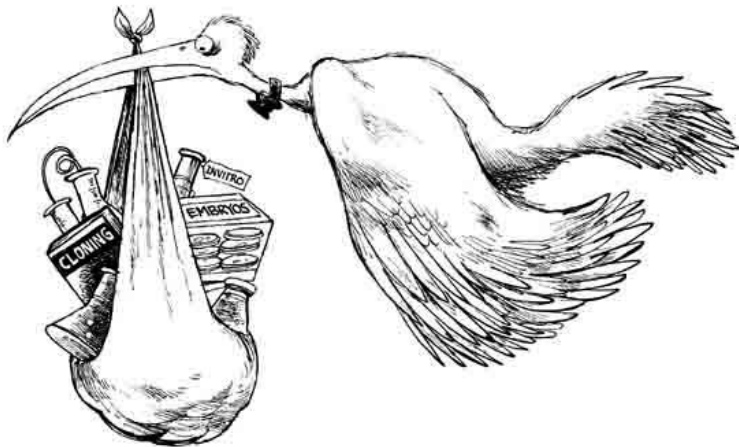
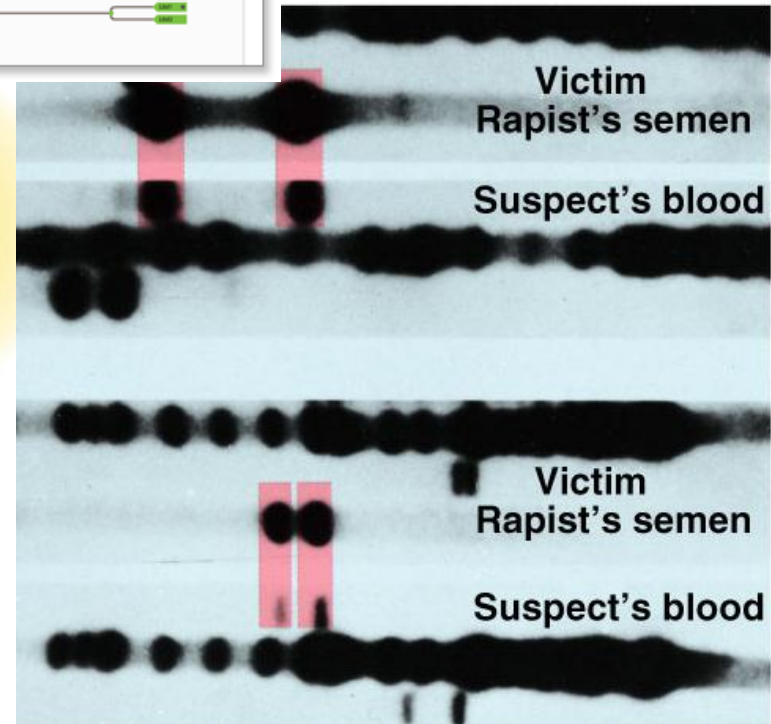
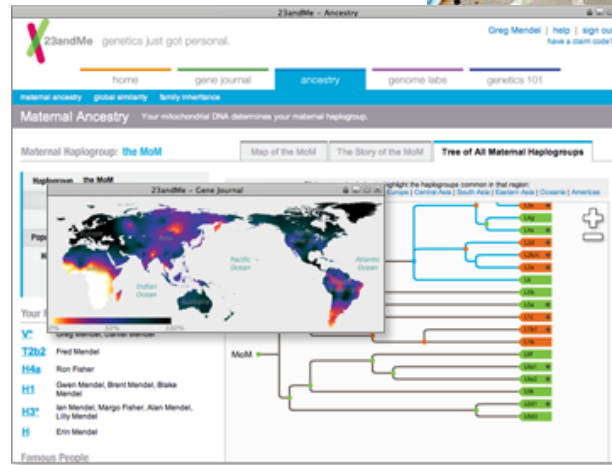
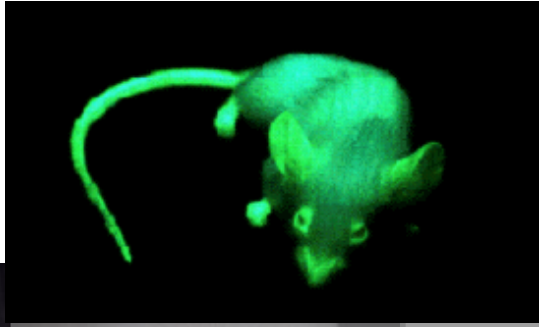
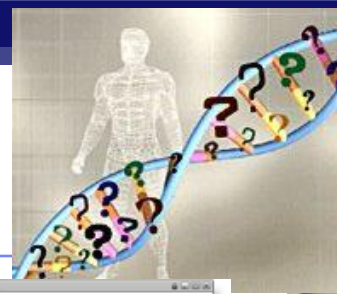


