BIOTECHNOLOGY

PREFACE

- BIOTECHNOLOGY-
  - manipulating organisms to make useful products
  - includes age old selective breeding
  - use of microorganisms to produce food/other products
  - includes modern genetic engineering
  - has sequenced the entire human genome
  - will continue to have direct impacts on human society

PREFACE

- THE FOCUS OF THIS UNIT INCLUDES:
  - Techniques used to manipulate DNA
  - Techniques used to analyze gene expression
  - Techniques used in cloning
  - Techniques used in producing stem cells
  - Survey practical applications of biotechnology
  - Consider social and ethical issues of biotechnology

BIOTECHNOLOGY

I. Main Idea:
To work with specific genes scientists have developed methods for preparing well-defined segments of DNA in multiple identical copies, DNA cloning.

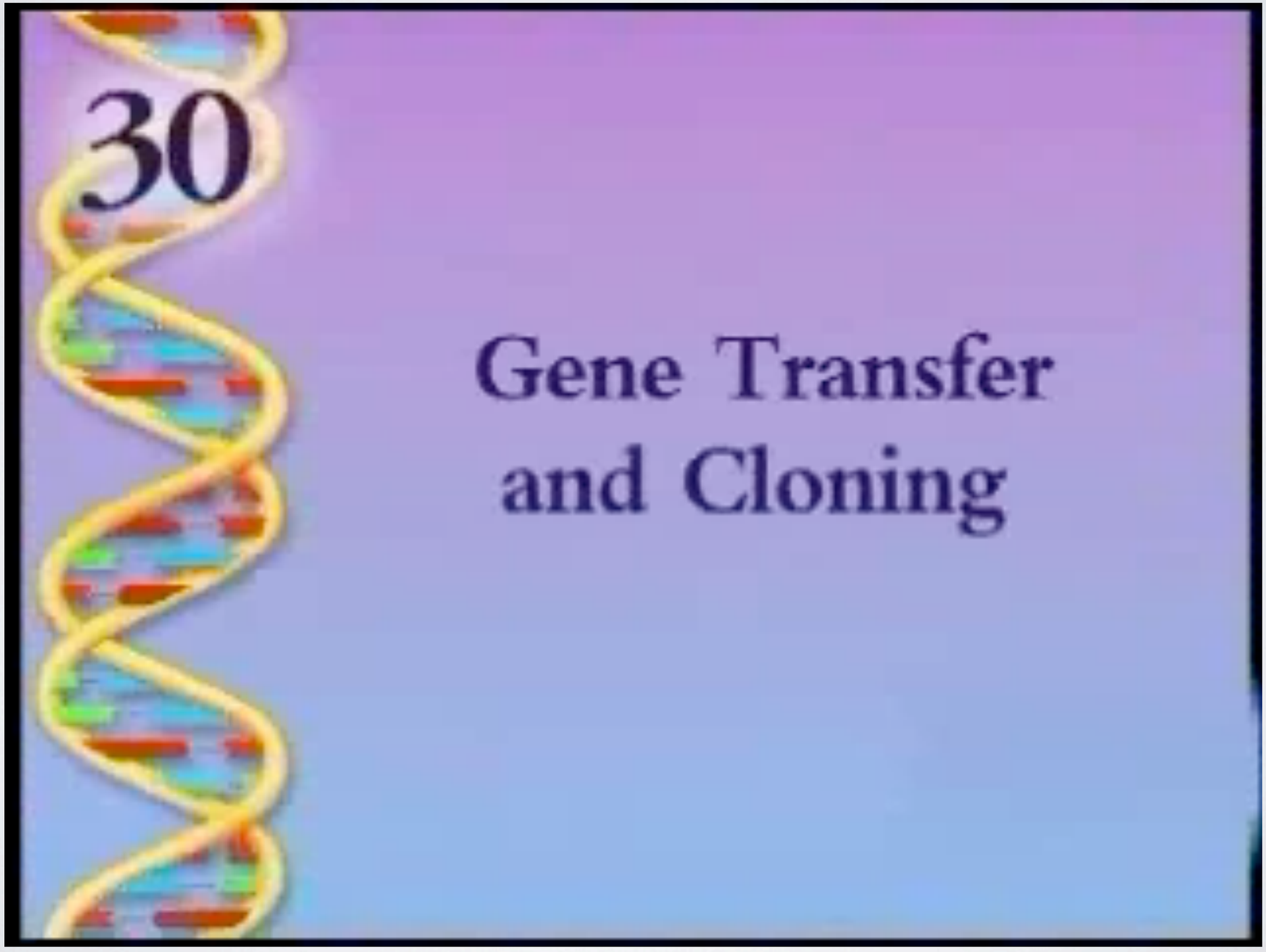
DNA CLONING

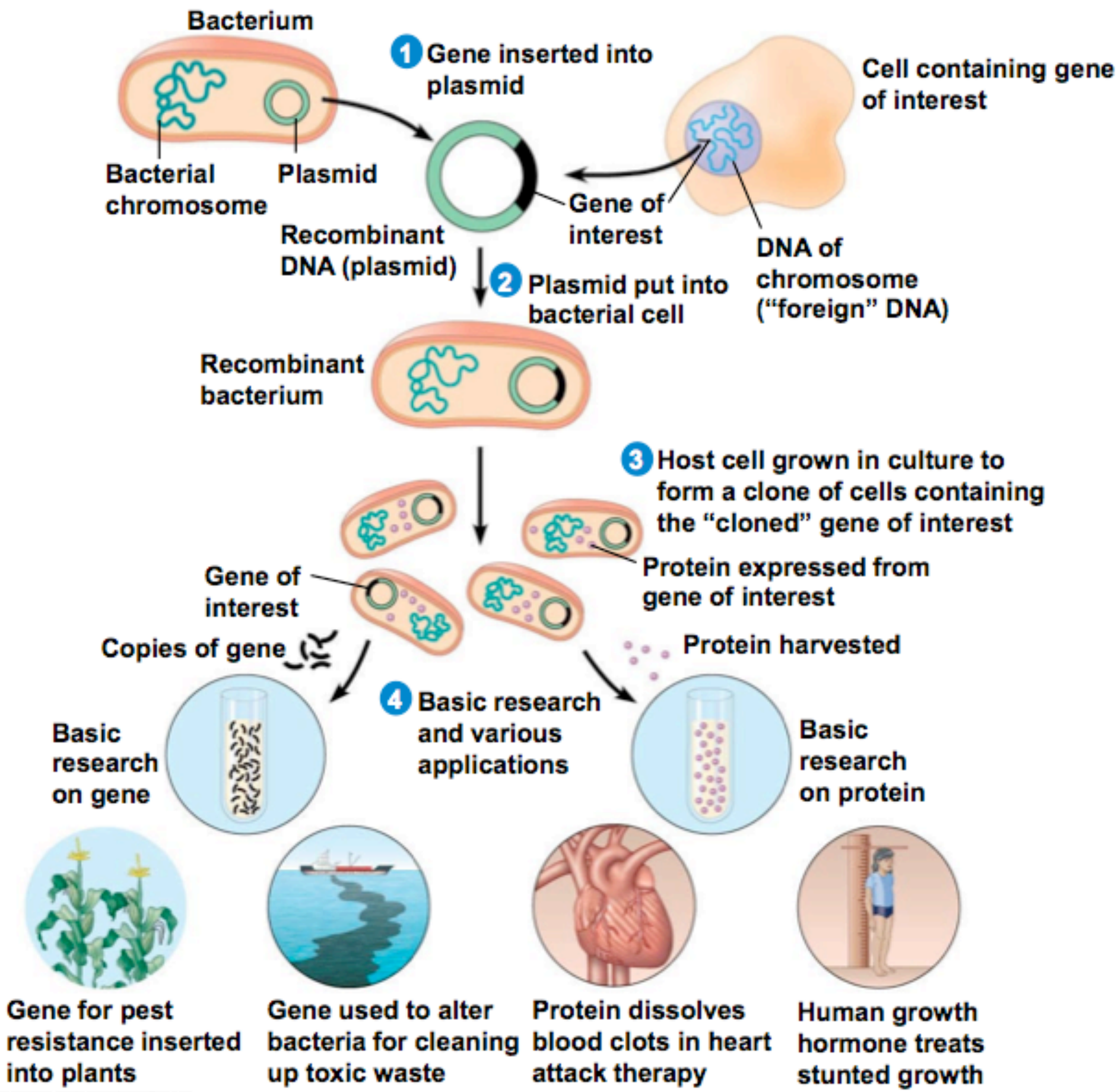
- Most DNA cloning techniques share common features
- **Gene cloning**- is the production of multiple copies of a single gene
- Gene cloning is useful for two reasons: 1. to make more copies of gene OR 2. to produce a protein product.

