

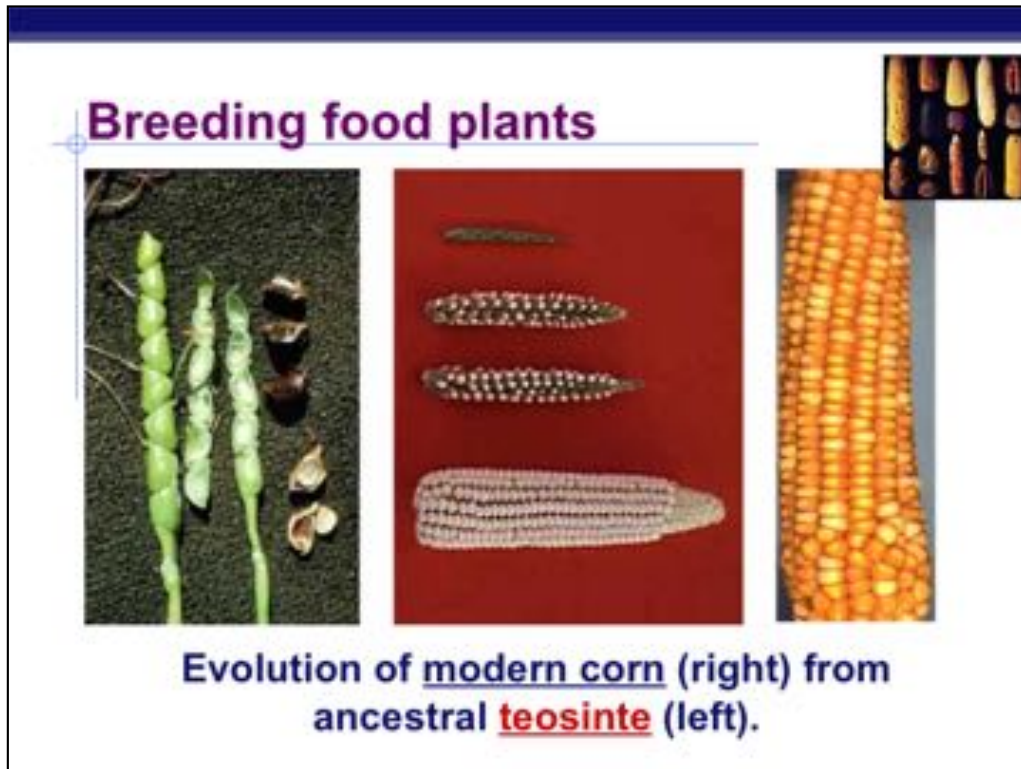
**Genetic Engineering
Biotechnology**

Genetics and the Environment

We have been manipulating DNA for generations!

- Artificial breeding





Maize and TeosinteMaize is indigenous to Central and South America... its ancestor seems to be teosinte, which grows wild in highland Mexico. It is known that teosinte and maize have similar DNA and can be crossbred.

It is theorized that small groups of hunter-gatherers began to plant teosinte along route of seasonal rounds. Maize had little initial impact on nomadic life as development of teosinte into maize too 1000+ years. Thus the development of maize was not necessarily "purposeful", but occurred as a result of harvesting the best "ears" and planting them as opposed to the seed of the sparser plants.

The code is universal

- All living organisms...
 - ◆ use the same DNA
 - ◆ read their genes the same way

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

Strong evidence for a single origin in evolutionary theory.

We can mix genes from one creature with another's.



Mixing genes for medicine...

- ◆ bacteria producing **human insulin**
- ◆ bacteria producing **human growth hormone**

Humulin®



Despite its name, Humulin [insulin](#) does *not* come from human

Instead, it is made in a factory using a chemical process called recombinant DNA technology,

but is identical in chemical structure to human insulin.

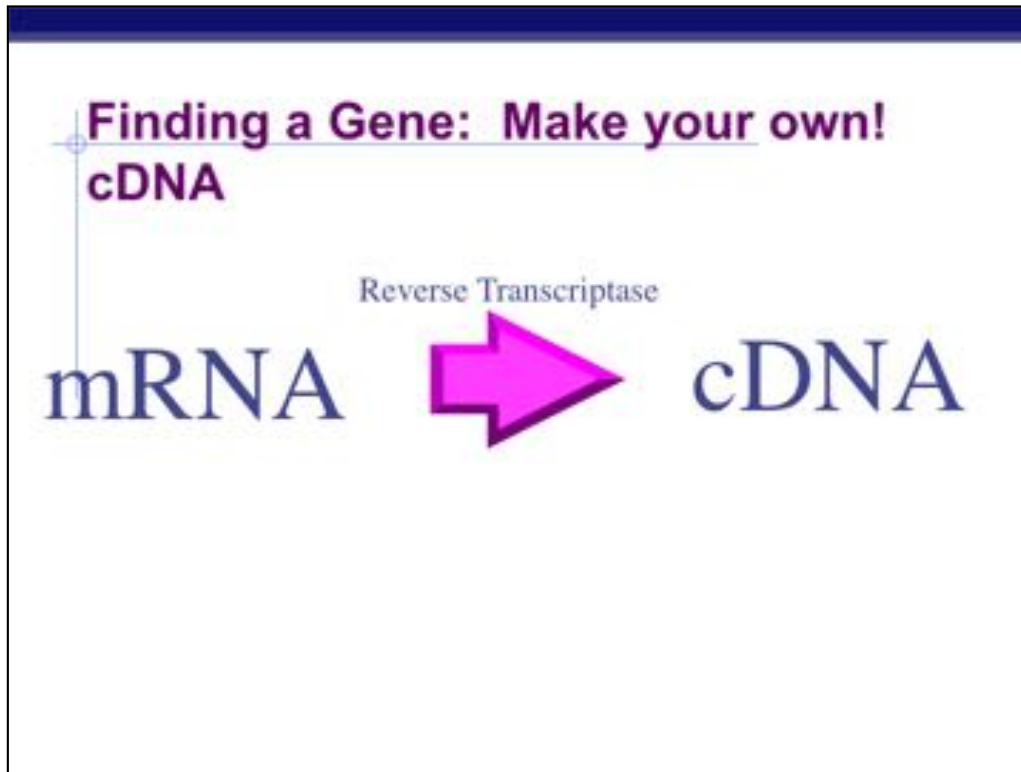
There are several types of Humulin insulin, and certain types are available in both a vial and a disposable Prefilled Pen.

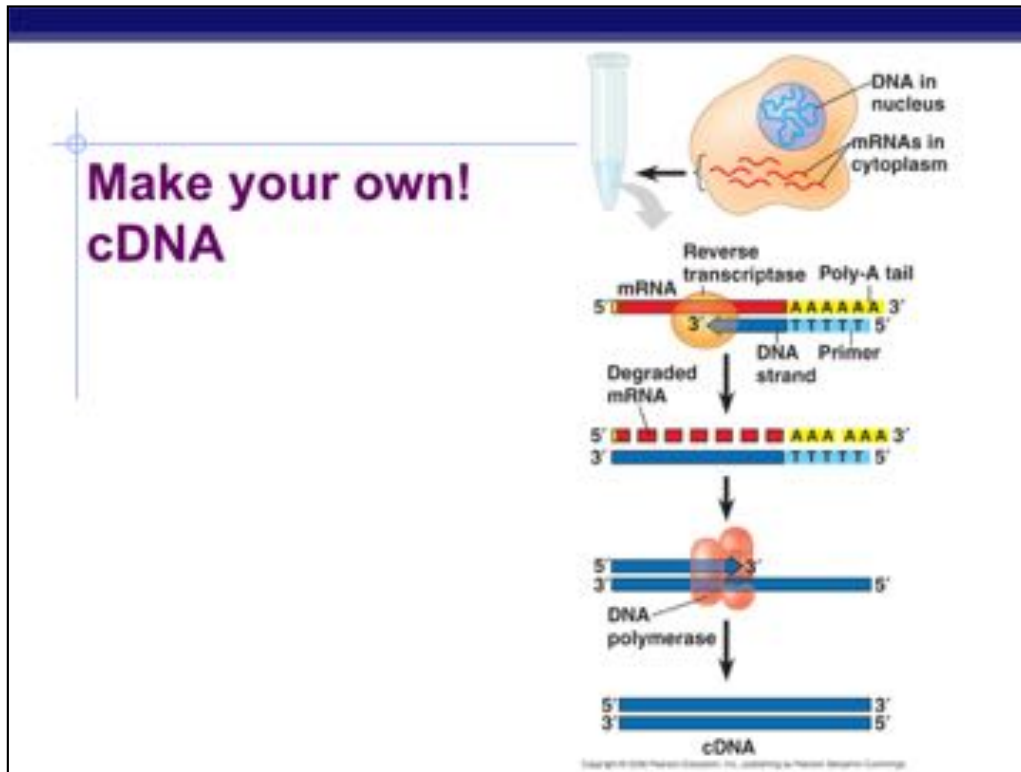
How do we mix genes?

