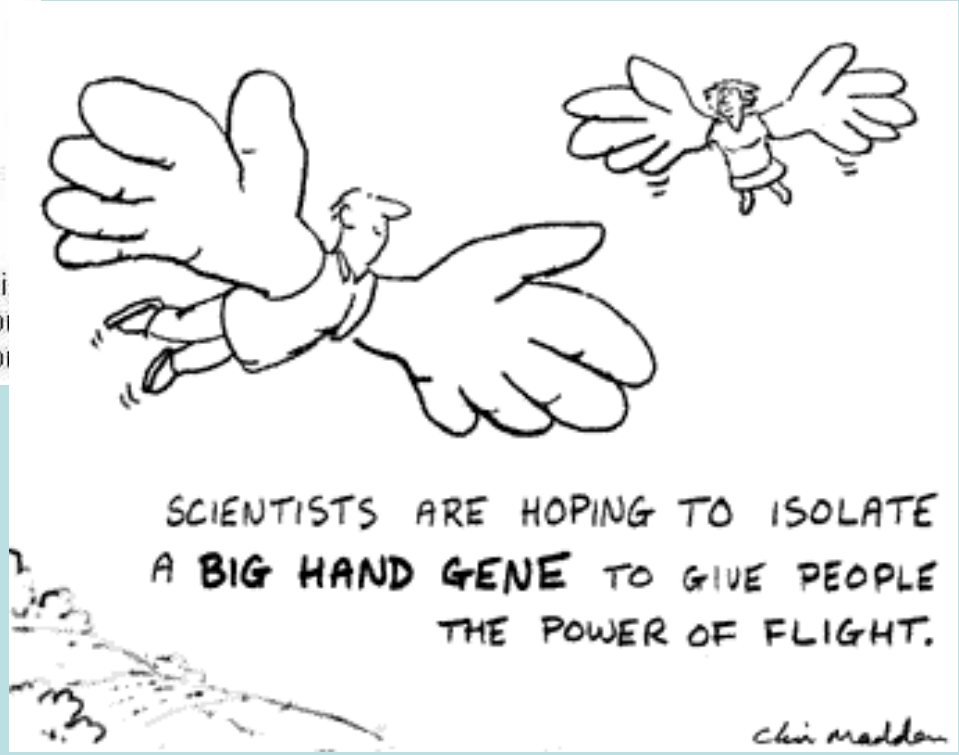
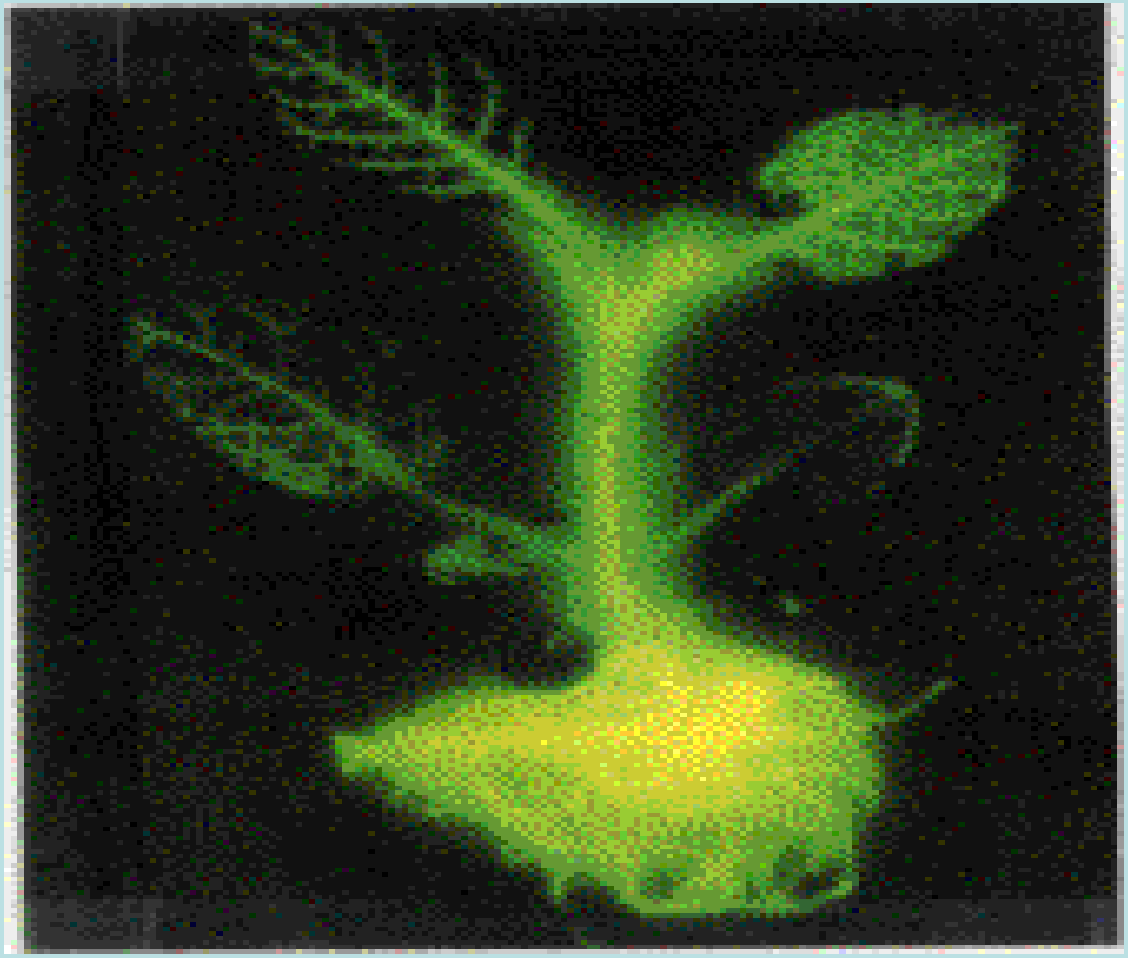
Chapter 13 Genetic Engineering



This genetically engineered plant Glows-in-the-Dark!



A genetically engineered mouse that can grow a human ear!

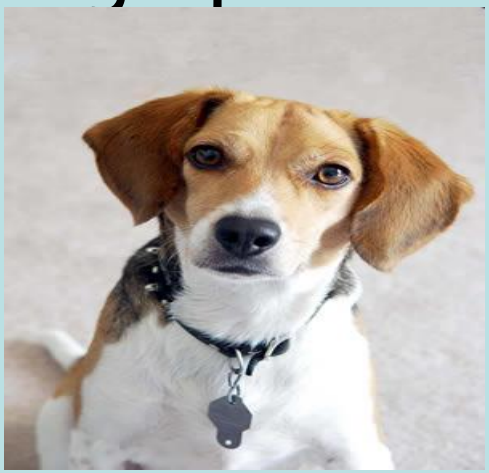
13-1 Changing the Living World

Humans use selective breeding, which takes advantage of naturally occurring genetic variation in plants, animals, and other organisms, to pass desired traits to the next generation of organisms.

Selective breeding allows only those organisms with desired characteristics to produce the next generation.

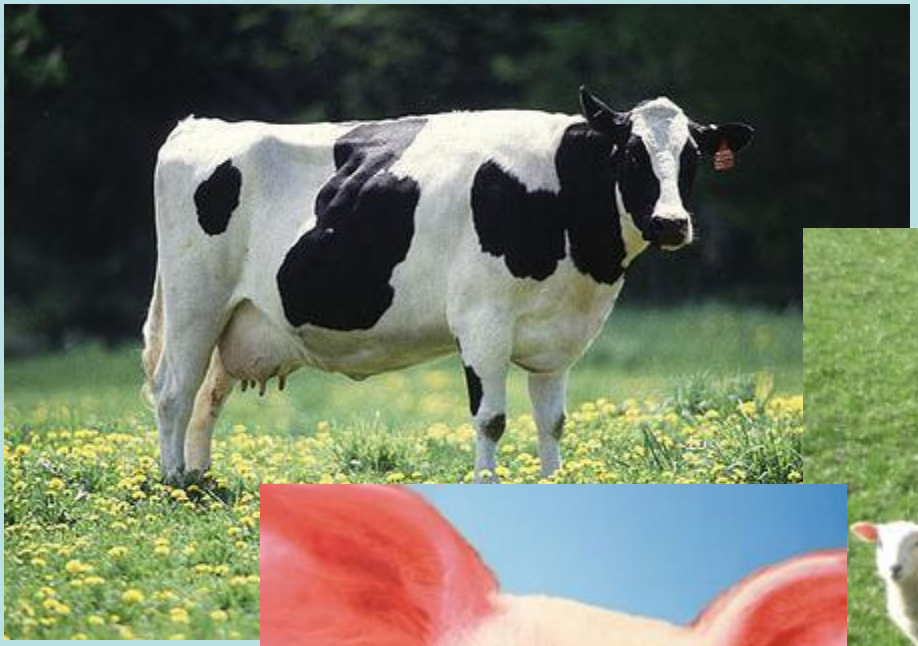
Nearly all domestic animals and most crop plants have been produced by selective breeding.

Ex: dog breeding, horse breeding – choose parents for their traits, attempts to make disease resistant plants that have a high production rate



HORSES

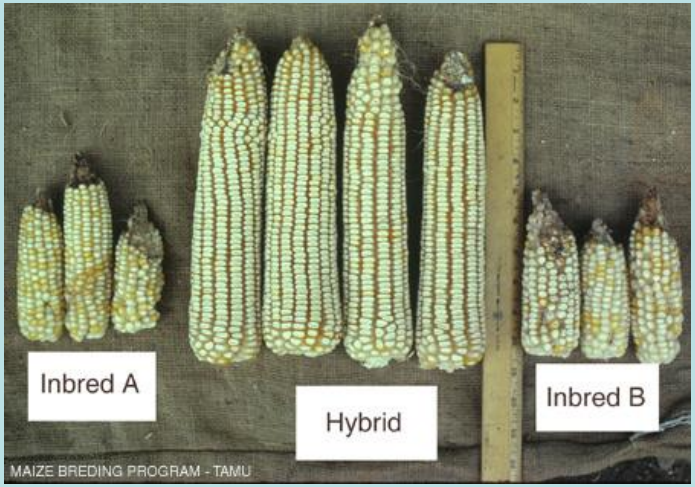
Even Cows, Sheep, & Pigs



All Products of Selective Breeding (Artificial Selection)

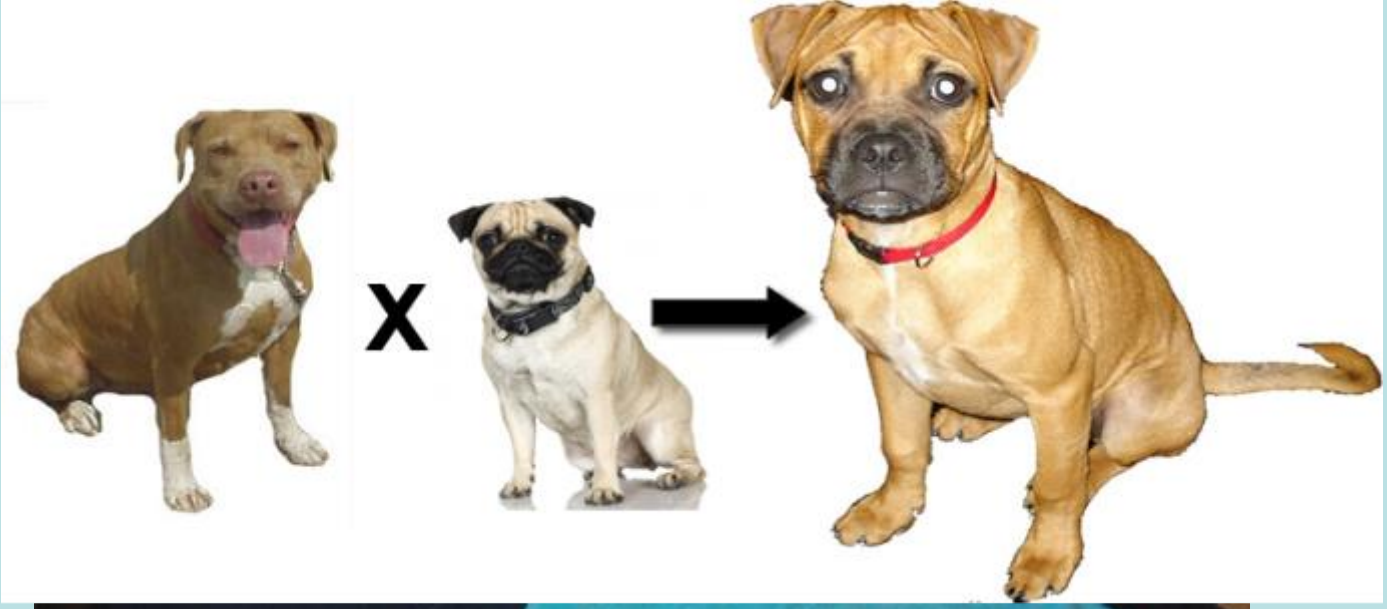
Hybridization

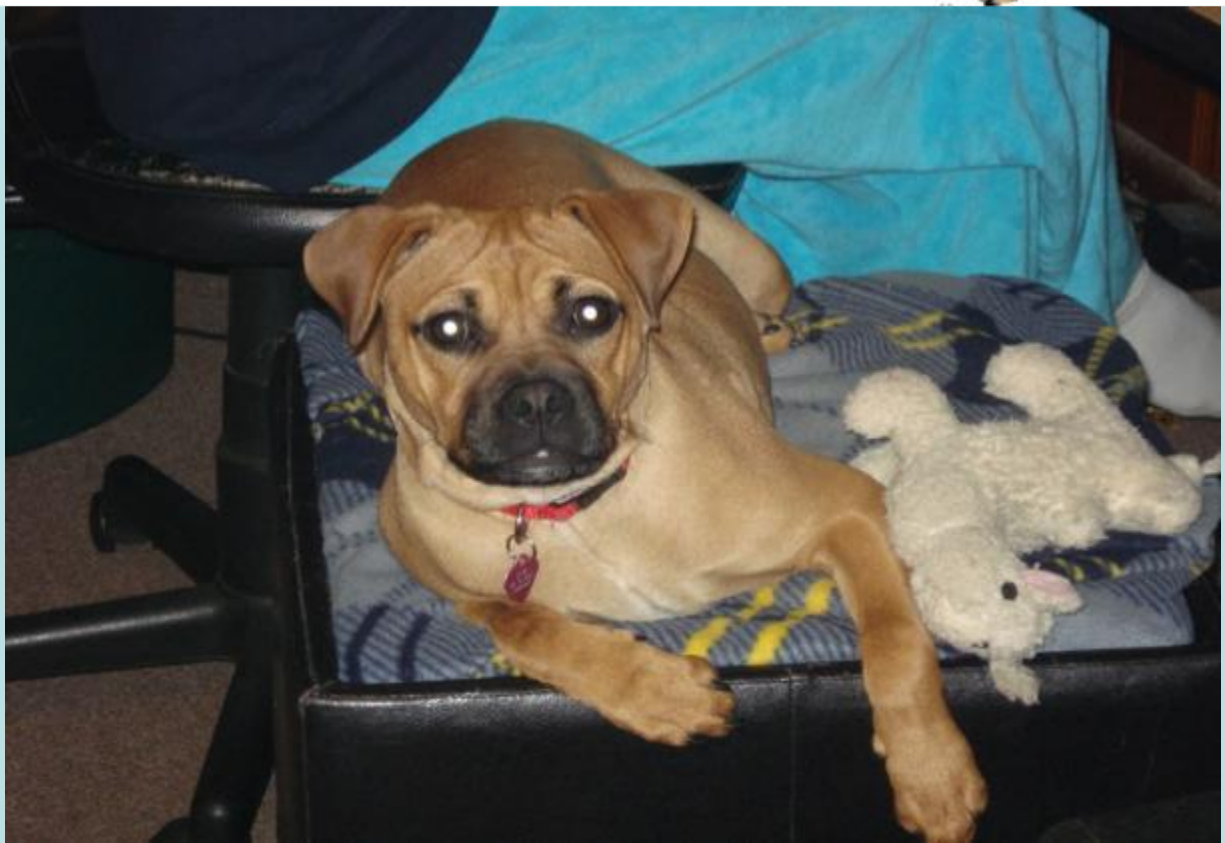
- Crossing (reproducing) different individuals to bring together the best of both organisms
- Produce a Hybrid which are often hardier than parents.
- Hybrids can be different species crossed together or different types within the same species.



Golden Doodle, Puggle

Designer breeds or mutts?


X

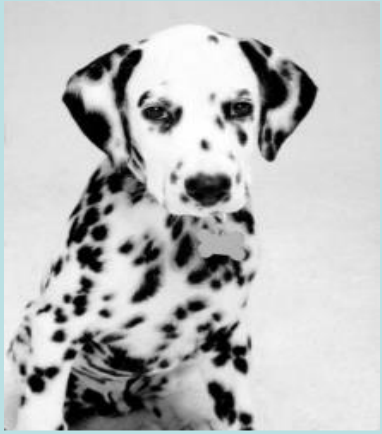


Not to be confused with hybrid cars..

INBREEDING

Inbreeding = continued breeding of similar individuals with similar characteristics to maintain these characteristics
(ex. pure breed dogs)
Has risks… increases breed's susceptibility to disease & deformities

Golden retrievers - epilepsy
Dalmatians - hereditary deafness



First cross puppies

Inbreeding

Persian Cat
flat face= breathing problems

Basset hound
Droopy, baggy eyes=
Prone to eye infection

Shar-pei
Huge rolls of skin=
Skin disease

Increasing Variation

Breeders can increase variation in a population by inducing mutations

Mutation = any change in DNA

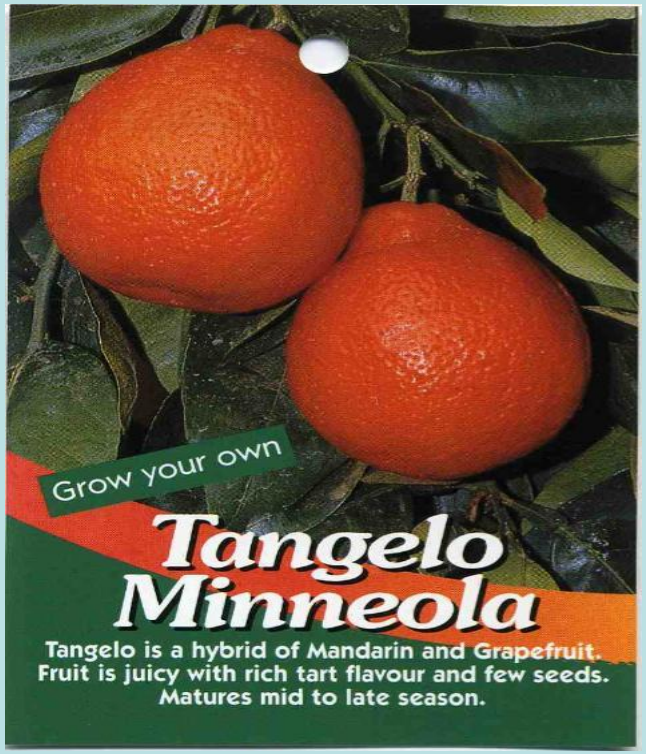
Mutations can happen randomly, as in this Scottish fold cat. Cat enthusiasts bred these cats from a single cat with a mutation for the ears.