Biotechnology

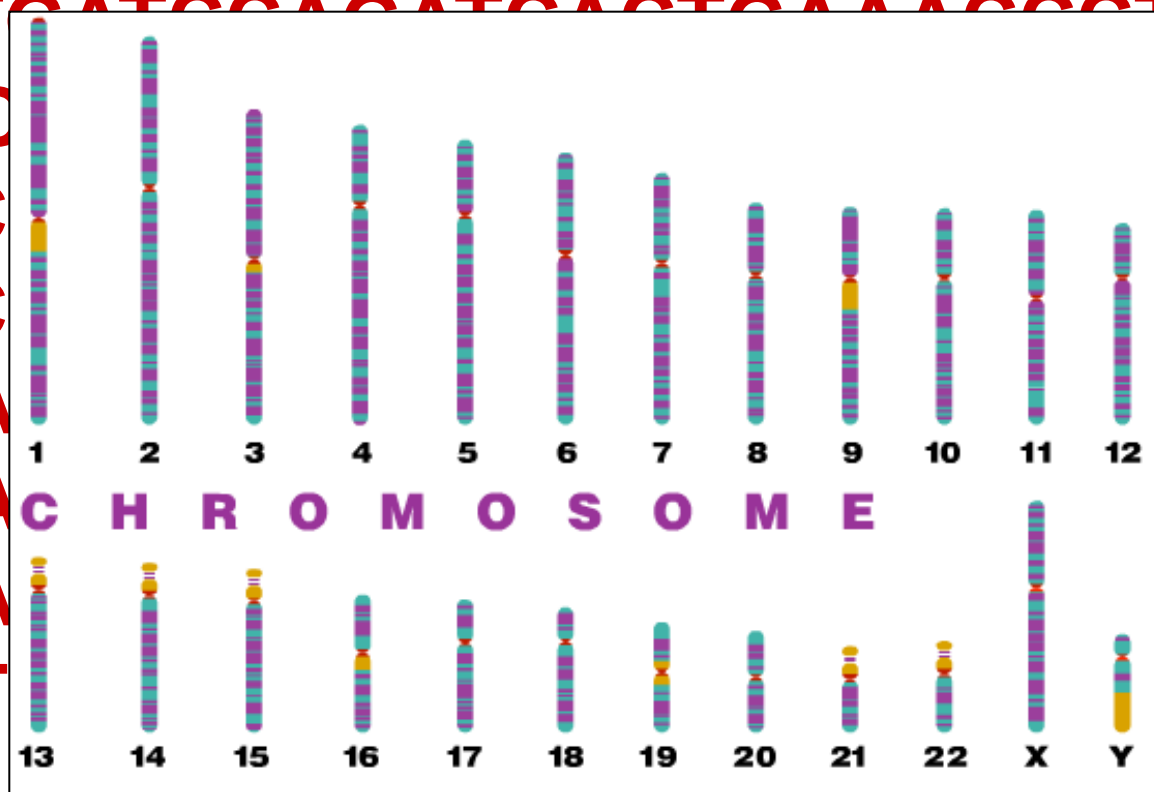


A Brave New World



human genome

3.2 billion bases



Biotechnology today

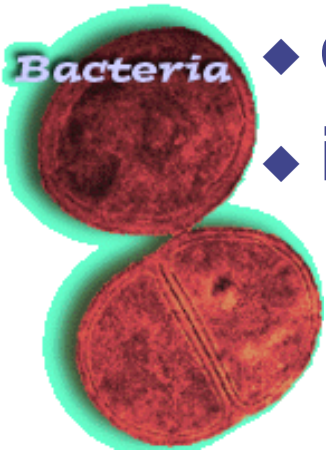
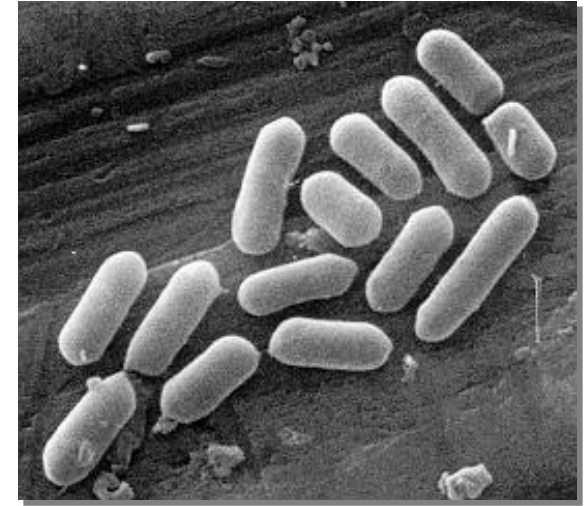
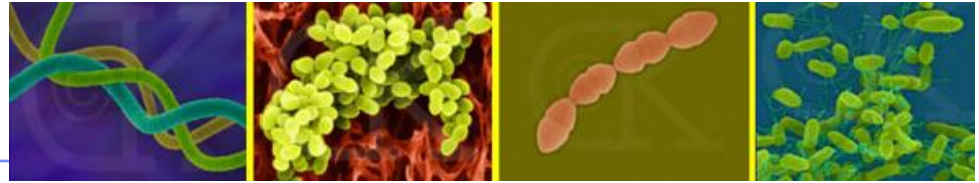
- Genetic Engineering
 - ◆ manipulation of DNA
 - ◆ if you are going to engineer DNA & genes & organisms, then you need a set of tools to work with
 - ◆ this unit is a survey of those tools...