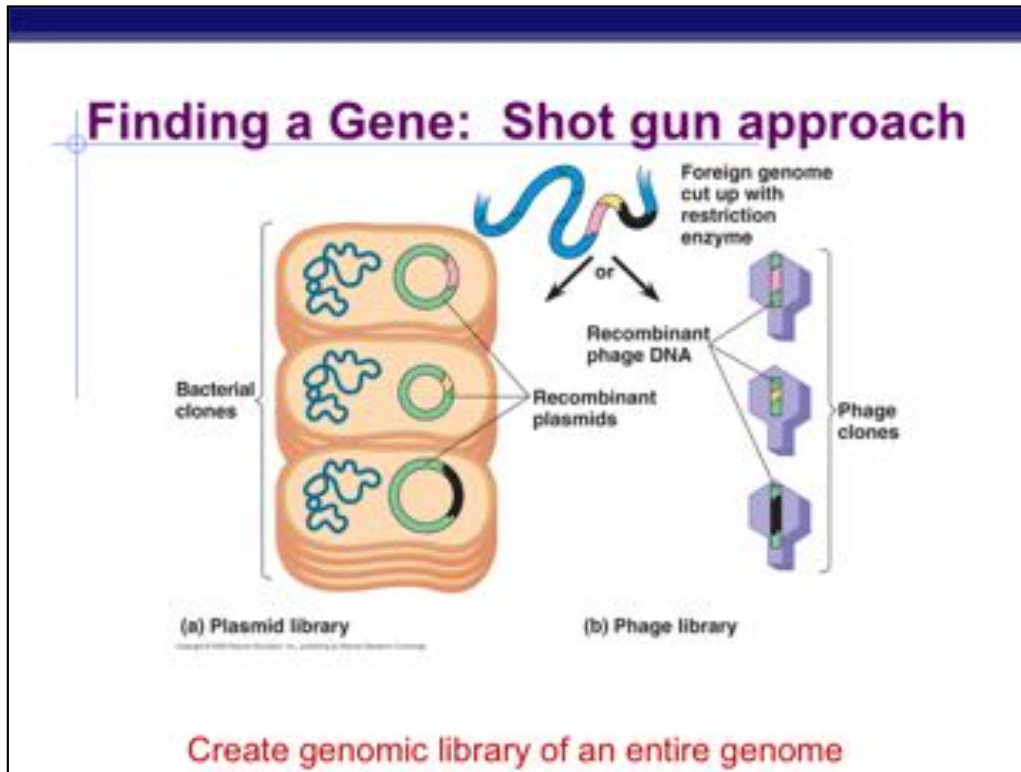
▪ Genetic engineering

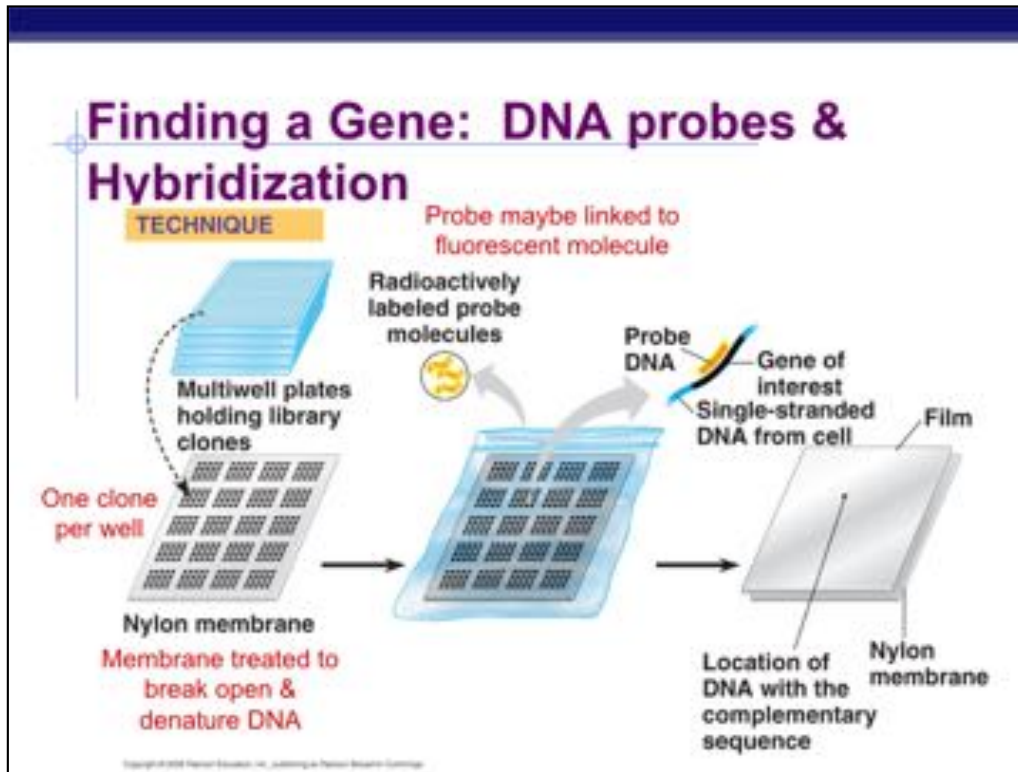
- ◆ find gene
- ◆ **cut** DNA in both organisms
- ◆ **paste** gene from one creature into other creature's DNA
- ◆ **insert** new chromosome into organism
- ◆ organism **copies** new gene as if it were its own
- ◆ organism **reads** gene as if it were its own
- ◆ **organism produces NEW protein:**
Remember: we all use the same genetic code!











