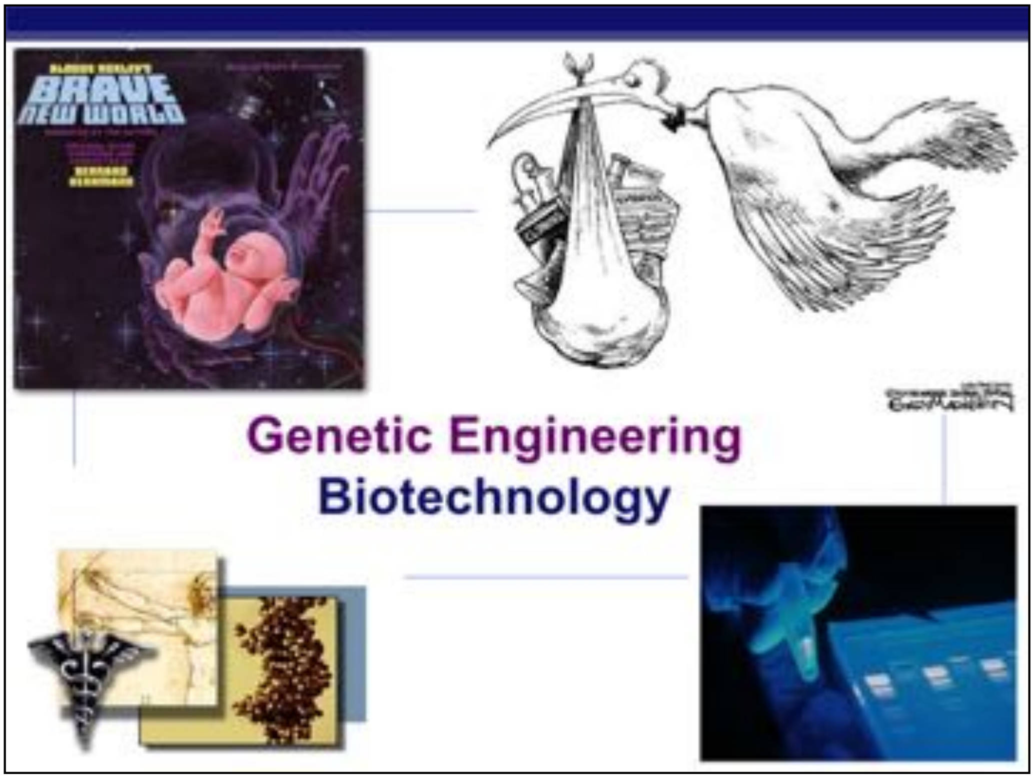
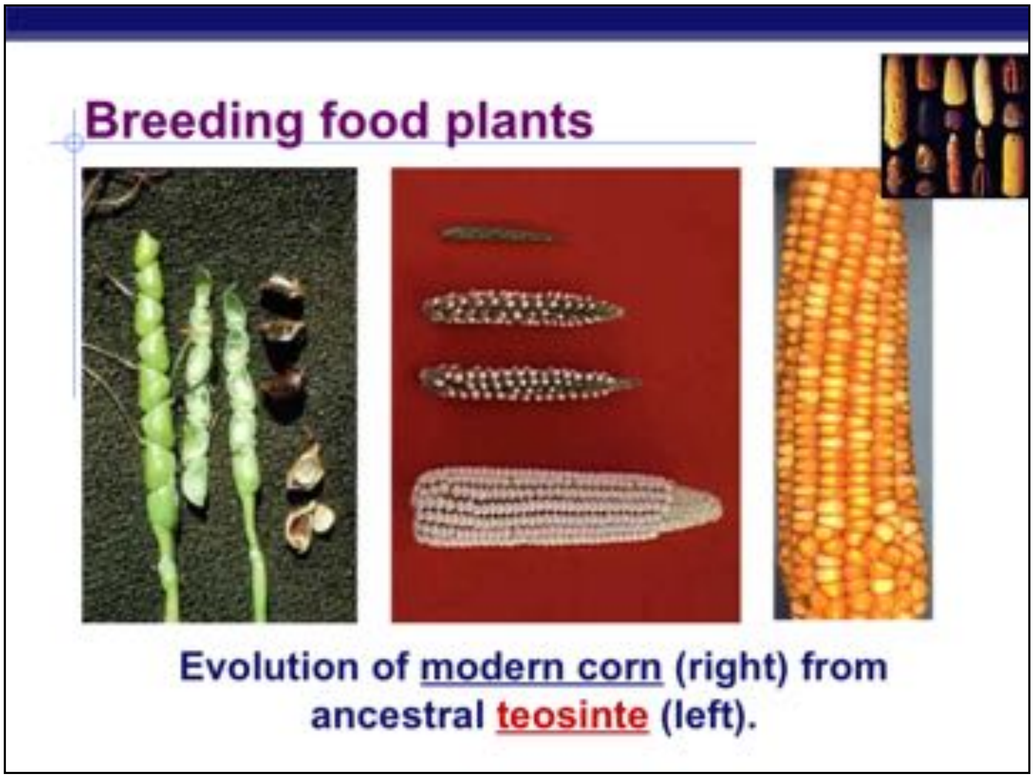
AP Biology: Chapter 20: Biotechnology

Margaret Bahe

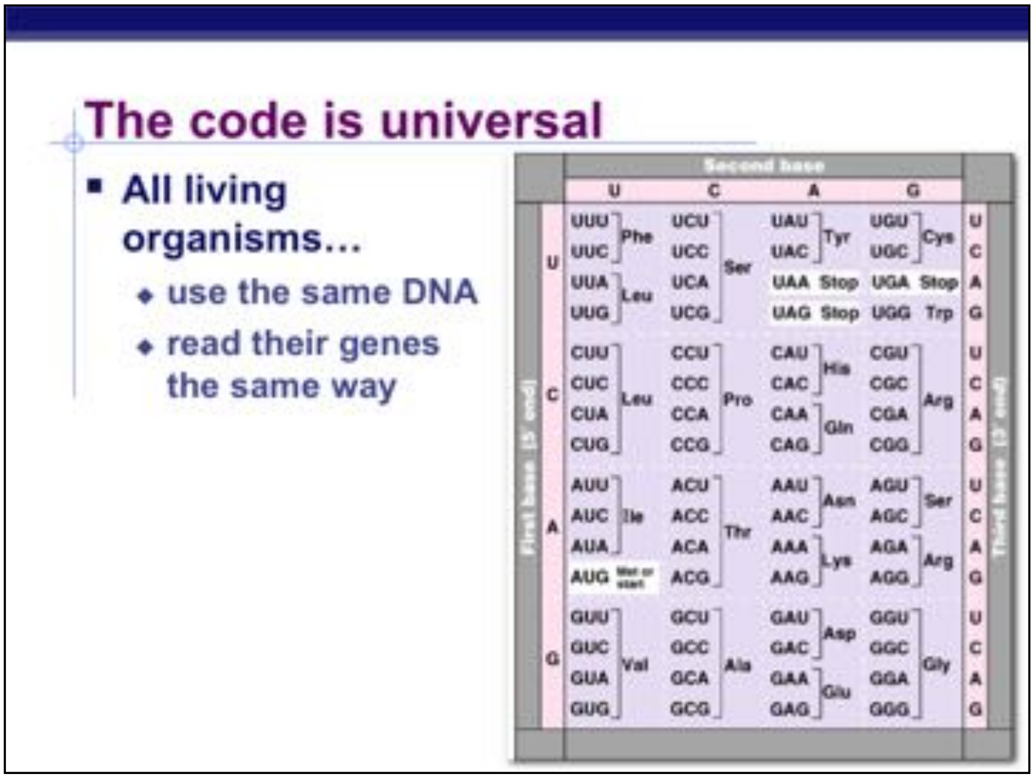



We have been manipulating DNA for generations!
Artificial breeding



Maize and Teosinte Maize 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 occured as a result of harvesting the best "ears" and planting them as opposed to the seed of the sparser plants.



Strong evidence for a single origin in evolutionary theory.


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

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.

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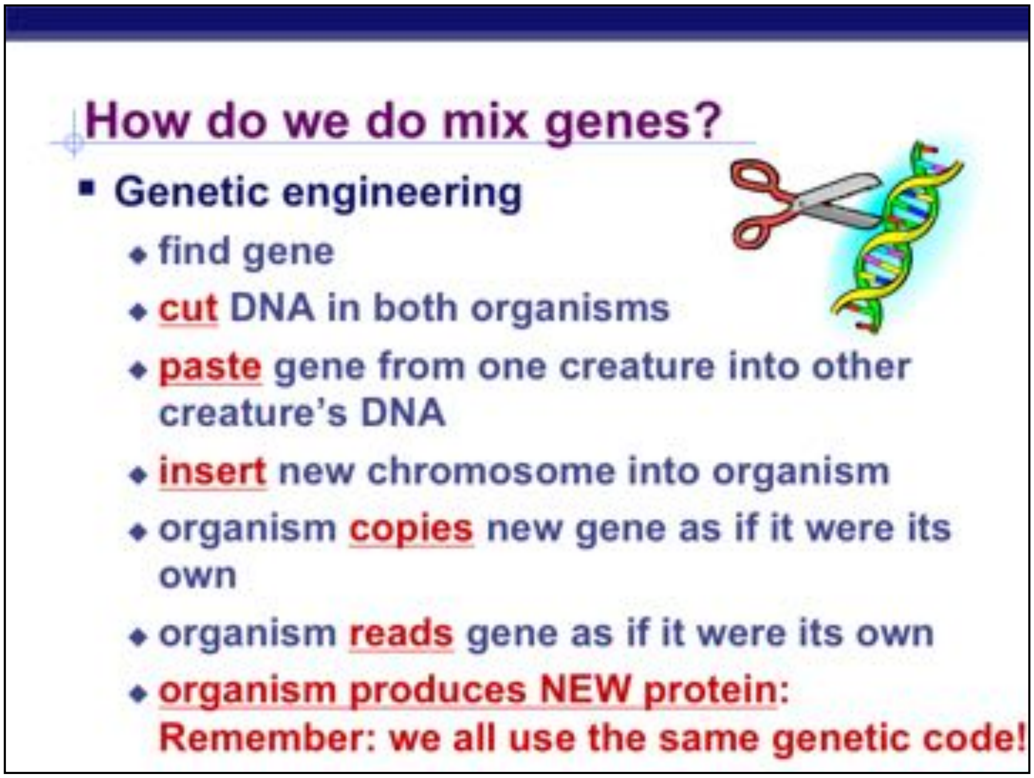
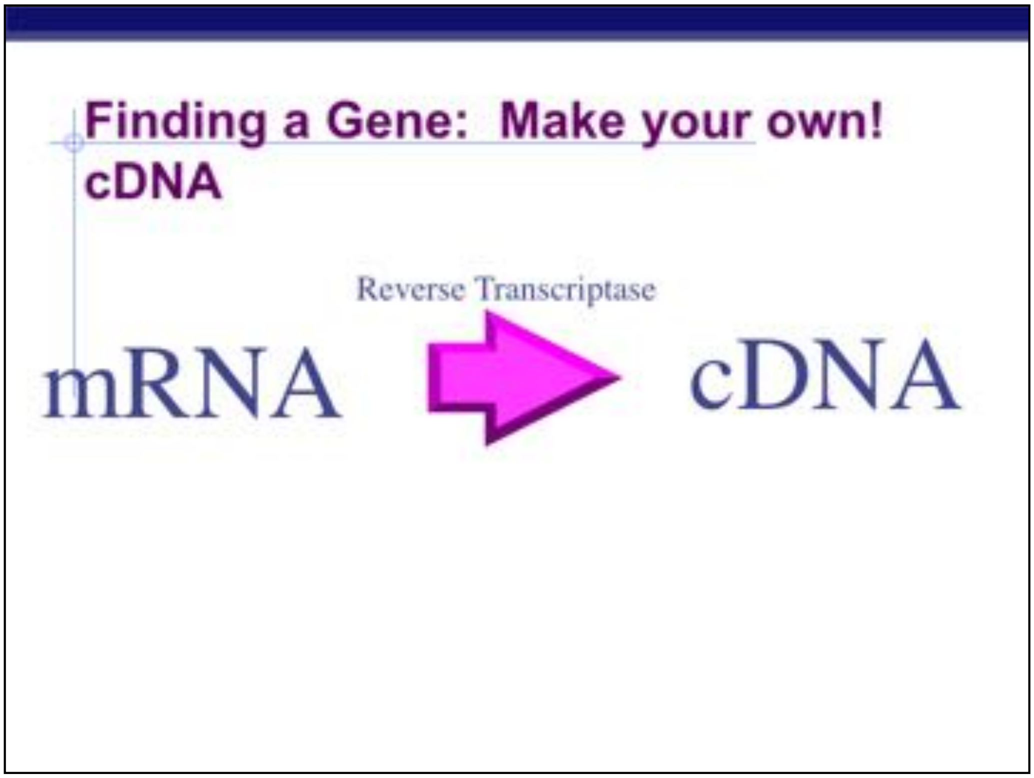
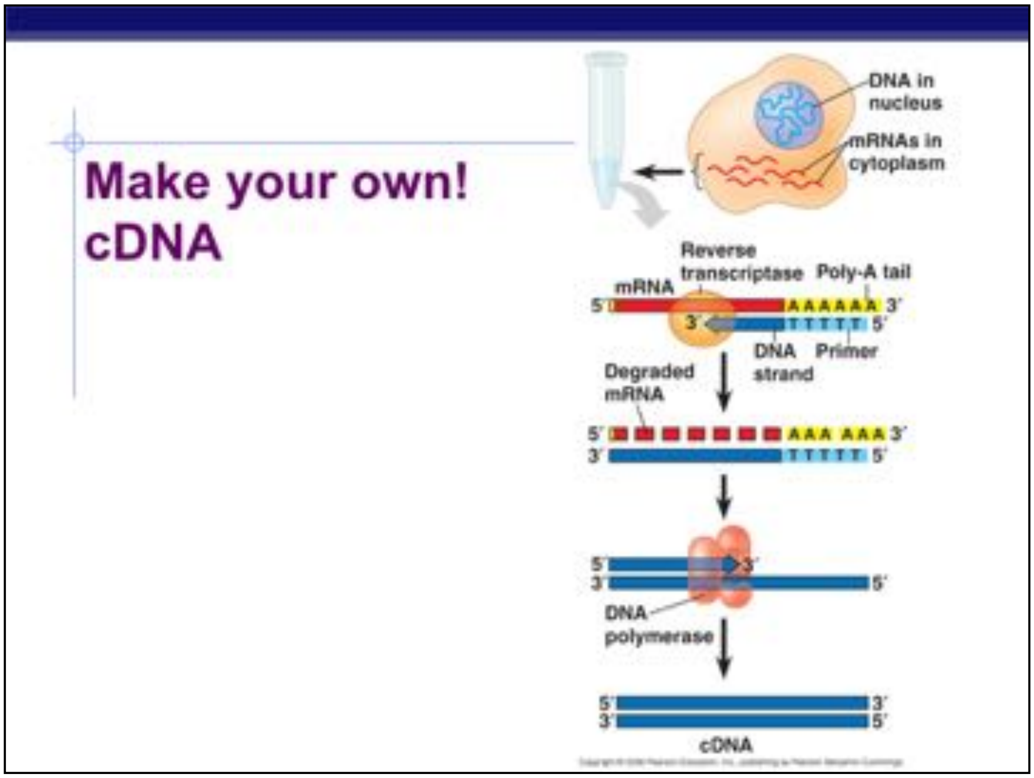
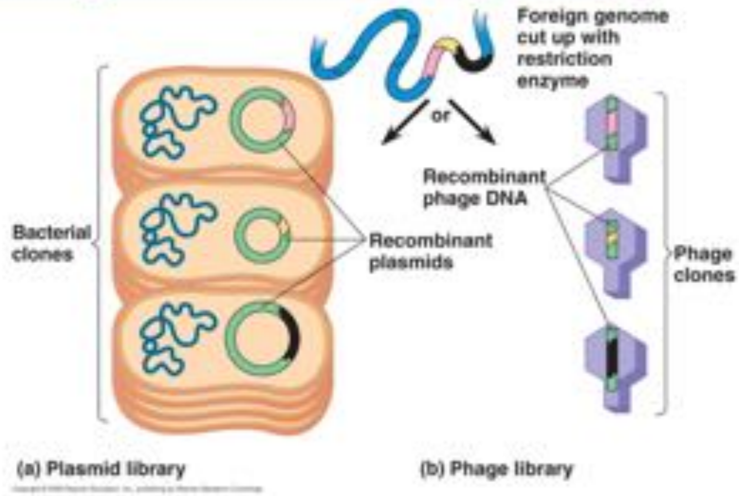