Mutations produce new kinds of bacteria
ex. oil-eating bacteria

Mutations produce new kinds of plants

Ex. day lilies, bananas, citrus fruits

Polyploid plants have multiple sets of chromosomes. Makes them larger and stronger



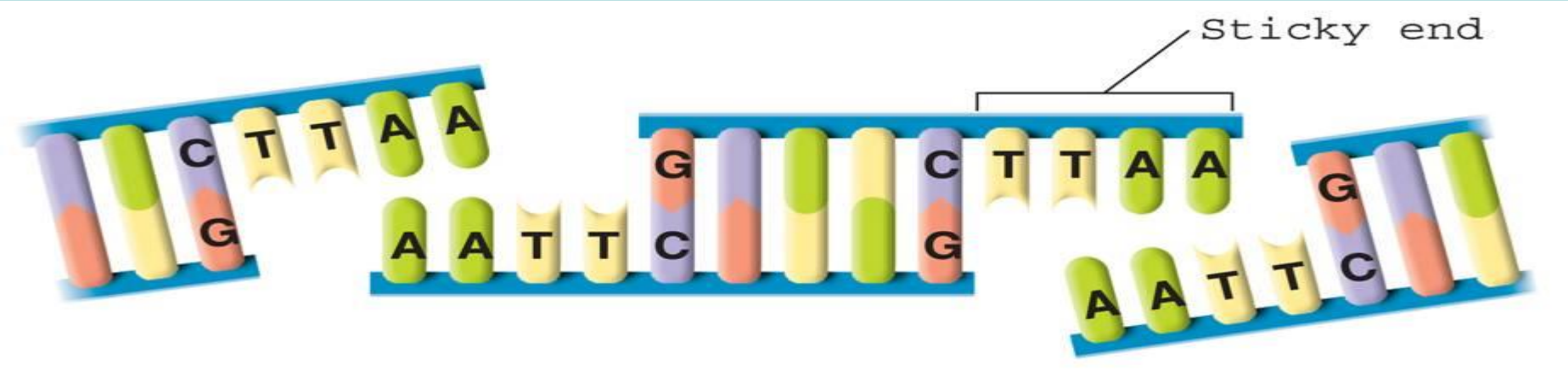
- Selective breeding=desired characteristics to produce next generation
- Hybridization = crossing dissimilar organisms...has resulted in a combination of organisms: puggle
- Inbreeding = continued breeding of individuals with similar characteristics… has resulted in the combination of recessive alleles with genetic defects: weepy eyes in dogs
- Increasing variation=mutation

13-2 Manipulating DNA

How are changes made to DNA?

Scientists use their knowledge of the structure of DNA & its chemical properties to study & change DNA molecules

*This is **GENETIC ENGINEERING**



Genetic engineering

= making changes in the DNA code of a living organism

Different techniques are used…

- to extract DNA from cells
- to cut DNA into smaller pieces
- to identify the sequence of bases in a DNA molecule
- to make unlimited copies of DNA

Some tools we use to change DNA:

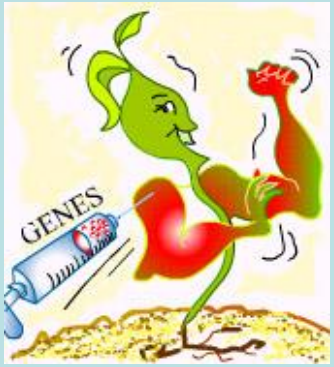
DNA Extraction – taking DNA out of the cell

Restriction enzymes: are special enzymes that cut DNA in specific locations by recognizing certain base sequences ...*small pieces are easier to work with, study and identify*

Gel electrophoresis: separates DNA fragments through a gel using electricity

Cutting and Pasting: we can make new DNA by combining DNA from different sources→ this is called **Recombinant DNA**

Polymerase Chain Reaction **(PCR):** uses DNA polymerase to create thousands of copies of a gene



DNA Extraction

DNA can be extracted from most cells by a simple chemical procedure.

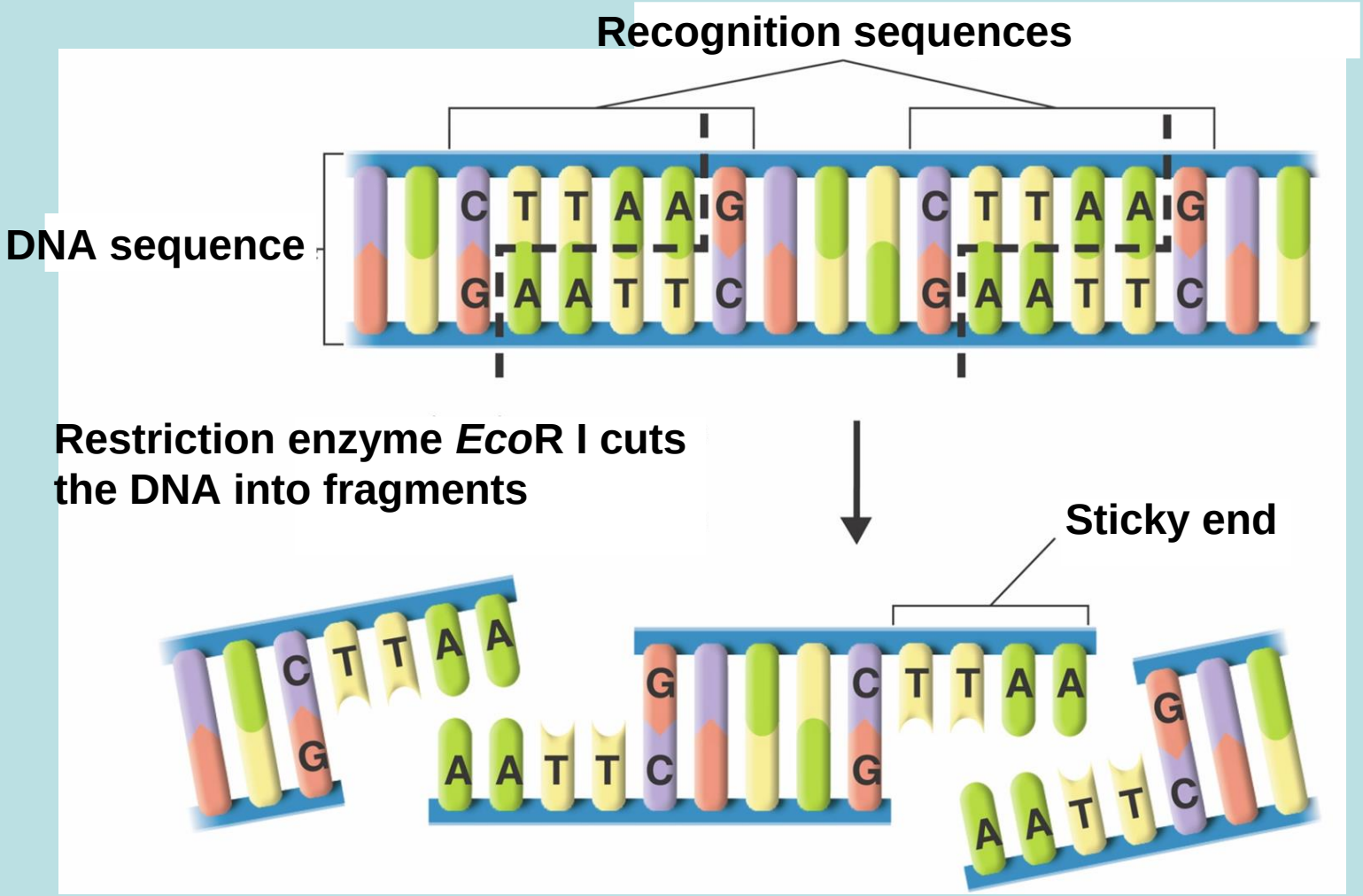
The cells are opened and the DNA is separated from the other cell parts.

Cutting DNA

Most DNA molecules are too large to be analyzed, so biologists cut them into smaller fragments using **restriction enzymes**.

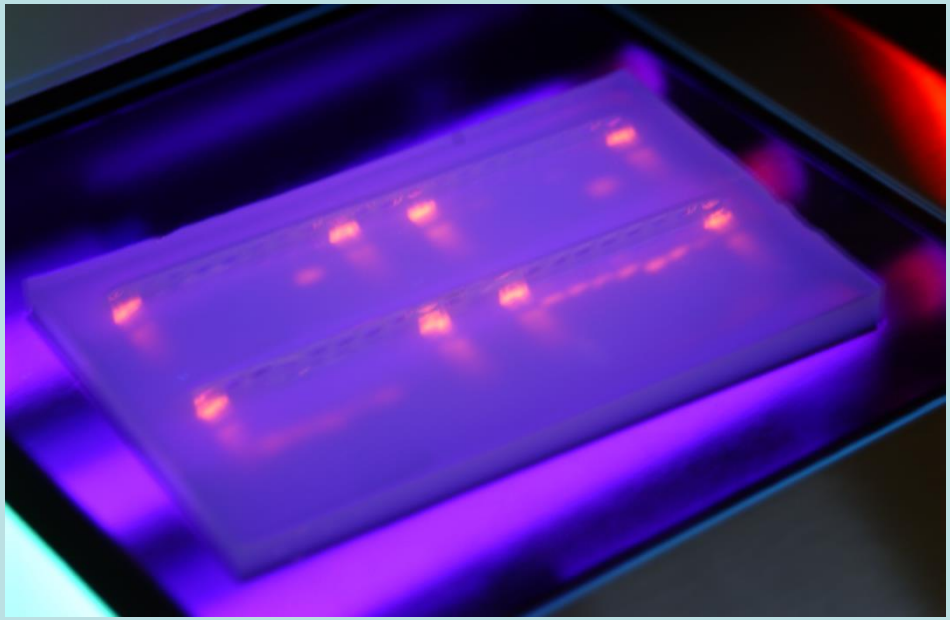
The restriction enzyme is so precise it will only cut a DNA sequence if it matches the sequence precisely.

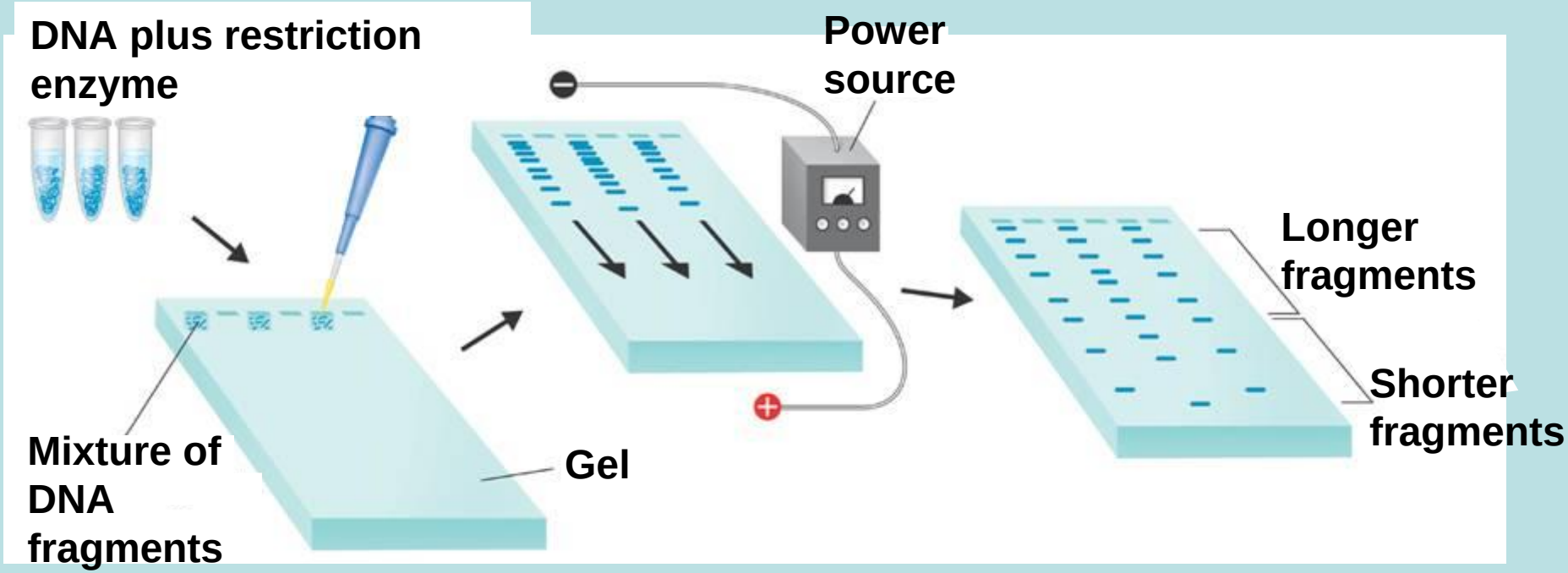
Each **restriction enzyme** cuts DNA at a specific sequence of nucleotides.



Separating DNA

In **gel electrophoresis**, DNA fragments are placed at one end of a porous gel, and an electric voltage is applied to the gel.

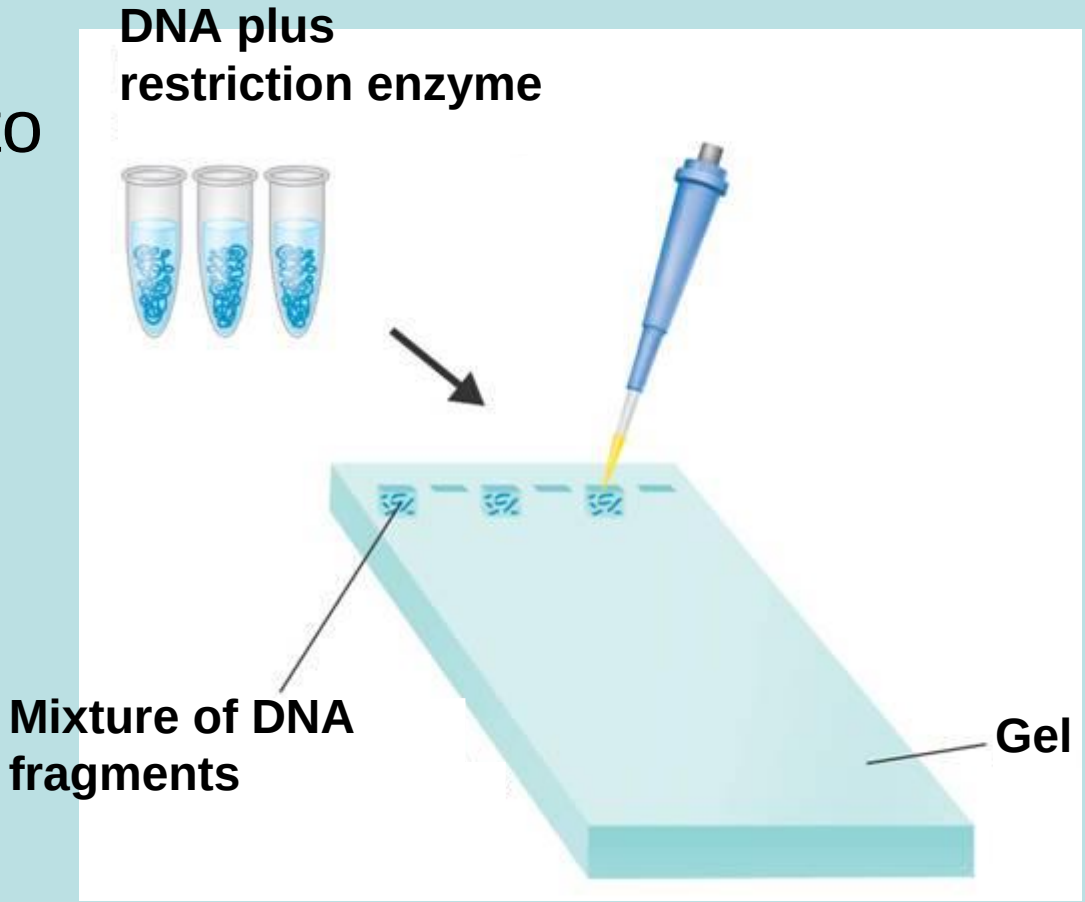




Gel Electrophoresis

First, restriction enzymes cut DNA into fragments.

The DNA fragments are poured into wells on a gel.

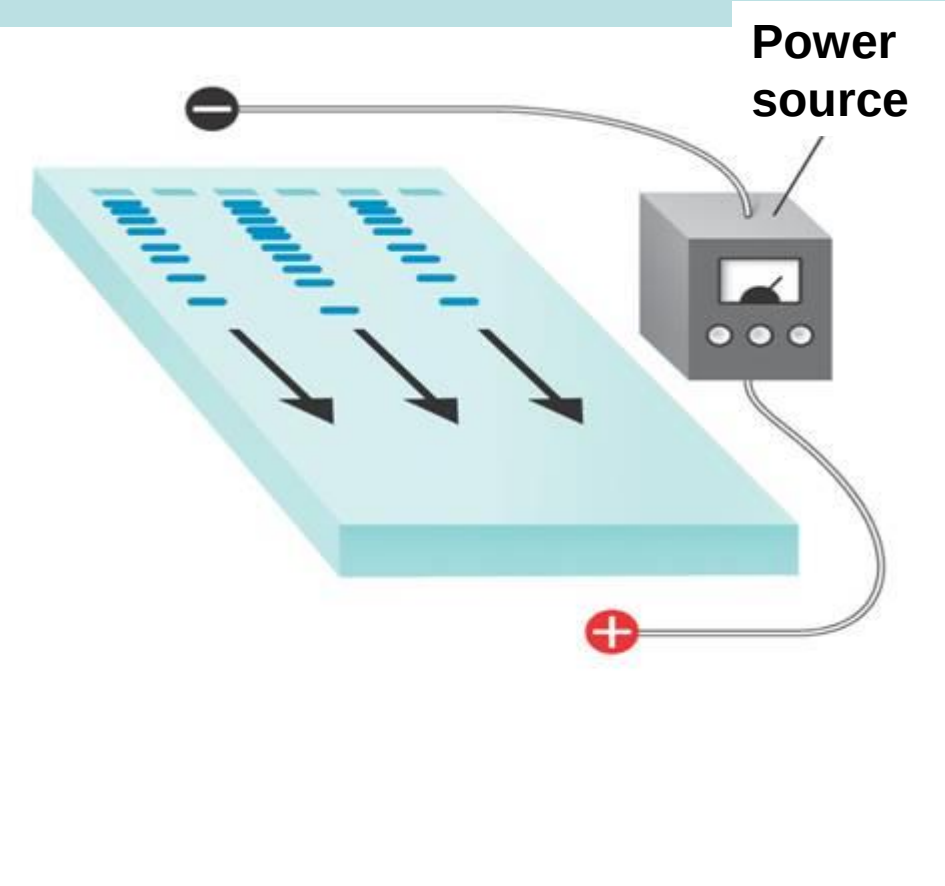


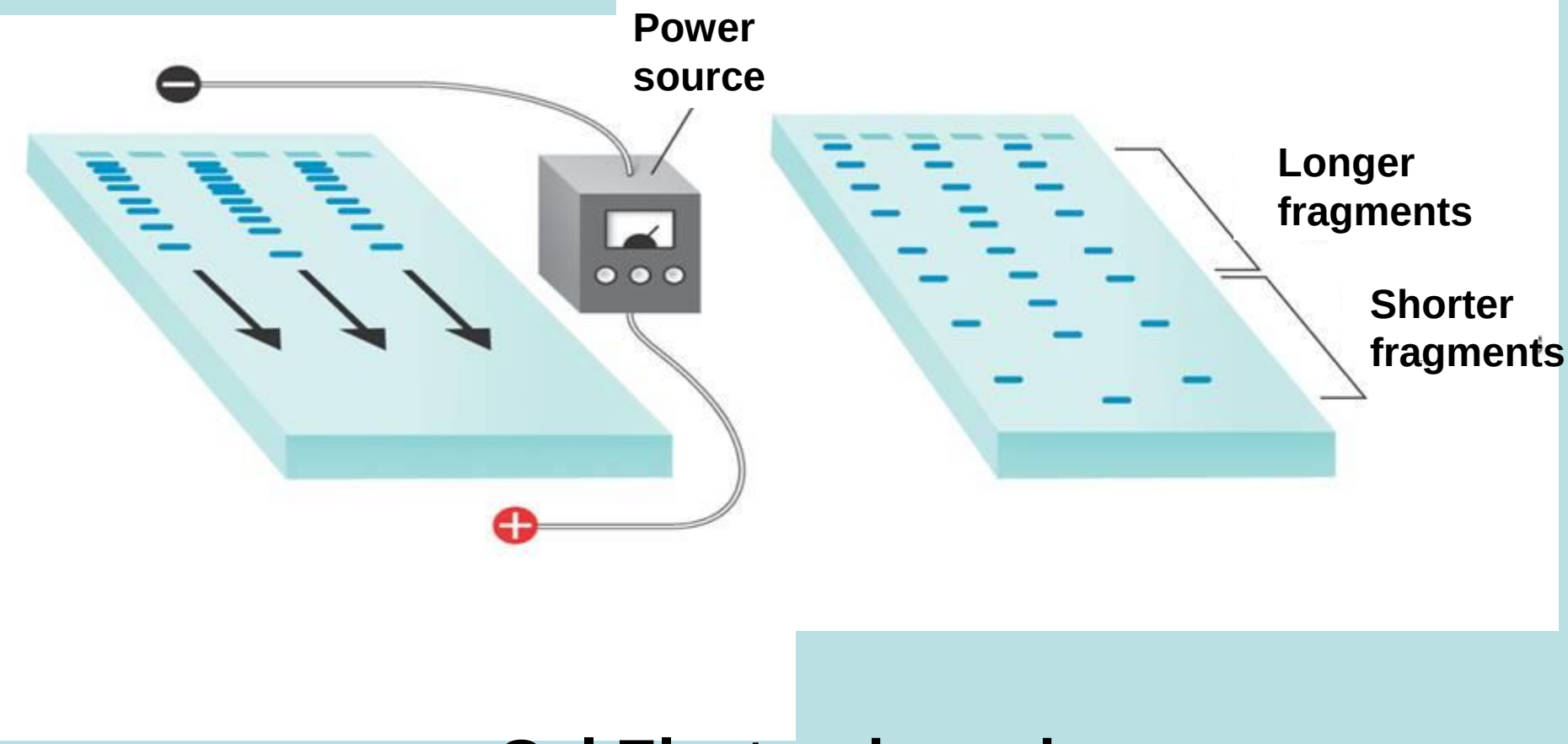
Gel Electrophoresis

An electric voltage is applied to the gel.

