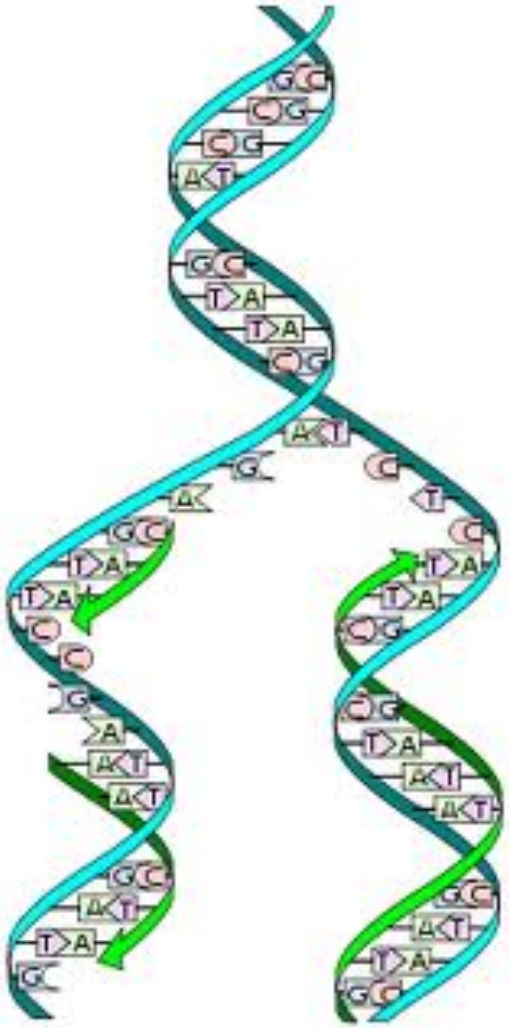
DNA

Chapter 26 and 28

Ted-Ed “Book of You”