Tools: Cutting DNA

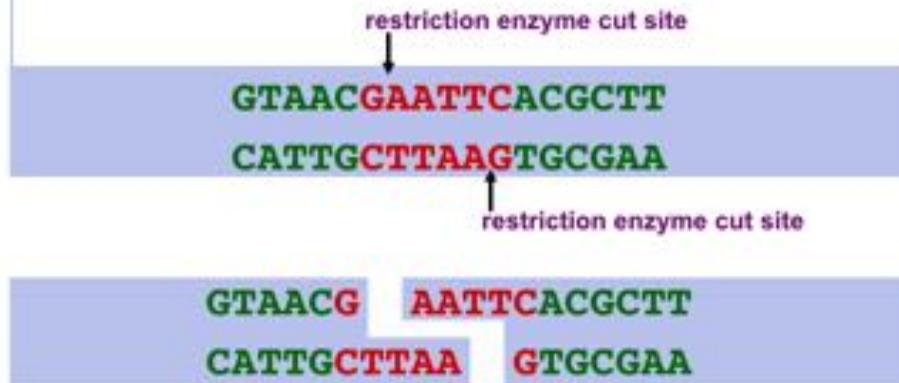
- DNA “scissors”
 - ◆ restriction enzymes
 - Found in bacteria
 - EcoRI, HindIII, BamHI
 - ◆ cut DNA at specific sites

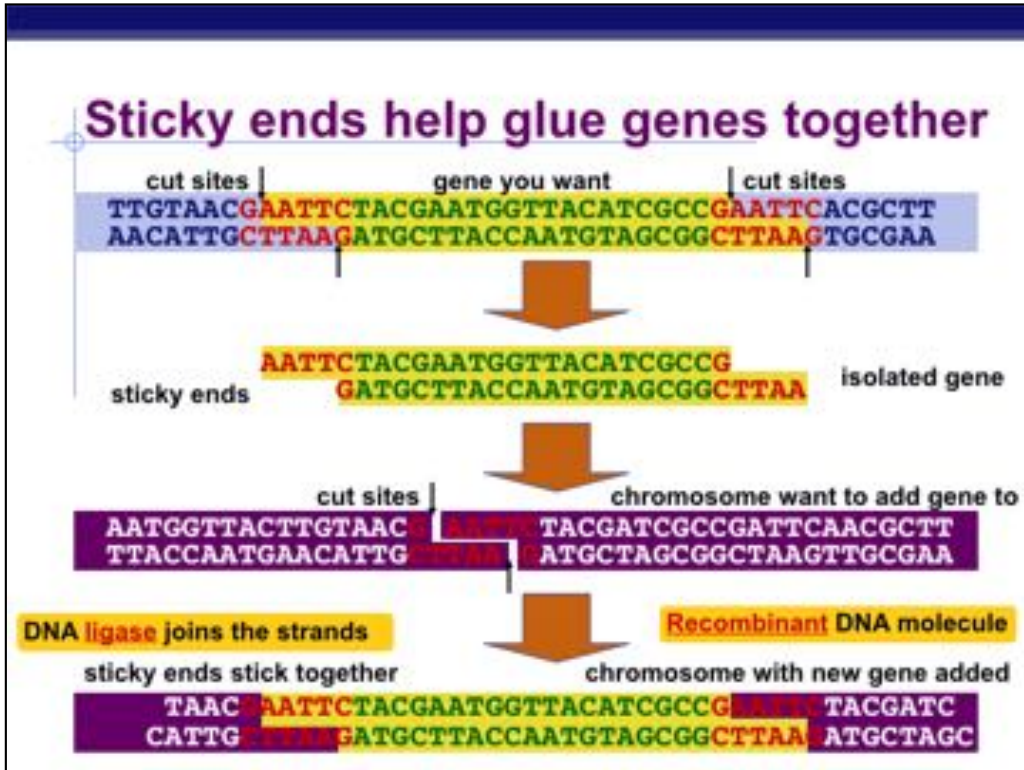


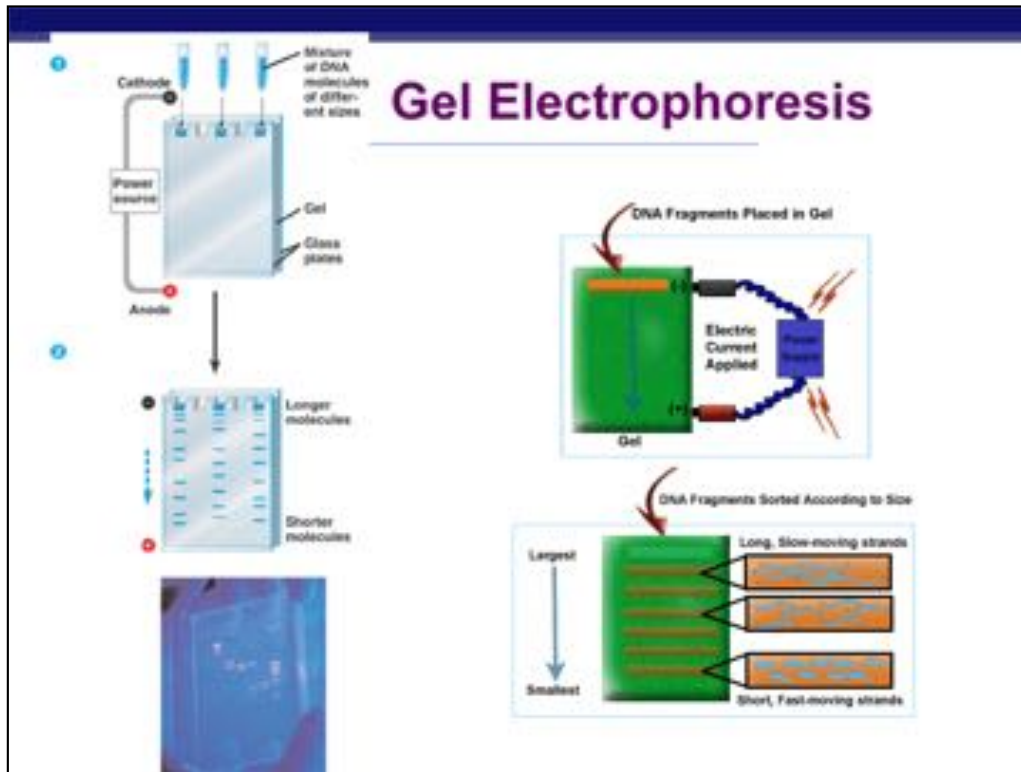
```
GTAACG | AATTCACGCTT
CATTGCTTAA | GTGCGAA
```

Restriction enzymes

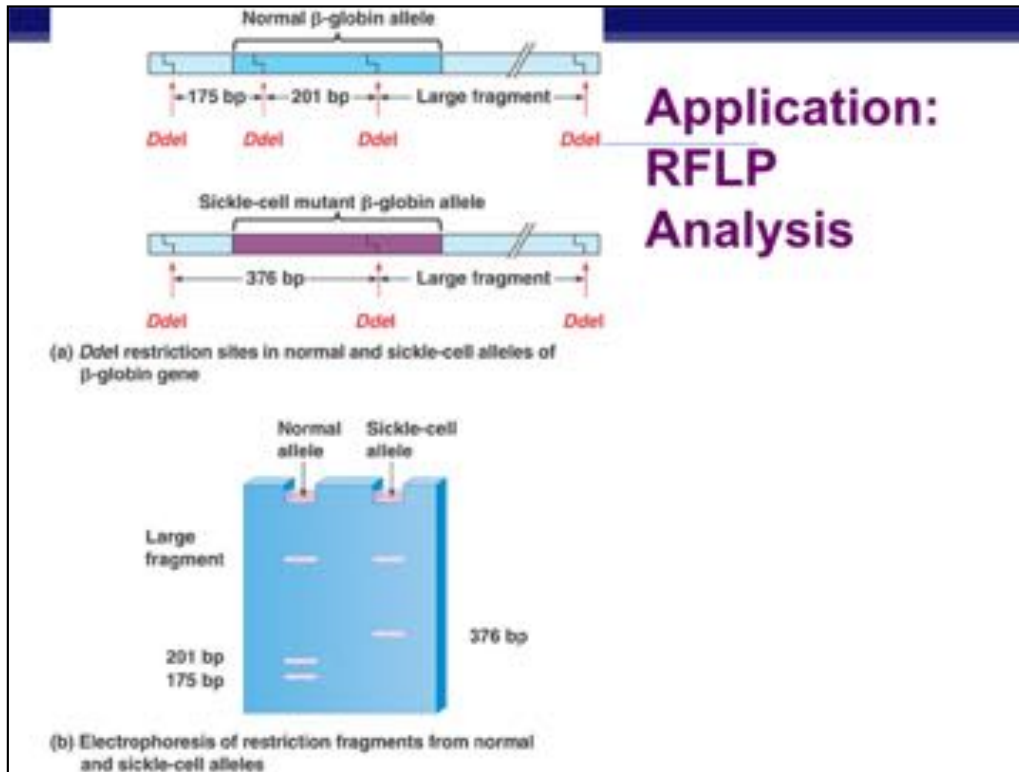
- Cut DNA at specific sites
 - ◆ leave "sticky ends"