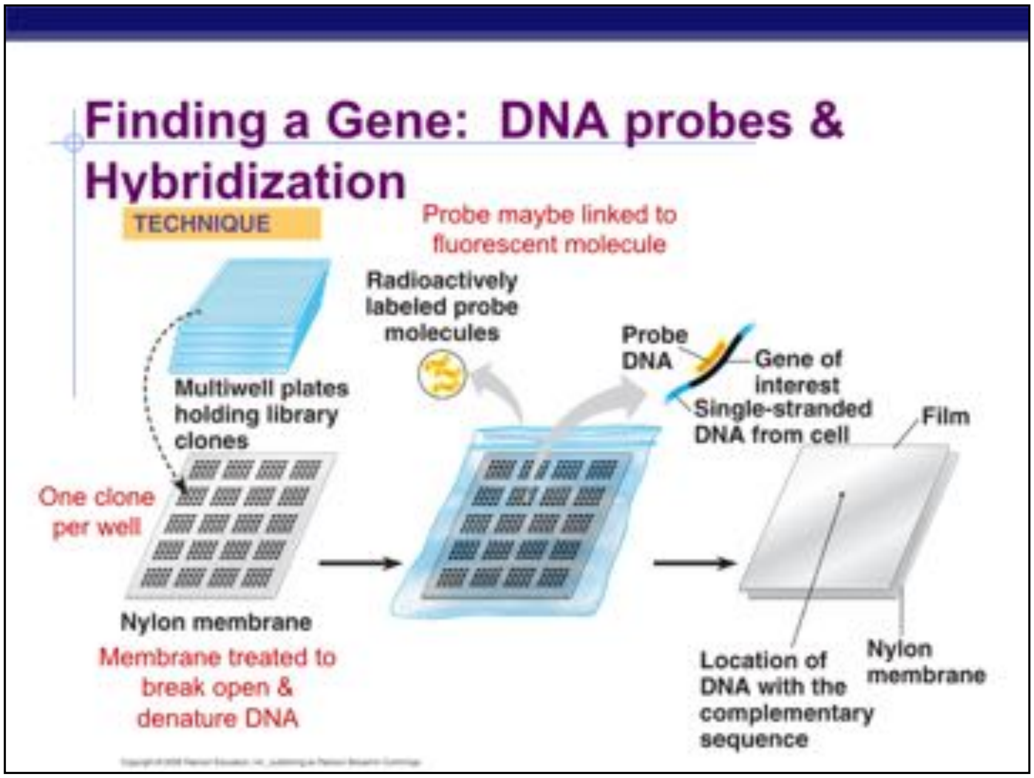

Finding a Gene: Make your own! cDNA
Reverse Transcriptase
mRNA
cDNA


Make your own! cDNA
DNA in nucleus
mRNAs in cytoplasm
Reverse transcriptase
Poly-A tail
mRNA
5′
AAAAAA 3′
3′
TTTTT 5′
DNA strand
Primer
Degraded mRNA
5′
AAA AAA 3′
3′
TTTTT 5′
5′
3′
3′
5′
DNA polymerase
5′
3′
3′
5′
cDNA

Finding a Gene: Shot gun approach



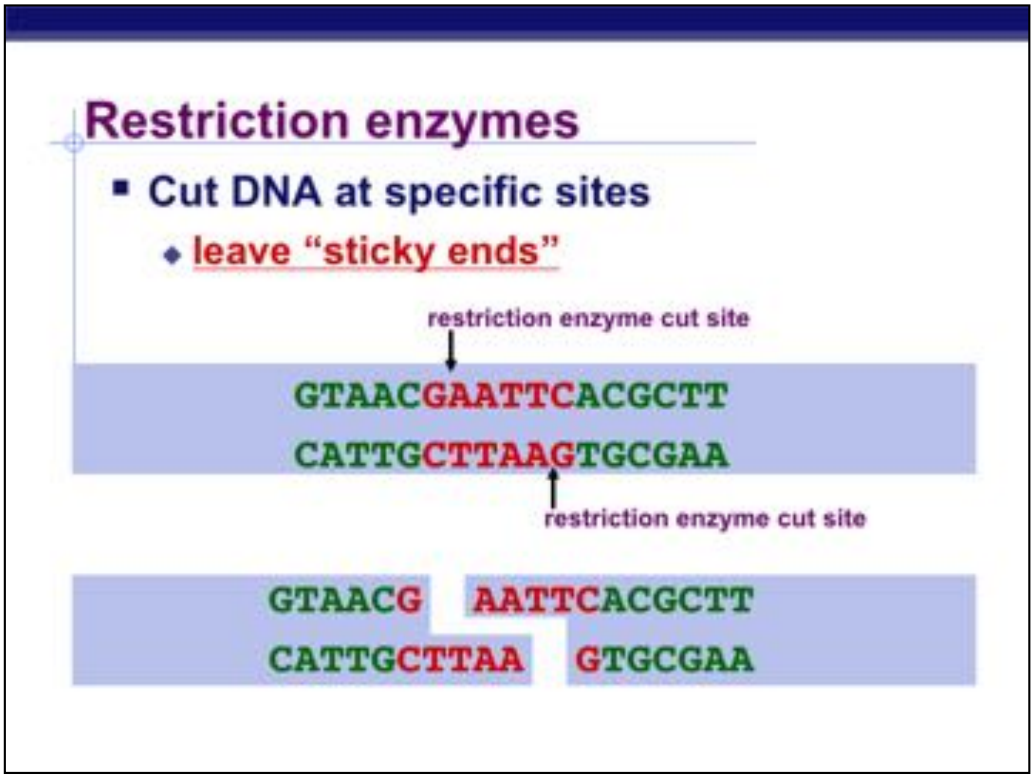
Create genomic library of an entire genome


Finding a Gene: DNA probes & Hybridization
TECHNIQUE
Probe maybe linked to fluorescent molecule
Radioactively labeled probe molecules
Probe DNA
Gene of interest
Single-stranded DNA from cell
Film
Multiwell plates holding library clones
One clone per well
Nylon membrane
Membrane treated to break open & denature DNA
Location of DNA with the complementary sequence
Nylon membrane

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"
restriction enzyme cut site
GTAACGAATTCACGCTT
CATTGCTTAAGTGCGAA
restriction enzyme cut site
GTAACG
AATTCACGCTT
CATTGCTTAA
GTGCGAA