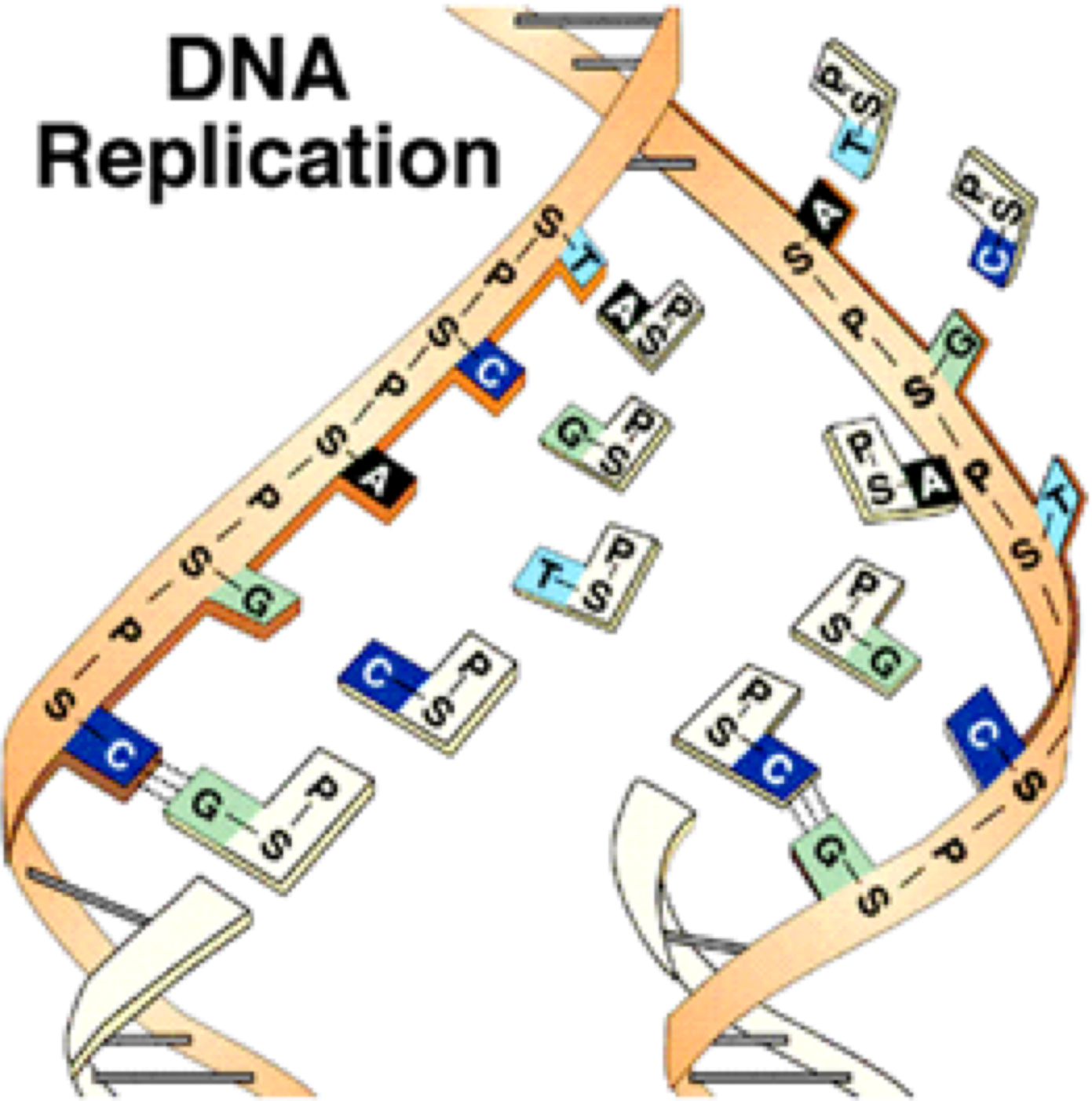
DNA Replication ANIMATION

- DNA making identical copies of itself

- Inherent in DNA's structure is a mechanism for reproducing itself. Before a cell can divide, all of the DNA must be **duplicated**.

- This duplication process is called **REPLICATION**.

DNA Replication



Region of parental DNA helix. (Both backbones are shown in dark color.)

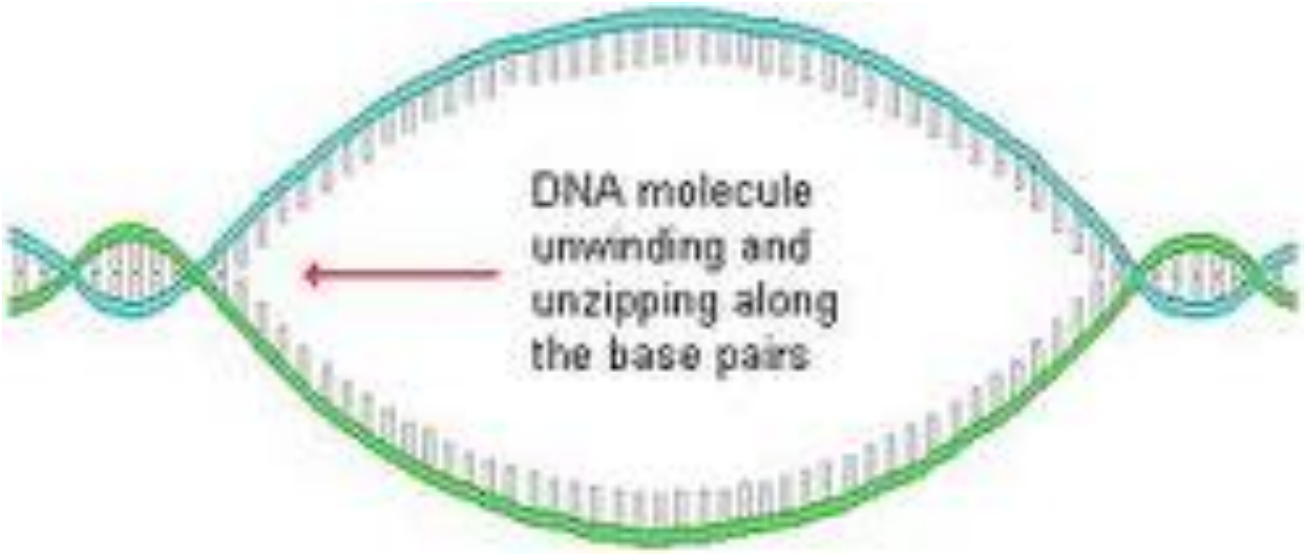
Region of replication (simplified). Parental DNA helix is unwound and unzipped. New nucleotides are pairing with those in parental strands.

Region of completed replication. Each double helix is composed of an old parental strand (dark) and a new daughter strand (light).

- each strand of DNA can be viewed as a template:
- like a potter's mold, it can produce a "reverse image" copy of itself (a complementary copy).
- Each new strand of DNA produced has a sequence of bases exactly complementary to the template strand