DNA molecules are negatively charged so they move toward the positive end of the gel.

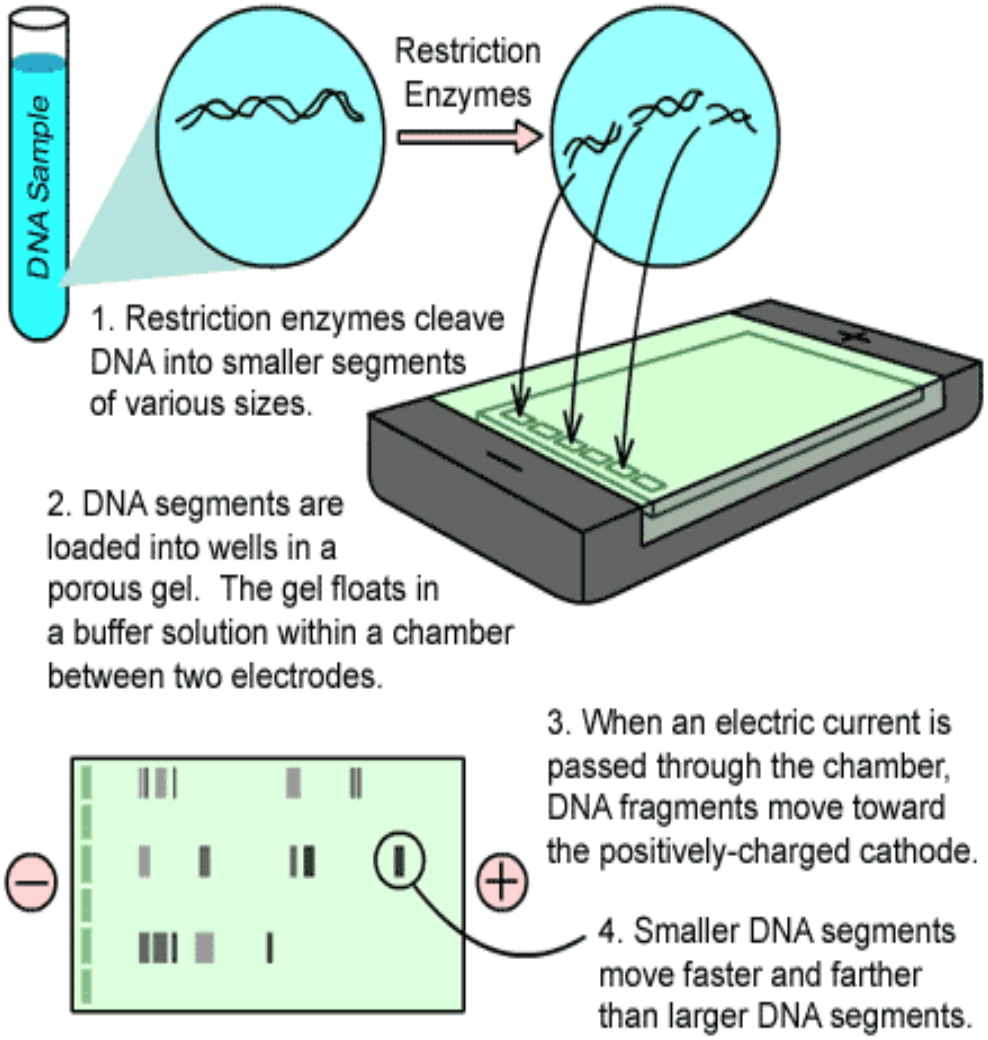
The smaller the DNA fragment, the faster and farther it will move across the gel.

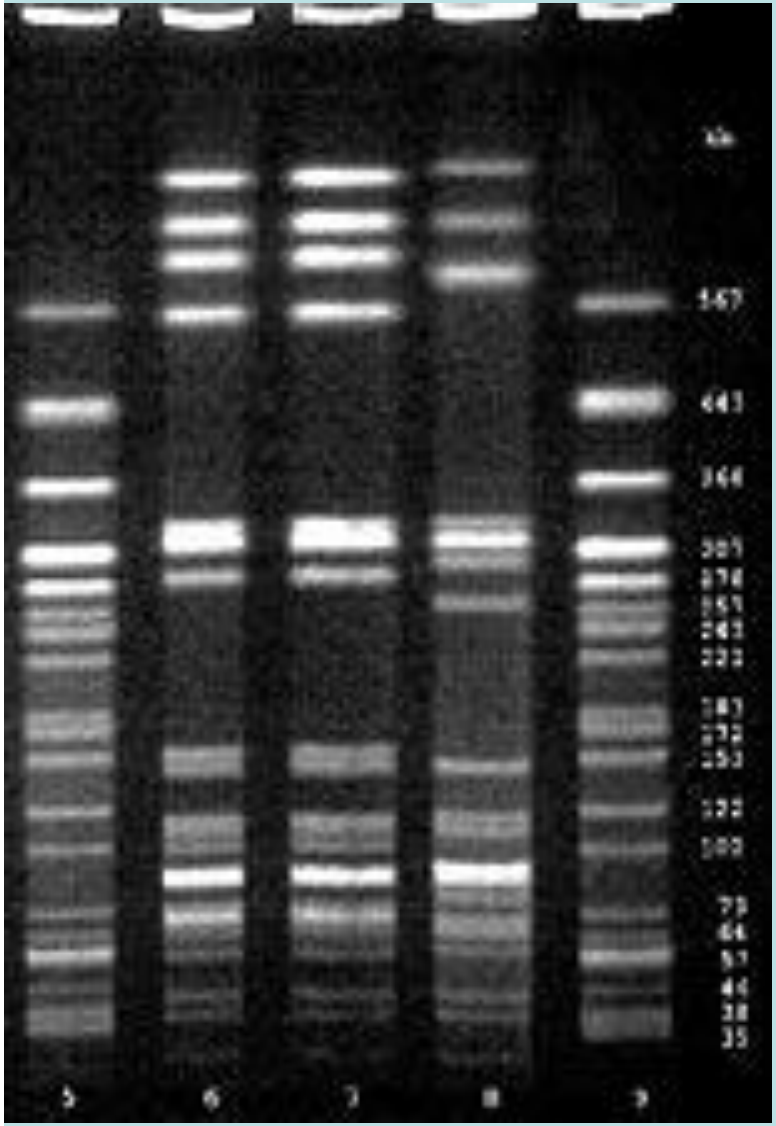




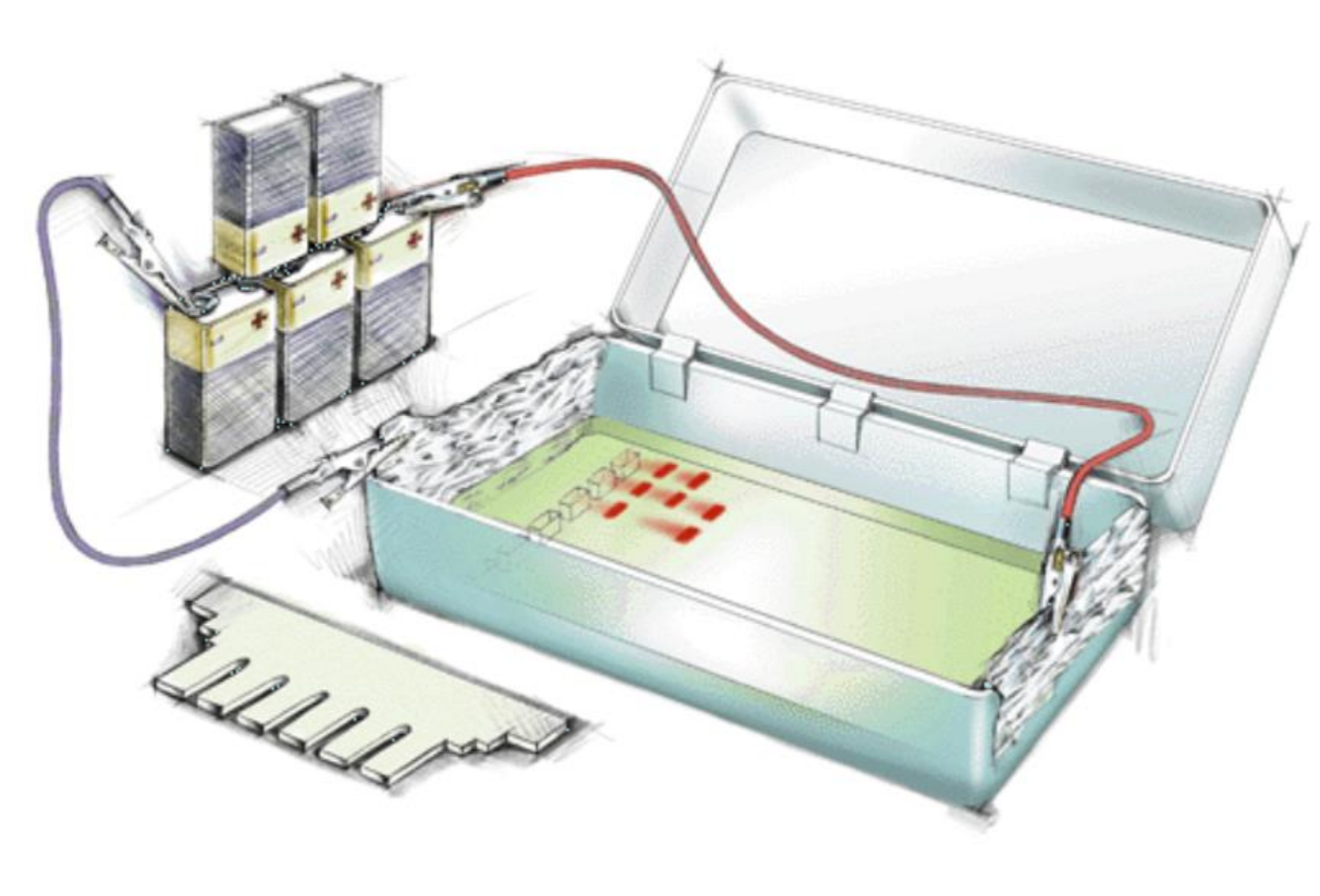
Gel Electrophoresis

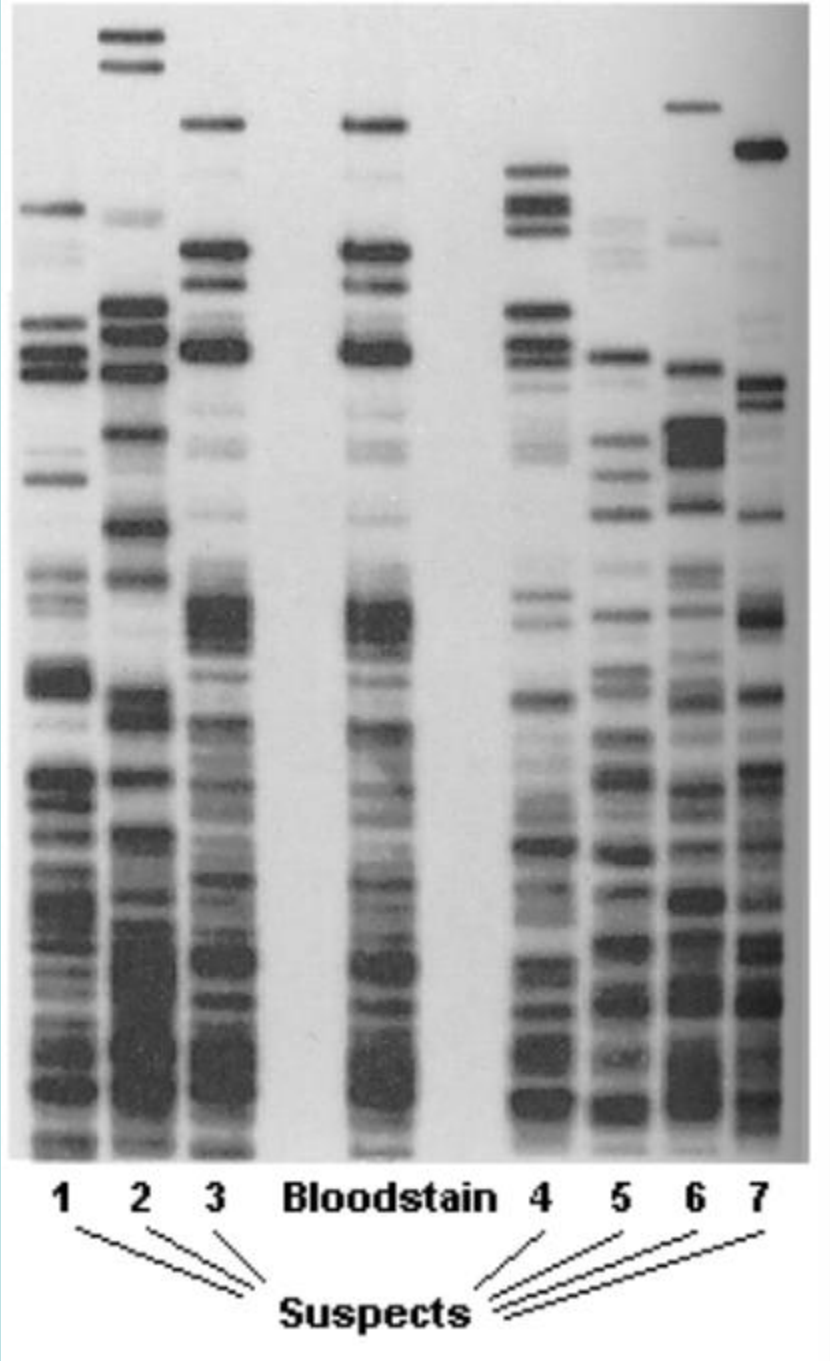
Figure S-2: Gel Electrophoresis

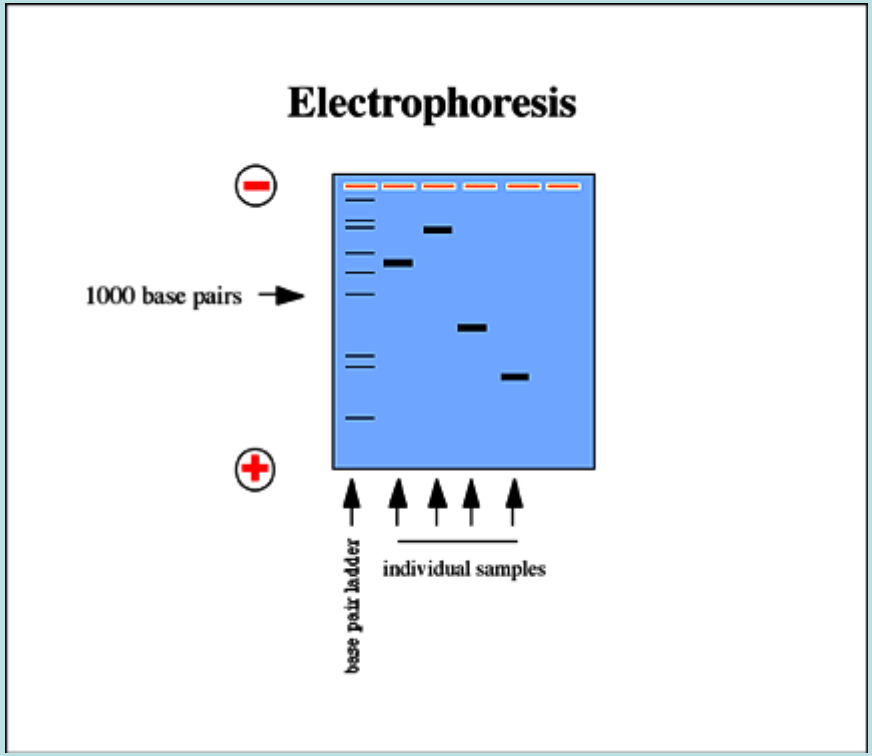





DANGER HIGH VOLTAGE
GREENCELL
10 12:31PM




1
2
3
Bloodstain
4
5
6
7
Suspects

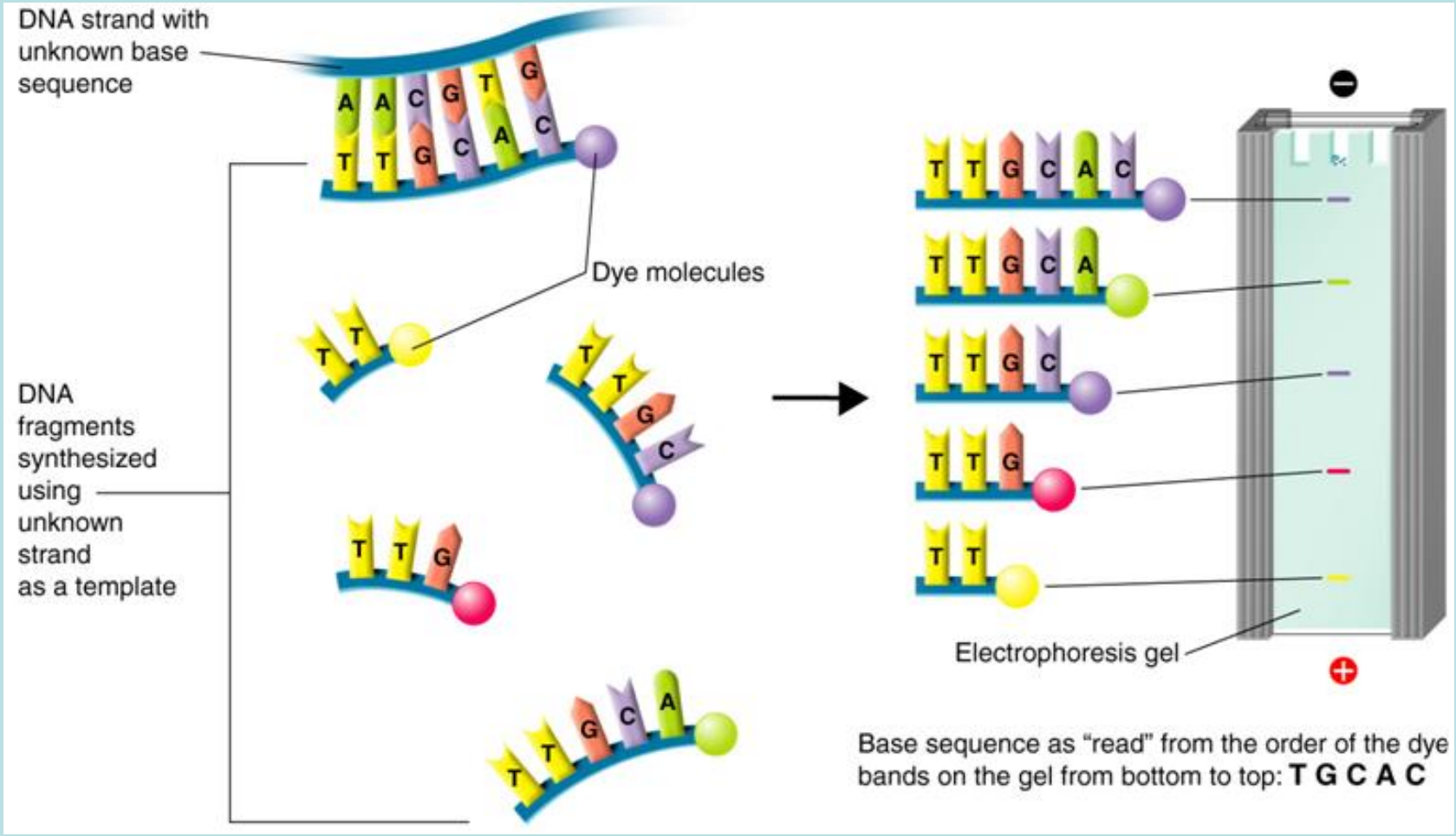

Electrophoresis
1000 base pairs
base pair ladder
individual samples

Reading the sequence

With the DNA fragments separated, DNA molecules can now be read, studied and changed.