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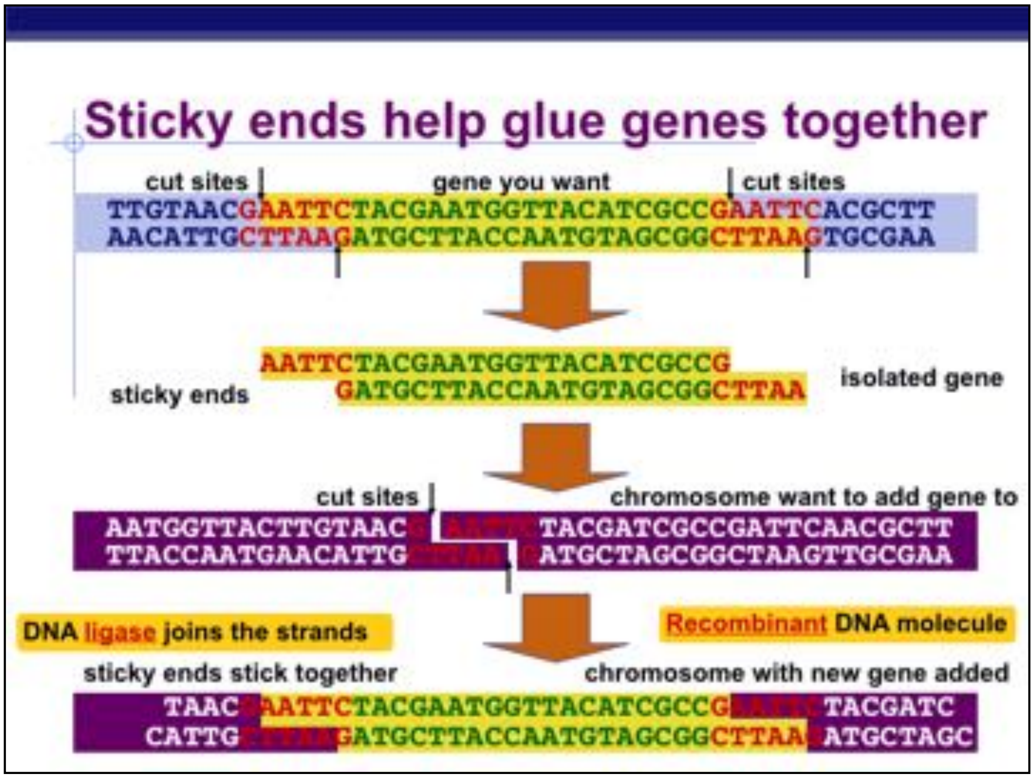
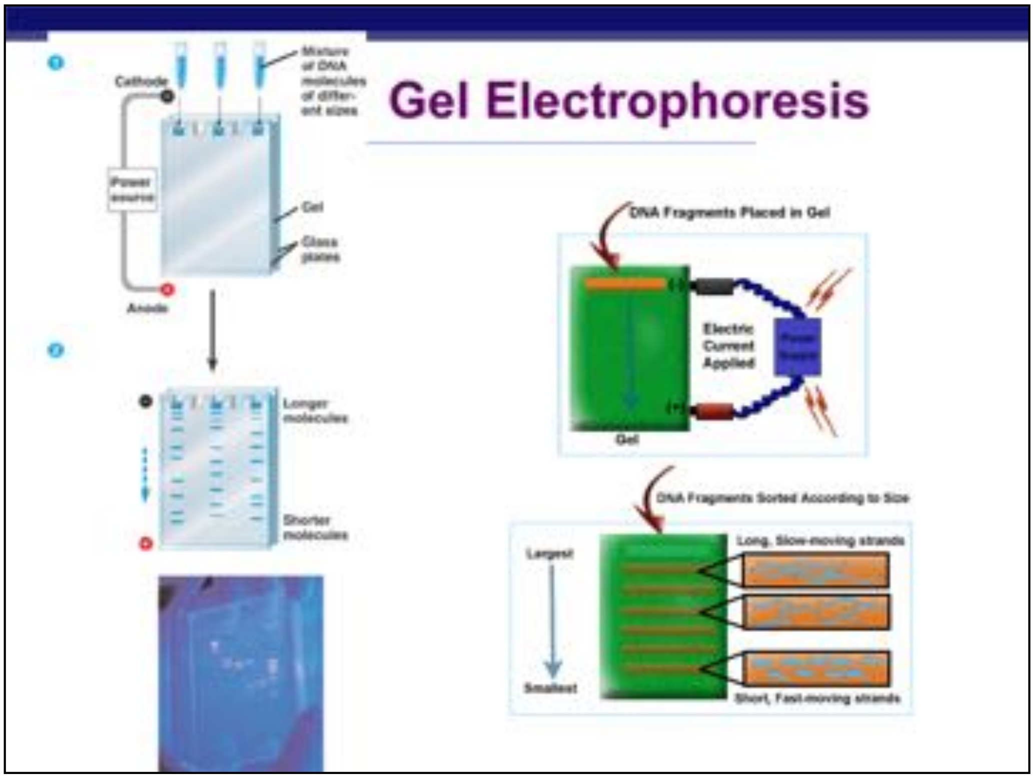
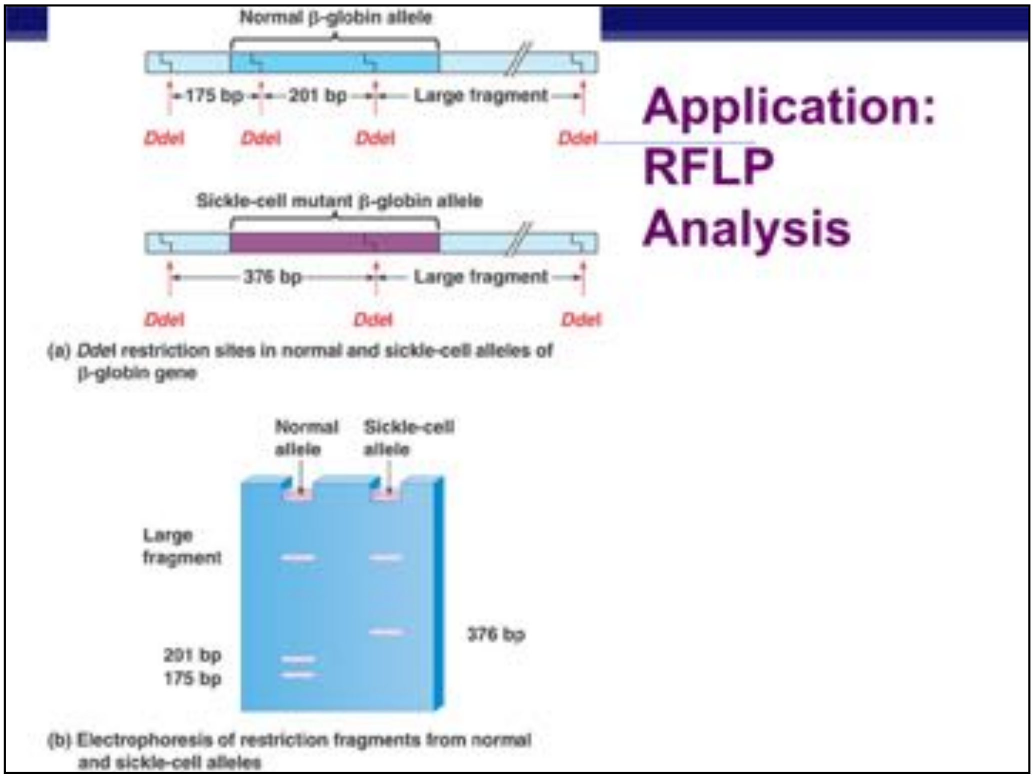


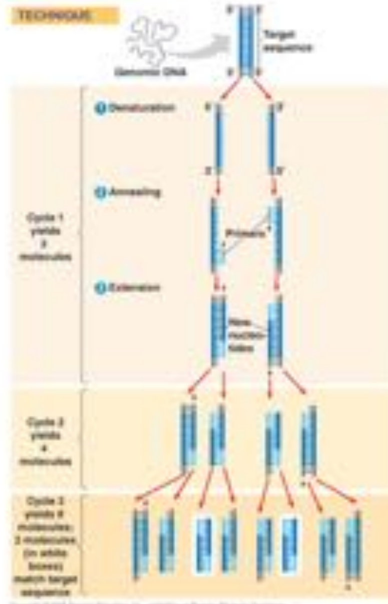


Explain how you would use this to identify a person who is a carrier for sickle cell anemia --See next slide


Normal β-globin allele
175 bp
201 bp
Large fragment
DdeI
DdeI
DdeI
DdeI
Sickle-cell mutant β-globin allele
376 bp
Large fragment
DdeI
DdeI
DdeI
(a) DdeI restriction sites in normal and sickle-cell alleles of β-globin gene
Normal allele
Sickle-cell allele
Large fragment
201 bp
175 bp
376 bp
(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles
Application: RFLP Analysis

Amplify your DNA: PCR

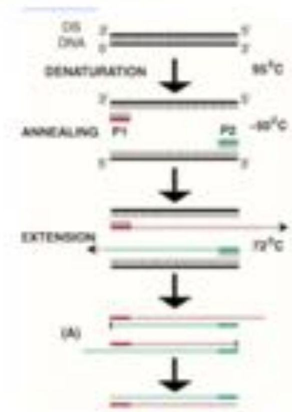
- **What if you have too little DNA to work with?**
- **Polymerase Chain Reaction**



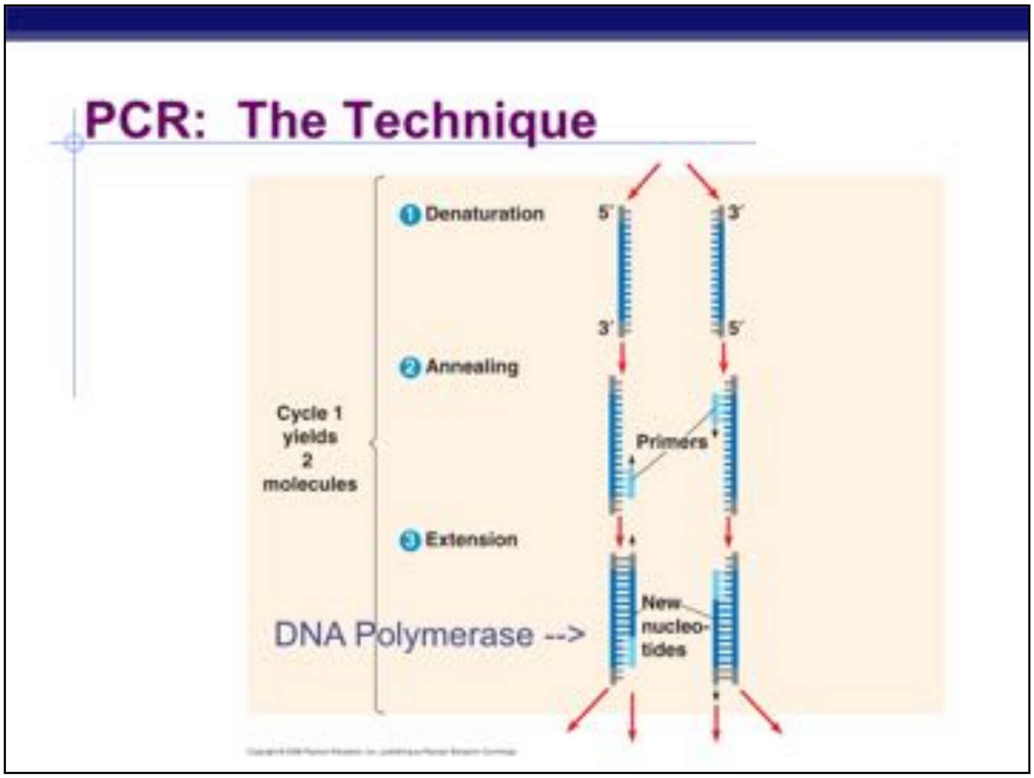
Copying DNA without bacteria or plasmids!

PCR Primers

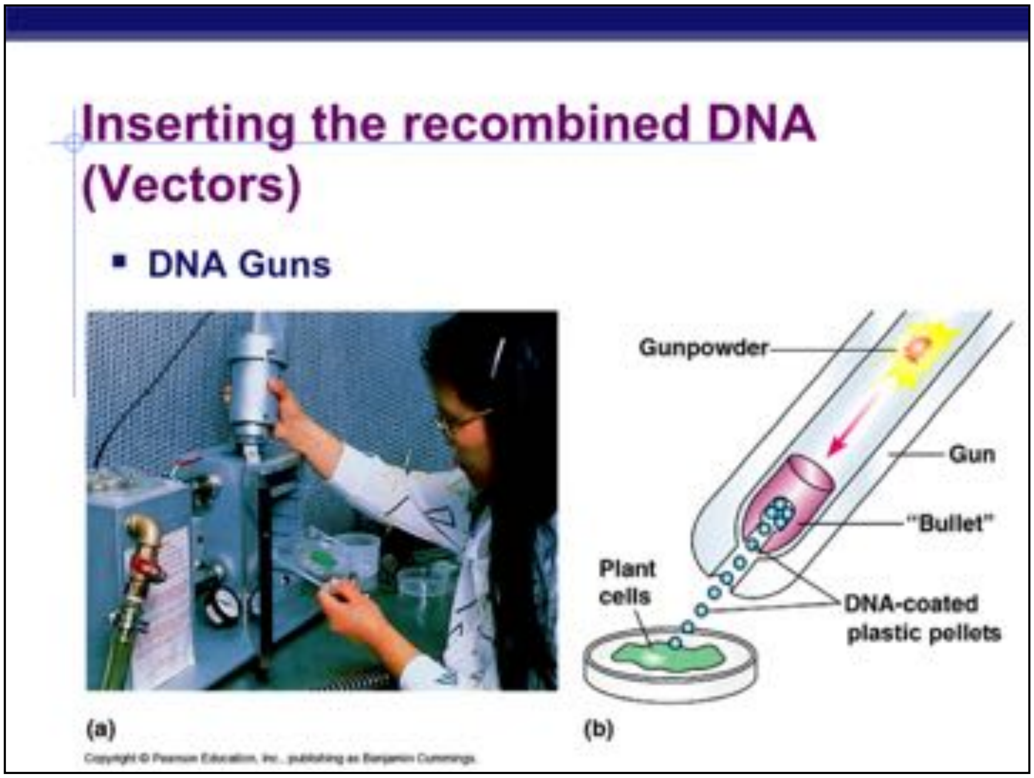
- **Critical!!**
  - Need to know sequence
  - Primers flank target DNA
  - Primers define the section of DNA to be cloned
- **Taq polymerase**
  - From hot springs
  - Why do we use it?