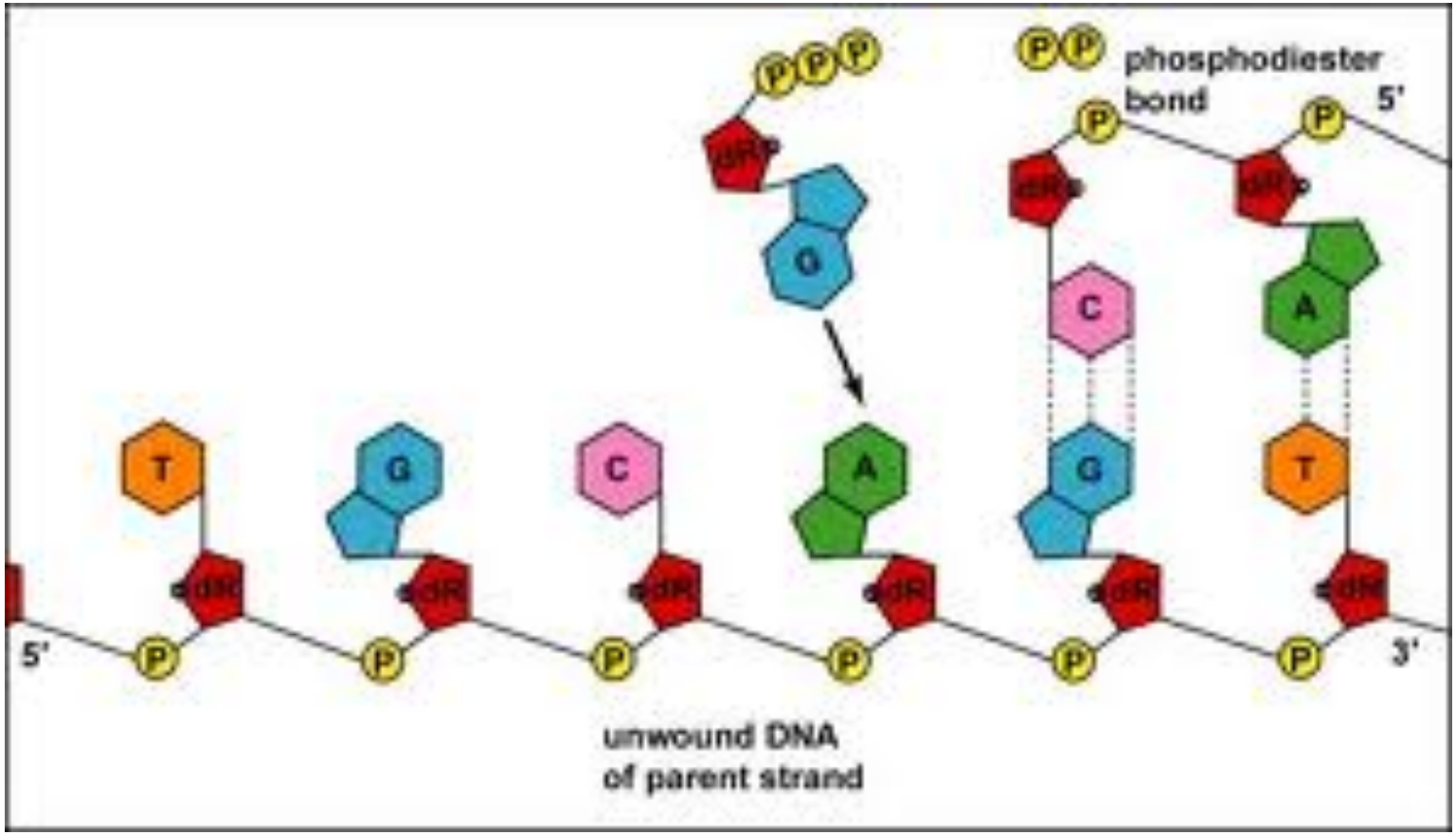
Sequence of Events in Replication:

1. **UNZIPPING:** the DNA double helix unwinds, and the two strands of DNA separate; hydrogen bonds between the bases break