Reading the sequence - The fragments that are separated in gel electrophoresis can be tagged with different dyes. The fragments are placed in a test tube with DNA polymerase, four nucleotide bases, and a small number of bases that have a chemical dye attached.

Since each base is labeled with a different color the result is a series of dye tagged DNA fragments of different lengths.
These fragments are separated according to length by gel electrophoresis.
The order of the colored bands on the gel tells the exact sequences of bases in DNA



Cutting and Pasting

Short sequences of DNA can be assembled using laboratory machines called DNA synthesizers. “Synthetic” DNA can be joined to natural DNA sequences using enzymes that splice them together.

These same enzymes make it possible to take a gene from one organism and attach it to the DNA of another chromosome. This DNA is sometimes called recombinant DNA.

Recombinant DNA – DNA produced by combining DNA from different sources.

Making Copies

In order to study genes biologists often need to make copies of a particular gene.

Polymerase chain reaction (PCR) is a technique that allows biologists to make copies of genes.

Small amounts of DNA can be multiplied making it easier to analyze.

Made possible by an enzyme found in a bacterium living in hot springs in Yellow Stone National Park.

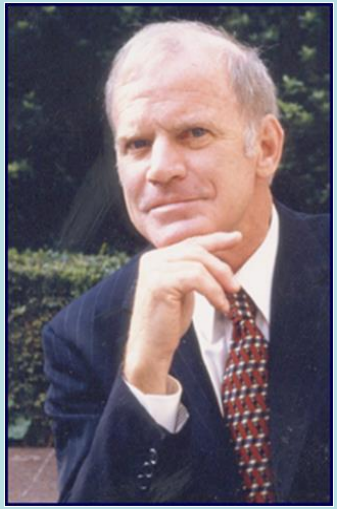
Making Copies

At one end of the piece of DNA scientists want to copy they will add a short piece of DNA that is complementary. That is done at the other end as well.

The short pieces are know as “primers” - they provide a place for DNA polymerase to start working

DNA is then heated to separate the strands, then cooled so the primers can bind to single stranded DNA.

DNA polymerase starts making copies of the region between the primers.



Making copies

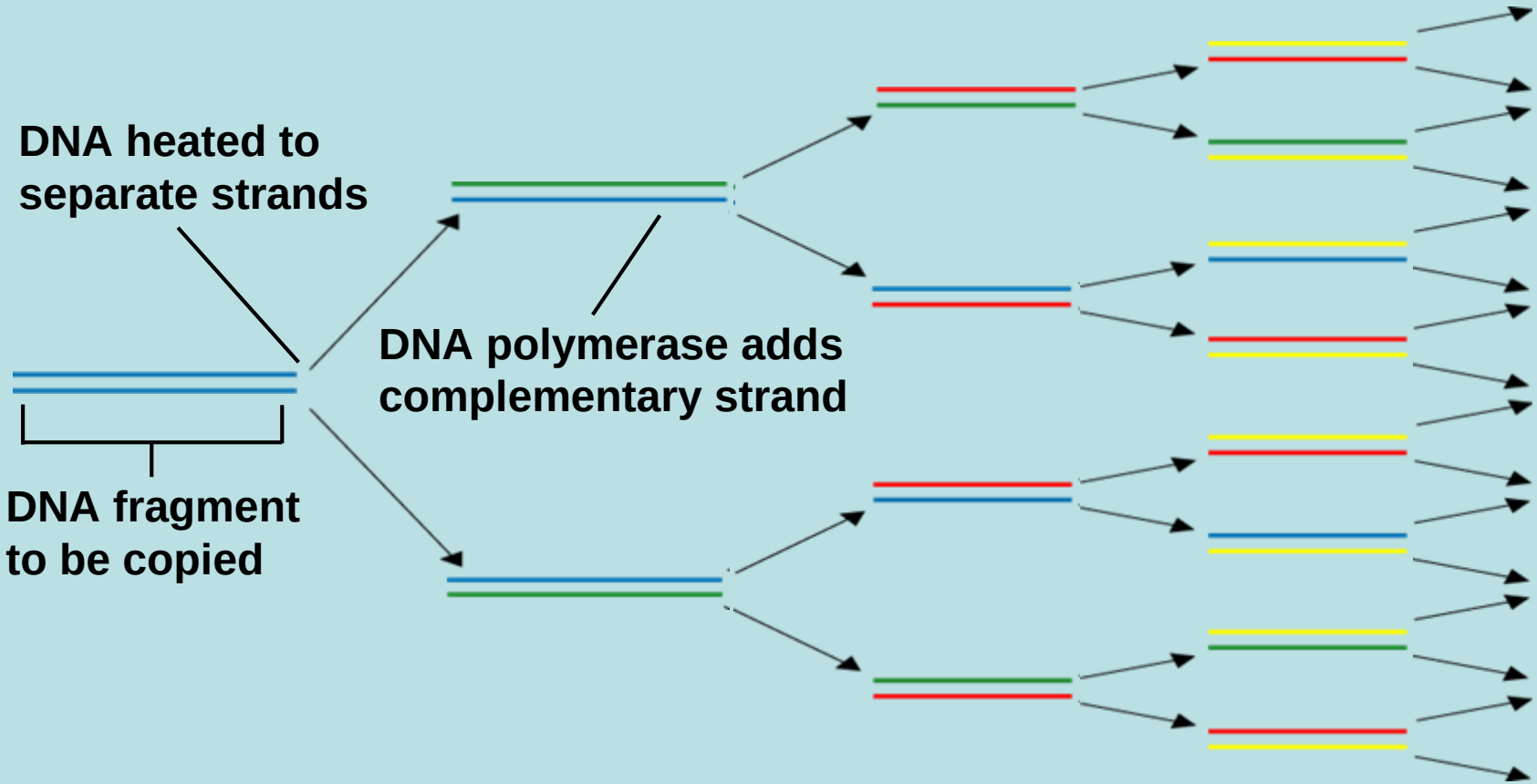
The copies also serve as templates to make more copies so a few cycles of replication can produce millions of copies of DNA between the primers.

Kary Mullis - American inventor of PCR

Had to find an enzyme that could stand repeated cycles of heating and cooling.

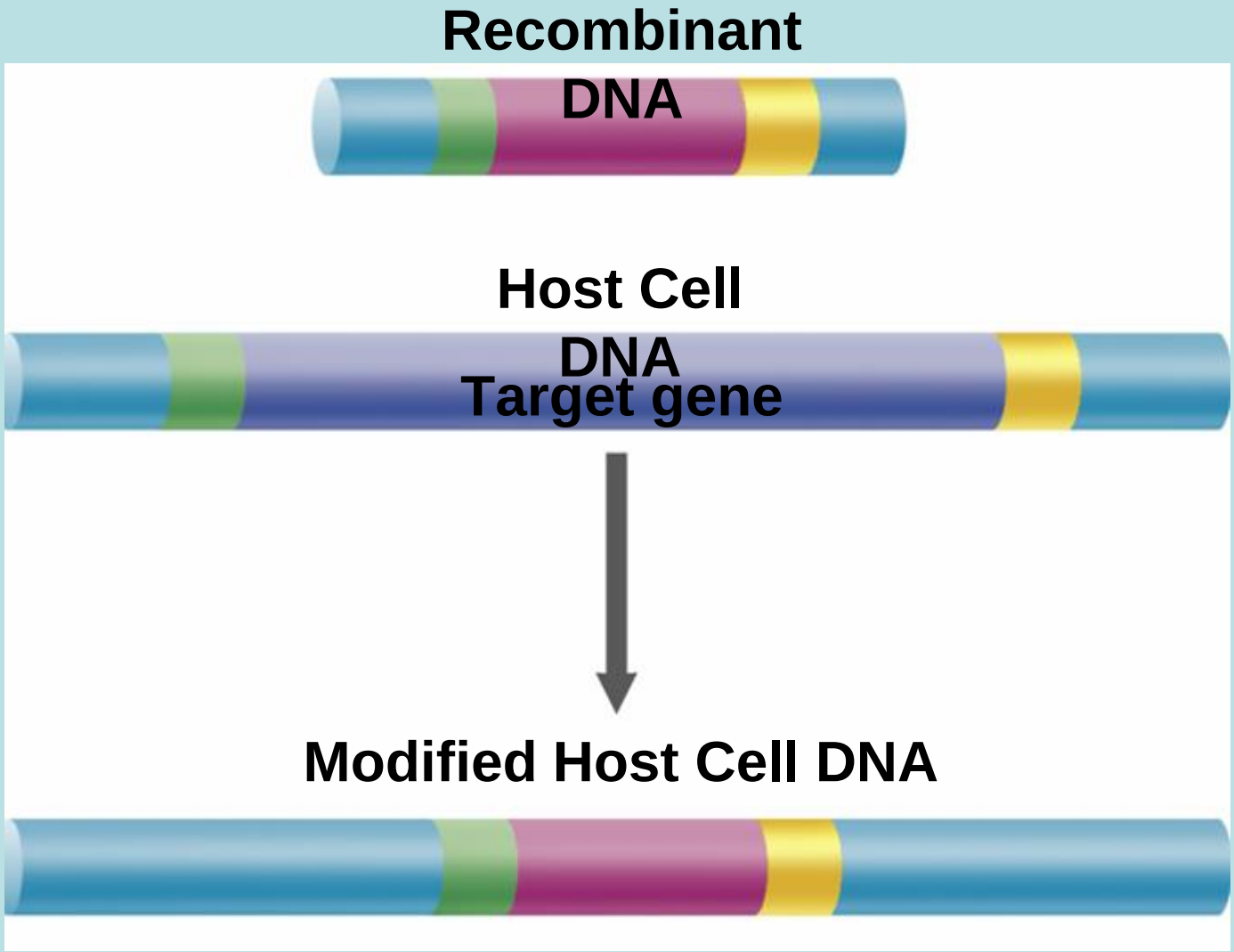
Found it in bacteria in the hot springs in Yellowstone National Park

Polymerase Chain Reaction (PCR)



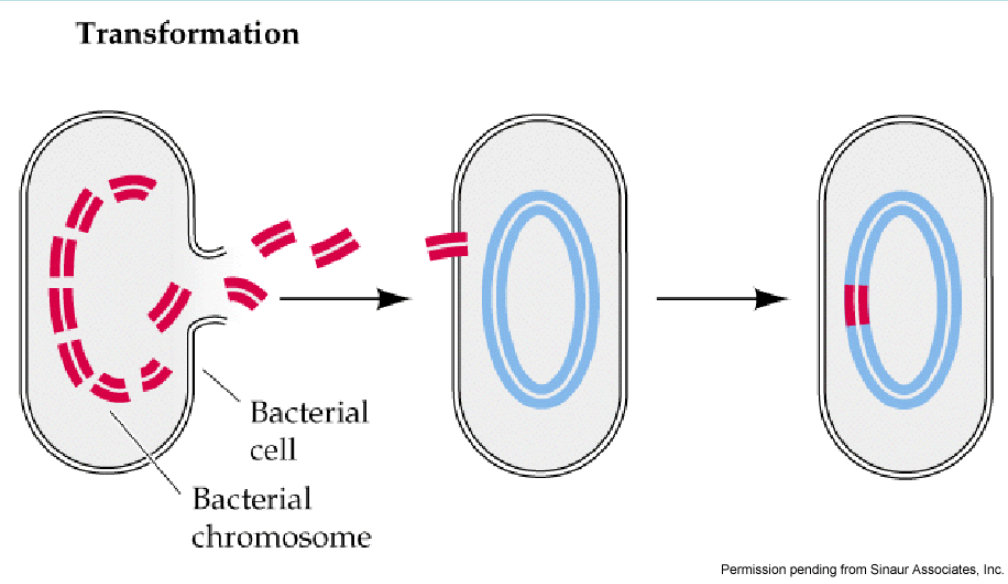
PCR cycles	1	2	3	4	5 etc.
DNA copies	1	2	4	8	16 etc.

13-3 Cell Transformation



What happens during cell transformation?

During transformation, a cell takes in DNA from outside the cell. The external DNA becomes a component of the cell's DNA.

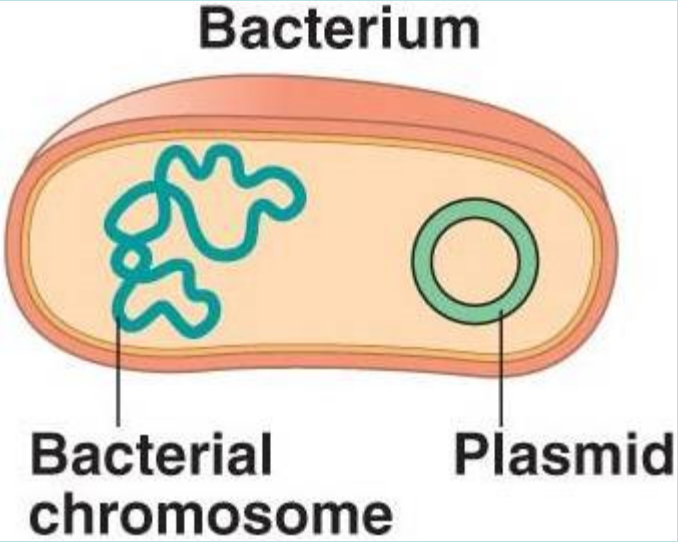


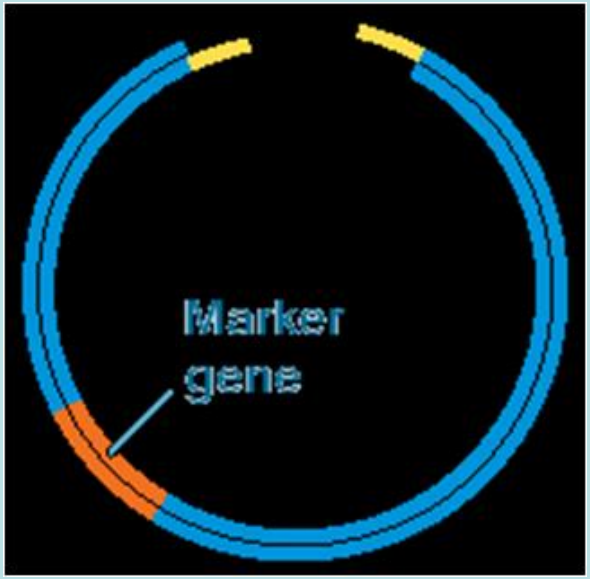
Transforming Bacteria

Foreign DNA is first joined to a small, circular DNA molecule known as a **plasmid**.

Plasmids are found naturally in some bacteria and have been very useful for DNA transfer.

The plasmid has a **genetic marker**—a gene that makes it possible to distinguish bacteria that carry the plasmid (and the foreign DNA) from those that don't.



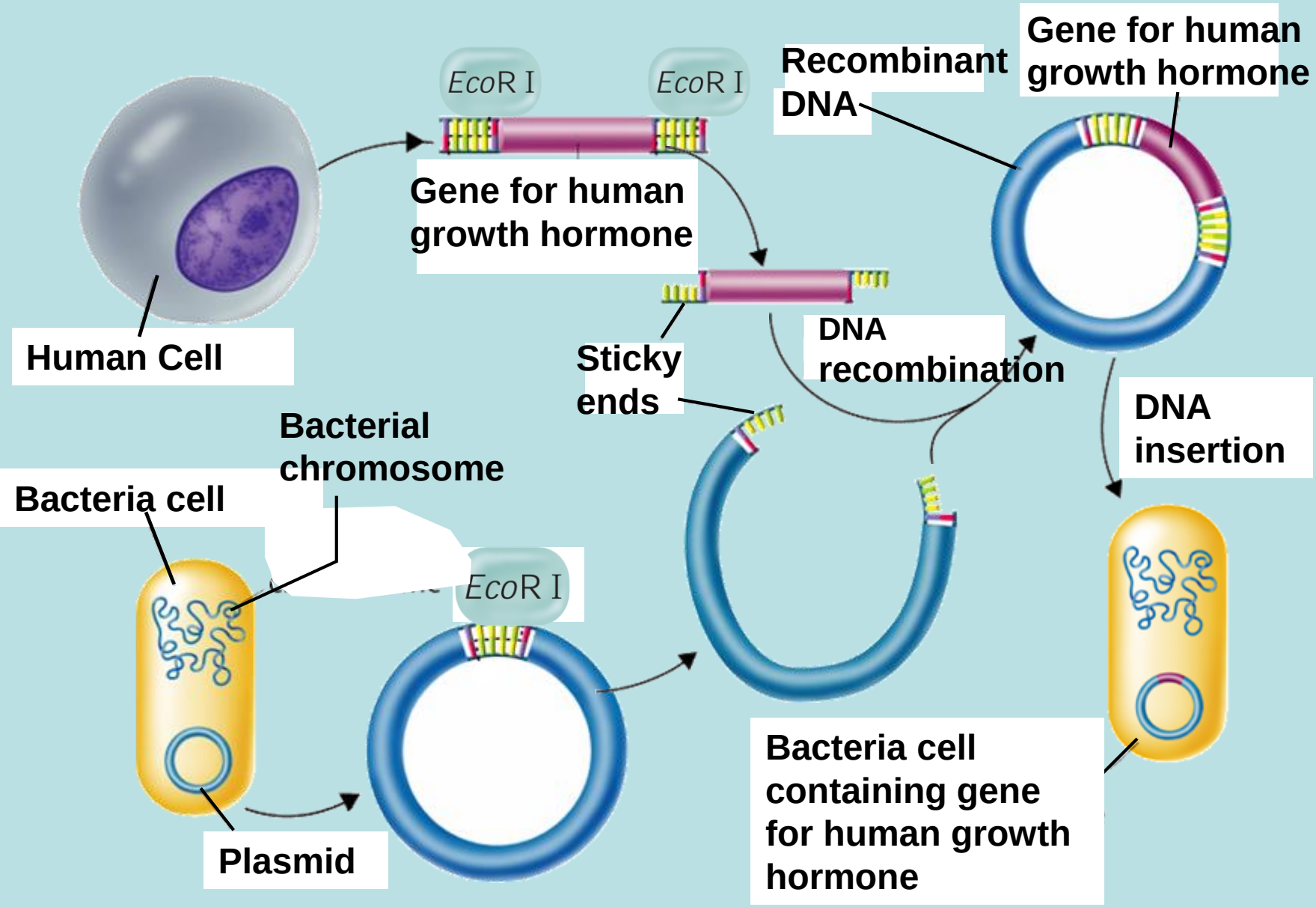


Steps to Transforming Bacteria

1. Take the gene of interest out of the DNA from a human cell
2. Cut it out using restriction enzymes (act like scissors!!)

