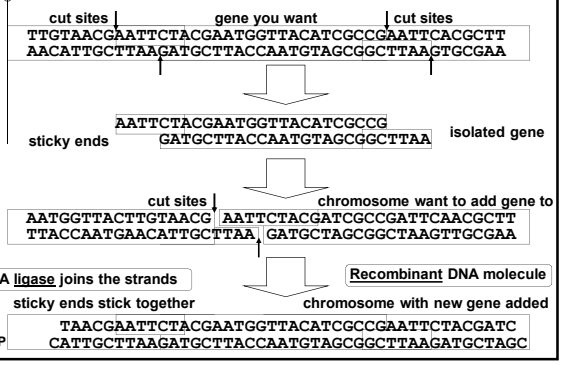
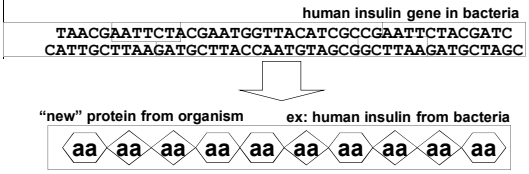
Sticky ends help glue genes together

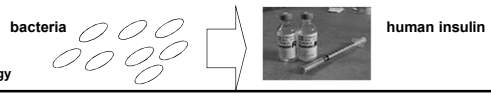


AP

Why mix genes together?

- Gene produces protein in different organism or different individual





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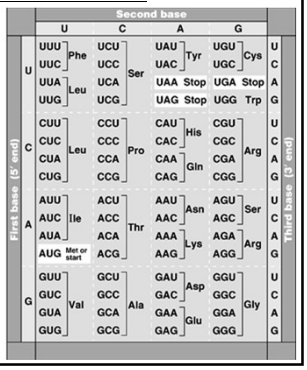
The code is universal

- Since all living organisms…
  - use the same DNA
  - use the same code book
  - read their genes the same way



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Common Restriction Enzymes (with "sticky" ends)

•EcoRI: 5' GAATTC 3'
3' CTTAAG 5'

HindIII: 5' AAGCTT 3'
3' TTCGAA 5'

•BamHI: 5' GGATCC 3'
3' CCTAGG 5'

Common Restriction Enzymes (with "blunt" ends)

•AluI: 5' AGCT 3'
3' TCGA 5'

•SmaI: 5' CCCGGG 3'
3' GGGCCC 5'

- DNA fragments are put back together by DNA ligase (forms phosphodiester bonds between 5' and 3' ends of nucleotides)
- Restriction enzymes and ligase are used in cloning.
- In cloning, a piece of DNA is put into a plasmid/vector of a bacterium. It is allowed to become recombinant and then it is placed back into the host cell.
- After restriction, DNA fragments are often separated by gel electrophoresis.

Complete Activity 1

Gel Electrophoresis

- The standard method for separating DNA fragments through agarose gels.
- Agarose—polysaccharide like agar; dissolves in boiling water, and then gels as it cools.
- DNA is applied to the gel/agarose, and then an electric current is applied across the gel.
- DNA has a negative charge; therefore, it migrates toward the positive electrode.

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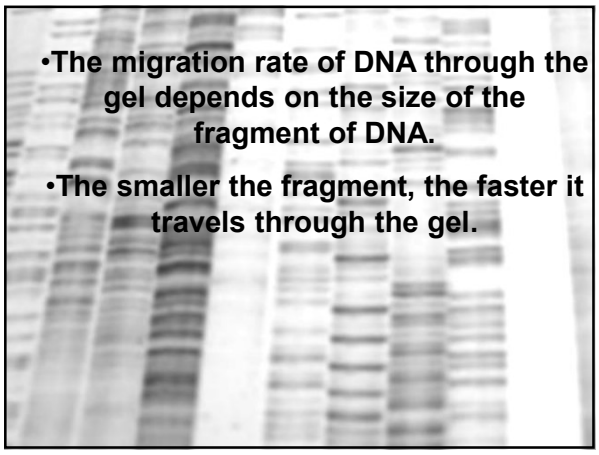
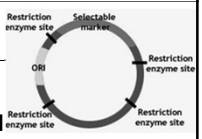


Copy (& Read) DNA



- Transformation
  - insert recombinant plasmid into bacteria
  - grow recombinant bacteria in agar cultures
    - bacteria make lots of copies of plasmid
    - "cloning" the plasmid
  - production of many copies of inserted gene
  - production of "new" protein
    - transformed phenotype

DNA → RNA → protein → trait

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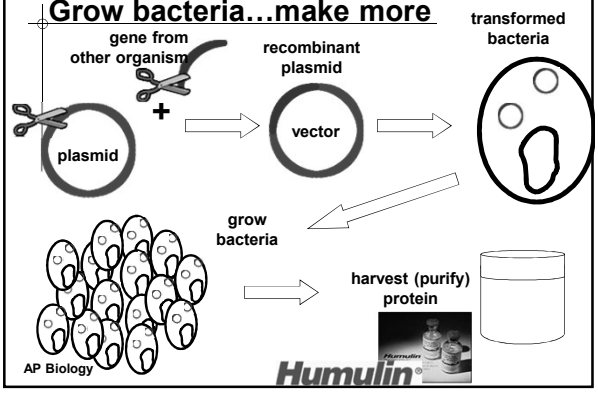
Grow bacteria…make more



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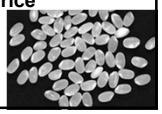
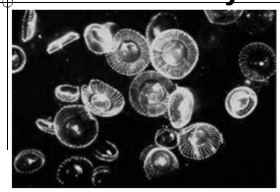
Uses of genetic engineering

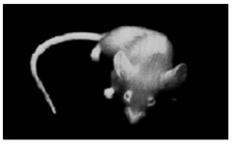
- Genetically modified organisms (GMO)
  - enabling plants to produce new proteins
    - Protect crops from insects: BT corn
      - corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)
    - Extend growing season: fishberries
      - strawberries with an anti-freezing gene from flounder
    - Improve quality of food: golden rice
      - rice producing vitamin A improves nutritional value



Green with envy??

Jelly fish "GFP"





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Transformed vertebrates

Cut, Paste, Copy, Find…

- Word processing metaphor…
  - cut
    - restriction enzymes
  - paste
    - ligase
  - copy
    - plasmids
      - bacterial transformation
    - is there an easier way??
  - find
    - ????

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