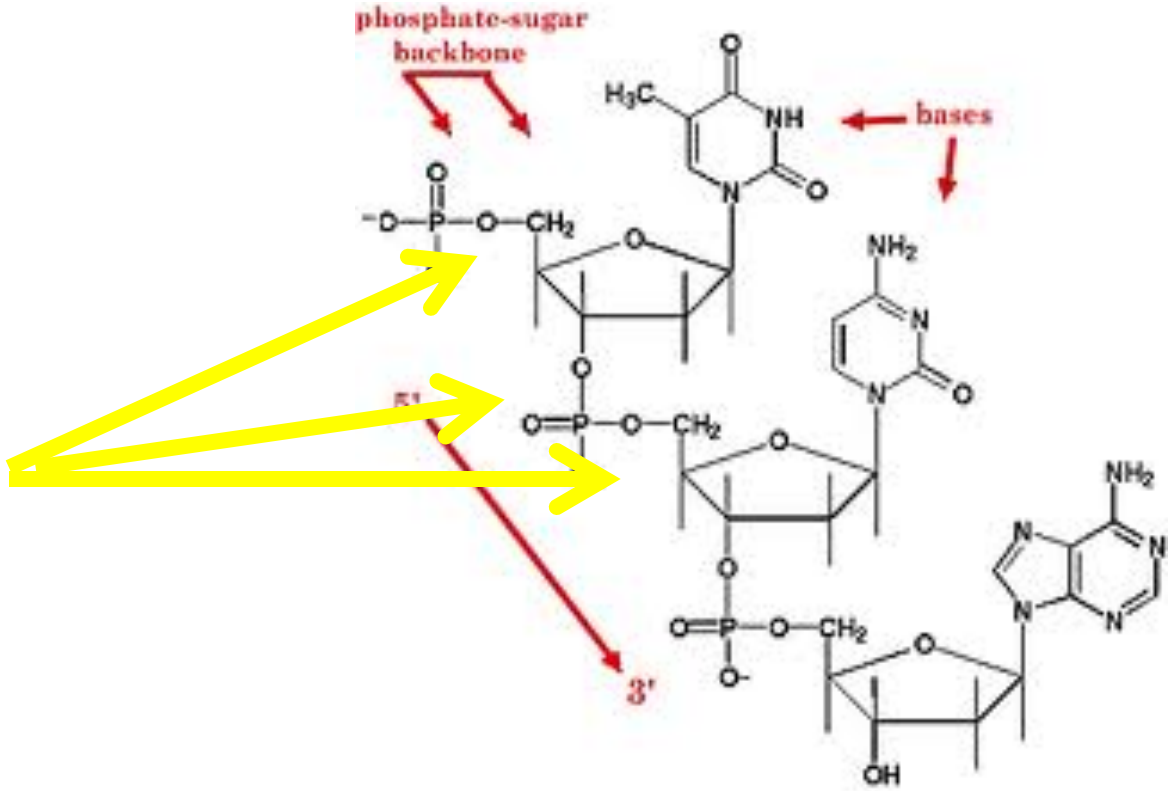
2. COMPLEMENTARY BASE PAIRING:

- new nucleotides move in to pair up with bases of each template strand of DNA. These new nucleotides are always floating around within the nucleoplasm.