DNA CLONING

- **Gene cloning** involves using bacteria to make multiple copies of a gene
- Foreign DNA is inserted into a plasmid, and the recombinant plasmid is inserted into a bacterial cell
- **Plasmids** are small circular DNA molecules that replicate separately from the bacterial chromosome
- Reproduction in the bacterial cell results in cloning of the plasmid including the foreign DNA
- This results in the production of multiple copies of a single gene

DNA CLONING: INTRODUCTION "THE BASIC CONCEPTS"




Bacterium
1 Gene inserted into plasmid
Cell containing gene of interest
Bacterial chromosome
Plasmid
Gene of interest
Recombinant DNA (plasmid)
DNA of chromosome ("foreign" DNA)
2 Plasmid put into bacterial cell
Recombinant bacterium
3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest
Protein expressed from gene of interest
Gene of interest
Copies of gene
Protein harvested
4 Basic research and various applications
Basic research on gene
Basic research on protein
Gene for pest resistance inserted into plants
Gene used to alter bacteria for cleaning up toxic waste
Protein dissolves blood clots in heart attack therapy
Human growth hormone treats stunted growth

RESTRICTION ENZYMES

- Gene cloning and genetic engineering rely on **restriction enzymes** that cut DNA at limited and specific locations.

- *Restriction enzymes are used by bacteria to protect themselves from phages by cutting up the foreign DNA before it damages the bacteria*

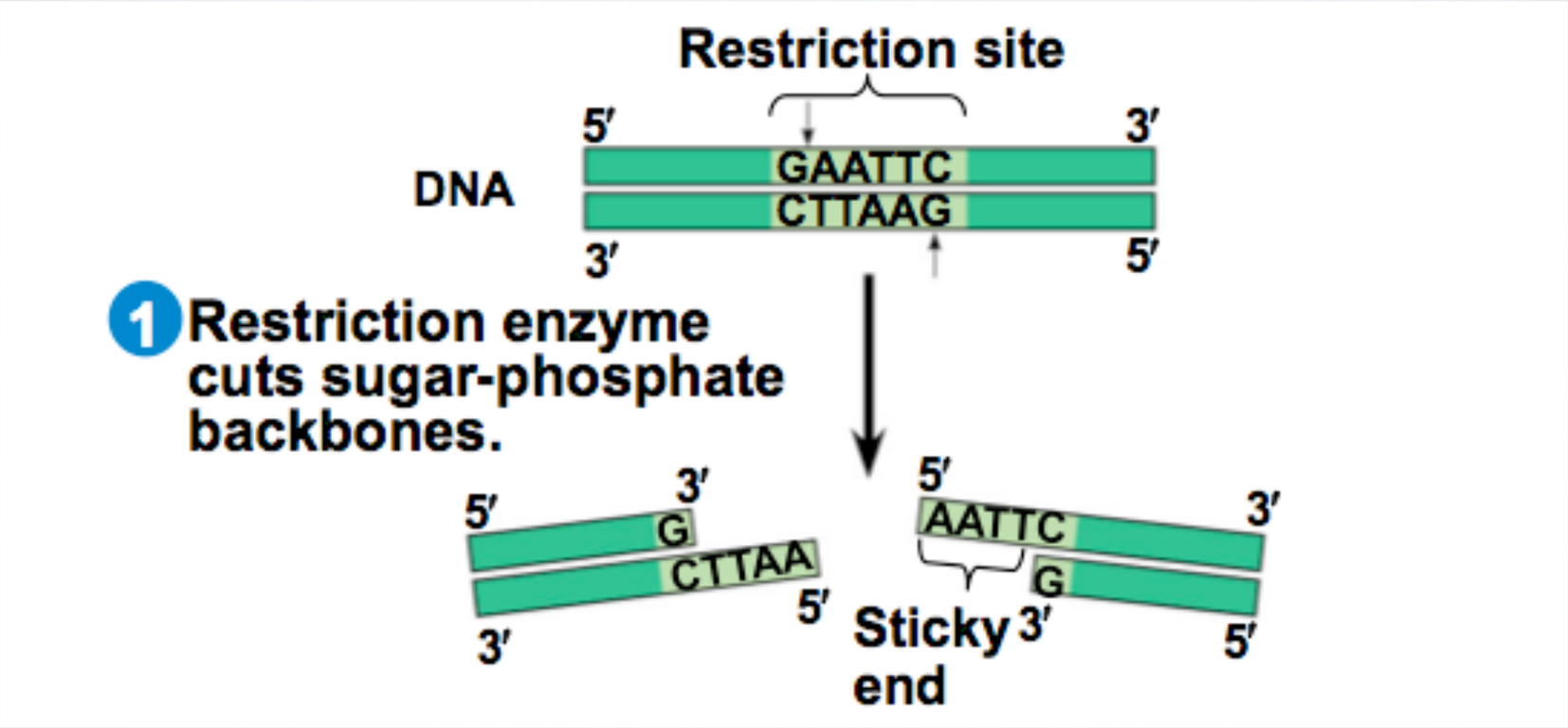
- *Bacteria protects its own DNA from restriction enzymes by methylating its DNA*