20-30 cycles
3 steps/cycle
30 sec/step


PCR: The Technique
1 Denaturation
2 Annealing
3 Extension
Cycle 1 yields 2 molecules
5′
3′
3′
5′
Primers
New nucleo-tides
DNA Polymerase -->


PCR Song



Mini-chromosomes

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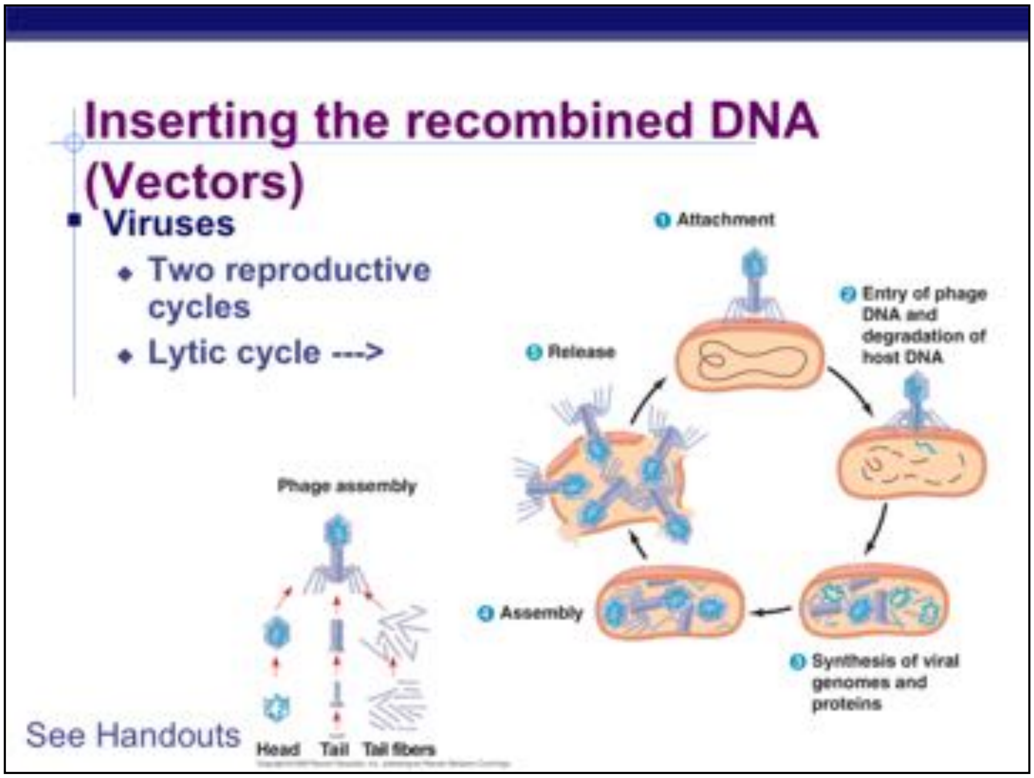

http://www.youtube.com/watch?v=O-EdX4MaMFE&feature=PlayList&p=B1AC4F7A9C99C847&index=14&playnext=2&playnext_from=PL

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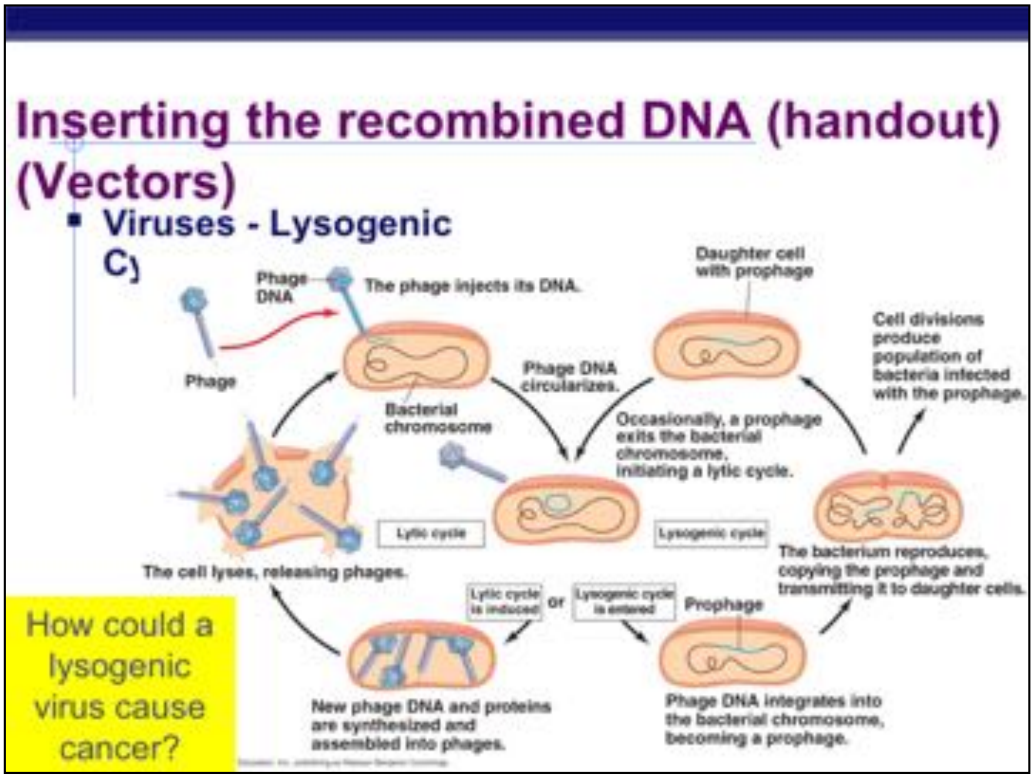
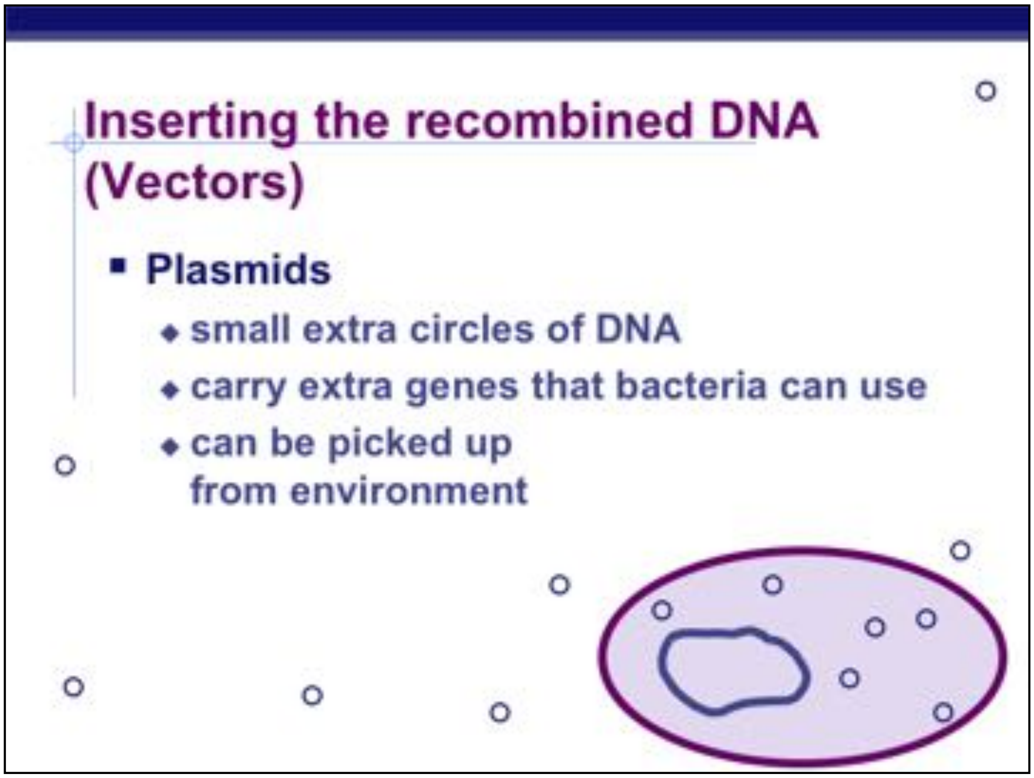


Mini-chromosomes



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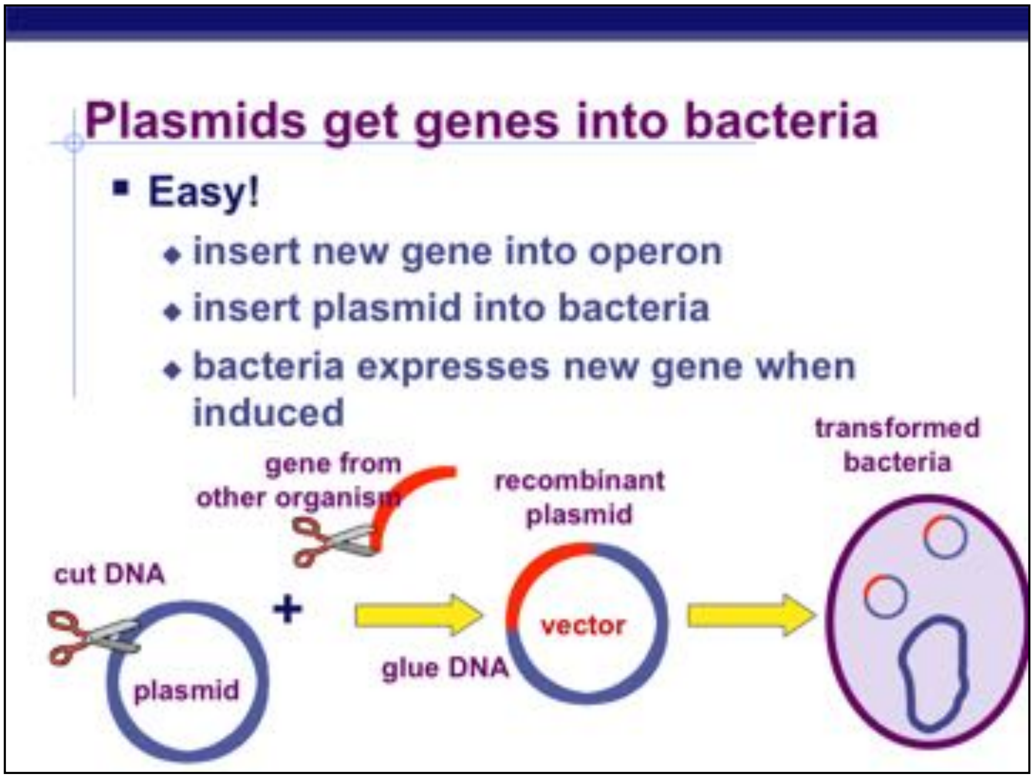
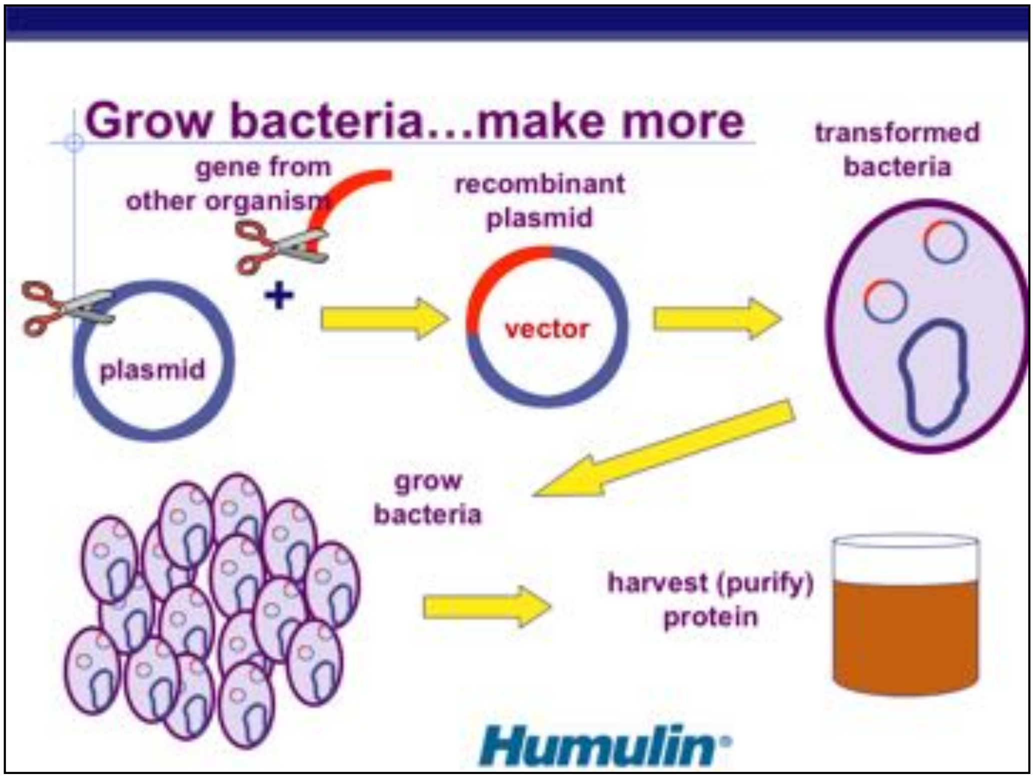
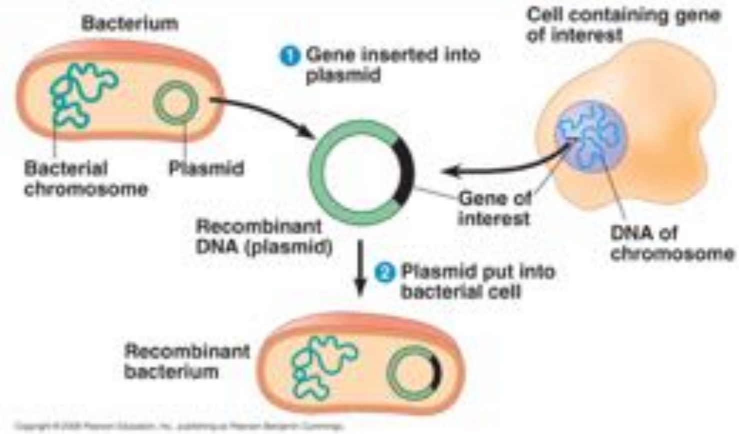