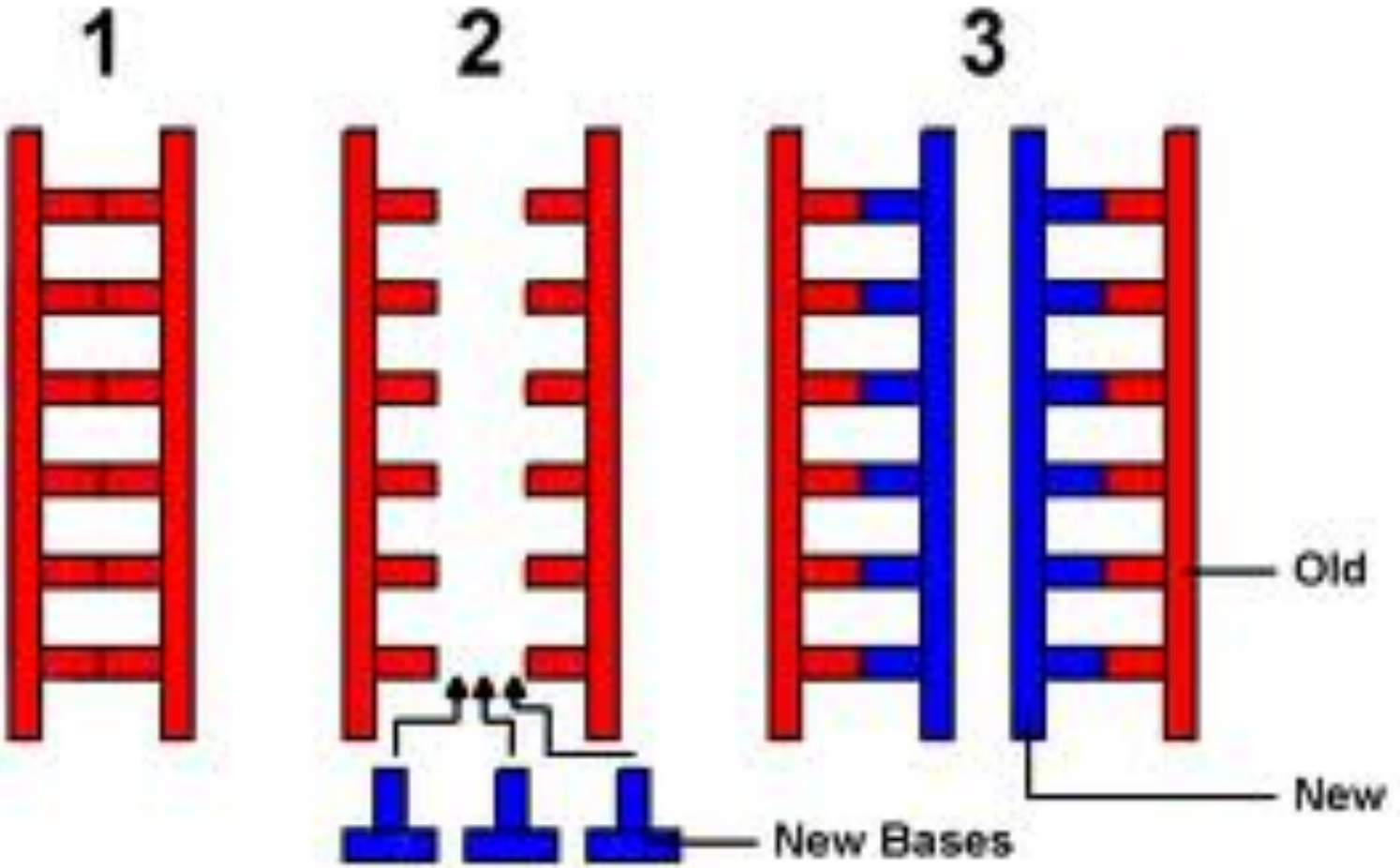


3. ADJACENT NUCLEOTIDES BOND:

- sugar-phosphate bonds form between adjacent nucleotides of the new strand to complete the molecule. The new molecule winds into a double helix.



- each new strand of DNA produced contains one "old" strand (the template) and one new strand. This is known as "SEMI-CONSERVATIVE" replication. Since half of the original molecule is conserved in each of the new molecules, this ensures that there will be very, very accurate replication of the parent molecule.
- this process proceeds by the action of several very specific enzymes (e.g. DNA Polymerases, gyrase, helicase)
- product of replication by one DNA molecule is two complete double-stranded DNA molecules, each with one new strand and one original stand that acted as a template for replication. ANIMATION


1
2
3
Old
New
New Bases

Ted-Ed “Human Genome”

Ted-Ed “Twisting Tale”

Ted-Ed “Chicken or the Egg?”

D3-D4: Recombinant DNA

I. Recombinant DNA

In a Nutshell: CRSPR

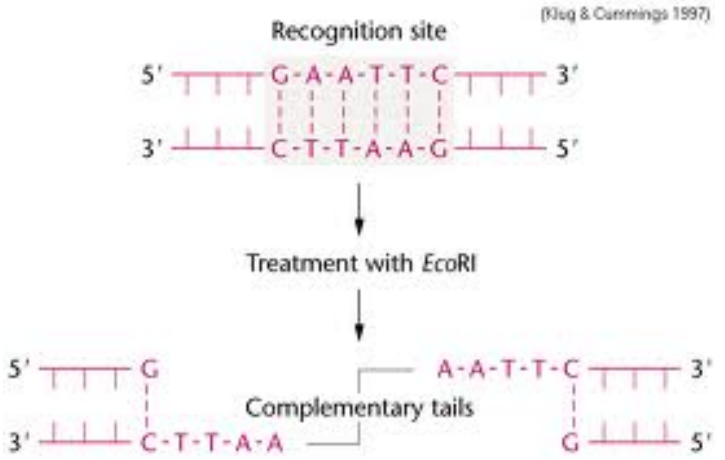
A. Use of various techniques and enzymes to recombine DNA from different organisms

B. Genes from one species can be cut out and inserted into the DNA of an entirely different species

C. The new gene can then be expressed by the recipient species

D. Recombinant DNA technology involves the use of special enzymes as tools:

1. Restriction enzymes cleave DNA at specific sites



2. Other enzymes such as DNA polymerase, Ligase, Reverse transcriptase

II. Uses for Recombinant DNA

A. There are many possibilities for uses of recombinant DNA:

1. Protein production

a. It is possible to isolate a gene from one organism (e.g. Human insulin), and using recombinant DNA techniques, insert that gene into a different organism (e.g. *E. coli*)

b. The new organism can then produce that protein

c. By culturing large quantities of the bacteria it is possible to collect large amounts of Human insulin inexpensively

d. Many other useful human proteins are being produced in this manner (interferon, Growth Hormone, interleukins etc.)