Bacteria

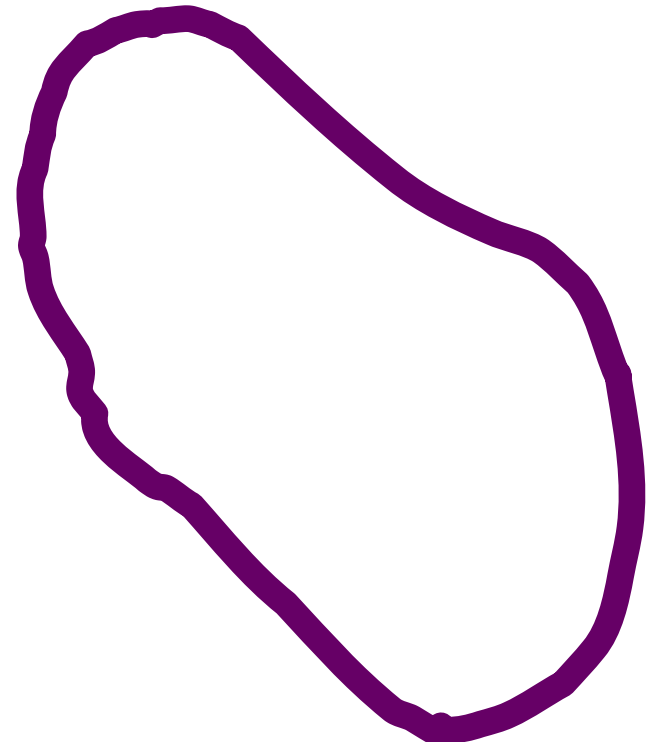
■ Bacteria review

- ◆ 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

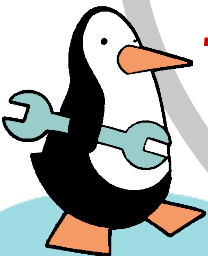


Bacterial genome

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

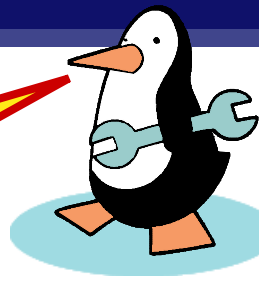


How have these little guys gotten to be so diverse??



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



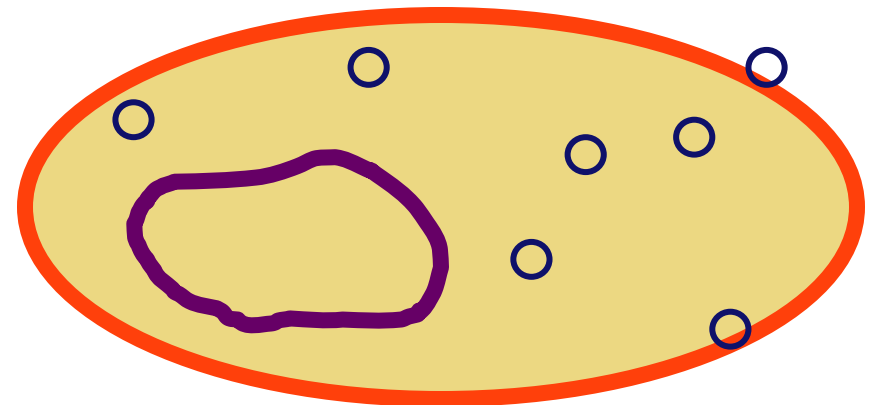
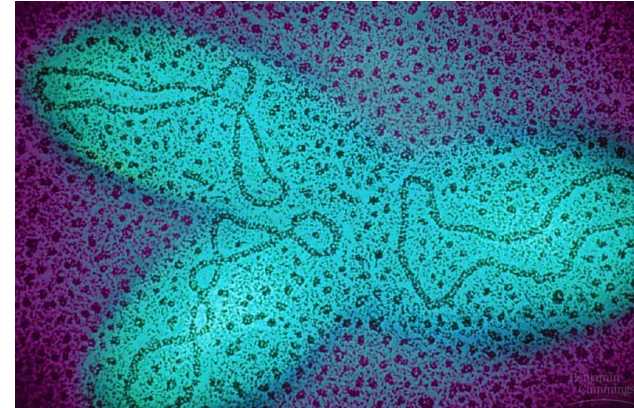
mice die