Plasmids get genes into bacteria
Easy!
insert new gene into operon
insert plasmid into bacteria
bacteria expresses new gene when induced
gene from other organism
recombinant plasmid
transformed bacteria
cut DNA
plasmid
+
glue DNA
vector

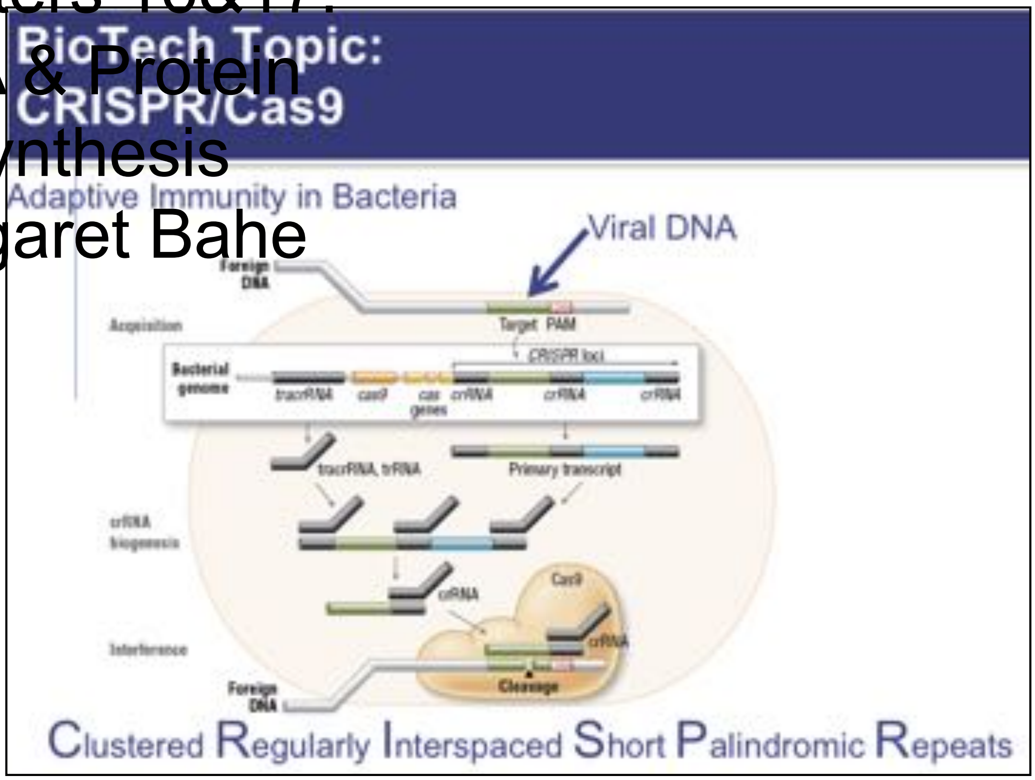

Grow bacteria…make more
gene from
other organism
recombinant
plasmid
transformed
bacteria
+
plasmid
vector
grow
bacteria
harvest (purify)
protein
Humulin

Summary: Gene Cloning



Enzyme Song

Now Binder 117



Virus infects a bacerial cell by injecting it's DNA.

Bacteria have a defense:

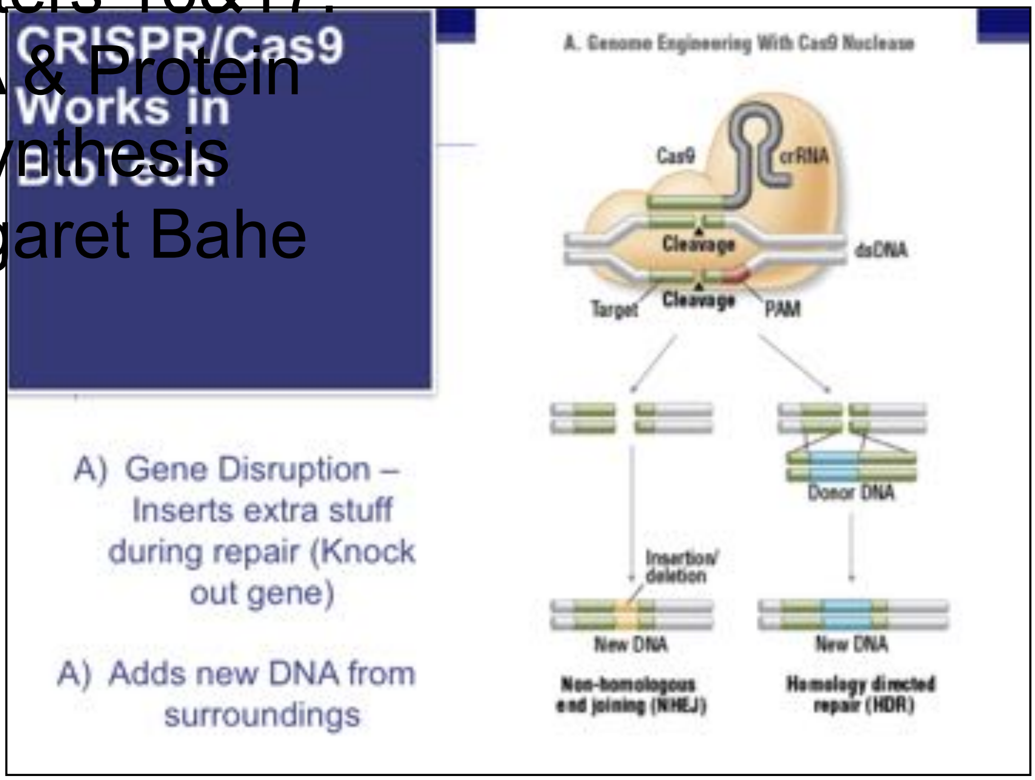
The incorporate pieces of the viral DNA into their own genome separated by repeating segments of DNA. This is the CRISPR system.

The Viral pieces are transcribed into RNA (single stranded) and this RNA is then associated with Cas9. these pices 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.