2. Gene therapy

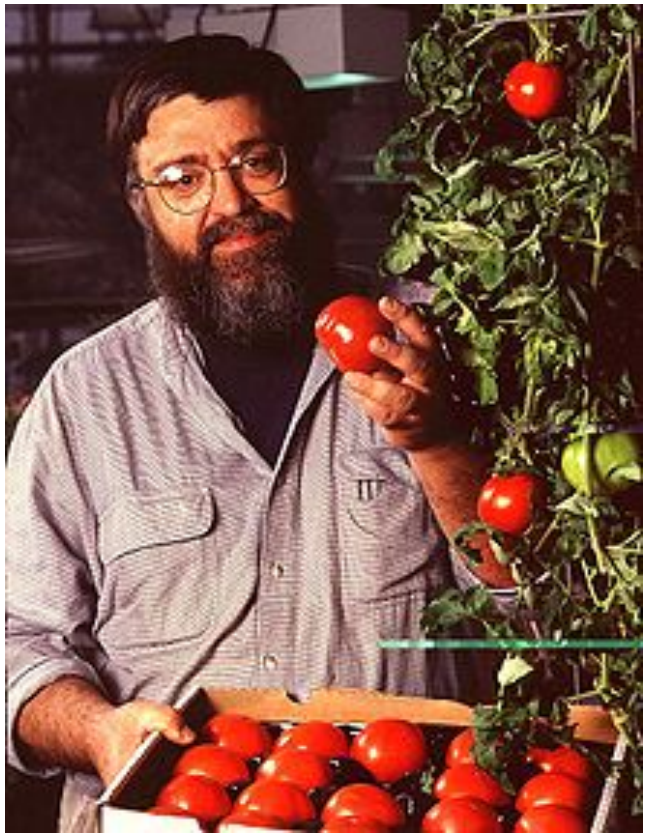
a. It is possible to correct genes in individuals that have non-functional (mutated) genes

b. Example: the corrected gene for the protein that, when mutant, causes Cystic Fibrosis has been inserted into a virus that infects human lung cells (the virulent parts of the virus genes have been deactivated)

c. The virus then injects the corrected cystic fibrosis gene into the cells of the cystic fibrosis patient, and their symptoms are greatly reduced! ANIMATION & ANIMATION

3. Transgenic organisms

a. Selected genes can be inserted into a plant to give it features that were not possible through breeding



The Flavrsavr tomato!
The first transgenic crop to be approved in US
The tomato was made more resistant to rotting by adding an antisense gene which interferes with the production of the enzyme polygalacturonase. The enzyme normally degrades pectin in the cell walls and results in the softening of fruit which makes them more susceptible to being damaged by fungal infections. The modified tomatoes are picked before fully ripened and are then artificially ripened using ethylene gas which acts as a plant hormone.

b. Example: a bacterial insect toxin (*Bacillus thuringiensis*) gene can be inserted into a plant (eg. potato) so the plant is now toxic to insects, and fewer insecticides are needed in order to grow it!

NewLeaf^{tm} Potato is resistant to the Colorado Potato Beetle

4. Safer Vaccines

4. Safer Vaccines

a. vaccines: historically made with treated pathogens

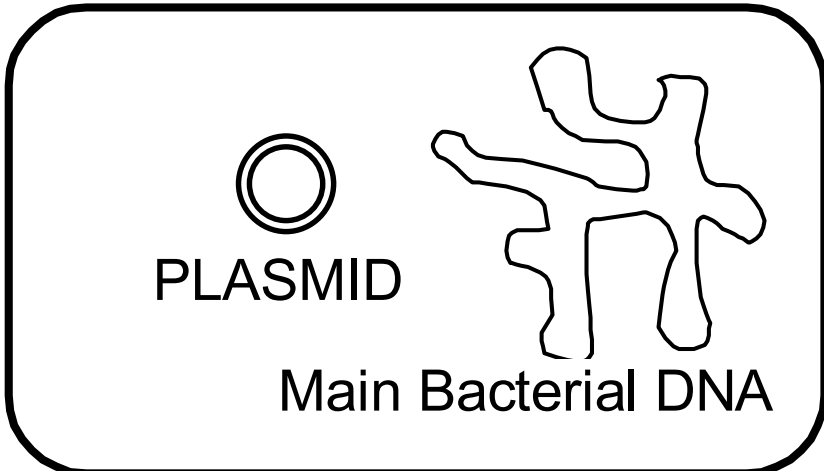
b. some "treated" vaccines not attenuated enough, and caused the disease!

c. can make vaccines out of only the surface proteins from these pathogens (which is what human antibodies would normally bind to, anyway) to train immune system to 'recognize' and attack these pathogens without risking exposure to the actual pathogen