Explain how you would use this to identify a person who is a carrier for sickle cell anemia --See next slide

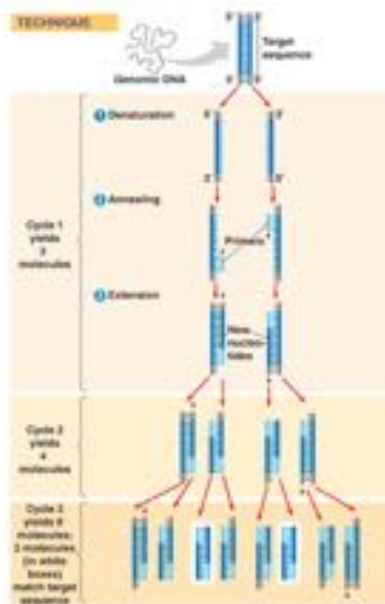


Amplify your DNA: PCR

- What if you have too little DNA to work with?
- Polymerase Chain Reaction

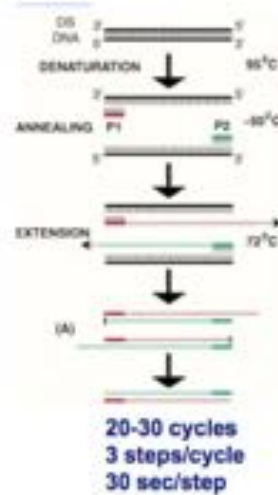


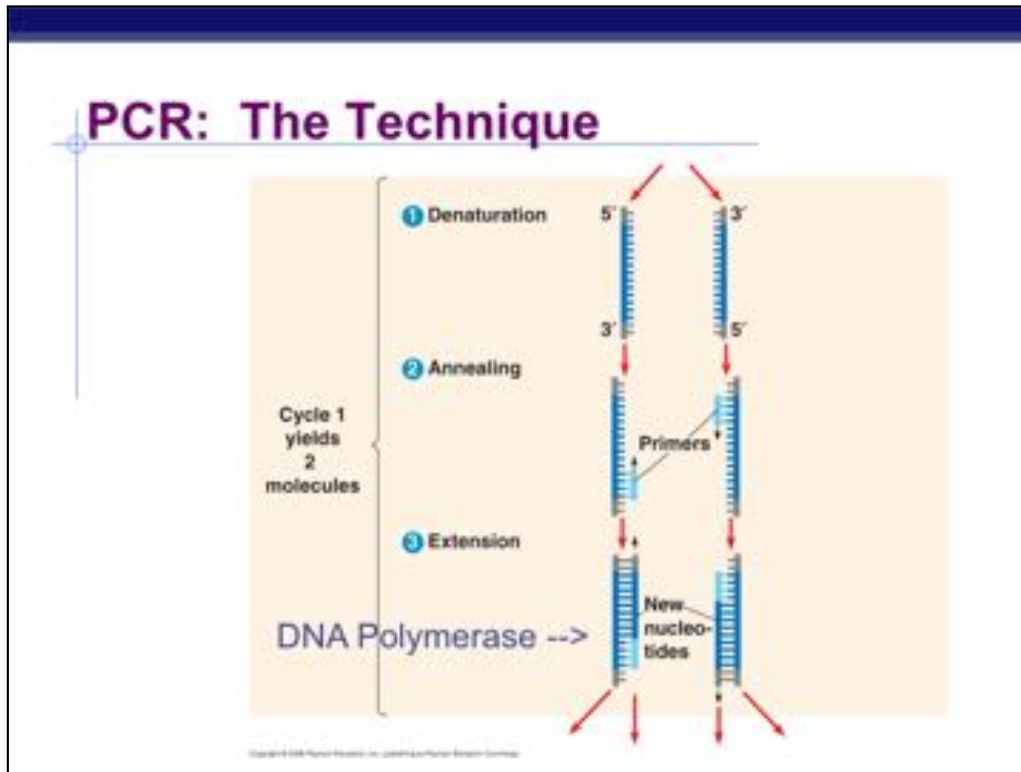
Copying DNA without bacteria or plasmids!



PCR Primers

- **Critical!!**
 - ◆ Need to know sequence
 - ◆ Primers flank target DNA
 - ◆ Primers define the section of DNA to be cloned
- **Taq polymerase**
 - ◆ From hot springs
 - ◆ **Why do we use it?**





PCR Song



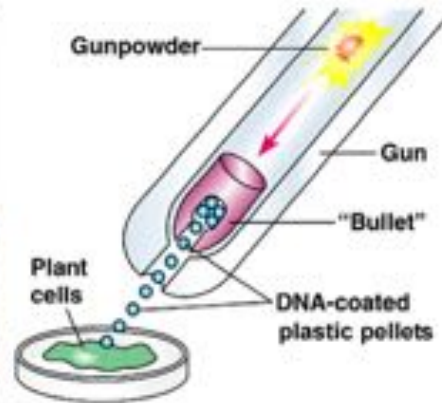
Inserting the recombined DNA (Vectors)

▪ DNA Guns



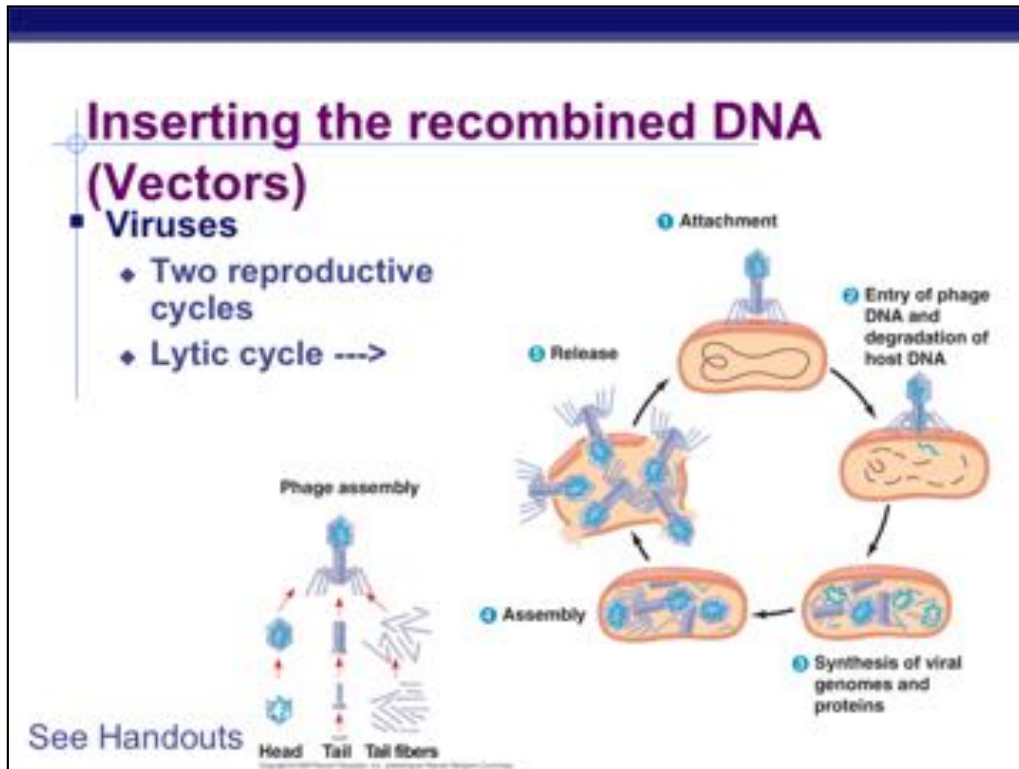
(a)