3. You are left with your “gene of interest”
4. Take a plasmid (ring of DNA) out of a bacterial cell, cut it with restriction enzymes.
5. Place the gene of interest in the plasmid, making a ring again
6. Put this ring back into a bacteria and let it reproduce!!
   ...and bacteria will reproduce really fast!!!!

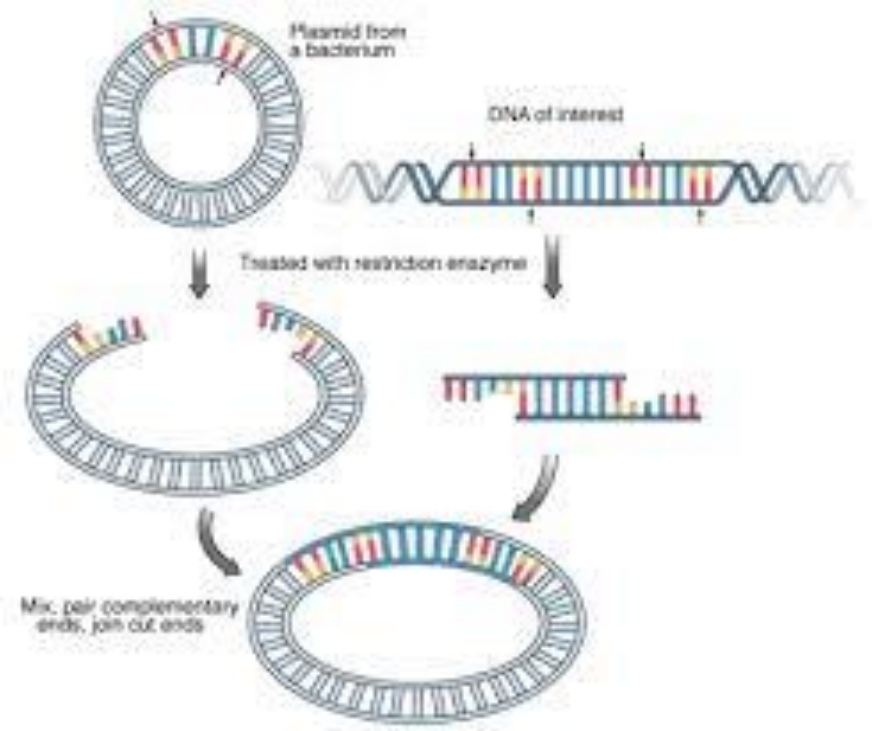
Transforming Bacteria



Cell Transformation

How can you tell if a transformation experiment has been successful?

If transformation is successful, the recombinant DNA is integrated into one of the chromosomes of the cell.



Transforming Plant Cells

In nature, a bacterium exists that produces tumors in plant cells.

Researchers can inactivate the tumor-producing gene found in this bacterium and insert a piece of foreign DNA into the plasmid.

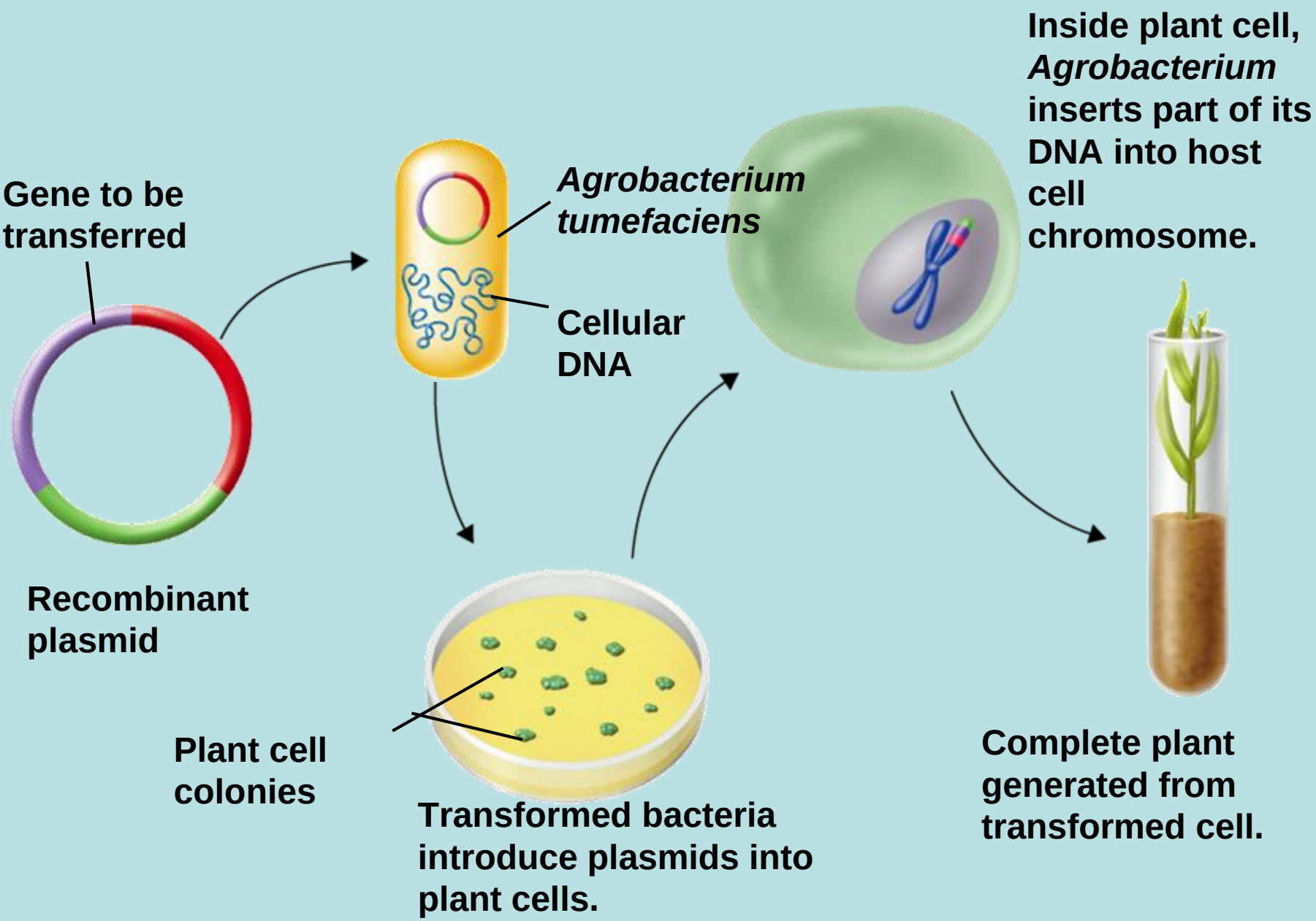
The recombinant plasmid can then be used to infect plant cells.

When their cell walls are removed, plant cells in culture will sometimes take up DNA on their own.

DNA can also be injected directly into some cells.

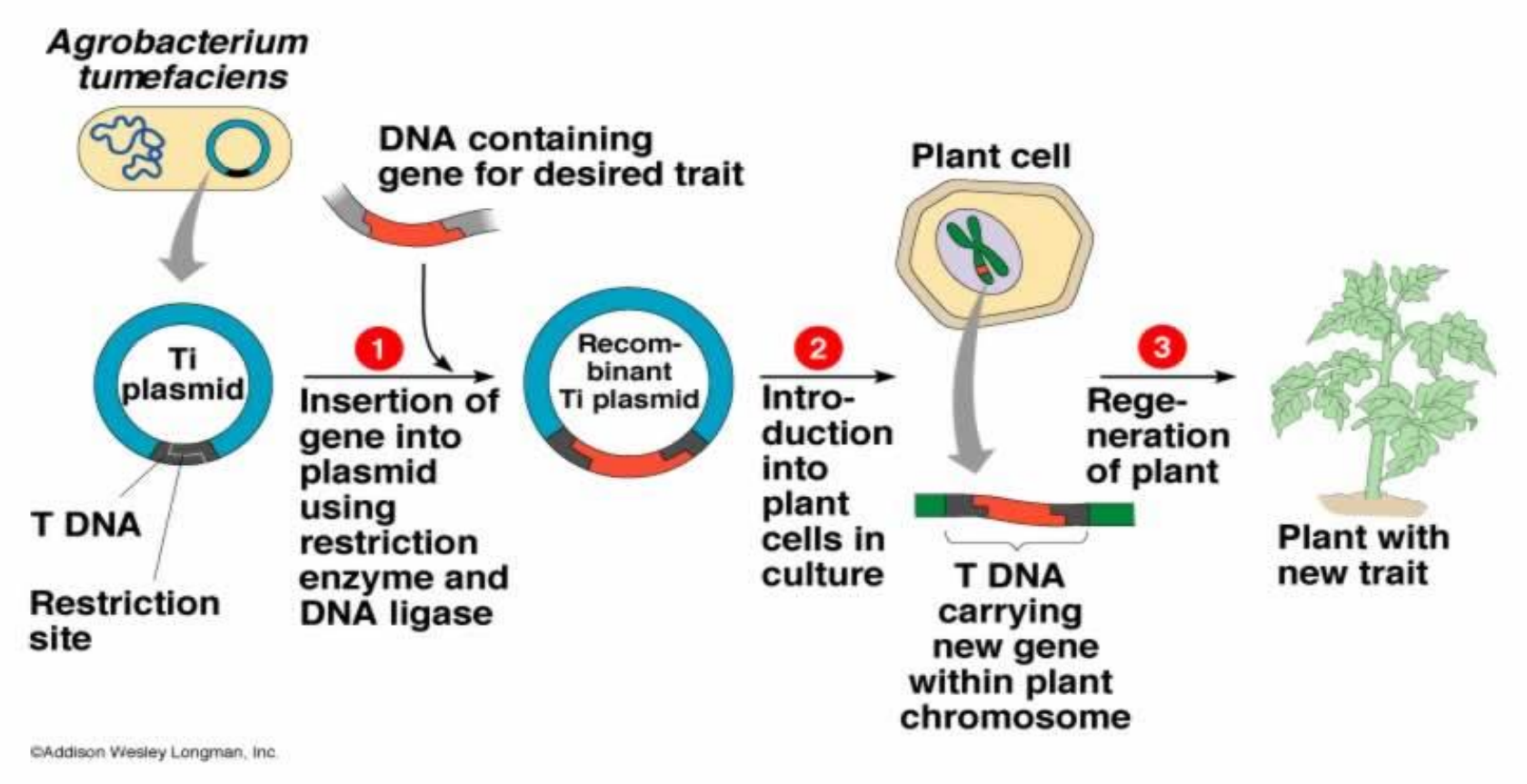
Cells transformed by either procedure can be cultured to produce adult plants.

Transforming Plant Cells



Transforming Plant Cells

Ex. Plants resistant to pesticides

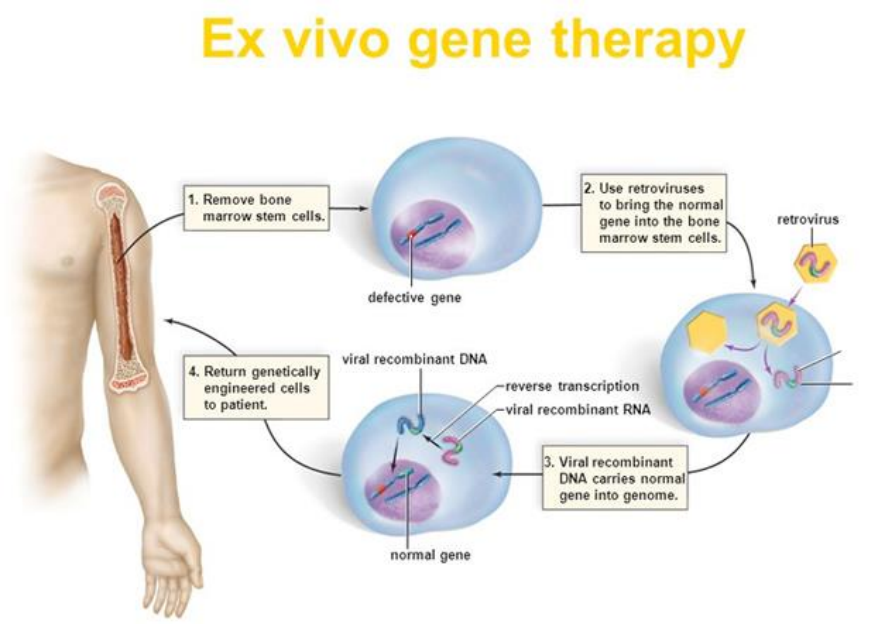


Transforming Animal Cells

Many egg cells are large enough that DNA can be directly injected into the nucleus.

Enzymes may help to insert the foreign DNA into the chromosomes of the injected cell.

DNA molecules used for transformation of animal and plant cells contain marker genes.



Transforming Animal Cells

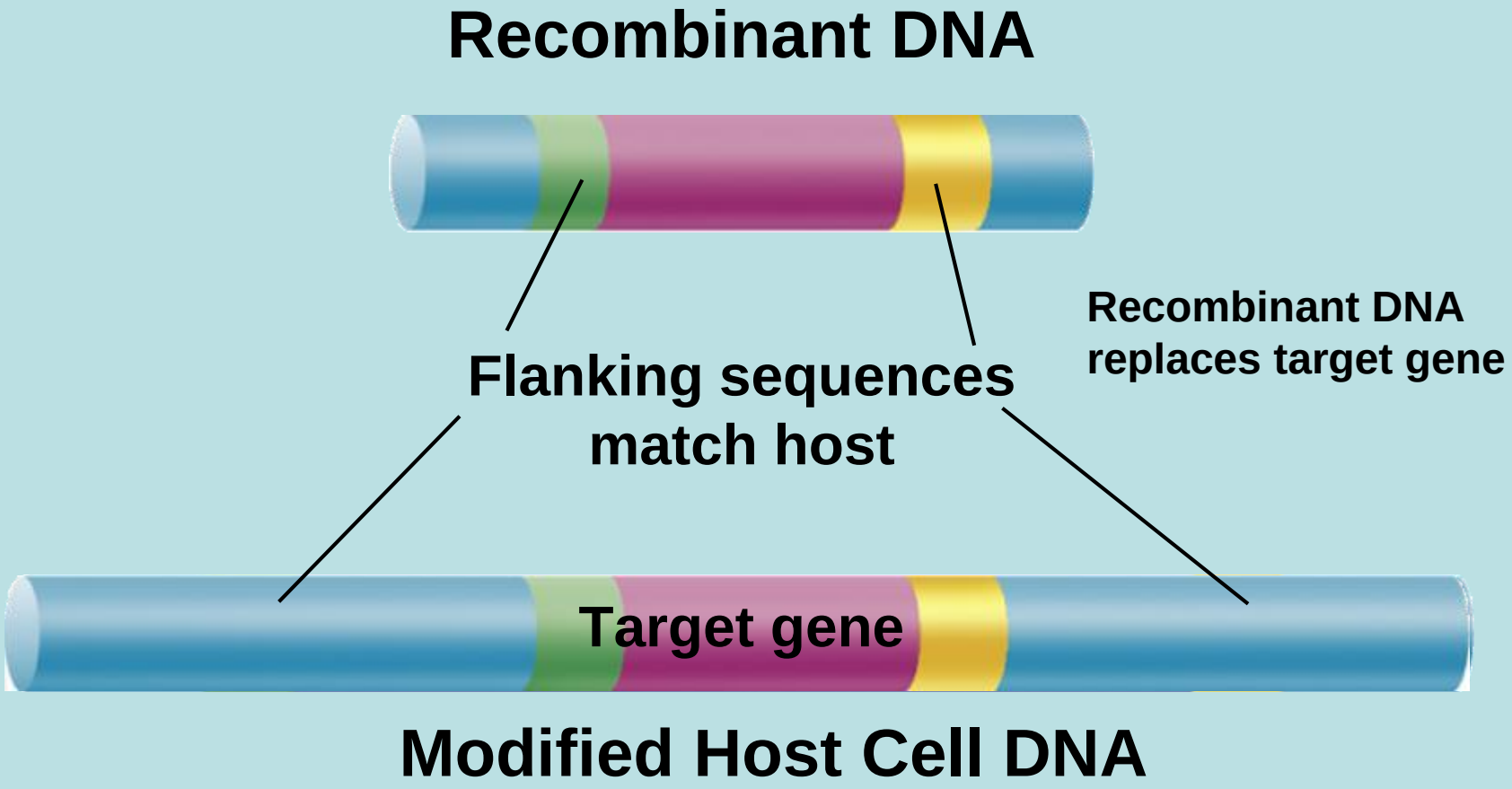
DNA molecules can be constructed with two ends that will sometimes recombine with specific sequences in the host chromosome.

The host gene normally found between those two sequences may be lost or replaced with a new gene.

“Knock out genes”

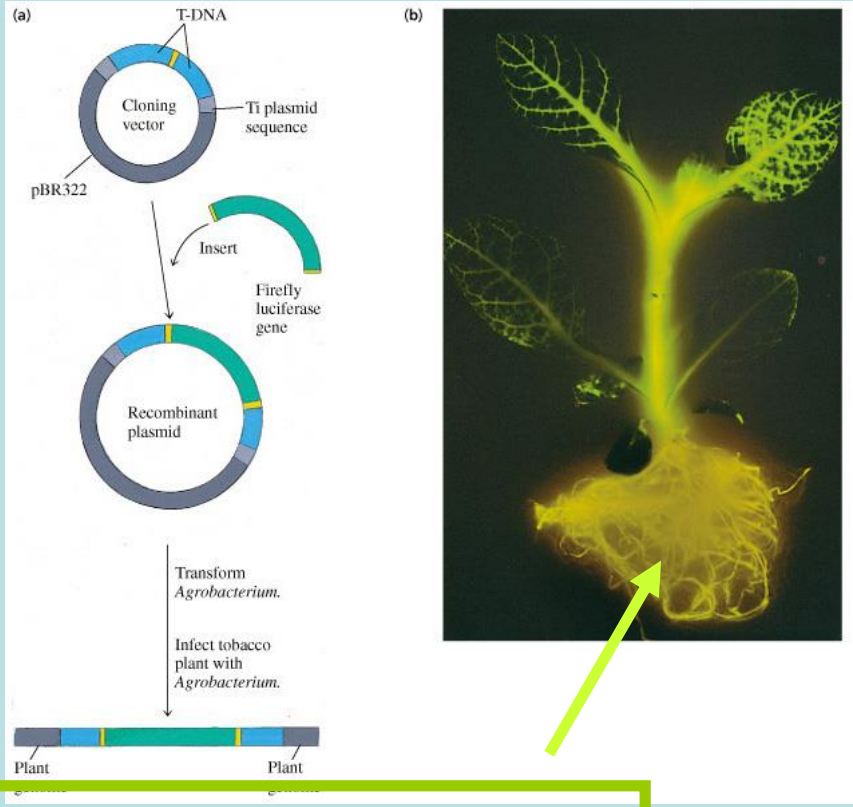
Recombinant DNA can replace a gene in an animal’s genome. When recombinant DNA is inserted into the target location, the host cell’s original gene is lost or “knocked out” of its place.