III. Techniques of Genetic Engineering

1. A "vector" is something that can get the DNA from one species into the other species' DNA.

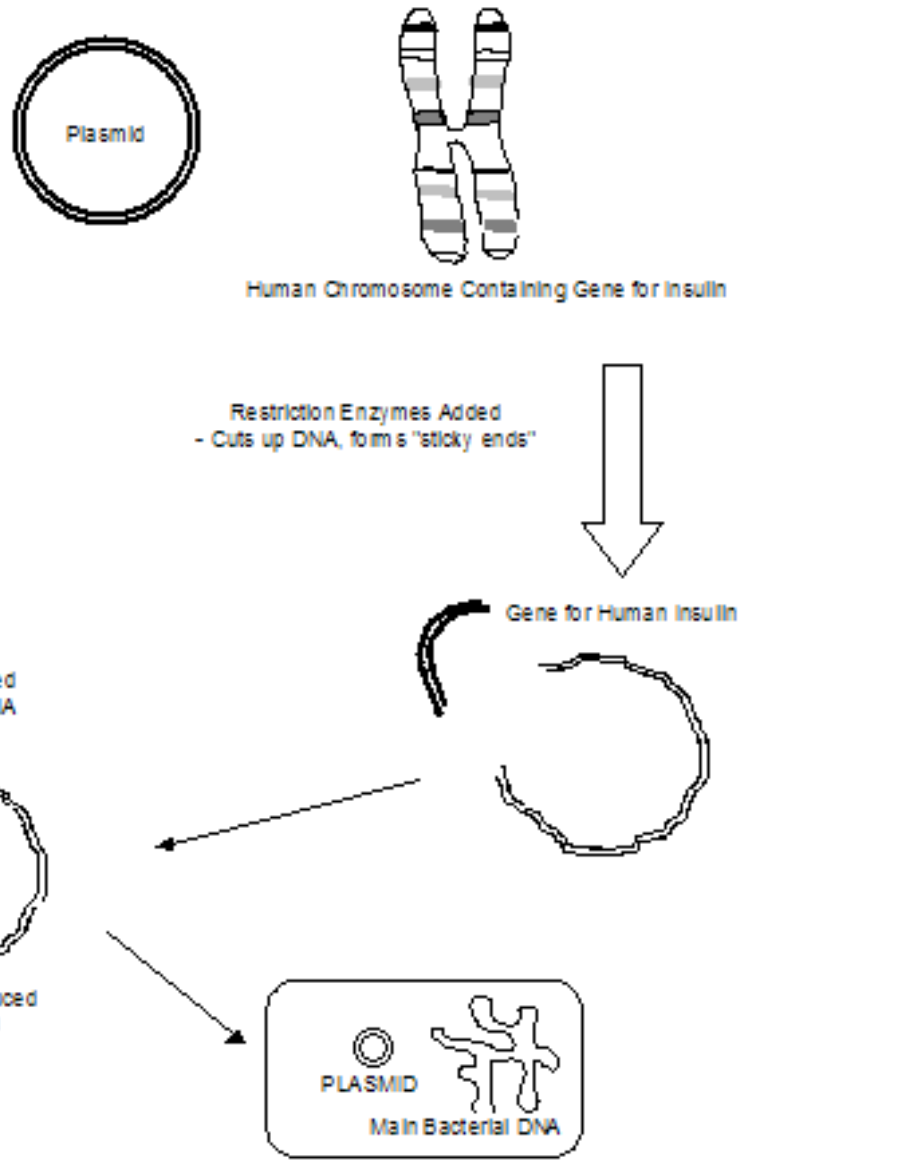
2. Often, this can be a "plasmid", a circular piece of DNA found in some bacteria.



3. A human gene, such as the gene for insulin, is inserted into the plasmid and then the plasmid is taken up by bacteria. The bacteria reproduces the plasmid along with its own DNA when it reproduces, and translates the human gene, producing human protein.

4. This technique can be used to produce cloned DNA by allowing the bacteria to multiply themselves. ANIMATION

Ted-Ed: How CRSPR Works


Plasmid
Human Chromosome Containing Gene for Insulin
Restriction Enzymes Added
- Cuts up DNA, forms "sticky ends"
Gene for Human Insulin
DNA Ligase added
- joins human DNA
into plasmid
Plasmid is introduced
back into bacteria
PLASMID
Main Bacterial DNA
Bacteria can now produce "cloned" DNA
Bacteria can also translate pure human insulin

B. Polymerase Chain Reaction (PCR)

1. PCR can also make large amounts of a desired gene or piece of DNA.

2. PCR can be done without bacteria, inside test tubes, and can amplify billions of times samples with very little DNA (e.g. a single hair from a crime scene, or inside some fossils).

3. PCR makes huge amounts of any gene, quickly by:

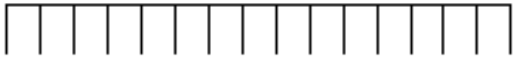
a. Heat the DNA to about 93°C, which unwinds the DNA and separates the two strands.

b. Add some replication primers, and allow to cool

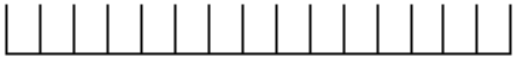
c. Add heat resistant DNA polymerase (Taq polymerase) (the replication enzyme) and free nucleotides. The DNA will copy itself.