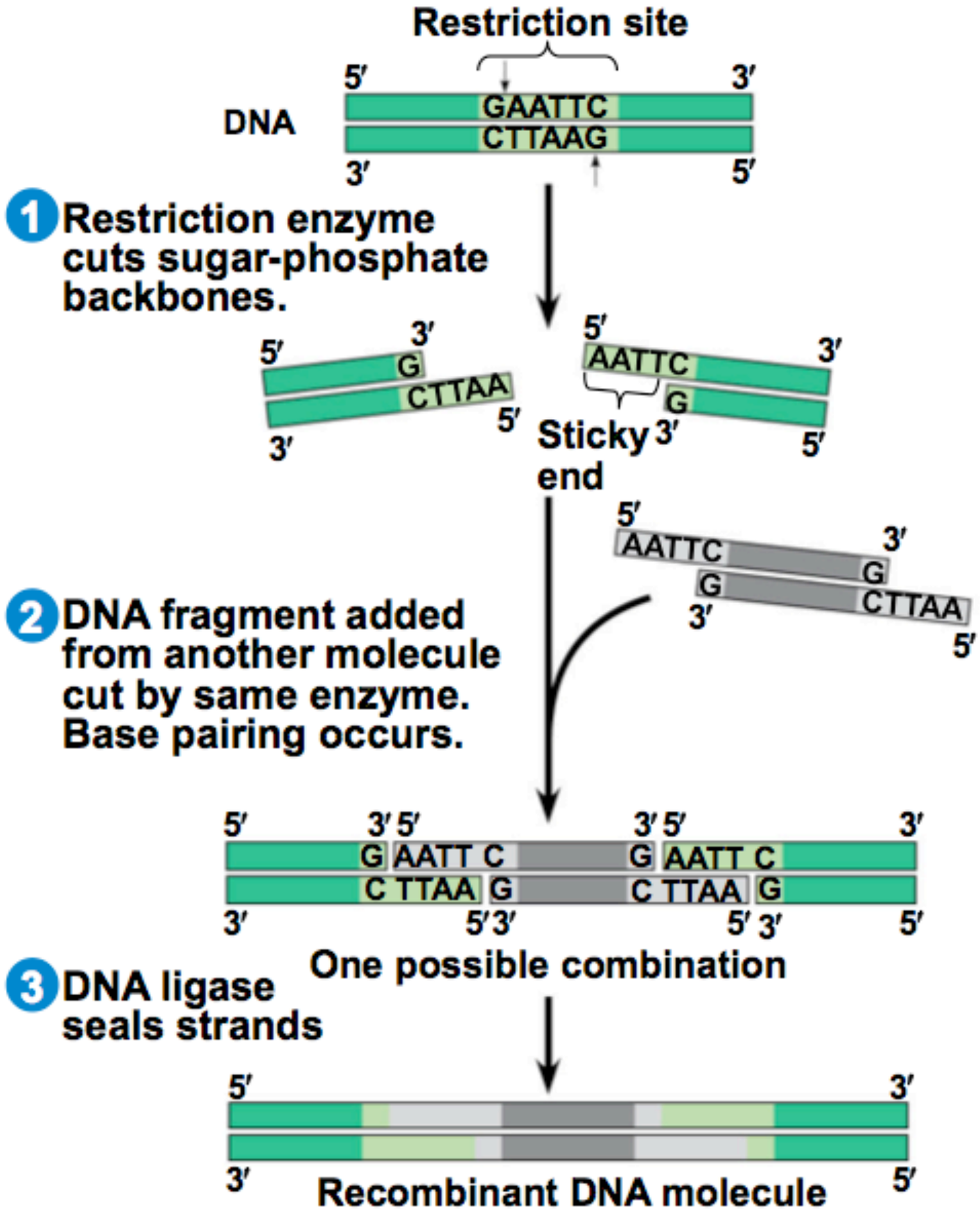
- *Hundreds of restriction enzymes have been identified and isolated, each recognizing a specific restriction site.*