Transforming Animal Cells



Other Uses for Recombinant DNA??

- DNA produced by combining DNA from different sources.
  - Genetically engineered cells contain recombinant DNA

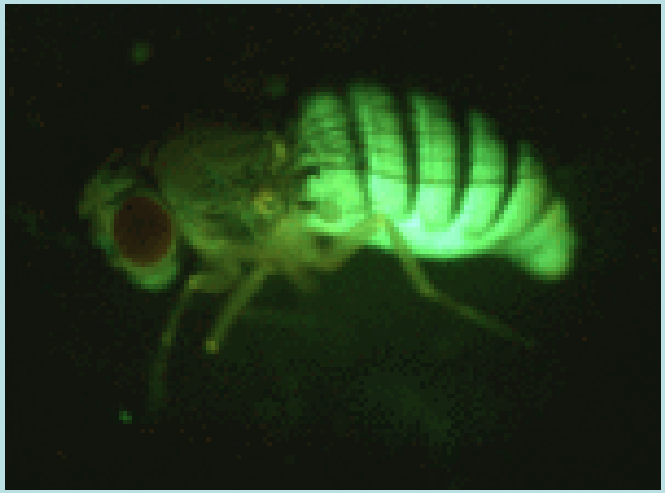


Firefly gene + tobacco plant = glowing tobacco plant!

13-4 Applications of Genetic Engineering: Biotechnology

- New technology has created a new field of study: Biotechnology
- We can create **transgenic organisms** – that contain genes from other organisms

Glowing bumblebee:



Modified Pigglets!

Transgenic Organisms

An organism described as transgenic, contains genes from other species.

Transgenic Organisms

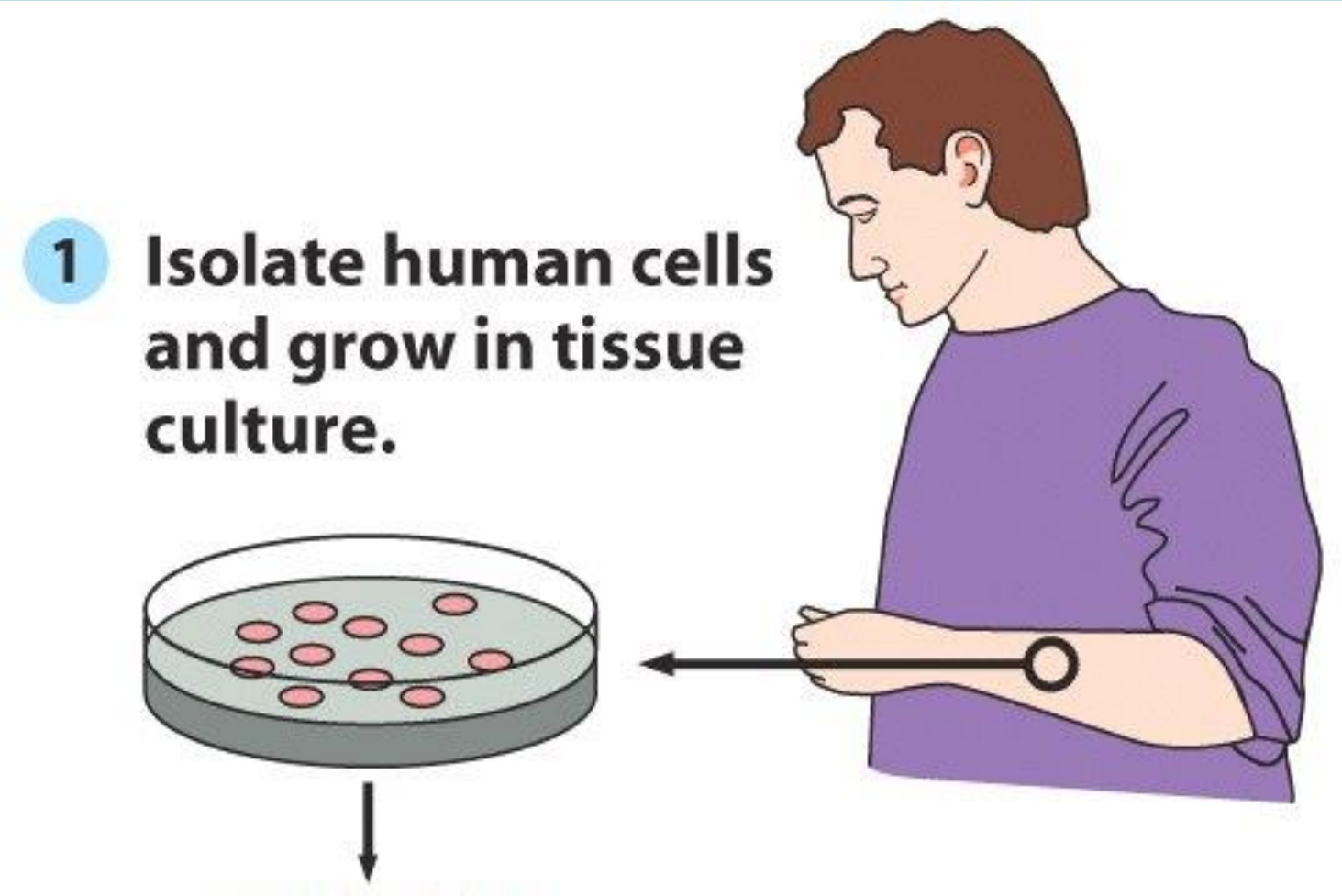
How are transgenic organisms useful to human beings?

Transgenic Microorganisms

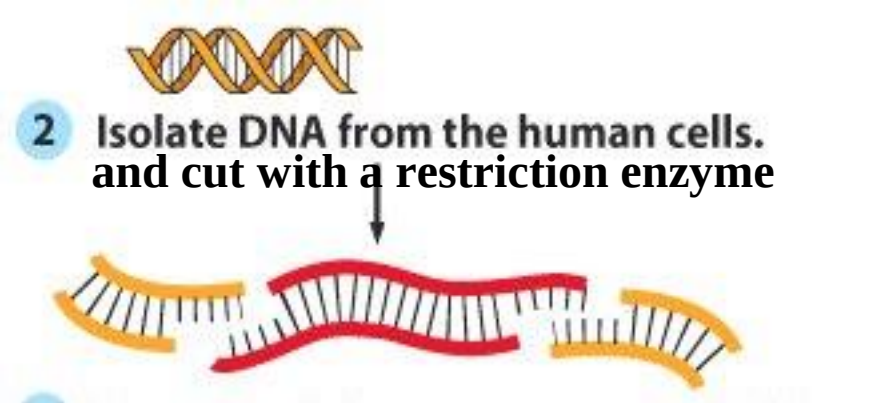
Transgenic bacteria produce important substances useful for health and industry. Transgenic bacteria have been used to produce:

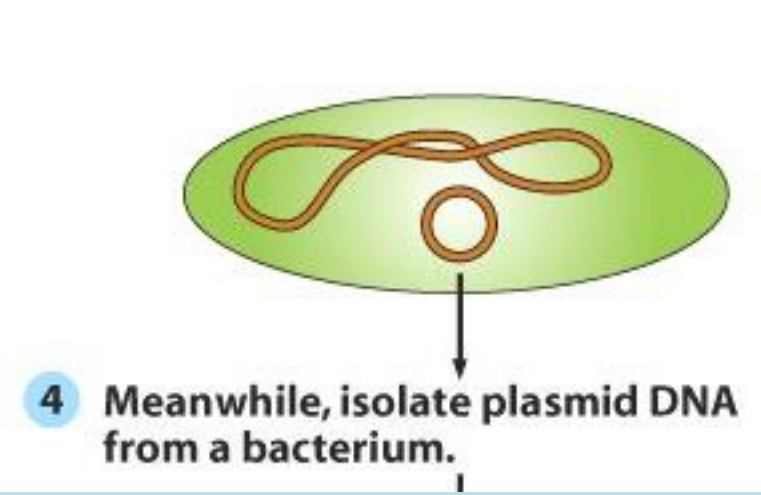
- insulin
- growth hormone
- clotting factor

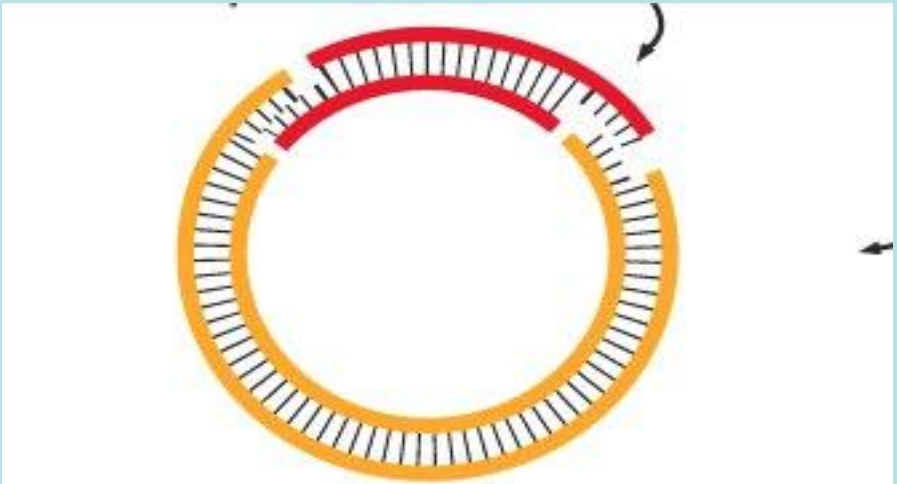
Harnessing the Power of Recombinant DNA Technology – Human Insulin Production by Bacteria



Human Insulin Production by Bacteria

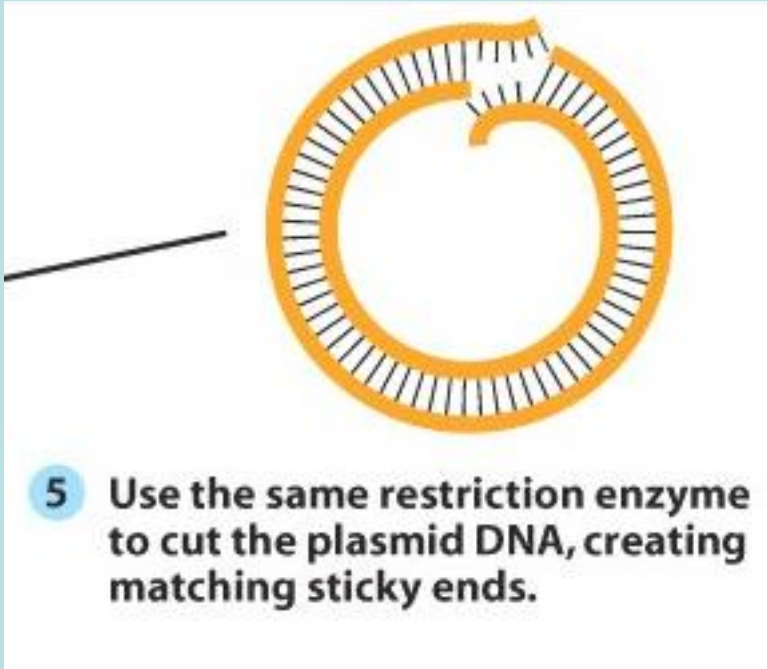




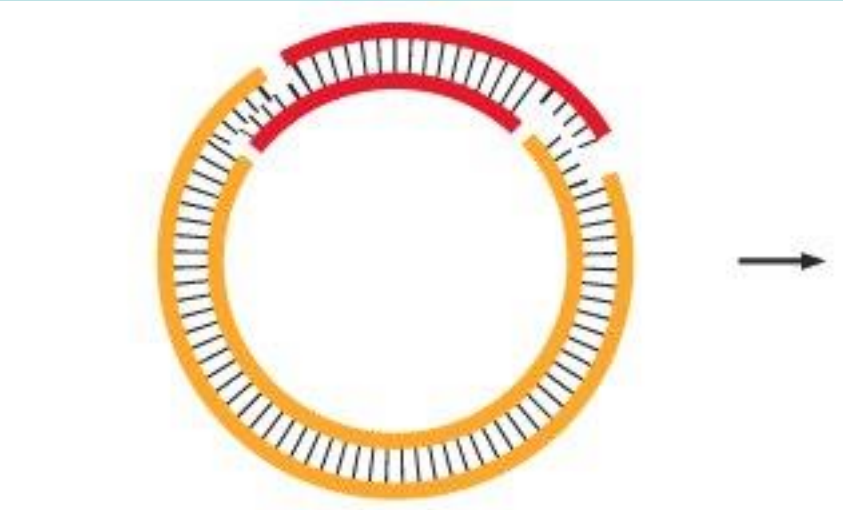


6) join the plasmid and human fragment

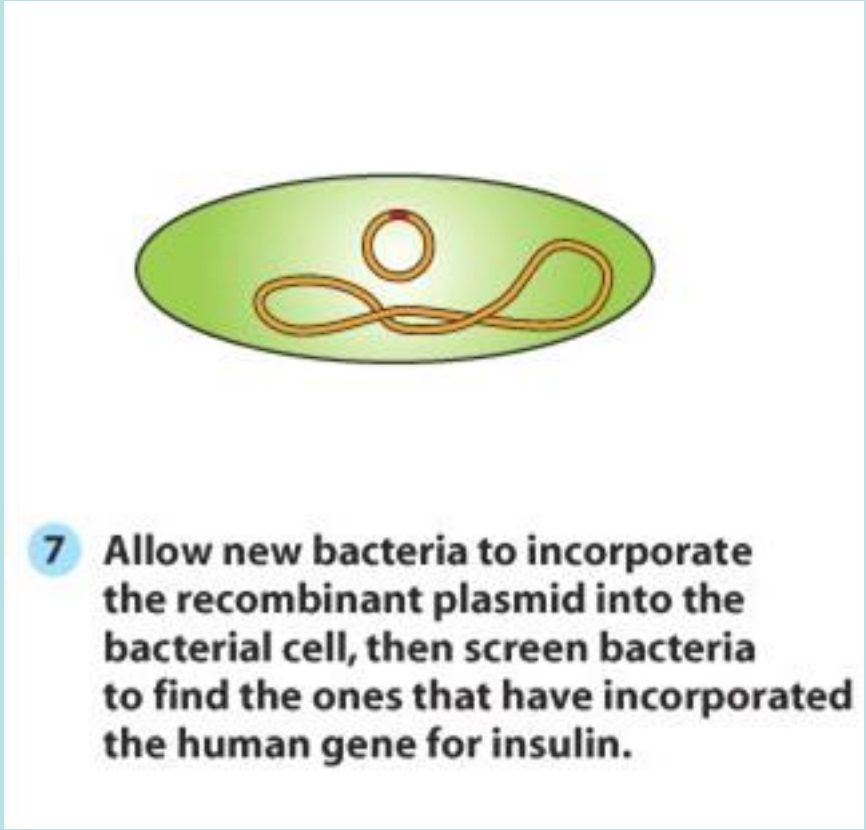
Figure 4-3 (2) Biology Today, 3/e (© 2004 Garland Science)



Human Insulin Production by Bacteria

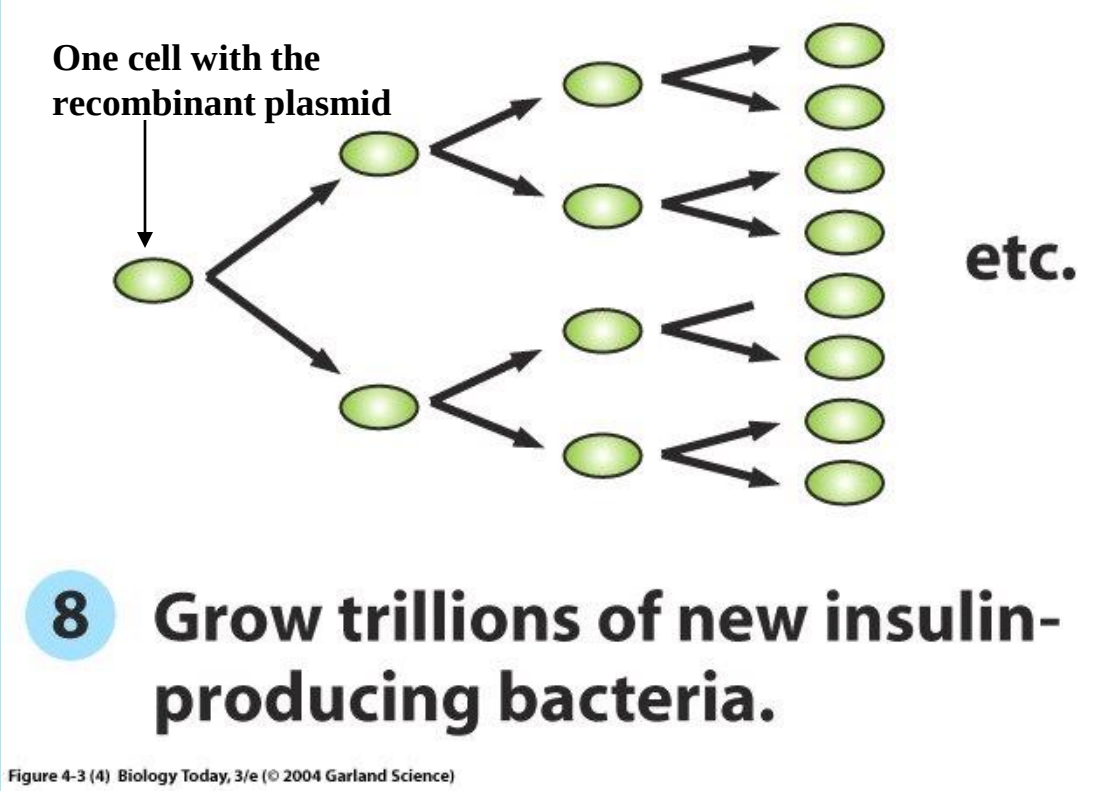


Mix the recombinant plasmid with bacteria.



Screening bacterial cells to learn which contain the human insulin gene is the hard part.

Route to the Production by Bacteria of Human Insulin



A fermentor used to grow recombinant bacteria.

This is the step when gene cloning takes place.

The single recombinant plasmid replicates within a cell.

Then the single cell with many recombinant plasmids produces trillions of like cells with recombinant plasmid – and the human insulin gene.