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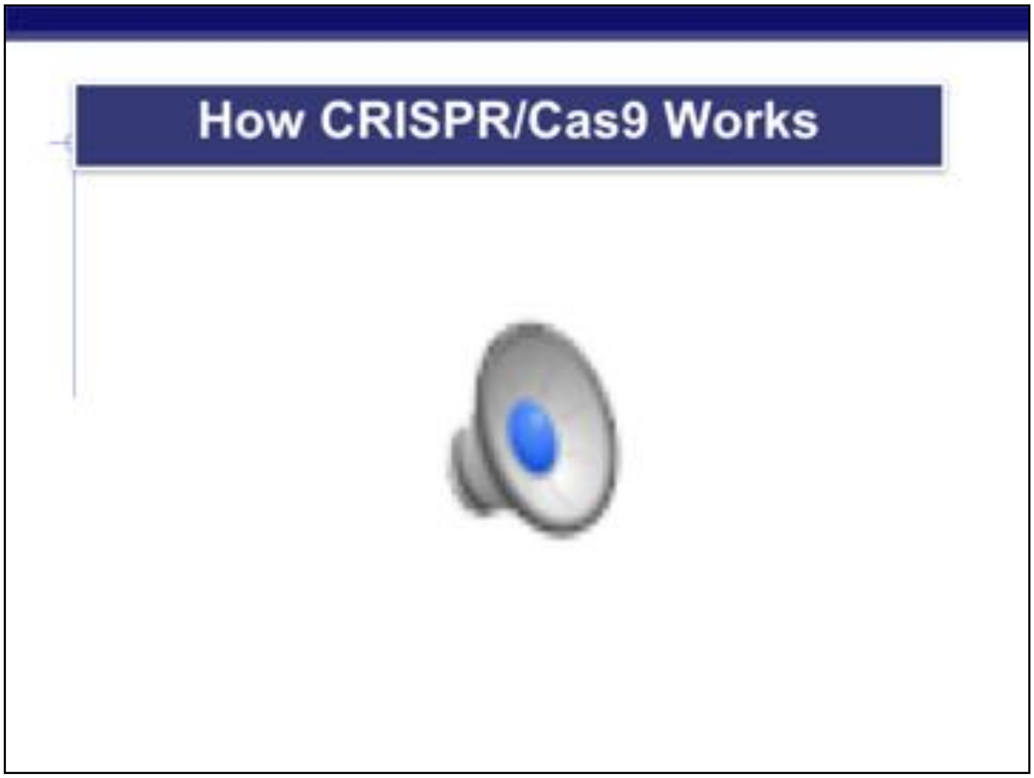
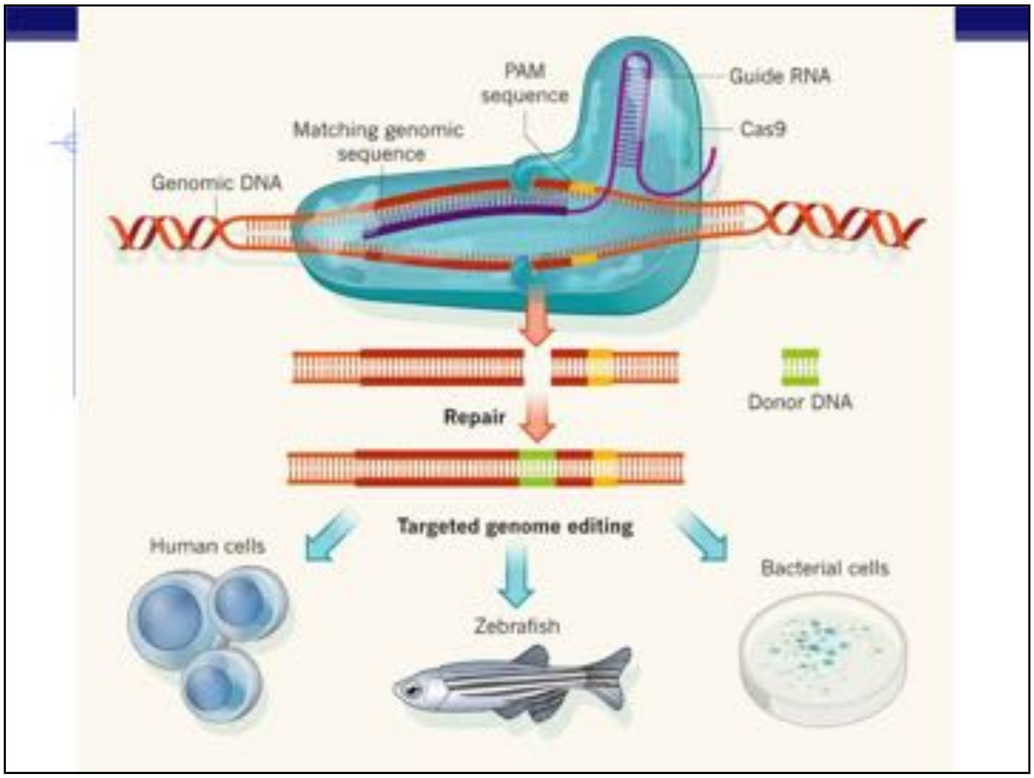
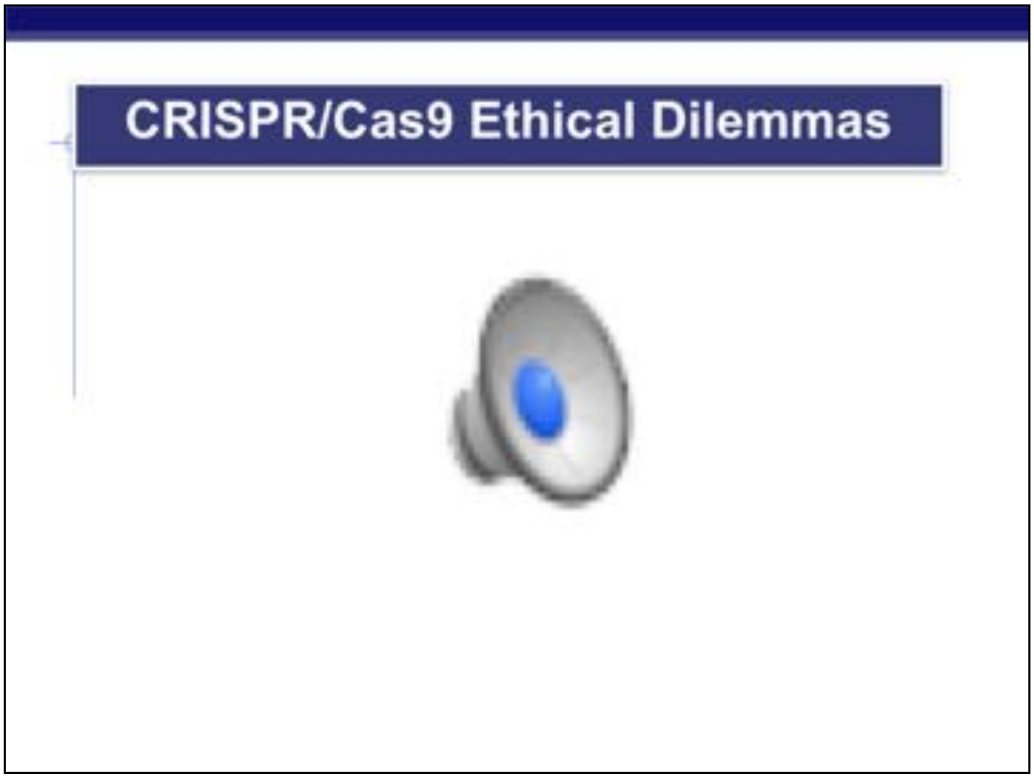
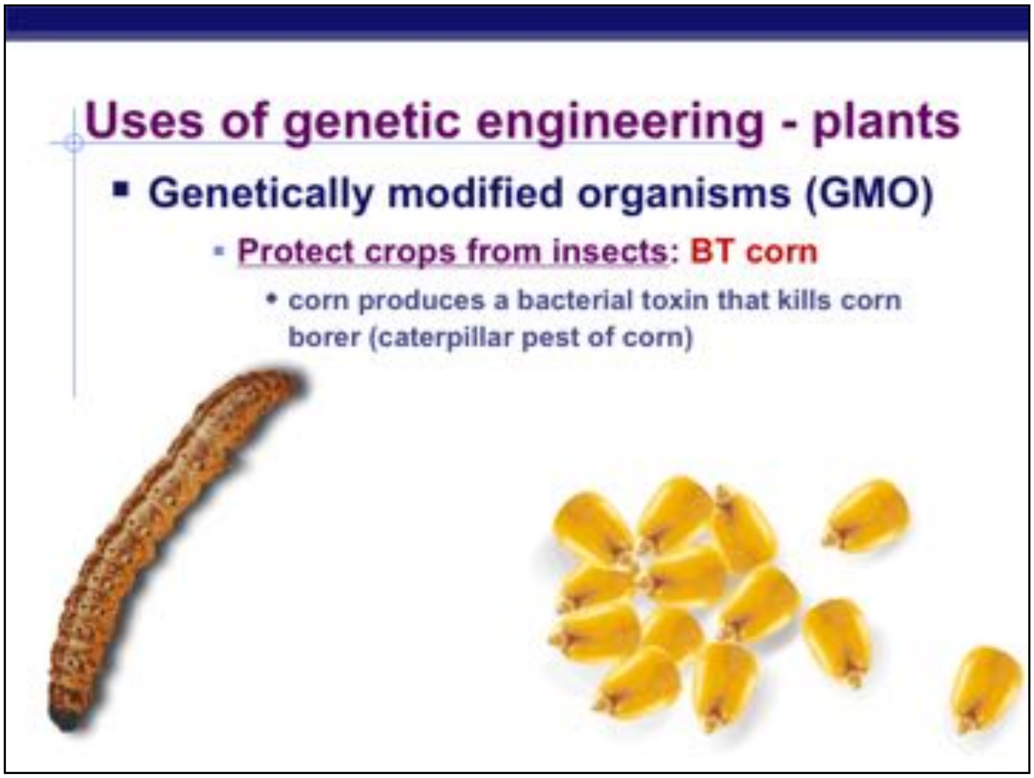

How CRISPR/Cas9 Works


PAM sequence
Guide RNA
Cas9
Matching genomic sequence
Genomic DNA
Donor DNA
Repair
Targeted genome editing
Human cells
Zebrafish
Bacterial cells

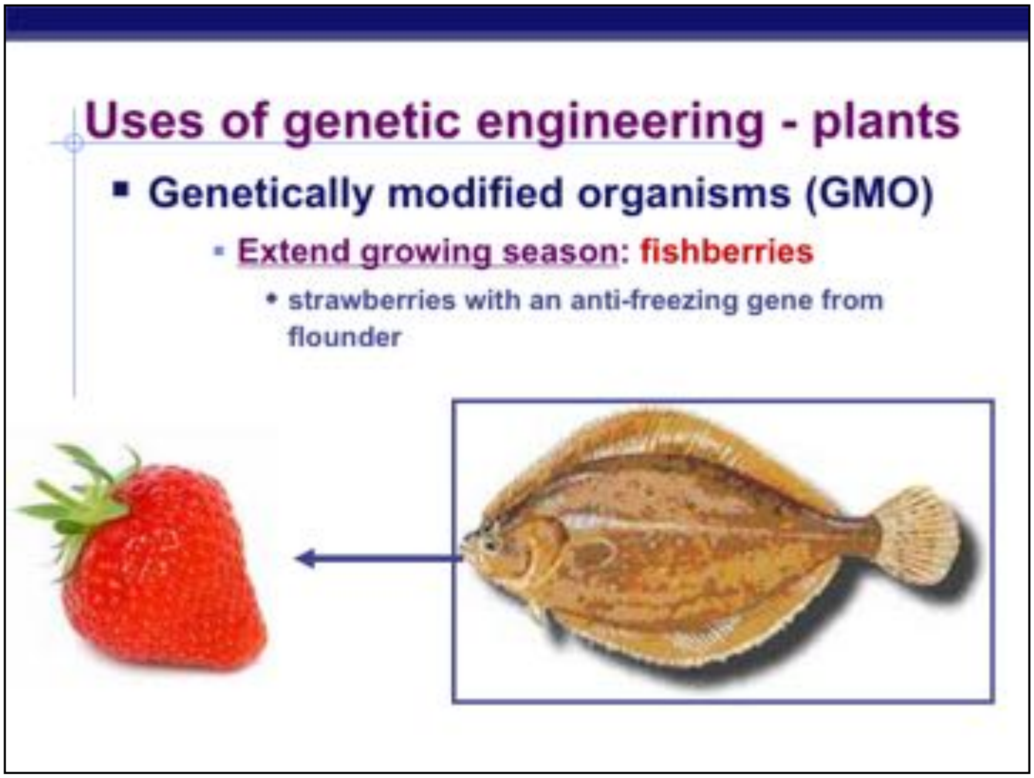

Video: The Most Important Story of 2015


CRISPR/Cas9 Ethical Dilemmas



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.



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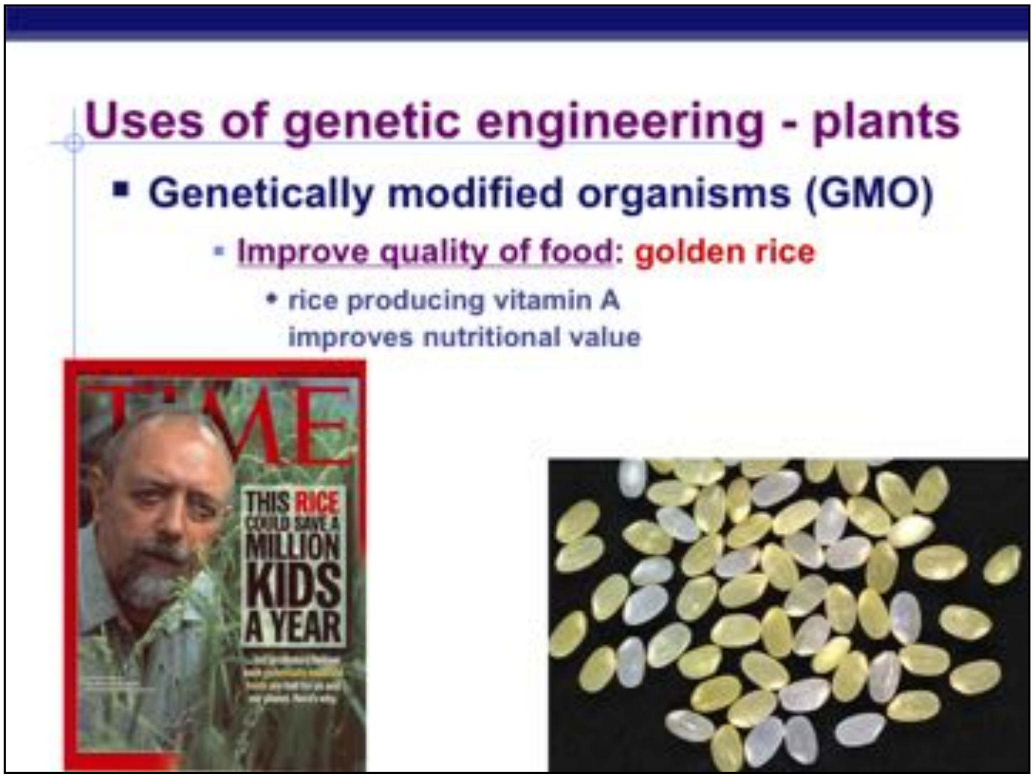

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- Humans use beta-carotene to make vitamin A.
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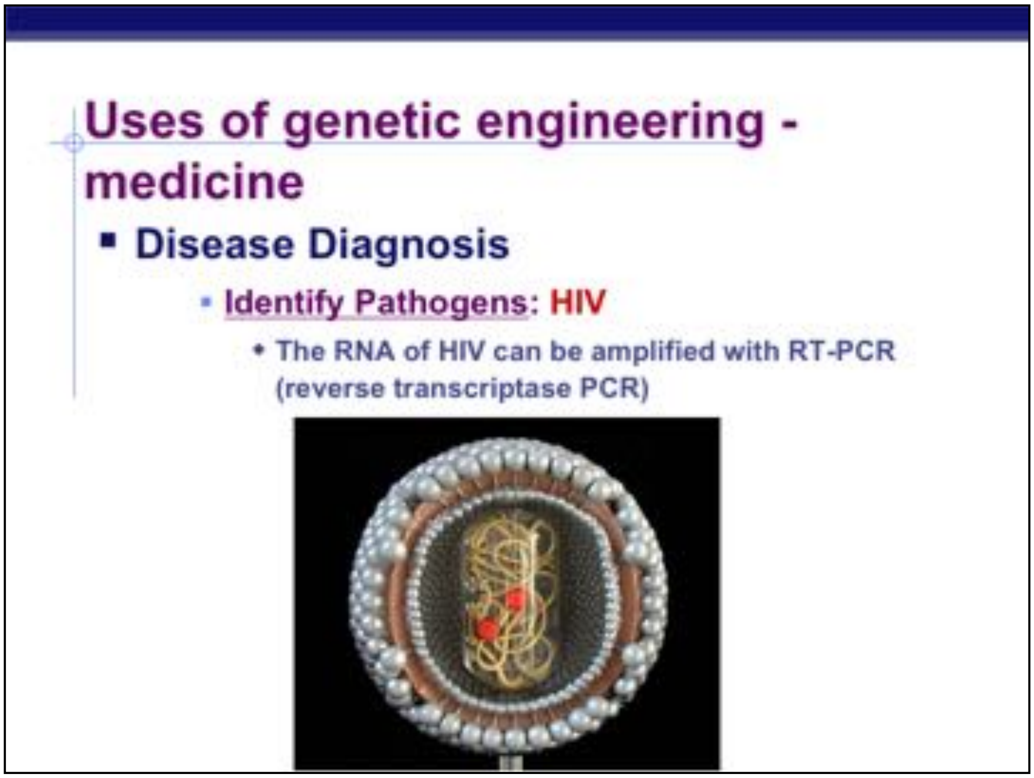
Geranylgeranyl diphosphate (GGPP) is made in the grain endosperm of white rice but is not converted to phytoene because the rice phytoene synthase is not expressed in this part of the plant. The original Golden Rice used a phytoene synthase from daffodil (*Narcissus psuedonarcissus*) and a carotene desaturase (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.