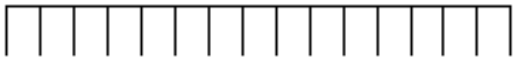
d. Heat and repeat. The DNA will go on doubling itself each "generation".

DNA

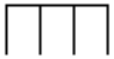


Heat to separate





Add Primers, nucleotides, DNA polymerase, let cool



Replication occurs -Two Strands Now Present

REPEAT

ANIMATION

4. After PCR has been performed, a variety of thing can be done:

   a. Sequence of bases on DNA can be determined (e.g. using the "Sanger Method") and is useful for:

      i. Study evolutionary relationships between organisms (e.g. humans and chimpanzees), and trace the origin of human races.

      ii. Map every single nucleotide on all human chromosomes ("the Human Genome Project")

b. DNA can be analyzed using a DNA probe

i. DNA probe is a specially synthesized single strand of radioactive DNA nucleotides that will bind to a complementary DNA strand on the DNA being tested.

ii. This can be used to detect viral infections, diagnose genetic disorders, and diagnose some cancers.

c. Comparing DNA from two different organisms by using RFLP analysis. (Restriction Fragment Length Polymorphisms)

i. This can provide a "DNA fingerprint" that is unique to each individual (except identical twins).

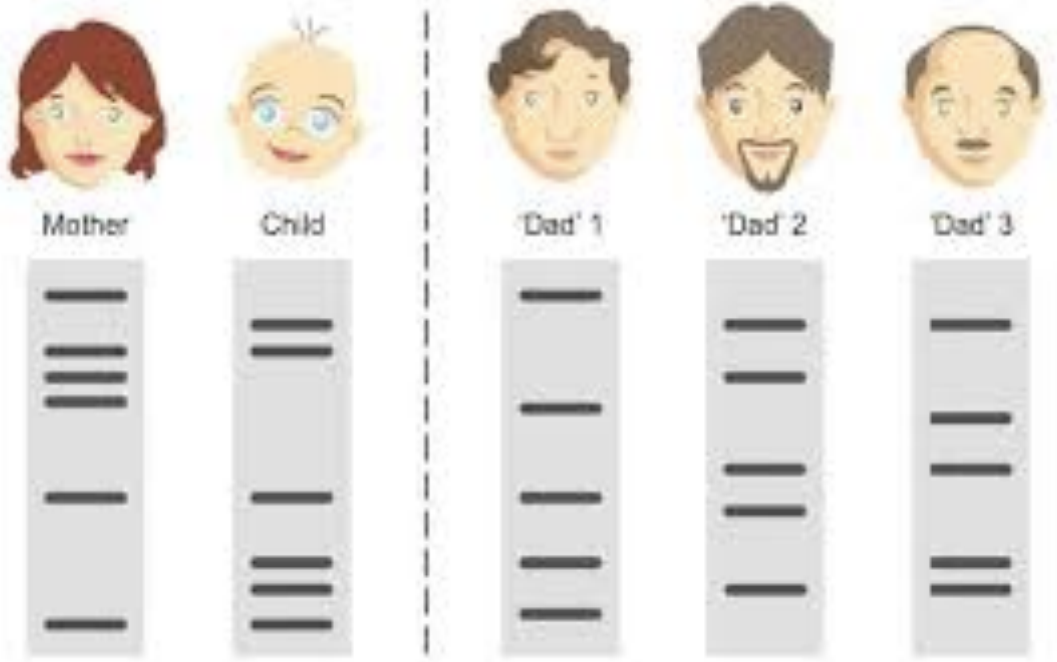
ii. RFLP analysis uses specific restriction enzymes that cut DNA at specific sequences.

iii. This produces fragments that, when separated using gel electrophoresis, produce patterns of bands that can be compared to another person's pattern of bands ANIMATION

iv. If the band pattern is identical, the DNA must have come from the same person.

iv. This can be used to identify a criminal from a blood or semen stain. It can also determine who the father of a child is, with a high degree of accuracy.

ANIMATION



Paternity testing by RFLP Analysis

Outcome 1 – alleged father excluded
Outcome 2 – alleged father excluded
Outcome 3 – Alleged father confirmed

vi. **RFLP analysis is also used to see whether a person carries a gene for a genetic disorder like cystic-fibrosis or sickle-cell anemia, and can be used for prenatal diagnosis.**

vii. **RFLP analysis also contributes to our knowledge of evolution and evolutionary relationships by comparing human and animal DNA.**

ETHICS of rDNA Tech (or later in unit)?

ELSI ANIMATION

Poll Everywhere Activity

Biology Ethics

Case Study: Vaccines