

AP Biology

Biotechnology

A Brave New World

Victim
Rapist's semen
Suspect's blood

Victim
Rapist's semen
Suspect's blood

human genome
3.2 billion bases

TACGCACATTTACGTACGCGGATGCCGCGACTATGATC
ACATAGACATGCTGTTCAGCTCTAGTAGACTAGCTGACT
CGACTAGCATGCTGTTCAGCTCTAGTAGACTAGCTGACT
GTACATCGA...
CTAGCTACTGACTCATGATCCAGATCACTGAAACCCTA
GATCGGG...
CATGCTA...
TCAATCA...
TGA CTGA...
ATTACAG...
TCGATCG...
TTTTTGC...
ACTCTGA...
TCATCCG...
H R O M O S O M E

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
 - 10⁸ (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

How have these little guys gotten to be so diverse??

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Transformation

promiscuous?

- 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

mix heat-killed pathogenic & non-pathogenic bacteria

mice die

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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

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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

gene from other organism

cut DNA

plasmid

glue DNA

recombinant plasmid

vector

transformed bacteria

Biotechnology

- Plasmids used to insert new genes into bacteria

gene we want

cut DNA

like what? ...insulin ...HGH ...lactase

cut plasmid DNA

ligase

recombinant plasmid

insert "gene we want" into plasmid... "glue" together

Cut DNA? DNA scissors?

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

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What do you notice about these phrases?

radar

racecar

palindromes

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

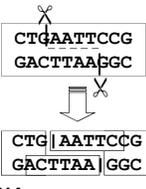
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Madam I'm Adam

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

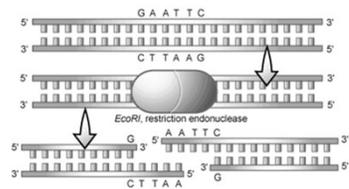
Discovery of restriction enzymes






Werner Arber Daniel Nathans Hamilton O. Smith

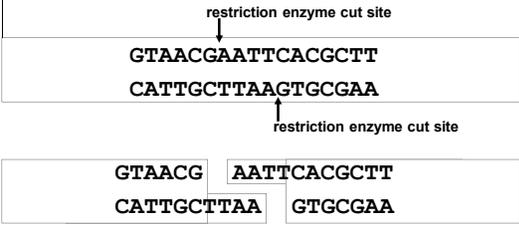
Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in *E. coli*



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Restriction enzymes

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



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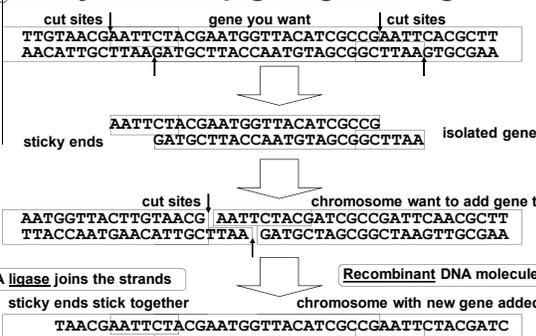
Sticky ends

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

GTAACG	AATTCACGCTT	gene you want
CATTGCTTAA	GTGCGAA	
GGACCTG	AATTCGGATA	chromosome want to add gene to
CCTGGACTTAA	GGCCTAT	
GGACCTG	AATTCACGCTT	combined DNA
CCTGGACTTAA	GTGCGAA	

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Sticky ends help glue genes together



DNA **ligase** joins the strands
sticky ends stick together

Recombinant DNA molecule
chromosome with new gene added

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Why mix genes together?

How can bacteria read human DNA?

- Gene produces protein in different organism or different individual

human insulin gene in bacteria

TACGAATTCTACGAATGGTTACATCGCCGAATTCTACGATC
CATTGCTTAAGATGCTTACCAATGTAGCGGCTTAAGATGCTAGC

"new" protein from organism ex: human insulin from bacteria

aa aa aa aa aa aa aa aa aa aa

bacteria

human insulin

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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

First base (5' end)	Second base				Third base (3' end)
	U	C	A	G	
U	UUU Phe UUC UCC UUA UCA UUG UCG	UCU Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

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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'