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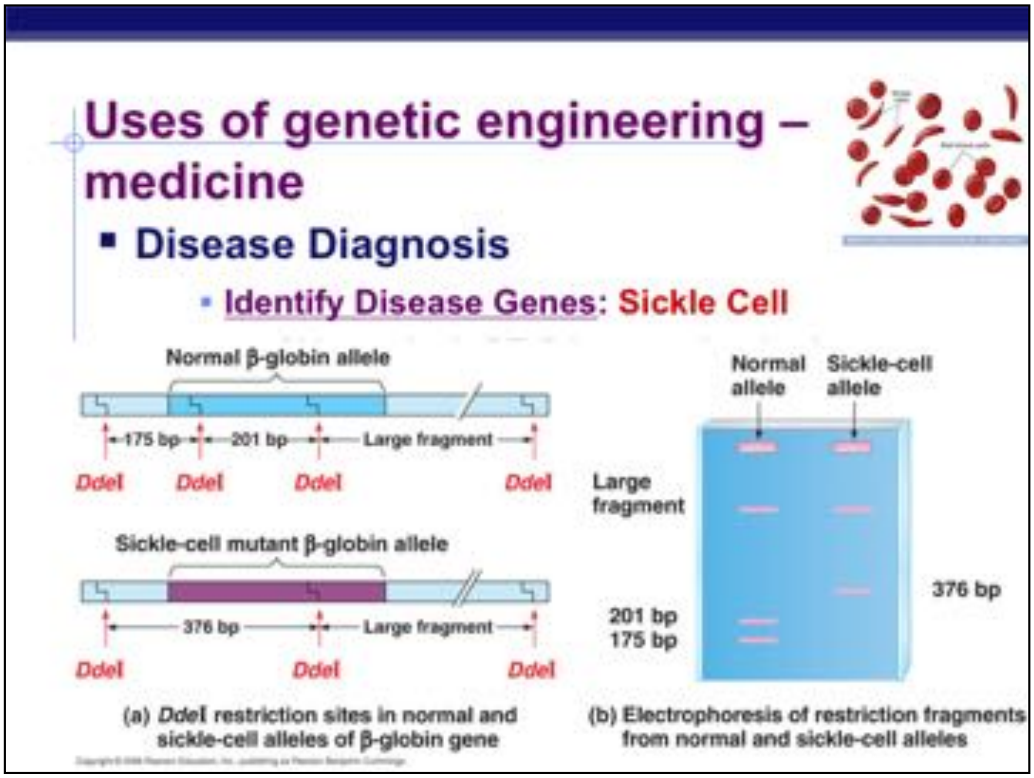
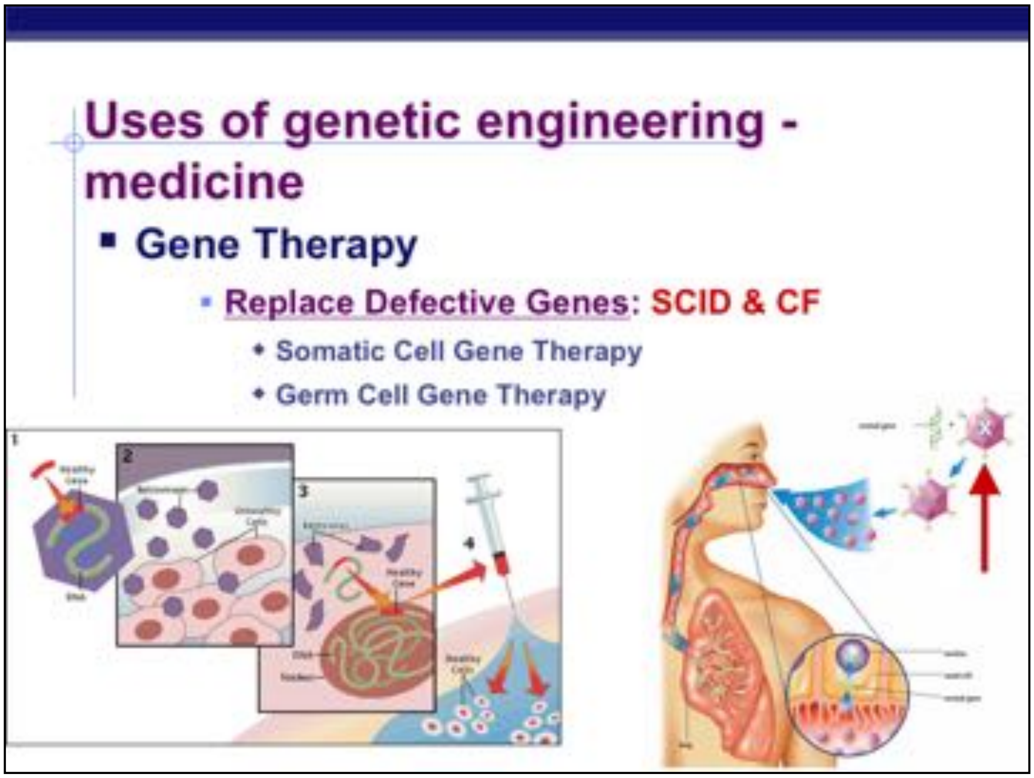
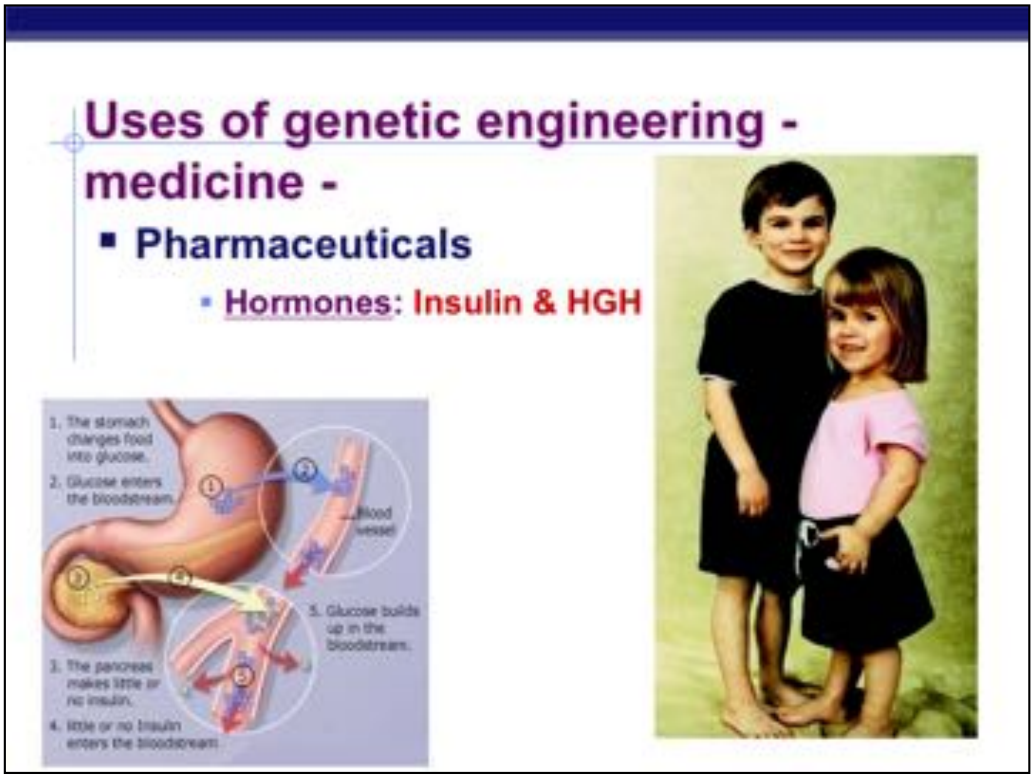


Single nucleotide polymorphisms (SNP)

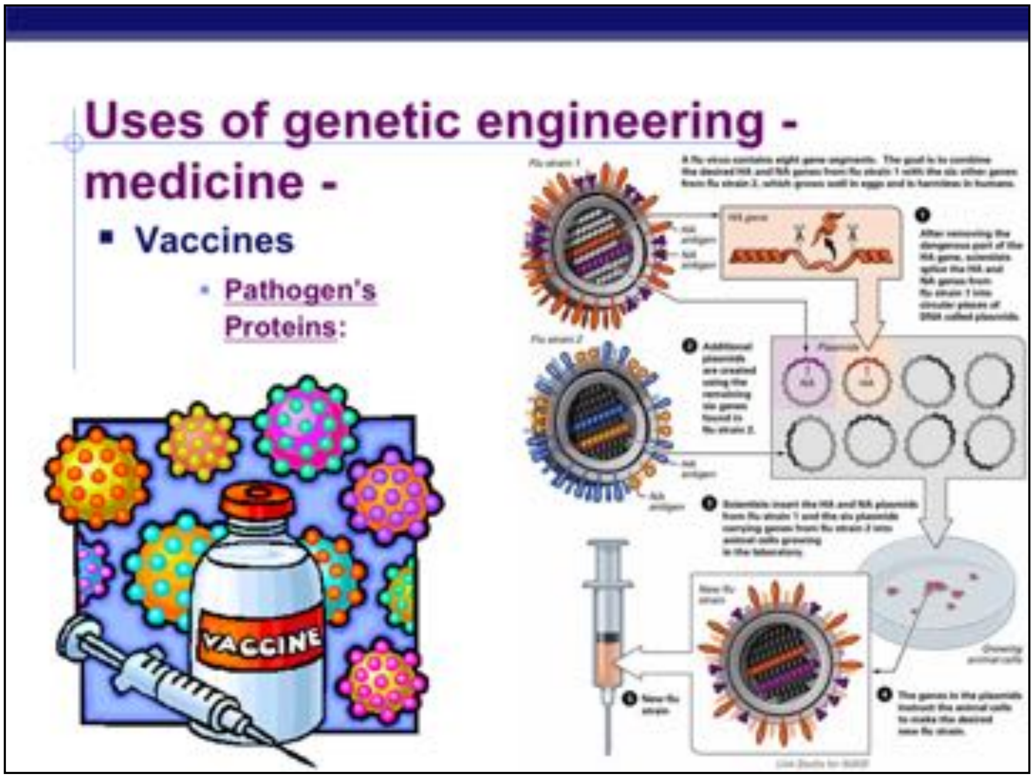
RFLP Restriction Fragment Length Polymorphism



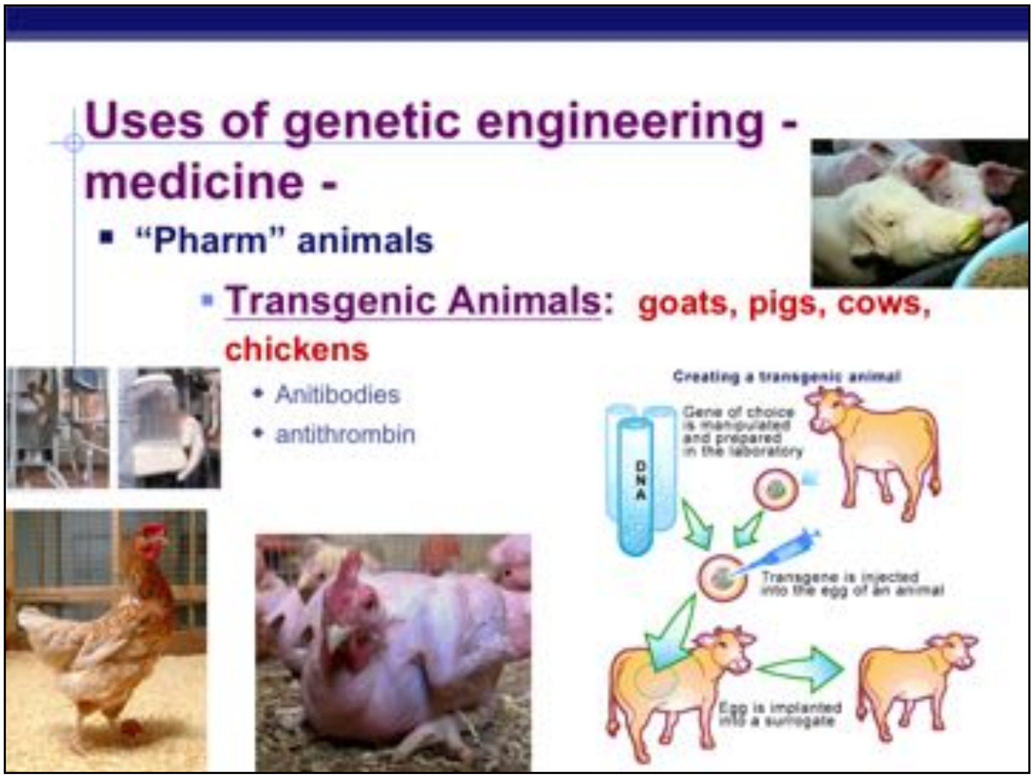
Adenovirus Used.