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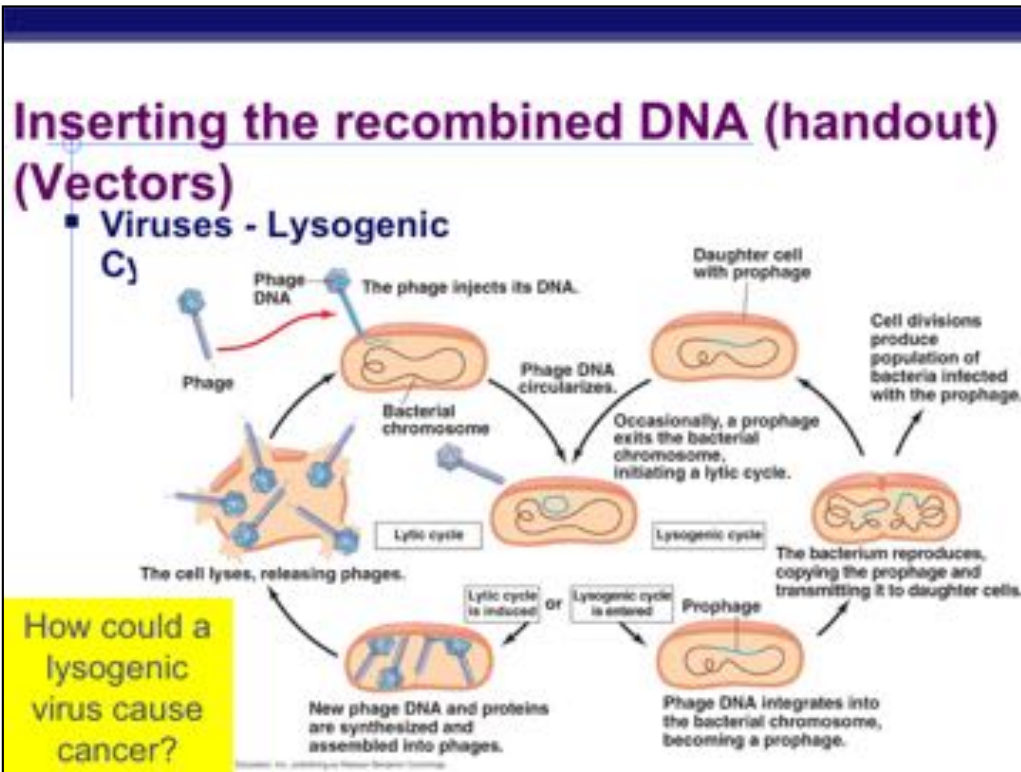


(b)

Mini-chromosomes



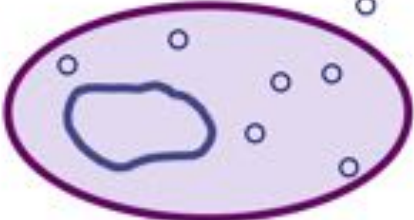
http://www.youtube.com/watch?v=O-EdX4MaMFE&feature=Playlist&p=B1AC4F7A9C99C847&index=14&playnext=2&playnext_from=PL



Mini-chromosomes

Inserting the recombined DNA (Vectors)

- **Plasmids**
 - ◆ small extra circles of DNA
 - ◆ carry extra genes that bacteria can use
 - ◆ can be picked up from environment



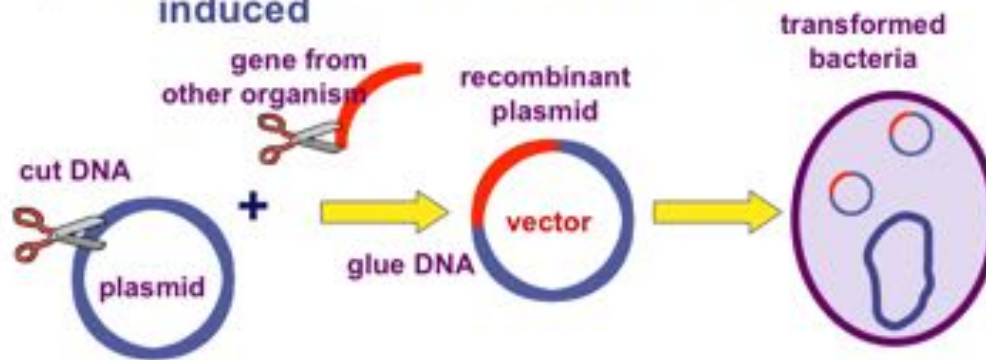
The diagram shows a purple oval representing a bacterial cell. Inside the cell is a larger, irregular blue shape representing the bacterial chromosome. Several small white circles with black outlines are scattered throughout the cell, representing plasmids. Some plasmids are inside the cell, while others are outside, illustrating that plasmids can be picked up from the environment.

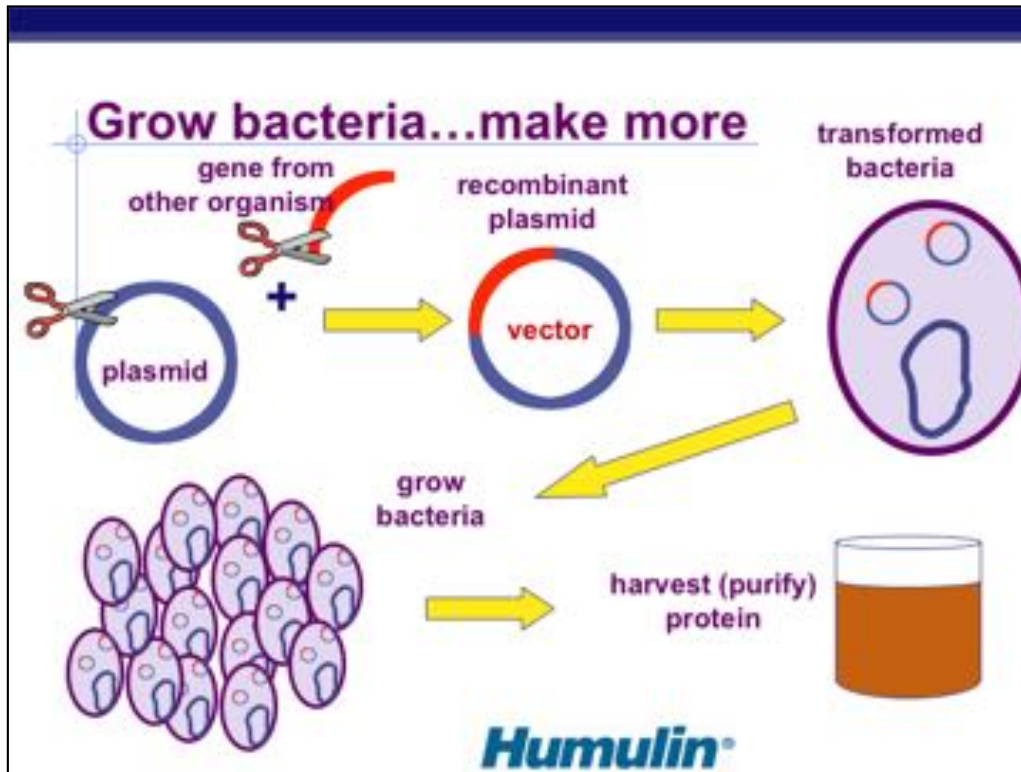
Mini-chromosomes

Plasmids get genes into bacteria

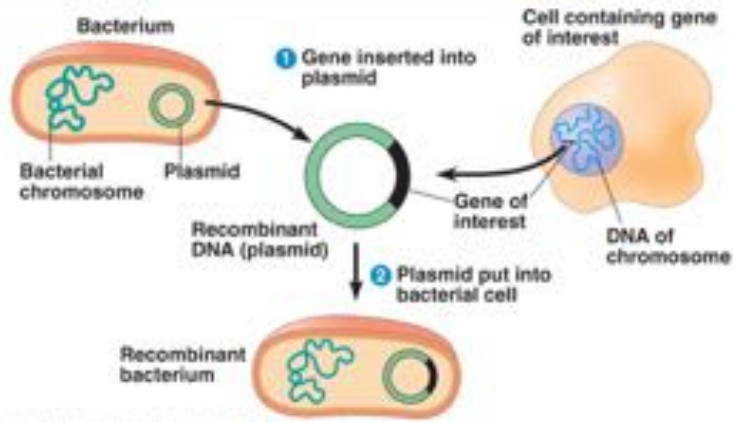
▪ Easy!