Plasmids

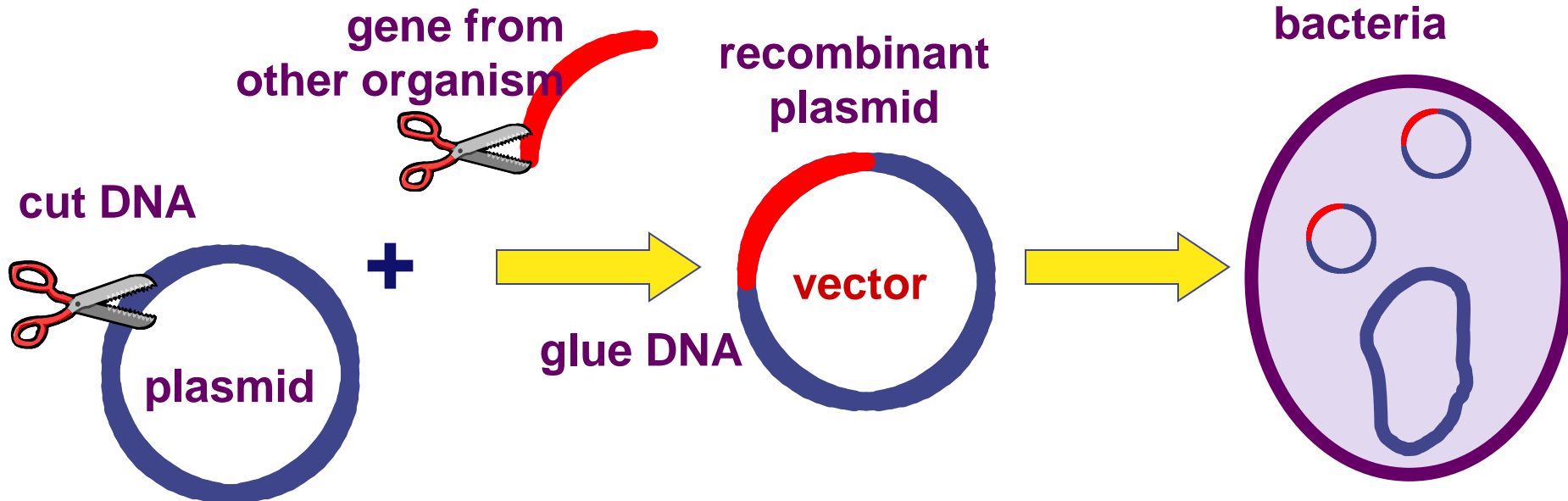
■ Small supplemental circles of DNA

- 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



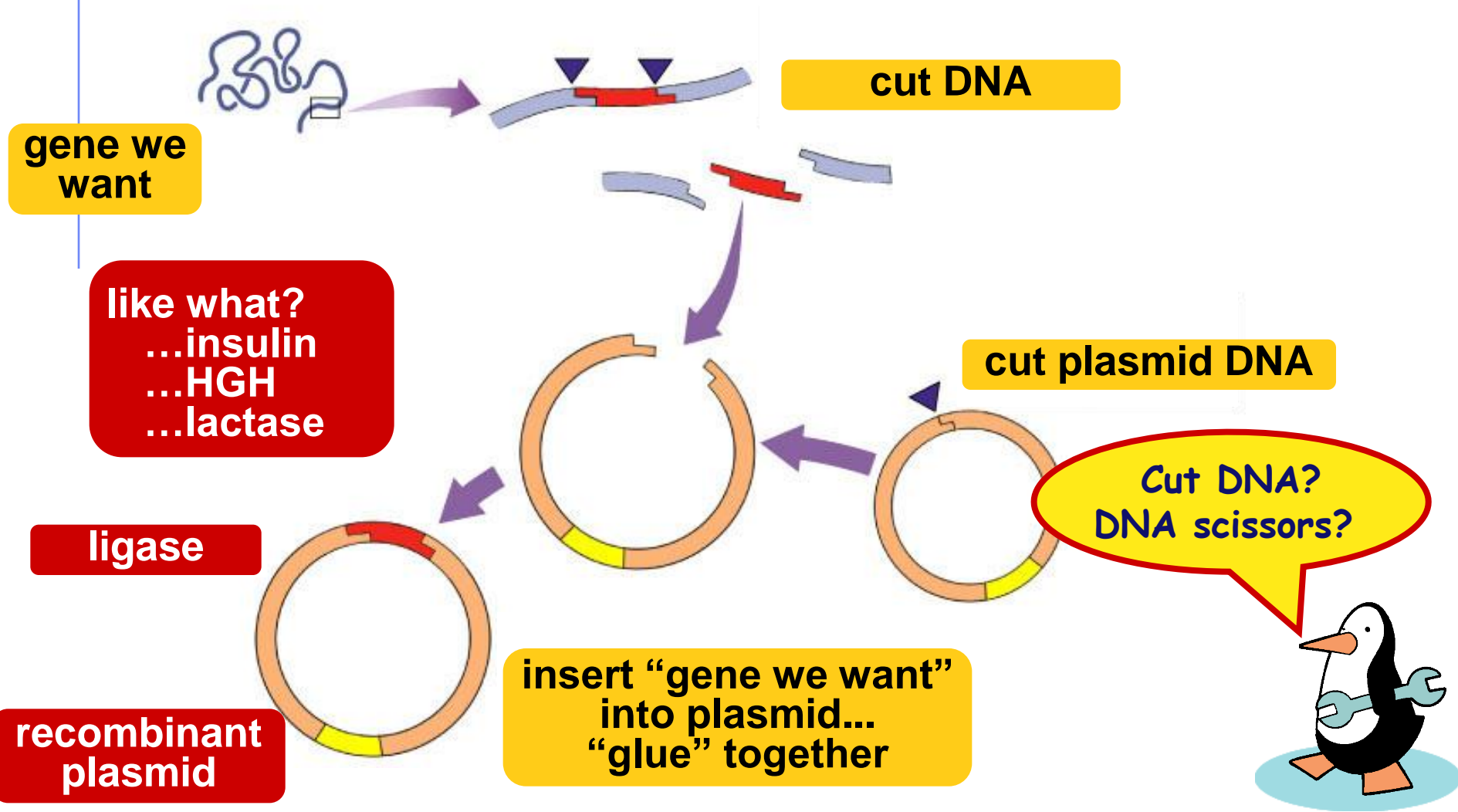
How can plasmids help us?