- Bacterial restriction enzymes cut DNA molecules at specific DNA sequences called **restriction sites**

RESTRICTION ENZYMES

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
- A restriction enzyme usually makes many cuts, yielding **restriction fragments**
- The most commonly used restriction enzymes recognize and cut DNA into sequences of 4-8 nucleotides in length





- The most useful restriction enzymes cut DNA in a staggered way, producing fragments with "**sticky ends**"
- Sticky ends can bond with complementary sticky ends of other fragments
- **DNA ligase** is an enzyme that seals the bonds between restriction fragments

RESTRICTION ENZYMES

- Restriction sites are specific but their number and location throughout an entire molecule is random.
- A restriction enzymes will cut every restriction site it finds.
- This results in hundreds or thousands of restriction fragments, each its own unique length and sequence but each with exactly the same "sticky ends".
- Imagine, only one of these fragments represents our "gene of interest", later we ill learn how to find our "needle in the haystack"

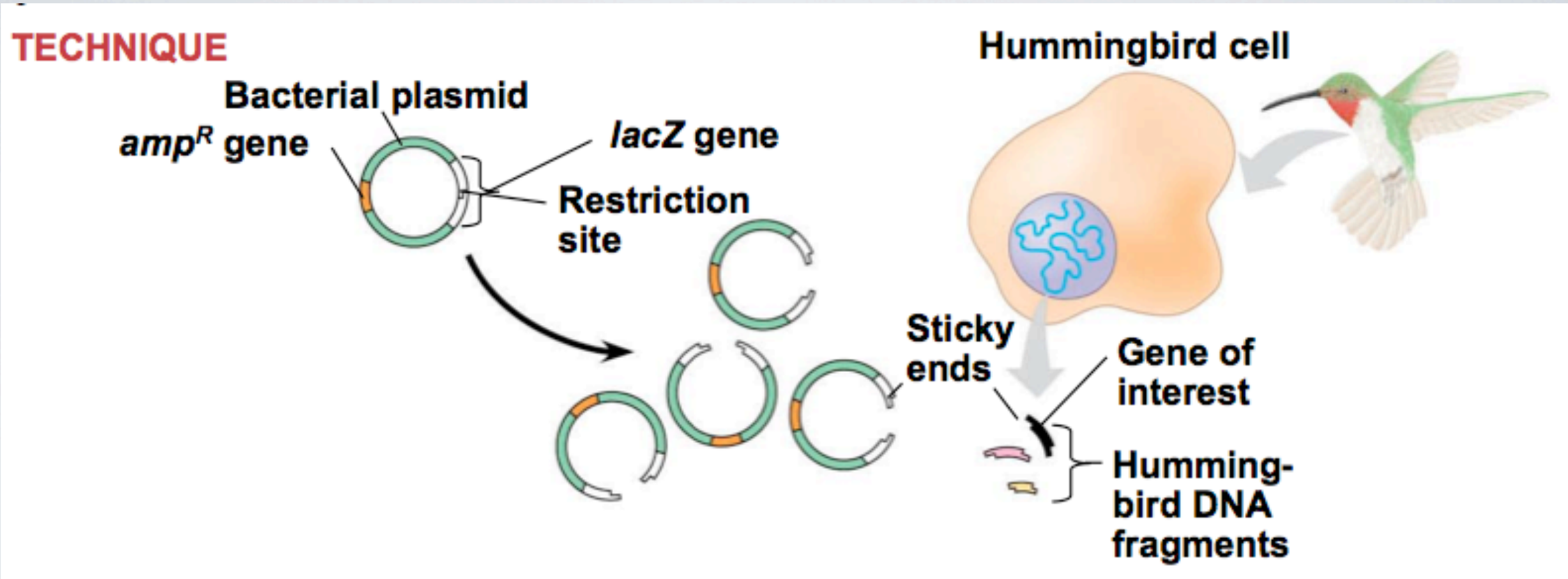
CLONING EUKARYOTIC GENES USING PLASMIDS

- Recall, the main idea behind gene cloning involves finding a gene of interest in one organism, cutting the gene out and then pasting this gene into another cell/organism.
- The restriction enzymes act like "scissors"
- DNA ligase acts like the "glue"

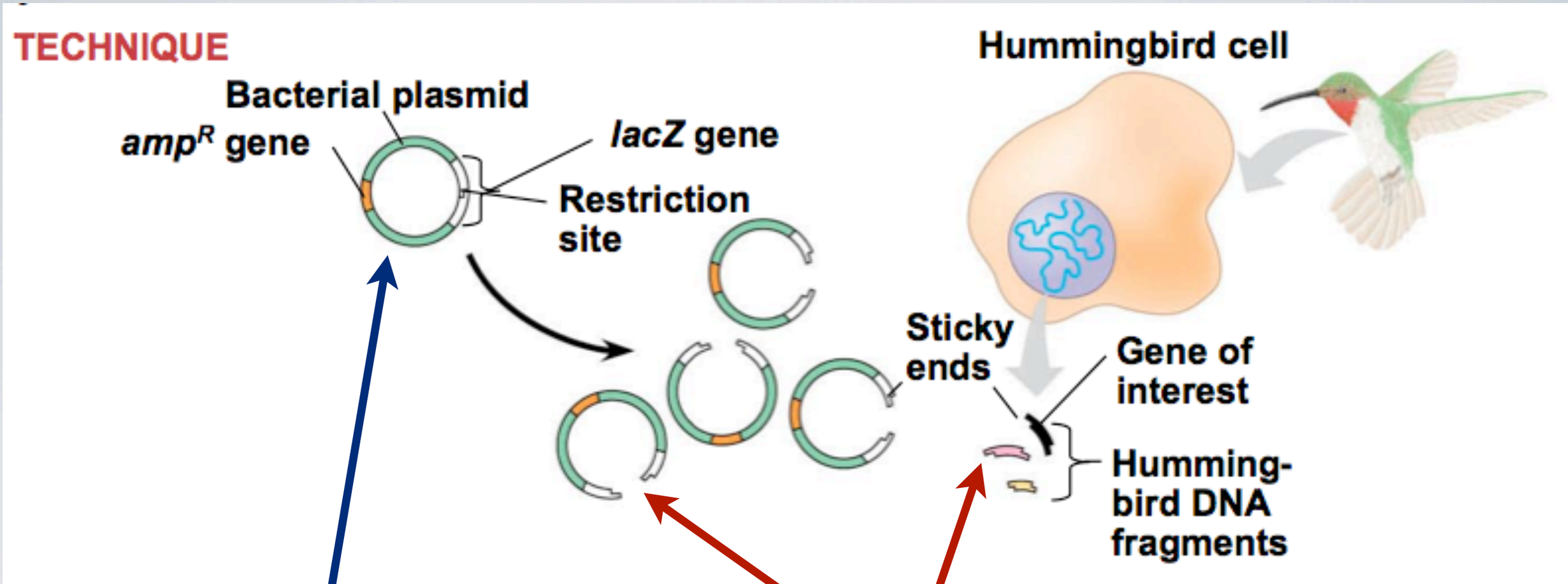
But we still need something to carry the gene into the host cell