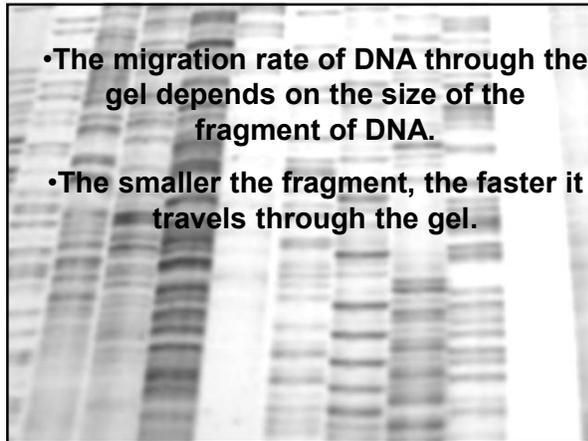
- DNA fragments are put back together by DNA ligase (forms phosphodiester bonds between 5' and 3' ends of nucleotides)
- Restriction enzymes and ligase are used in cloning.
 - In cloning, a piece of DNA is put into a plasmid/vector of a bacterium. It is allowed to become recombinant and then it is placed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

Complete Activity 1

Gel Electrophoresis

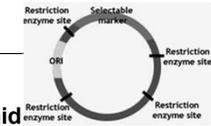
- The standard method for separating DNA fragments through agarose gels.
 - Agarose—polysaccharide like agar; dissolves in boiling water, and then gels as it cools.
- DNA is applied to the gel/agarose, and then an electric current is applied across the gel.
- DNA has a negative charge; therefore, it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

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- The migration rate of DNA through the gel depends on the size of the fragment of DNA.
- The smaller the fragment, the faster it travels through the gel.

Copy (& Read) DNA



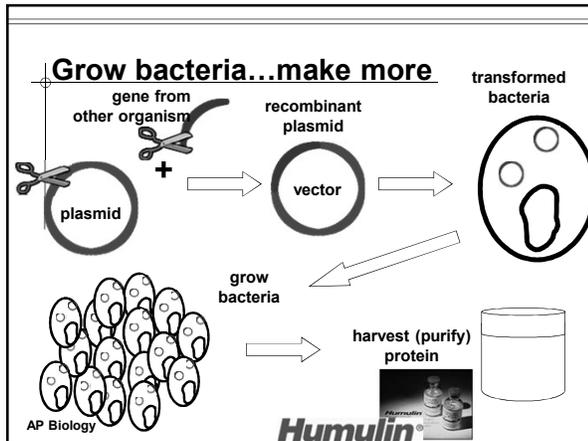
- **Transformation**
 - ♦ insert recombinant plasmid into bacteria
 - ♦ grow recombinant bacteria in agar cultures
 - bacteria make lots of copies of plasmid
 - “cloning” the plasmid
 - ♦ production of many copies of inserted gene
 - ♦ production of “new” protein
 - transformed phenotype



DNA → RNA → protein → trait

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Grow bacteria...make more

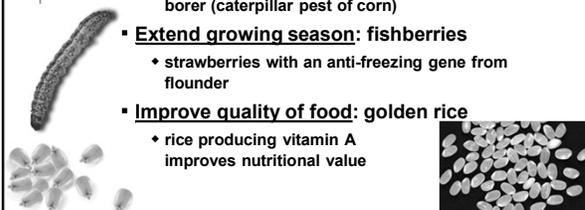


gene from other organism + plasmid → recombinant plasmid (vector) → transformed bacteria → grow bacteria → harvest (purify) protein → Humulin

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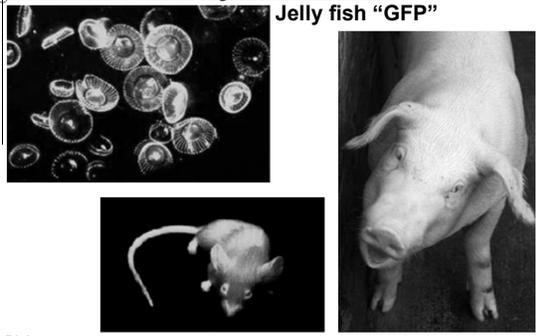
Uses of genetic engineering

- **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



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Green with envy??



Jelly fish “GFP”

Transformed vertebrates

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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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