Route to the Production by Bacteria of Human Insulin

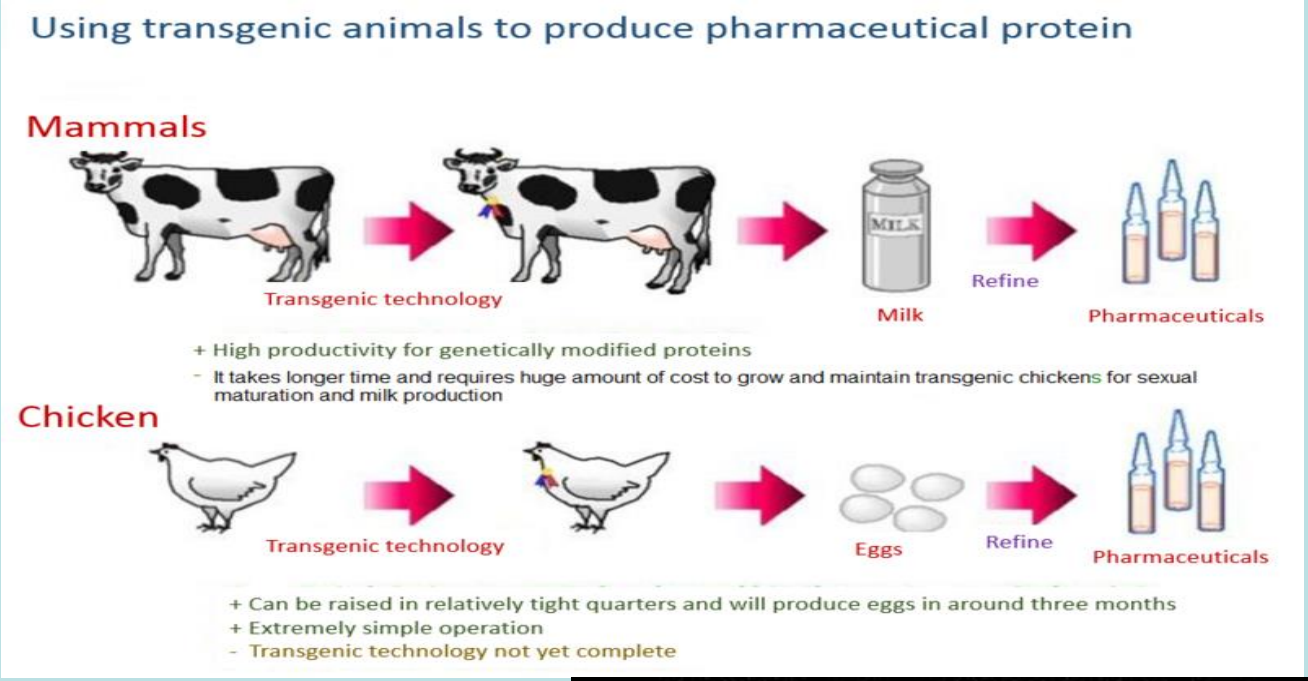
The final steps are to collect the bacteria, break open the cells, and purify the insulin protein expressed from the recombinant human insulin gene.

Transgenic Animals

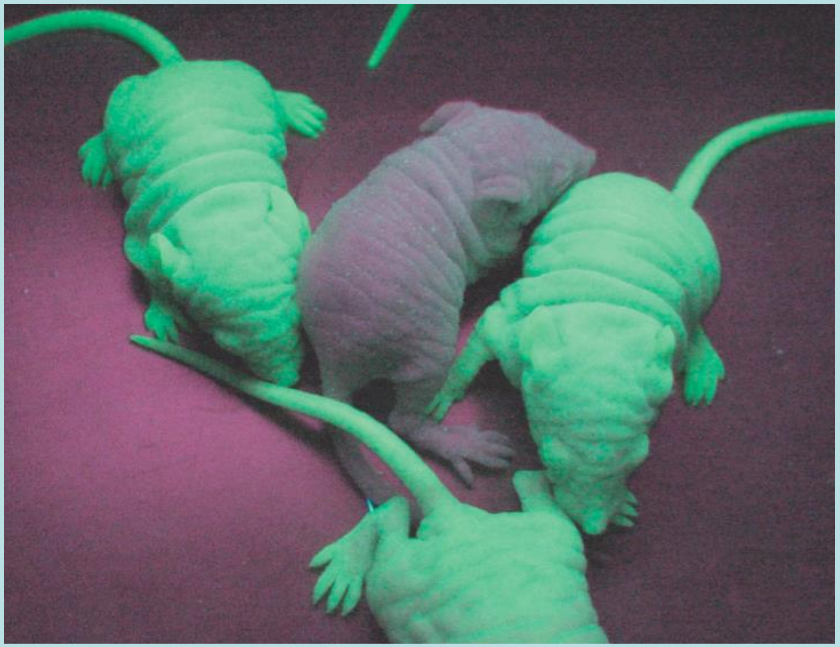
- Have been used to study genes and improve the food supply
- Mice have been produced with human genes that make their immune systems act similarly to those of humans. This allows scientists to study the effects of diseases on the human immune system.
- Research is under way where goats could be modified to produce spider silk proteins in their milk that could be used in the production of medical sutures and bullet-proof vests

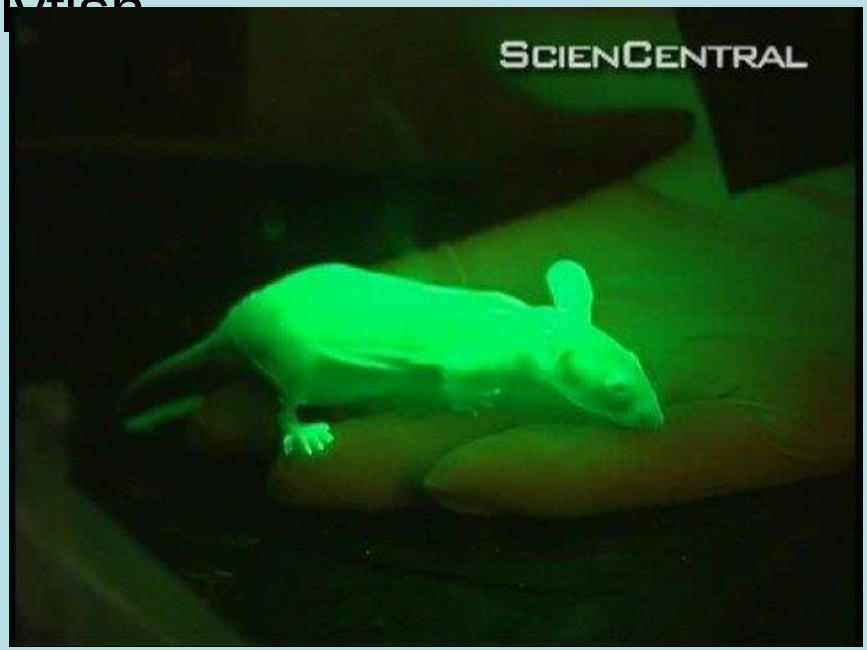
Transgenic Organisms

Researchers are trying to produce transgenic chickens that will be resistant to the bacterial infections that can cause food poisoning.



Gene causes these animals to glow in the dark.
Normally, the gene is found in jellyfish





Transgenic Plants

- Transgenic plants are now an important in our food supply!
- Many of these plants contain a gene that produces a natural insecticide, so plants don't have to be sprayed with pesticides.
- 25% of all corn grown in US is genetically modified

-actual data 89% (above is old book data)

- 52% of all soybeans grown in US is genetically modified

-actual data 94% (above is old book data)

- Ex. resist herbicides, vitamins

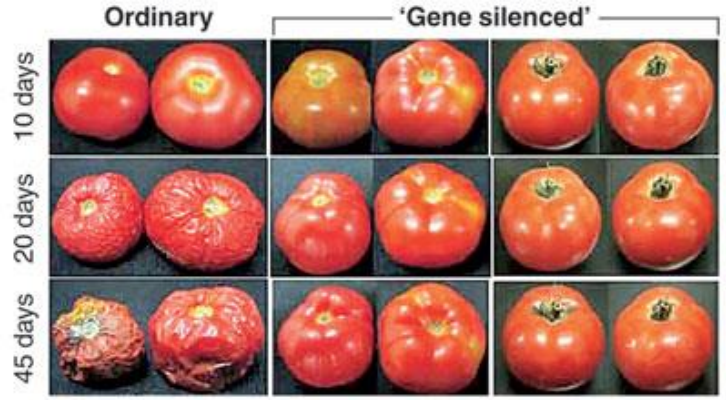
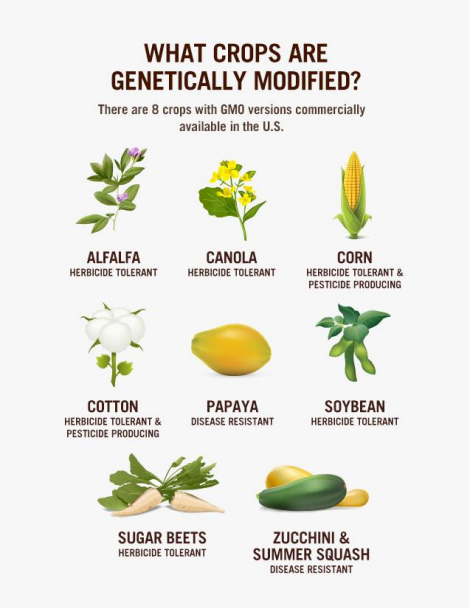


Image shows three sets of tomatoes. The ordinary control tomatoes (extreme left) soften and shrivel up, while texture of gene-silenced tomatoes remains intact for up to 45 days.

Photo credit: Asis Datta, Subhra Chakraborty, National Institute of Plant Genome Research, New Delhi



Cloning

A **clone** is a member of a population of genetically identical cells produced from a single cell.

In 1996, Ian Wilmut cloned a sheep called Dolly.

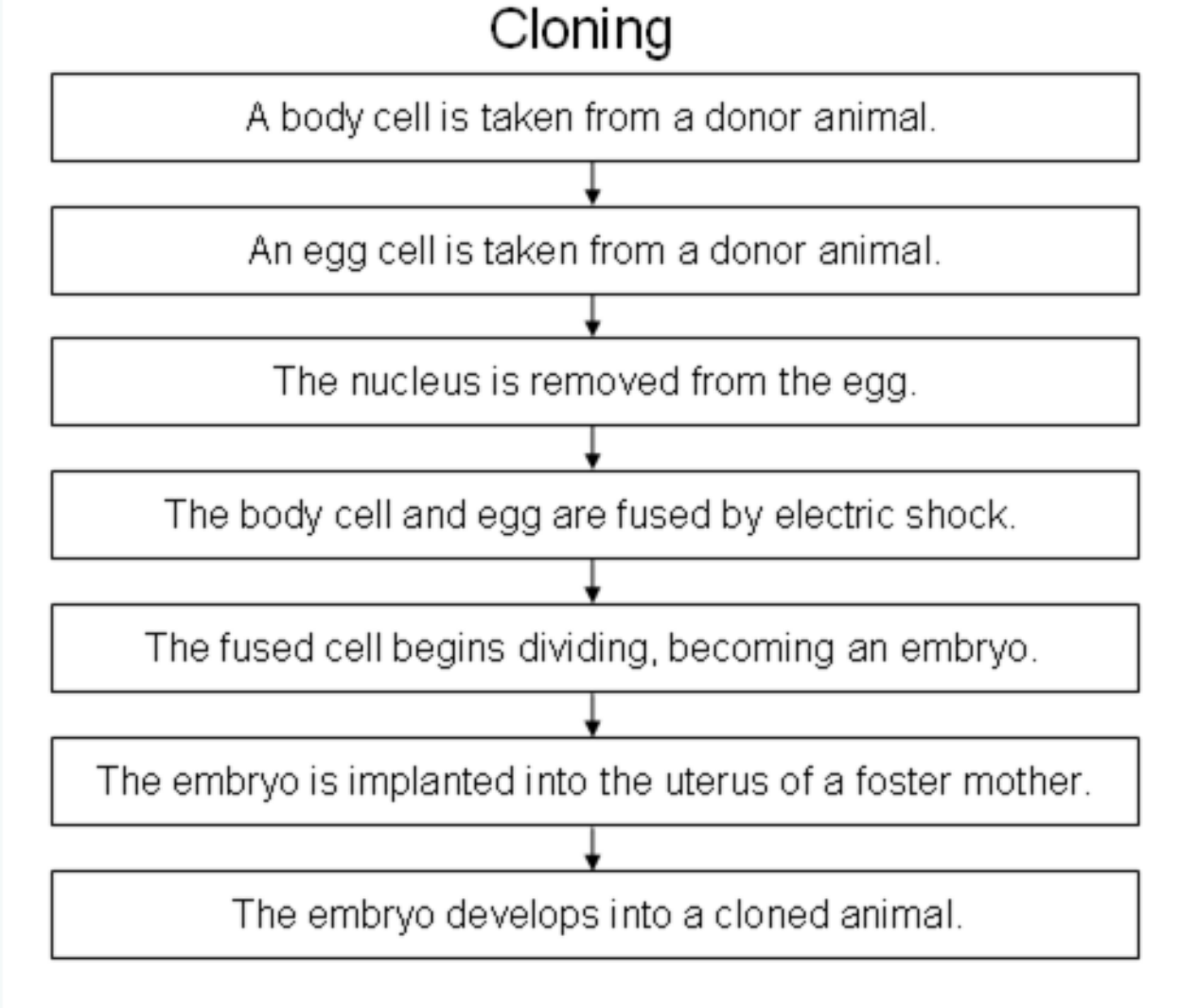
Dolly and Bonnie

- Bacteria naturally clone as do plants, multicellular organisms are more difficult;
- The possibilities raise many ethical and moral issues.

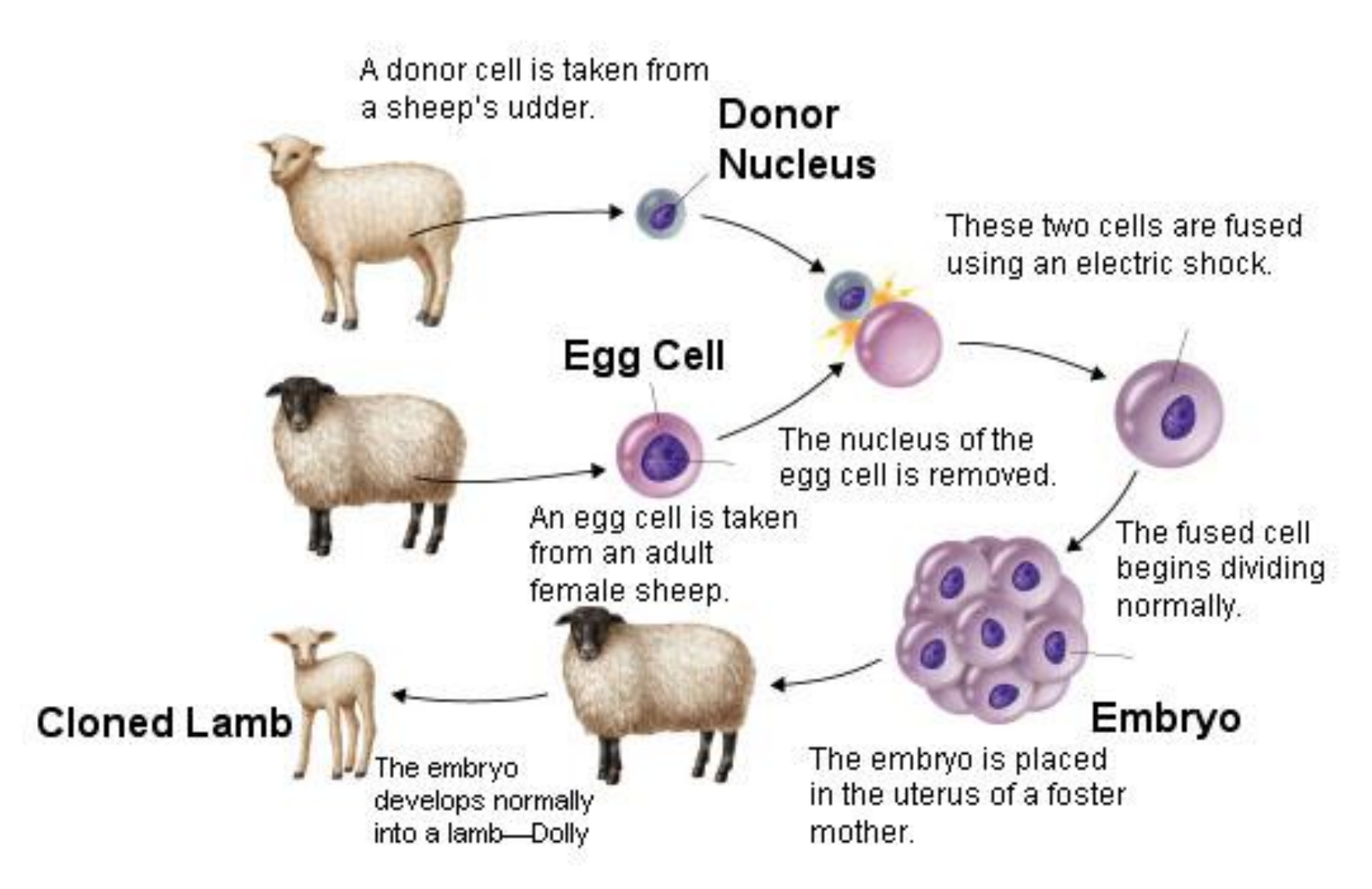
Pros and Cons of Cloning

- Pros
  - Replace vital organs
  - Solution to infertility
  - Genetic research to do away with genetic disorders
  - Used to give us certain advantages
    - Stronger immune system
- Cons
  - Decreases the diversity in gene pool
  - Errors will be made leading to malpractice
  - Will the cloned organs be cost effective
  - Devalue mankind
  - Playing God

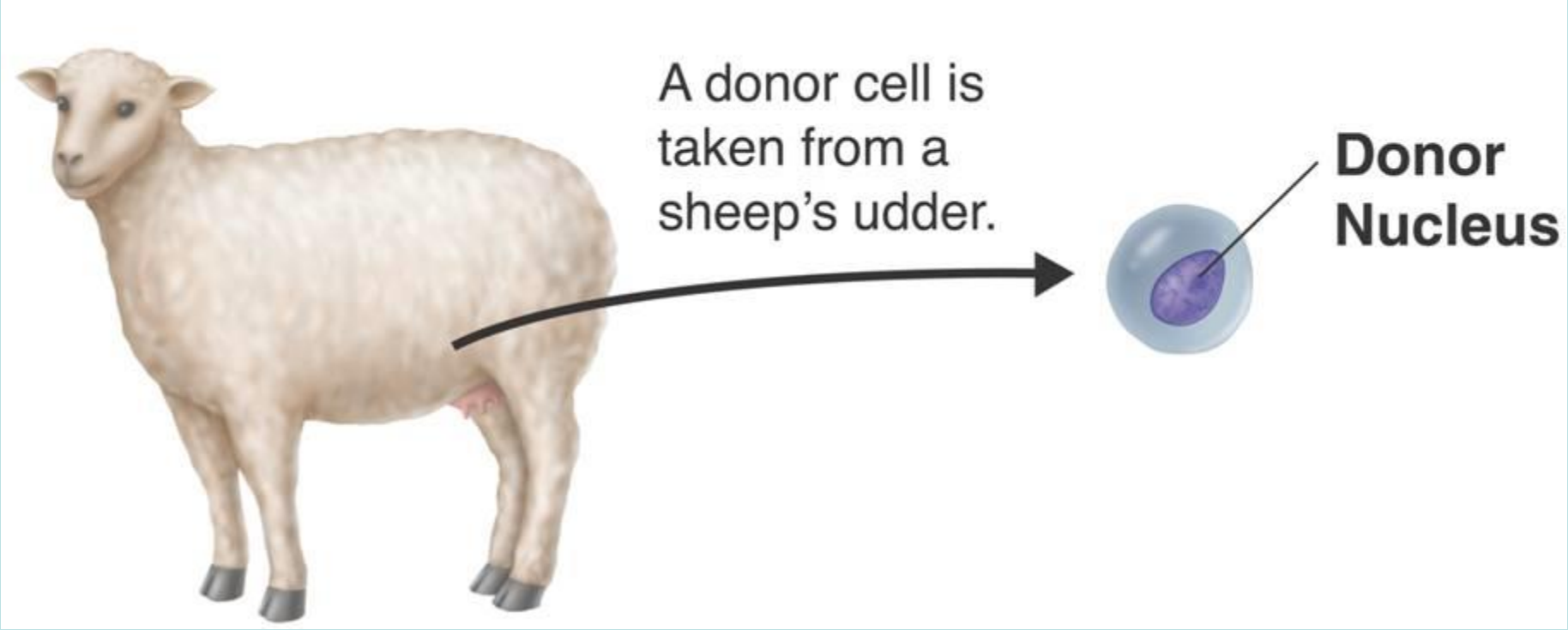
Clone = a member of a population of genetically identical cells produced from a single cell.