- A **cloning vector** is a molecule of DNA that can carry foreign DNA into a host cell and replicate there.
- Bacterial plasmids are widely used as cloning vectors

CLONING EUKARYOTIC GENES USING PLASMIDS

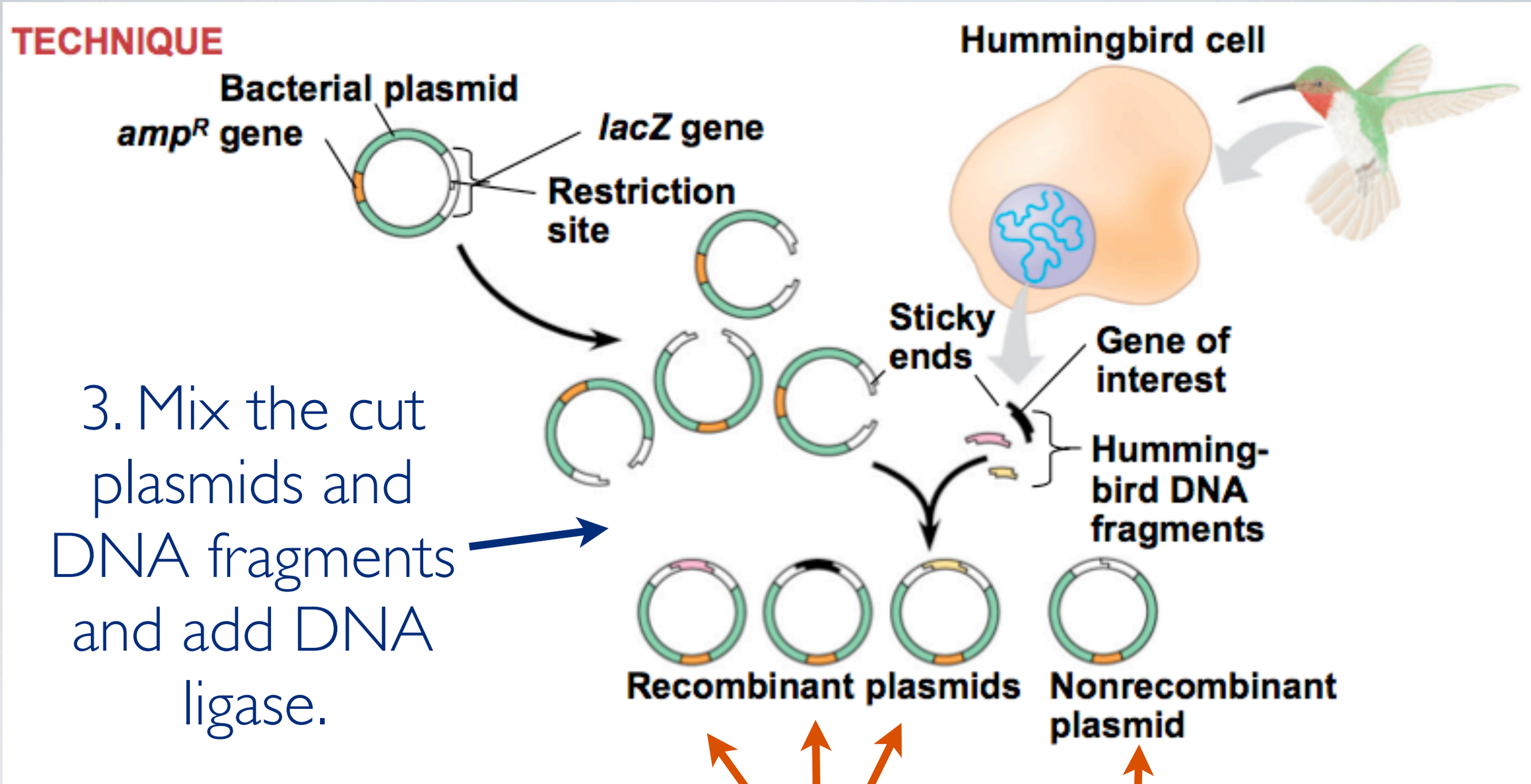


In this example we are going to learn how we might insert hummingbird genes into E. coli.