- 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

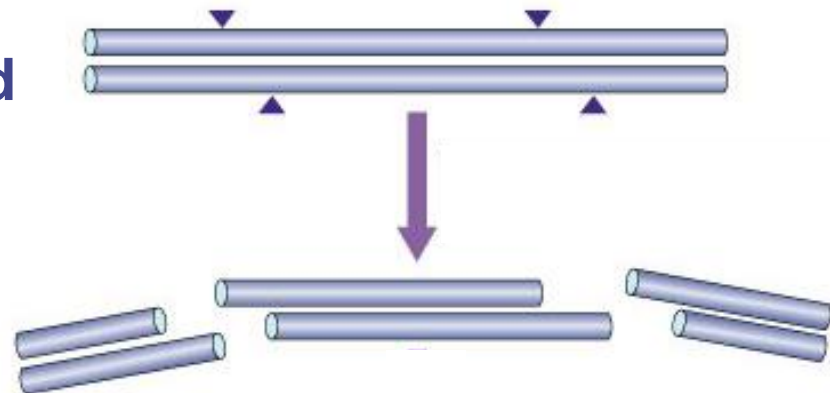
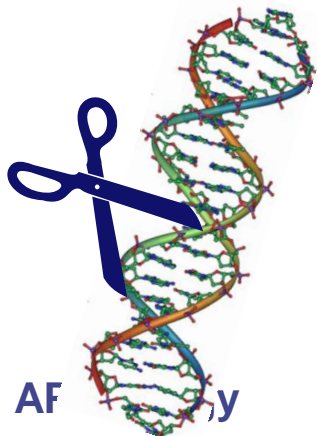
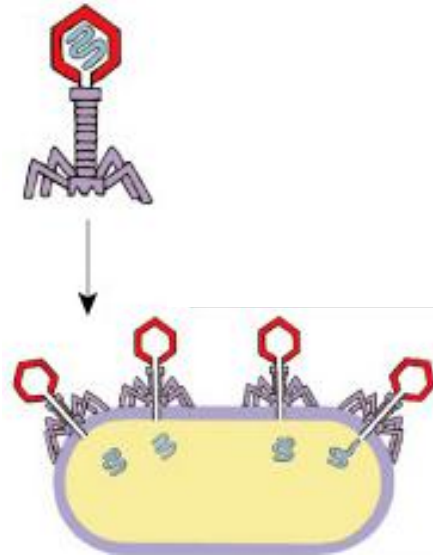
- Plasmids used to insert new genes into bacteria



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

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

Restriction enzymes

- **Action of enzyme**

- ◆ cut DNA at specific sequences

- restriction site

- ◆ symmetrical “palindrome”