- ◆ insert new gene into operon
- ◆ insert plasmid into bacteria
- ◆ bacteria expresses new gene when induced





Summary: Gene Cloning



Enzyme Song





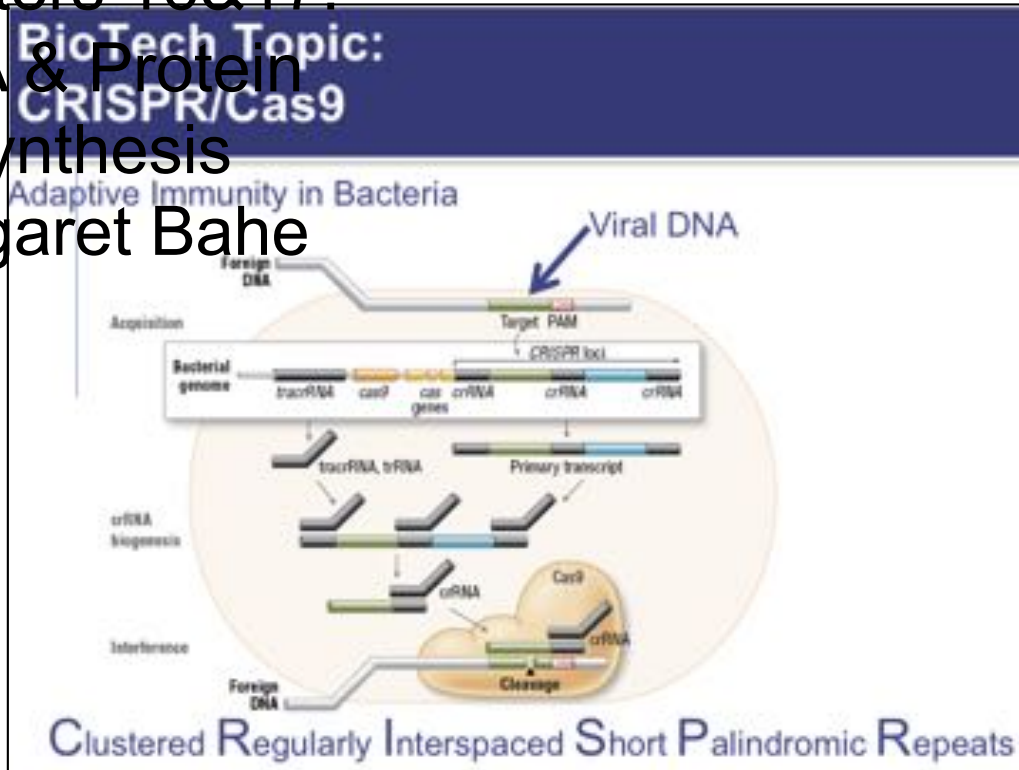
Now Binder 117



Chapters 16&17:

DNA & Protein Synthesis

Margaret Bahe



Virus infects a bacterial cell by injecting its DNA.

Bacteria have a defense:

The incorporate pieces of the viral DNA into their own genome separated by repeating segments of DNA. This is the CRISPR system.

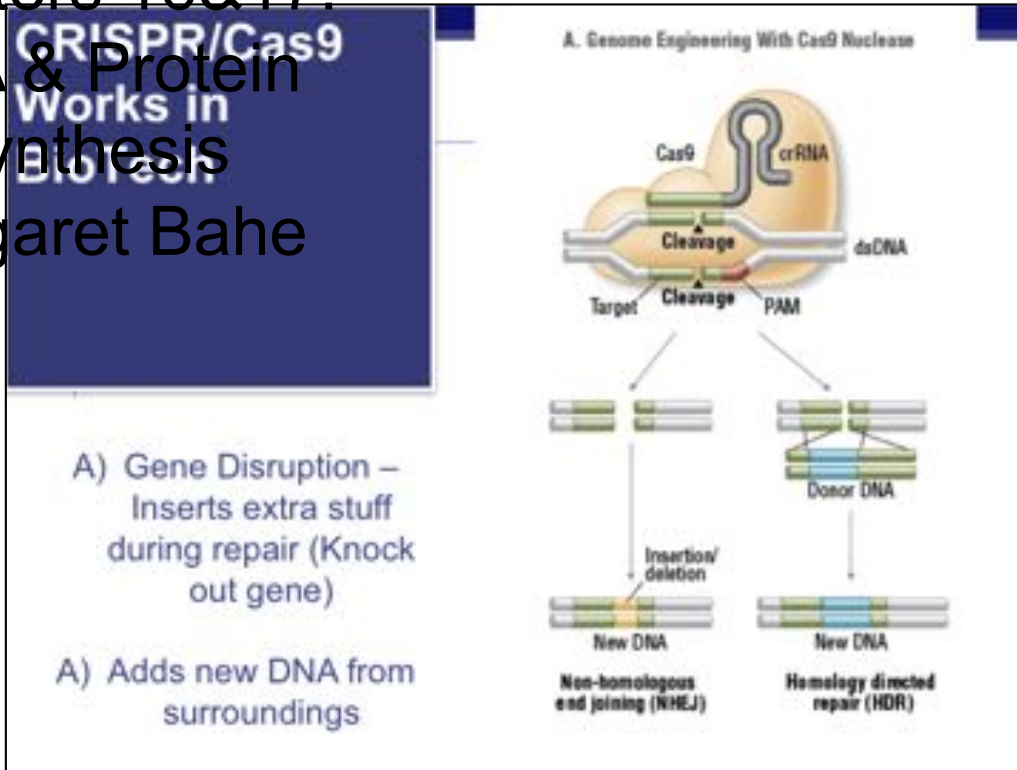
The Viral pieces are transcribed into RNA (single stranded) and this RNA is then associated with Cas9. these pieces of RNA are called crRNA (CRISPER-RNA) Cas9 is an enzyme that can chop up pieces of DNA straight through. The RNA show Cas9 where to cut.

Now, the crRNA/Cas9 are on the prowl looking for that exact sequence of DNA. When found (because the virus has invaded again), the Cas9 will cut the DNA precisely in the location matched by the crRNA.

This is the natural use of CRISPR/Cas9

Next slide → How BioTech can use this system.

