

MANIPULATING and ANALYZING DNA

- Discuss with the person next to you and then write an answer to the following question:
 - Where in life have you heard of biotechnology being used?

Biotechnology:

- Overall biotechnology involves manipulating and analyzing DNA
- This includes biological processes, organisms, or systems to manufacture products intended to improve the quality of human life.

We will discuss the following uses of Biotechnology

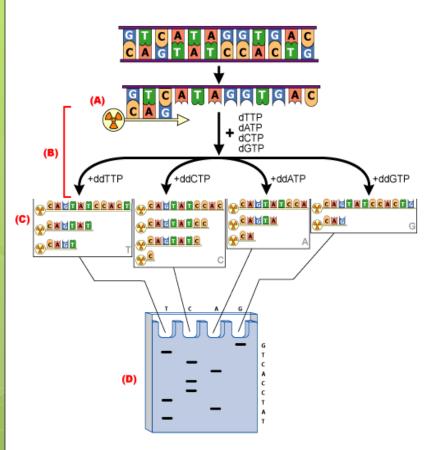
- Crime Scene Investigation
- In Vitro Fertilization and designer babies
- Genetically Modified Organisms
- Cloning
- Therapeutic cloning
- Stem Cell Research

•What do you think genetic engineering is? How does it relate to biotechnology?

Genetic Engineering

- One of the most common tools in biotechnology is the use of genetic engineering
- Definition: The technology including all processes of altering the genetic material of a cell to make it capable of performing desired functions, such as producing novel substances

To complete any genetic engineering, you must...



• DNA Sequencing:

reading or identifying the sequence of bases along the length of a DNA molecule AND understanding what that sequence codes for...

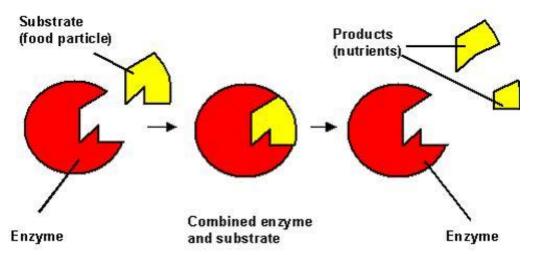
The basic tools of genetic engineering

- 1. restriction enzymes
- 2. plasmids
- 3. recombinant DNA
- 4. transformation

- oLet's review:
 - •What are enzymes? How do they function?

Recall...

- What defines function of a protein?
 - SHAPE DEFINES FUNCTION!!
- The most major class of proteins are enzymes
- Enzymes work like pieces of a puzzle...each is specifically shaped for whatever molecule it acts on