- ◆ produces protruding ends

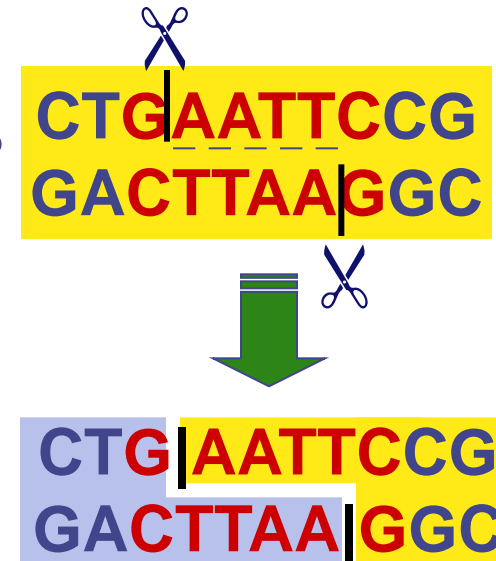
- sticky ends

- will bind to any complementary DNA

- **Many different enzymes**

- ◆ named after organism they are found in

- EcoRI, HindIII, BamHI, SmaI

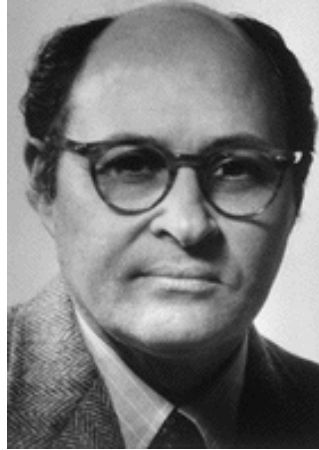


1960s | 1978

Discovery of restriction enzymes



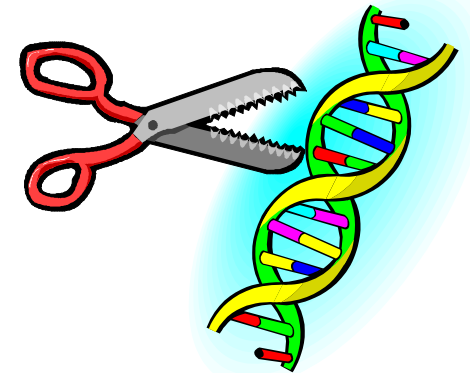
Werner Arber



Daniel Nathans

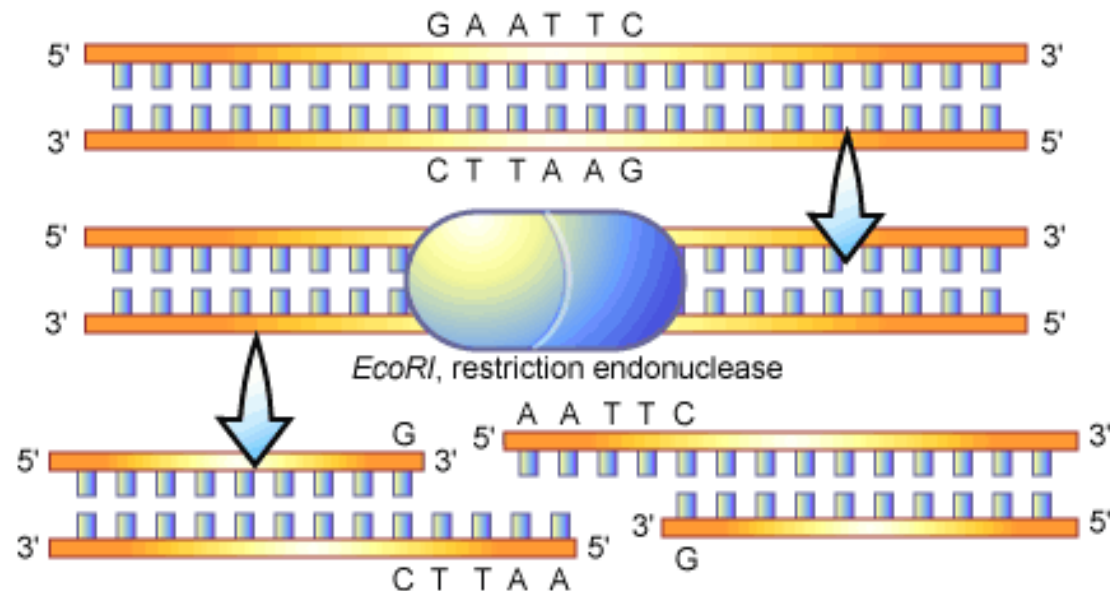


Hamilton O. Smith



Restriction enzymes are named for the organism they come from:

EcoRI = 1st restriction enzyme found in *E. coli*



Restriction enzymes

- Cut DNA at specific sites
 - ◆ leave “sticky ends”

restriction enzyme cut site
GTAACG **AATT** CACGC
↓
TT

CATTG **CTTAAG** TGCG
↑
AA restriction enzyme cut site

GTAACG **AATT** CACGCCTT
CATTG **CTTAAG** TGCGAA

Sticky ends

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

GTAACG AATTCACGCTT
CATTGCTTAA GTGCGAA

gene
you want

GGACCTG AATTCCTGGATA
CCTGGACTTAA GGCCTAT

chromosome
want to add
gene to

GGACCTG AATTCACGCTT
CCTGGACTTAA GTGCGAA

combined
DNA

Sticky ends help glue genes together

cut sites | gene you want | cut sites

TTGTAACGAATTCTACGAATGGTTACATCGCCGAATTCA
 AACATTGCTTAAGATGCTTACCAATGTAGCGGCTTAAGT

CGCTT
GCGAA

sticky ends AATTCTACGAATGGTTACATCGCCG
 GATGCTTACCAATGTAGCGGCTTA

isolated gene

cut sites | chromosome want to add gene to

AATTCATCGATCGCCGATTCAACGCTT
 TTACCAATGAACATTGCTTAAG

DNA ligase joins the strands

Recombinant DNA molecule

sticky ends stick together chromosome with new gene added

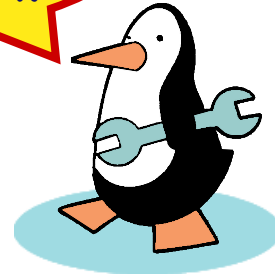
GAATTCATCGAATGGTTACATCGCCGAATTC
 CATTGCTTAAGATGCTTACCAATGTAGCGGCTTAAGATG

ATC

AP

Why mix genes together?

How can bacteria read human DNA?



- Gene produces protein in different organism or different individual

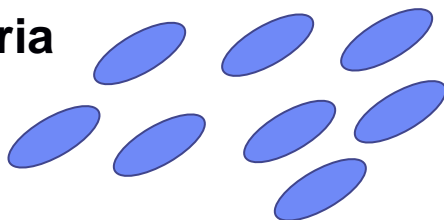


“new” protein from organism

ex: human insulin from bacteria



bacteria



human insulin

The code is universal

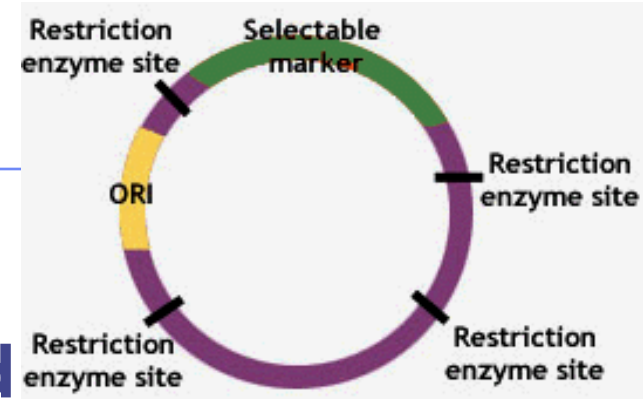
- Since all living organisms...
 - ◆ use the same DNA
 - ◆ use the same code book
 - ◆ read their genes the same way

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

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

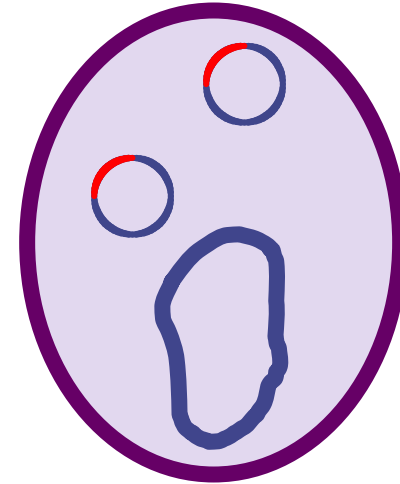
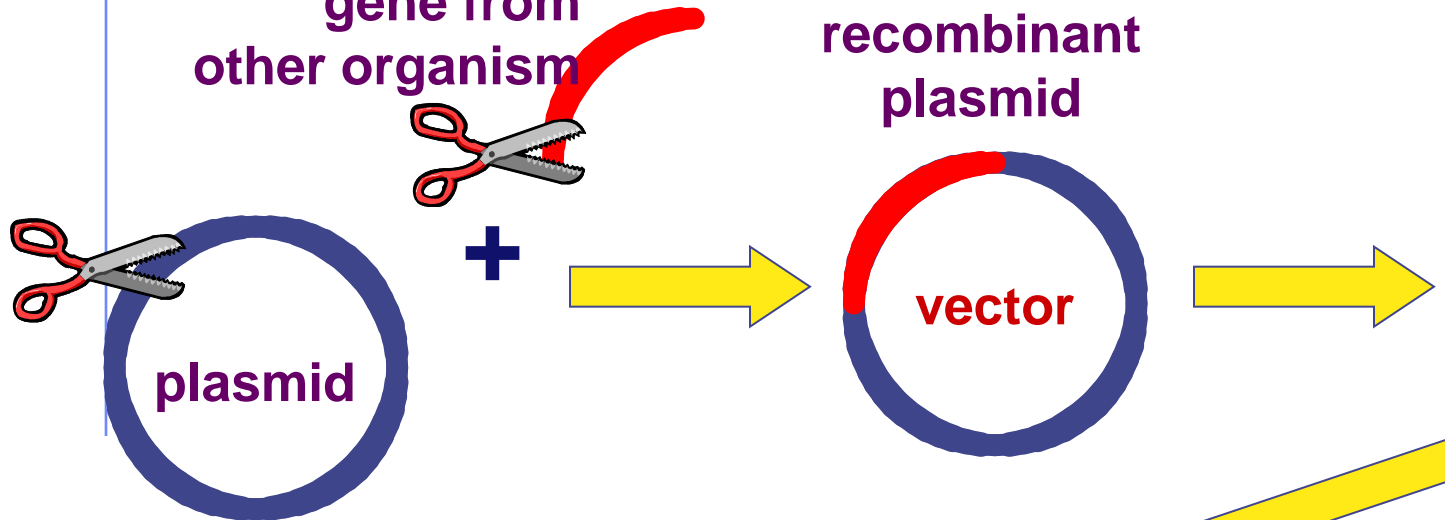