AP Biology:
Chapters 16&17:
DNA & Protein
Synthesis
Margaret Bahe



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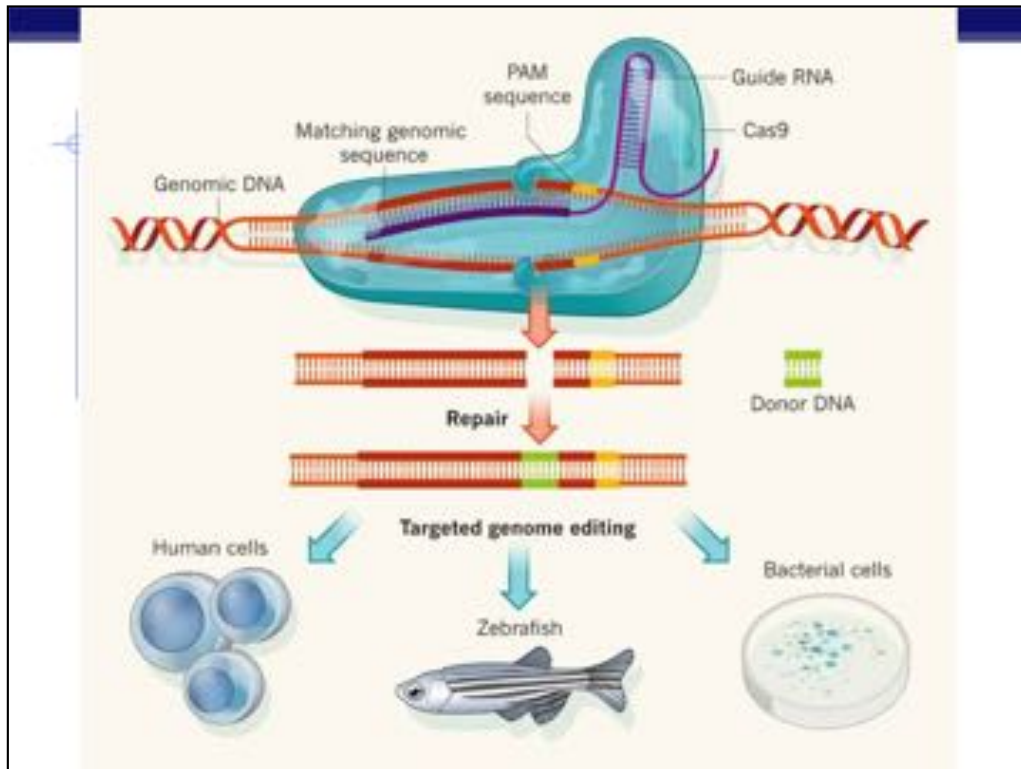
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How CRISPR/Cas9 Works





**Video: The Most Important Story
of 2015**




CRISPR/Cas9 Ethical Dilemmas



Uses of genetic engineering - plants

- **Genetically modified organisms (GMO)**
 - **Protect crops from insects: BT corn**
 - corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)

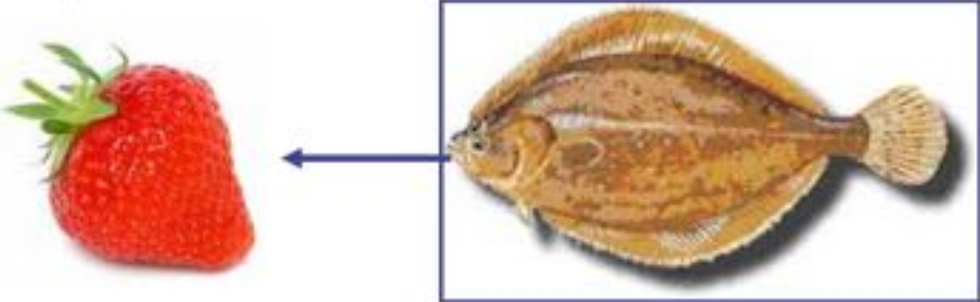
A photograph showing a brown caterpillar on the left and several yellow corn kernels on the right, illustrating the relationship between the pest and the crop.

For example, a transgenic rice plant has been developed that produces yellow grains containing beta-carotene.

- Humans use beta-carotene to make vitamin A.
- Currently, 70% of children under the age of 5 in Southeast Asia are deficient in vitamin A, leading to vision impairment and increased disease rates.

Uses of genetic engineering - plants

- **Genetically modified organisms (GMO)**
 - **Extend growing season: fishberries**
 - strawberries with an anti-freezing gene from flounder





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Uses of genetic engineering - plants

- Genetically modified organisms (GMO)
 - Improve quality of food: **golden rice**
 - rice producing vitamin A
 - improves nutritional value




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Geranylgeranyl diphosphate (GGPP) is made in the grain endosperm of white rice but is not converted to phytoene because the rice phytoene synthase is not expressed in this part of the plant. The original Golden Rice used a phytoene synthase from daffodil (*Narcissus pseudonarcissus*) and a carotene desaturase (CrtI) from the soil bacterium *Erwinia uredovora* to convert GGPP to colored carotenoids and yielded 1.6 g of total carotenoids per gram of dry weight. Paine *et al.* found that a maize (*Zea mays*) phytoene synthase dramatically increases flux towards phytoene. Golden Rice 2 contains up to 37 g of total carotenoids per gram.

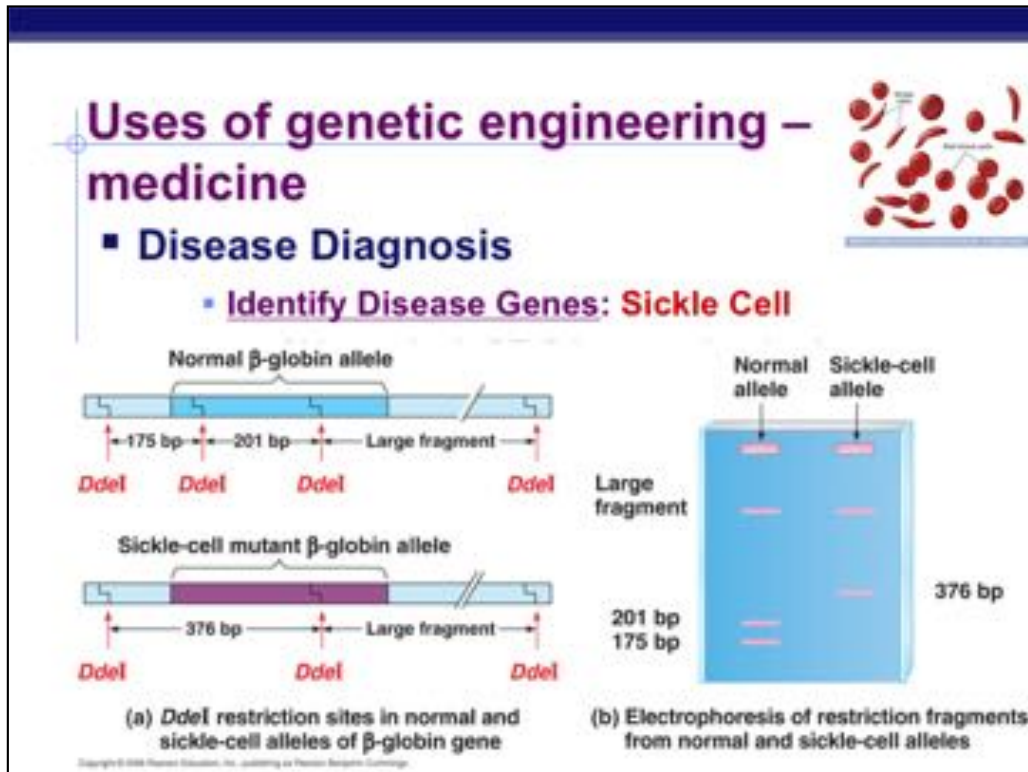
Uses of genetic engineering - medicine

- **Disease Diagnosis**
 - **Identify Pathogens: HIV**
 - The RNA of HIV can be amplified with RT-PCR (reverse transcriptase PCR)



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Single nucleotide polymorphisms (SNP)

RFLP Restriction Fragment Length Polymorphism

Uses of genetic engineering - medicine


- **Gene Therapy**
 - **Replace Defective Genes: SCID & CF**
 - Somatic Cell Gene Therapy
 - Germ Cell Gene Therapy

The diagram illustrates the process of gene therapy using adenovirus. It is divided into two main parts. The left part shows a four-step process: 1. A healthy gene is inserted into a plasmid. 2. The plasmid is inserted into a cell. 3. The cell produces healthy genes. 4. The healthy genes are injected into a patient. The right part shows a human torso with a magnified view of the respiratory system, where adenovirus particles are shown entering the lungs and delivering the healthy gene to the cells.

Adenovirus Used.