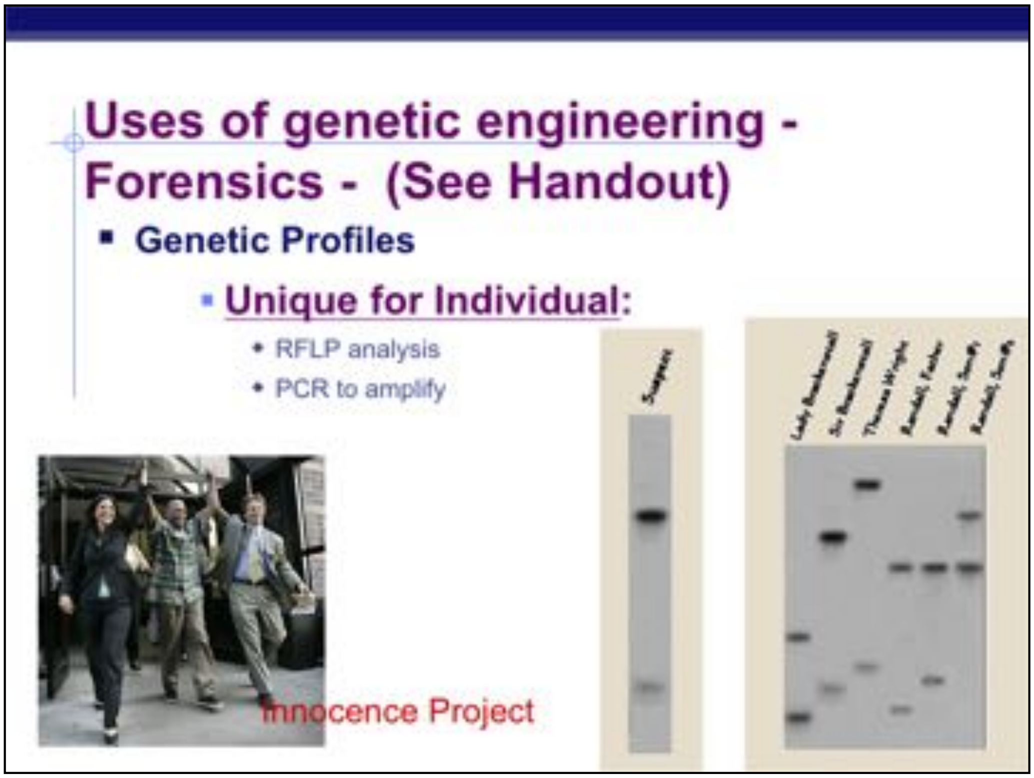
Adenovirus Used.



Adenovirus Used.



Adenovirus Used.



Adenovirus Used.

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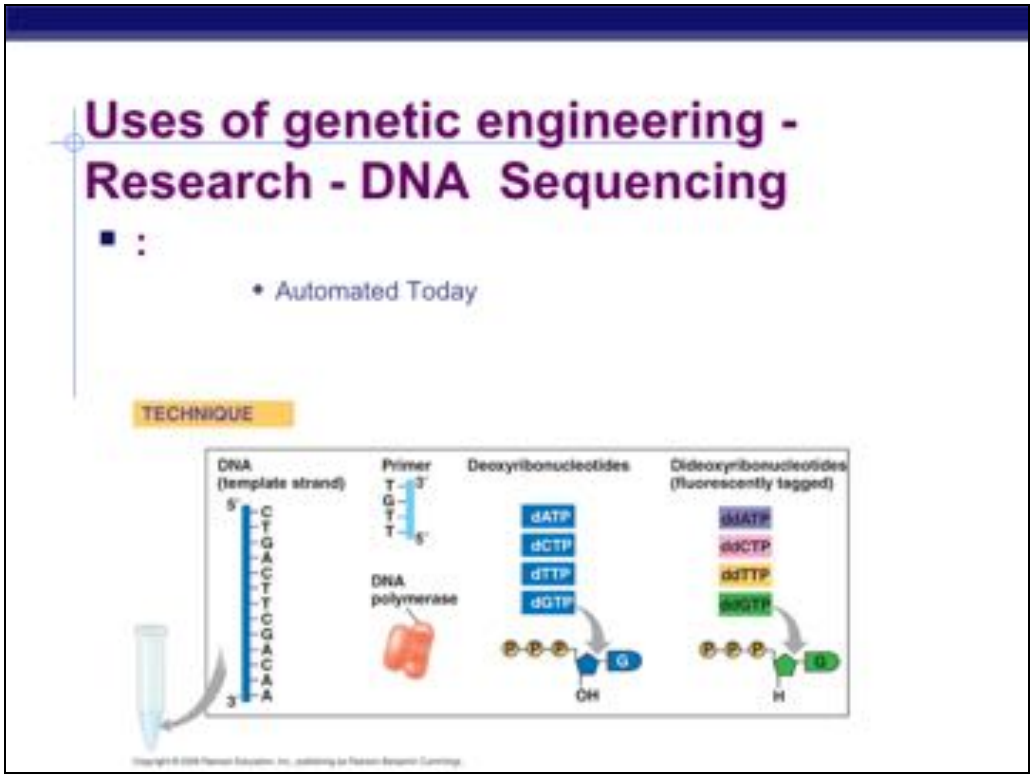
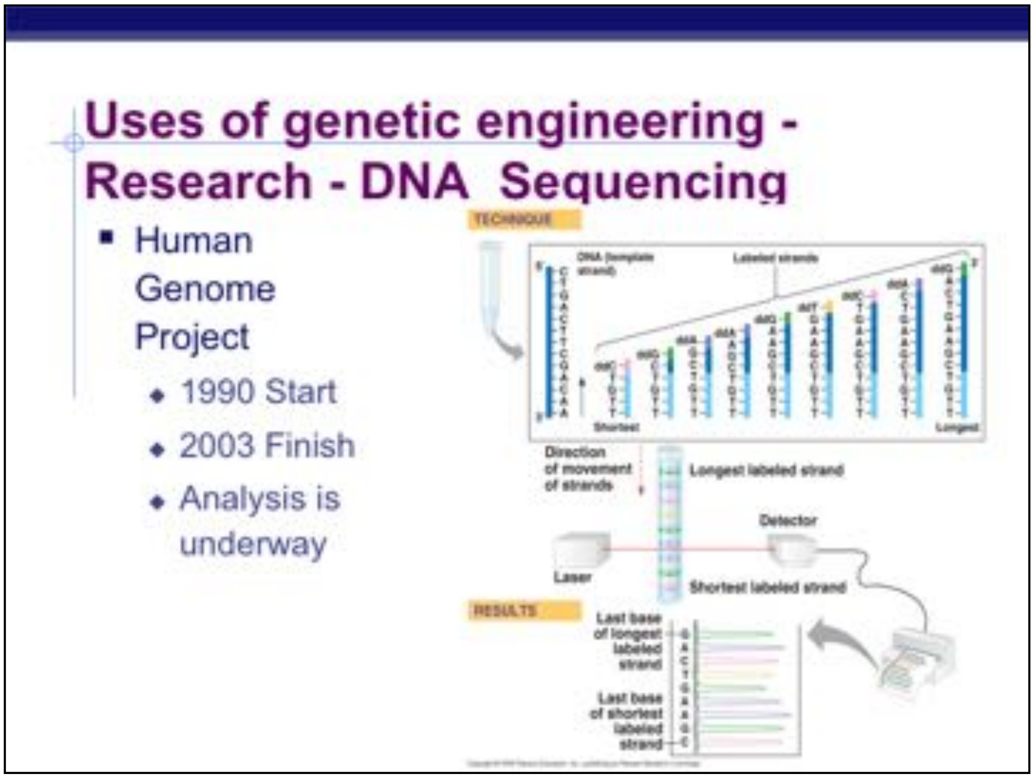


Adenovirus Used.



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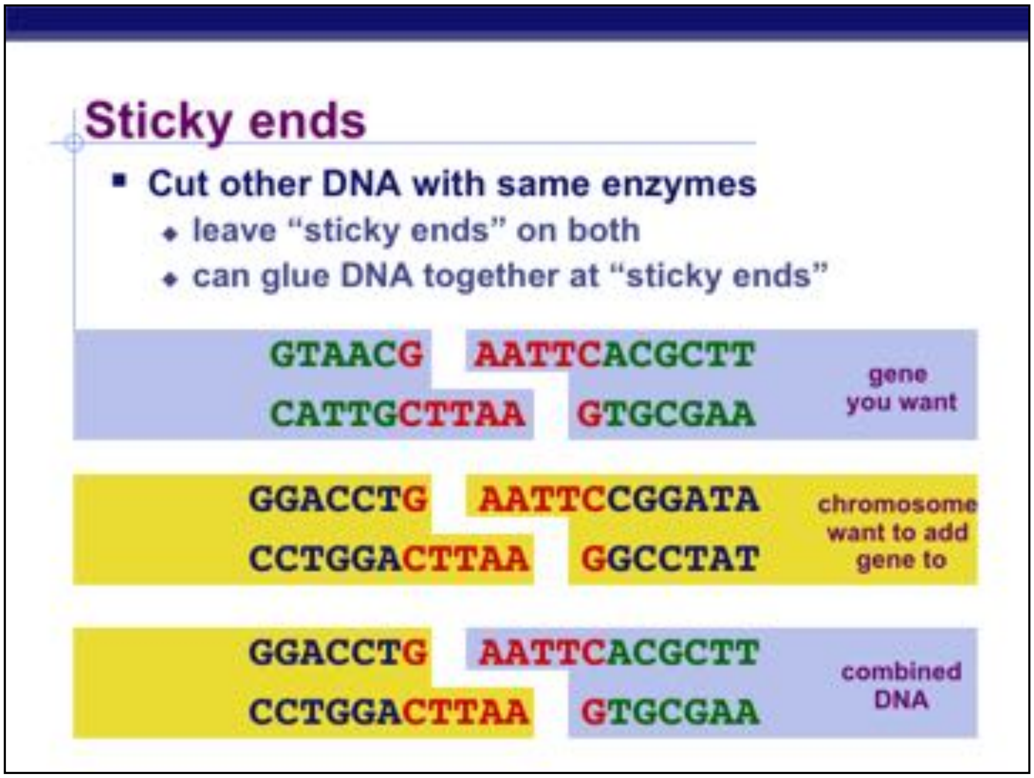
Sticky ends
Cut other DNA with same enzymes
leave "sticky ends" on both
can glue DNA together at "sticky ends"
GTAACG
AATTCACGCTT
CATTGCTTAA
GTGCGAA
gene you want
GGACCTG
AATTCCGGATA
CCTGGACTTAA
GGCCTAT
chromosome want to add gene to
GGACCTG
AATTCACGCTT
CCTGGACTTAA
GTGCGAA
combined DNA