How enzymes break down food into nutrients

Tool #1: Restriction Enzymes

```
5' ...A 6 C T... 3'
3' ...T C G A... 5'
Alul
              5' ...6 6 C C... 3'
3' ...C C 6 6... 5'
Haelll
              5' ... G G A T C C... 3'
3' ... C C T A G G... 5'
BamHI
               5' ...A A G C T T... 3'
3' ...T T C G A A... 5'
HindIII
               5' ...G A A T T C... 3'
3' ...C T T A A G... 5'
EcoRI
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- Enzymes originally found in bacteria that protected against viruses
- RE's chop DNA at specific
 sequences called recognition sites

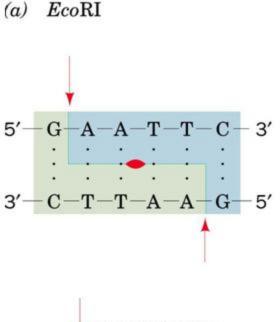
Which will produce blunt ends and which will produce sticky ends?

Alul and Haelli produce blunt ends

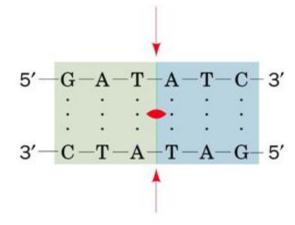
BamHI HindIII and EcoRI produce "sticky" ends

The "Ends"

- Two types of RE cuts...
 - "Sticky" ends leave exposed bases ready to hydrogen bond
 - o "Blunt" ends leave NO bases exposed
- DNA ligase joins "the ends" of cut DNA together



(b) EcoRV

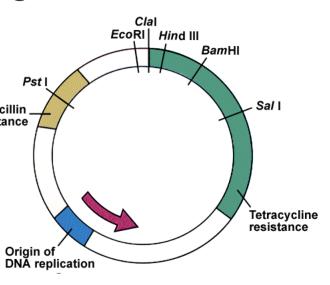


Cleavage site

Twofold symmetry axis

Tool #2: Plasmids

- Circular double-stranded DNA (bacterial)
- Used in biotechnology because:
 - often have multiple recognition sites
 - replicate on their own
 - Small
 - Have genetic markers resistance
- Used to create recombinant DNA



Tool #3: DNA Recombination

- DNA fragments that code for desired traits + bacterial plasmid = <u>Recombinant DNA</u>
- Using restriction enzymes, scientists extract the desired DNA from an organism and cut a plasmid and insert that DNA.
- Recombinant DNA cannot function all by itself
- They must become a part of the genetic material of LIVING cells before the genes they contain can be activated

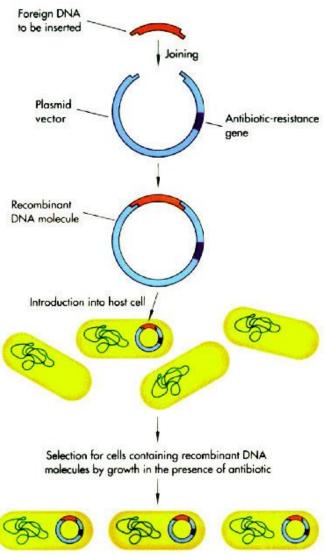
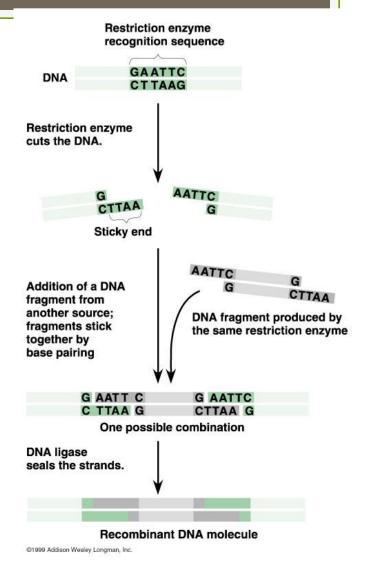


FIGURE 5-11
The cloning of DNA in a plasmid.



 Compare and contrast recombinant DNA and plasmids

Tool #4: Transformation

- Transformation is the process in which recombinant DNA is added into a living cell
- The living cell (usually a bacteria cell) will the express the new DNA

Putting it all together:

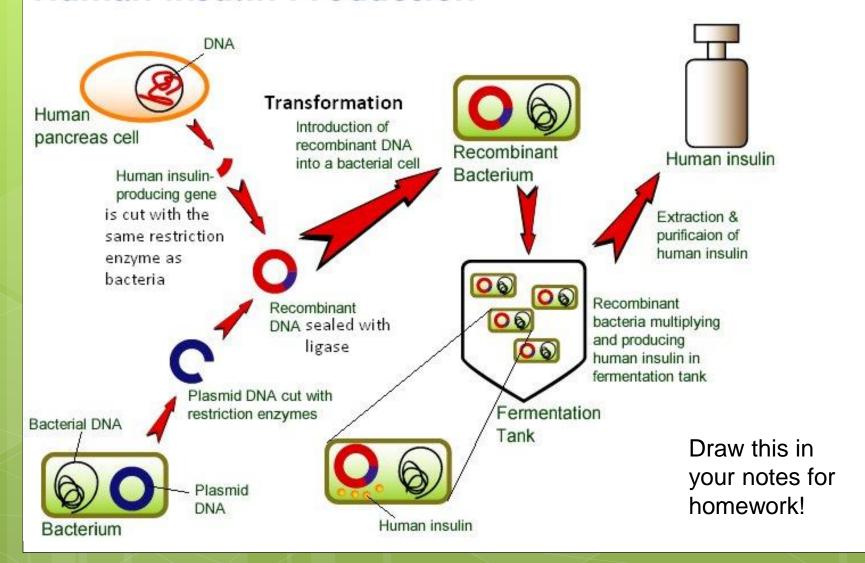
Creating Functioning Recombinant DNA

Steps: using insulin (for sufferers of diabetes) as an example

- 1. Cut open **plasmid** and **DNA with gene encoding for insulin** with same RE
- 2. Mix cut **plasmid** with cut **DNA** (they have the same *sticky* ends)
- 3. Seal with ligase
- 4. Transformation: Insert recombinant DNA into bacteria
- 5. Recombinant DNA replicates and bacteria divides
- 6. DNA is transcribed and translated = insulin

Putting it all together:

Human Insulin Production



•Working with the person next to you, write a summary of how the following are related: restriction enzymes, plasmids, recombinant DNA and transformation