Uses of genetic engineering - medicine -


- **Pharmaceuticals**
 - **Hormones: Insulin & HGH**



The diagram illustrates the process of glucose entering the bloodstream and the role of the pancreas. It includes the following steps:

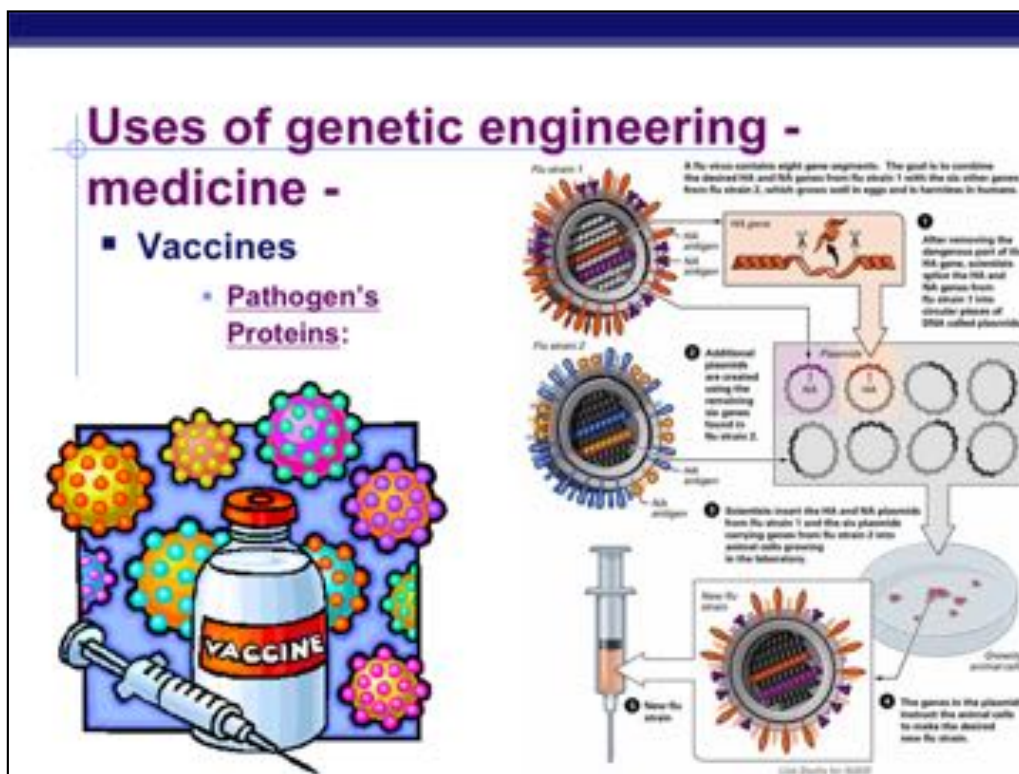
1. The stomach changes food into glucose.
2. Glucose enters the bloodstream.
3. The pancreas makes little or no insulin.
4. Little or no insulin enters the bloodstream.

Additional labels in the diagram include "blood vessel" and "Glucose builds up in the bloodstream."



A photograph showing two children standing side-by-side. The child on the left is significantly taller than the child on the right, illustrating the effects of growth hormone (HGH) treatment.





Adenovirus Used.



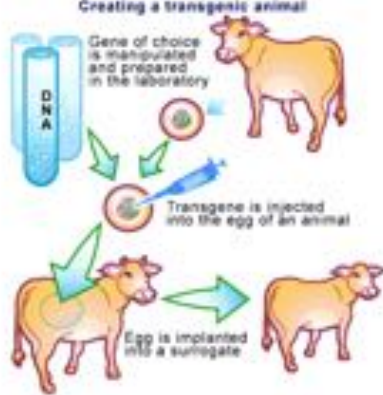
Adenovirus Used.

Uses of genetic engineering - medicine -

- “Pharm” animals
 - **Transgenic Animals:** goats, pigs, cows, chickens
 - Antibodies
 - antithrombin



Creating a transgenic animal



DNA

Gene of choice is manipulated and prepared in the laboratory

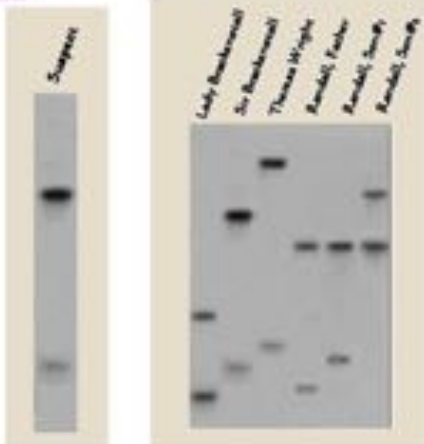

Transgene is injected into the egg of an animal

Egg is implanted into a surrogate

Adenovirus Used.

Uses of genetic engineering - Forensics - (See Handout)

- Genetic Profiles
 - Unique for Individual:
 - RFLP analysis
 - PCR to amplify




The gel electrophoresis image displays two lanes. The left lane is labeled 'Suspect' and shows a single, prominent dark band. The right lane contains six lanes, each labeled with a name: 'Lady Bushmonai', 'Sir Bushmonai', 'Thomas Wright', 'Randy Fisher', 'Randy Smith', and 'Randy Smith'. Each of these lanes shows a unique pattern of multiple dark bands, representing a genetic profile. The 'Suspect' lane's single band does not match any of the profiles in the right lane.

Adenovirus Used.

Uses of genetic engineering - Environmental Clean-up

- **Microbes**
 - **Degrade Oil**
 - Oil Spills
 - **Absorb Heavy Metals Copper, Lead**
 - Mining wastes

An aerial photograph showing a large-scale environmental cleanup operation in a wide body of water. The water is dark, and there are several large, light-colored barges or containment booms in the water. In the background, there are industrial structures and a coastline with buildings. The overall scene depicts a significant environmental remediation effort.

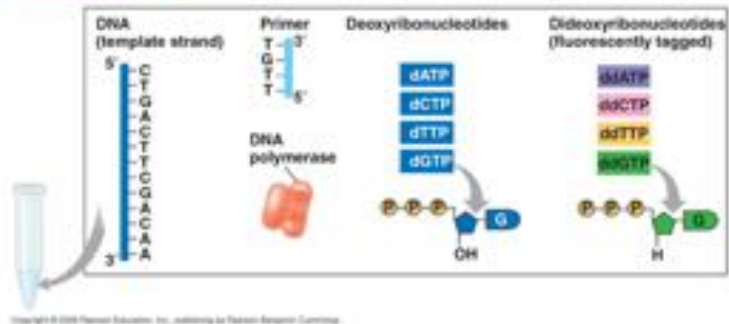
Adenovirus Used.

Uses of genetic engineering - Research - DNA Sequencing

■ :

- Automated Today

TECHNIQUE



Adenovirus Used.

Uses of genetic engineering - Research - DNA Sequencing

- Human Genome Project
 - ◆ 1990 Start
 - ◆ 2003 Finish
 - ◆ Analysis is underway

TECHNIQUE

The diagram illustrates the Sanger sequencing process. A DNA template strand (3' to 5') is shown on the left. To its right, several labeled strands are being synthesized in the 5' to 3' direction. These strands are of varying lengths, representing different stages of synthesis. The strands are labeled with their terminal bases: 'Shortest' (T), 'GAG', 'GAGG', 'GAGGG', 'GAGGGG', 'GAGGGGG', and 'Longest' (GAGGGGGG). A 'Direction of movement of strands' arrow points downwards. Below the strands, a 'Laser' beam passes through a 'Detector' to identify the labeled bases. The 'Shortest labeled strand' is shown with a red base (T), and the 'Longest labeled strand' is shown with a blue base (G).

RESULTS

The results are displayed as a sequence of bases. The 'Last base of longest labeled strand' is G, and the 'Last base of shortest labeled strand' is T. The resulting sequence is GAGGGGGT.

Adenovirus Used.



Sticky ends

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