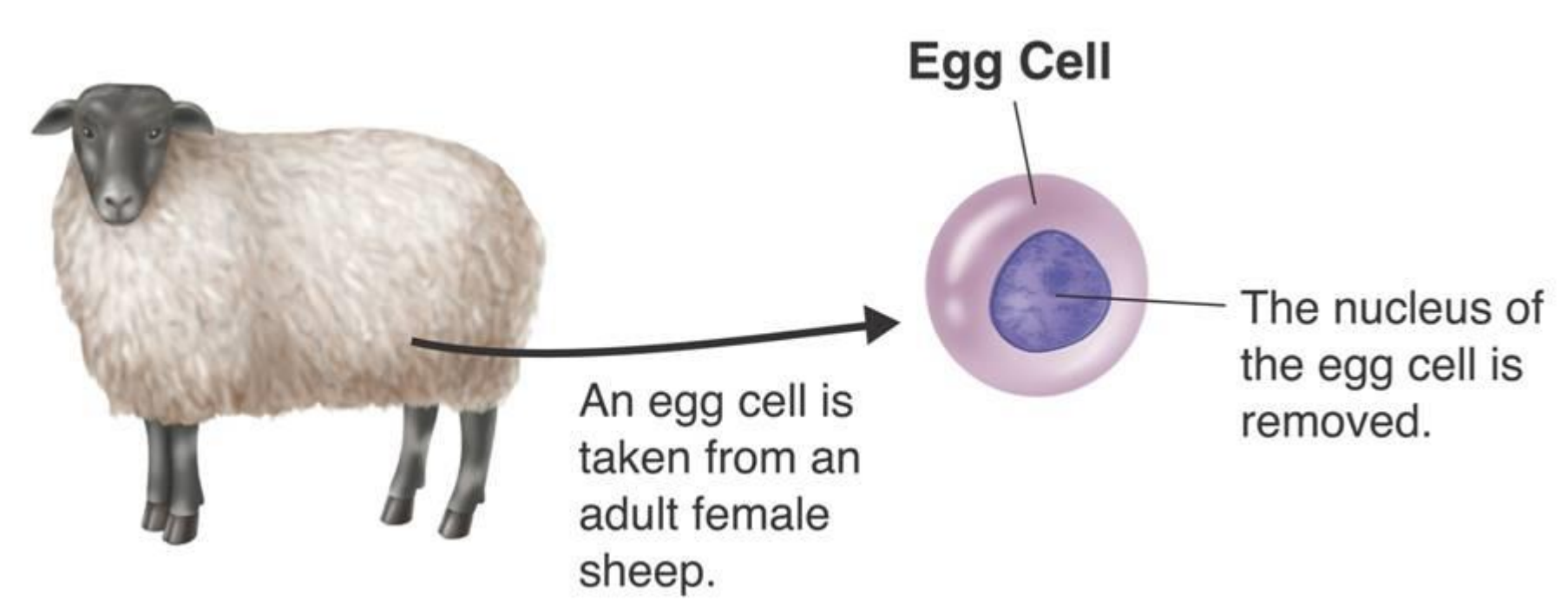
How do you make a clone?



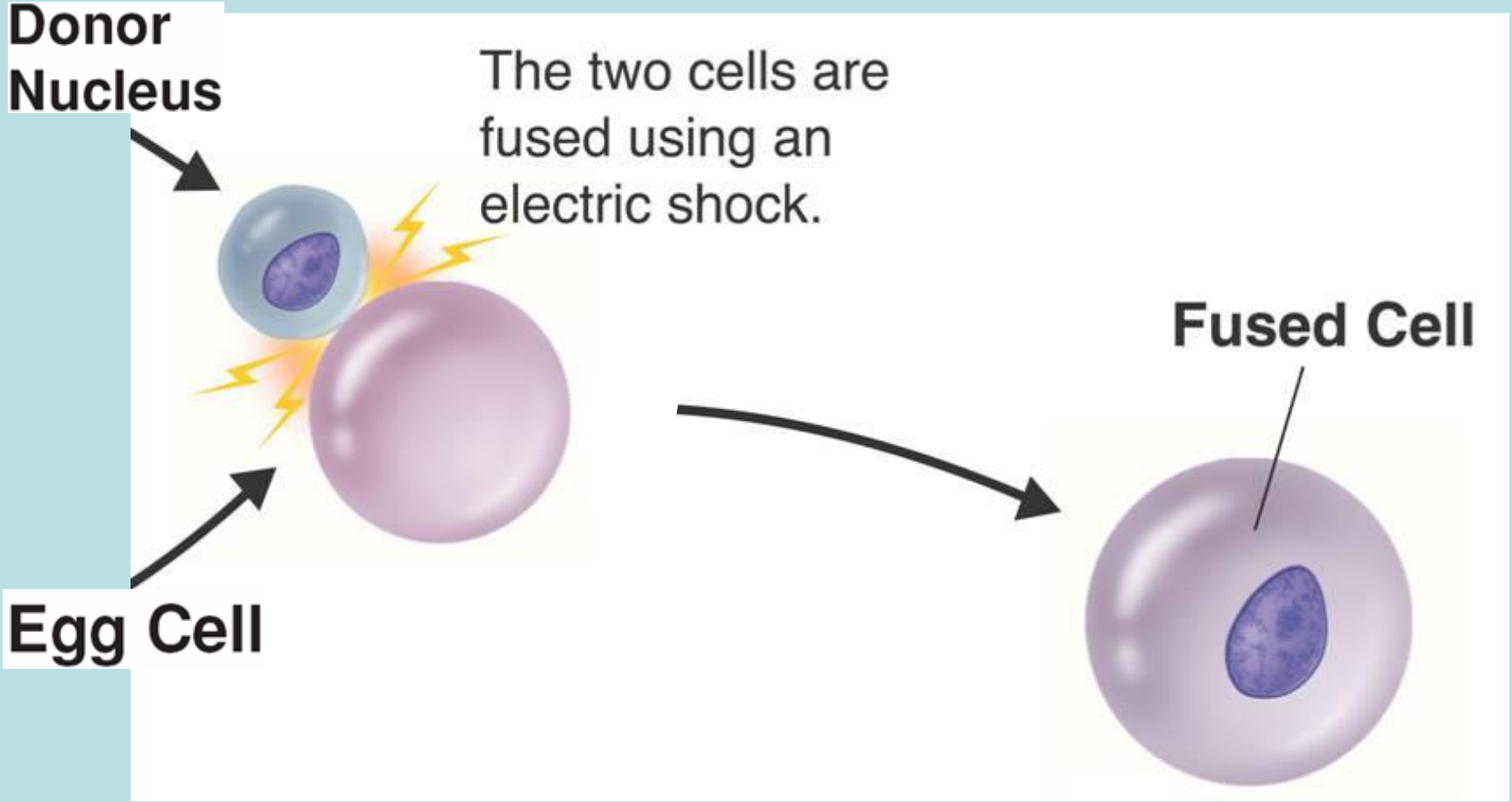
Cloning Dolly



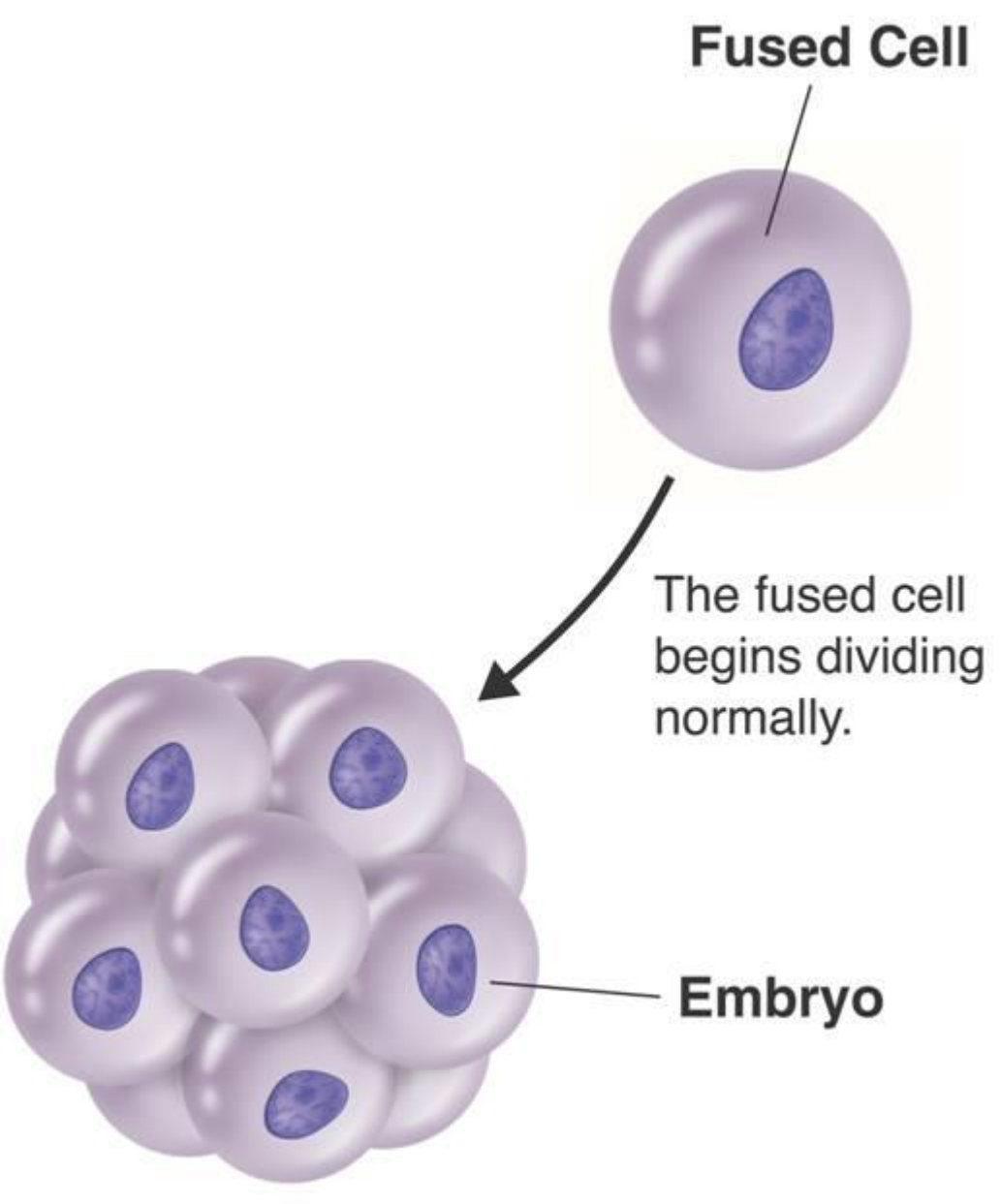
Cloning Dolly



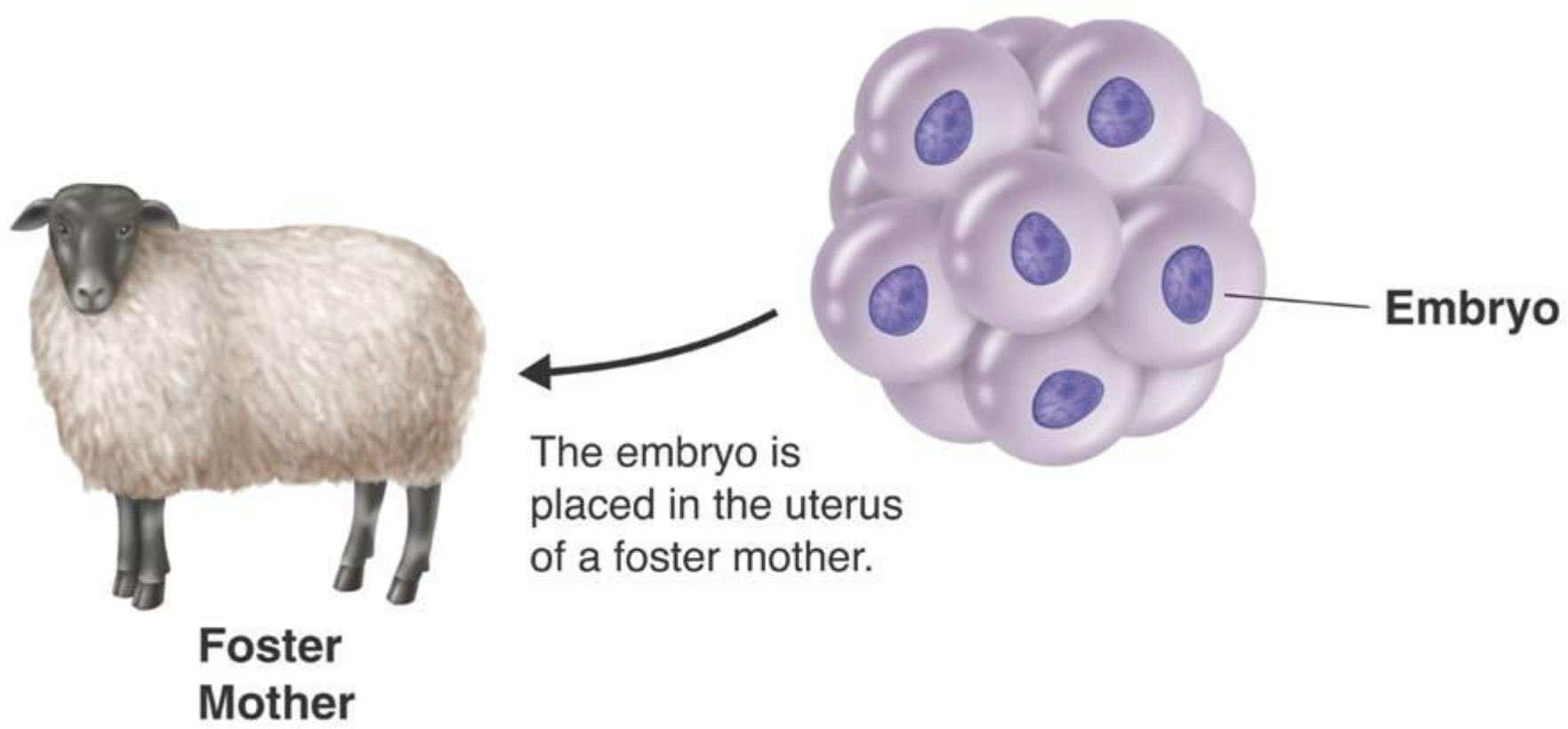
Cloning Dolly



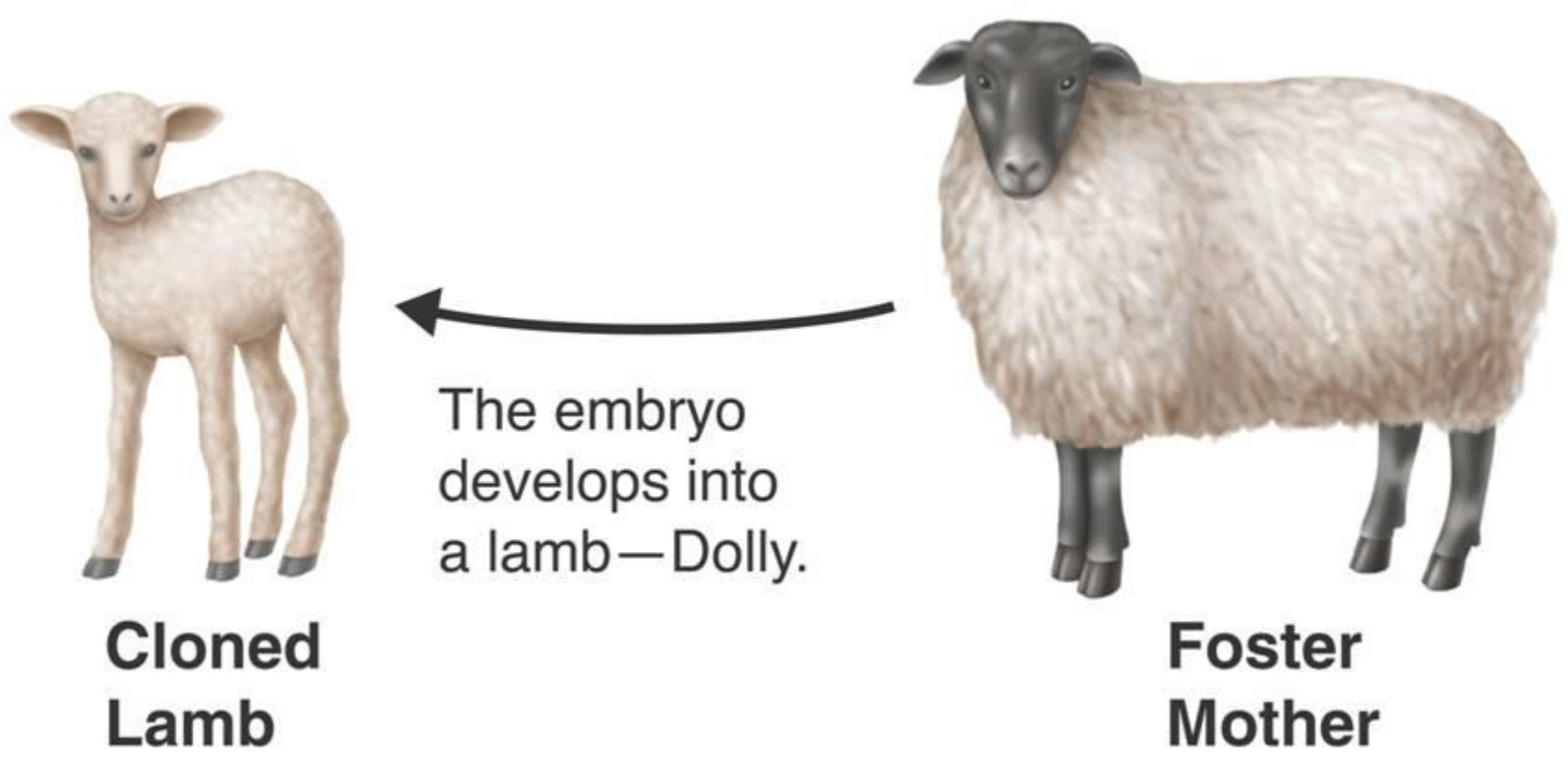
Cloning Dolly



Cloning Dolly



Cloning Dolly



Cloning

Researchers hope cloning will enable them to make copies of transgenic animals and help save endangered species.

Studies suggest that cloned animals may suffer from a number of genetic defects and health problems.

- Despite years of research, over 95% of cloning attempts fail, even with extensive veterinary intervention.
- Birth defects, physiological impairments, illness, and premature death continue to be the norm, not the exception, with cloning.
- Seemingly healthy clones have unexpectedly developed problems

Cloning a Human