Grow bacteria...make more

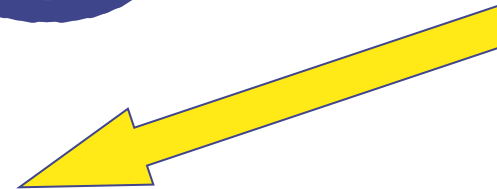
gene from
other organism

recombinant
plasmid

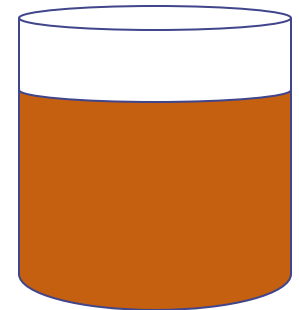
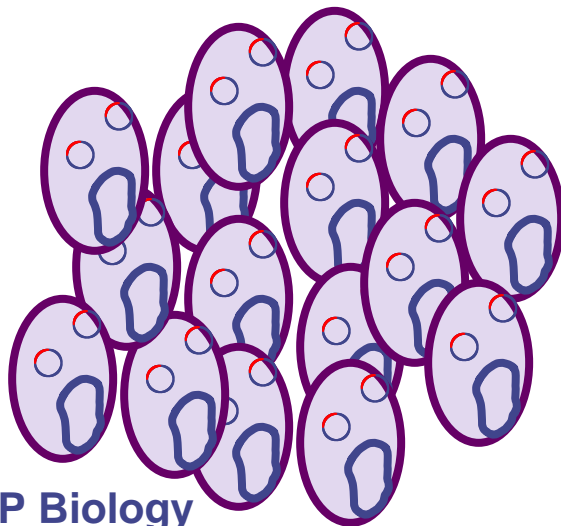
transformed
bacteria



grow
bacteria



harvest (purify)
protein



Uses of genetic engineering

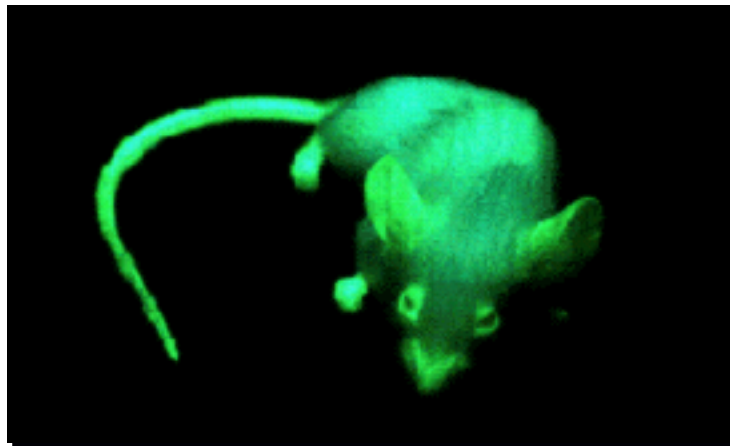
- **Genetically modified organisms (GMO)**
 - ◆ enabling plants to produce new proteins
 - Protect crops from insects: **BT corn**
 - ◆ corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)
 - Extend growing season: **fishberries**
 - ◆ strawberries with an anti-freezing gene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



Green with envy??



Jelly fish “GFP”



Cut, Paste, Copy, Find...

- Word processing metaphor...

- ◆ cut

- restriction enzymes

- ◆ paste

- ligase

- ◆ copy

- plasmids

- ◆ bacterial transformation

- is there an easier way??

- ◆ find

- ?????