1. Obtain an engineered plasmid, today this means purchasing it from a biotech supply company.

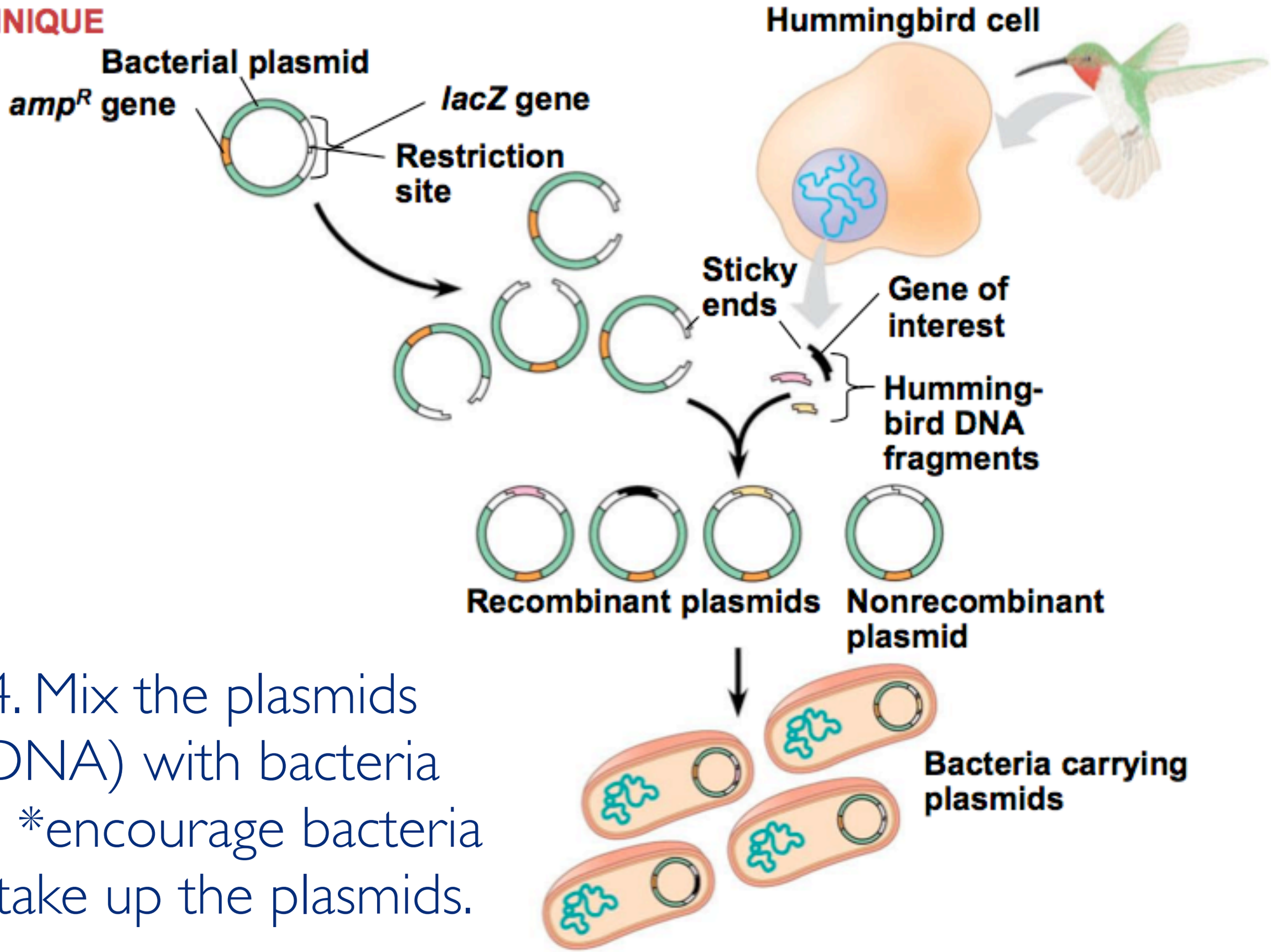
2. Cut plasmid and Hummingbird's DNA with the same restriction enzyme, one that makes a single cut in the Lac Z gene of the engineered plasmid .



3. Mix the cut plasmids and DNA fragments and add DNA ligase.

Some plasmids will be recombinants while others are not recombinants.

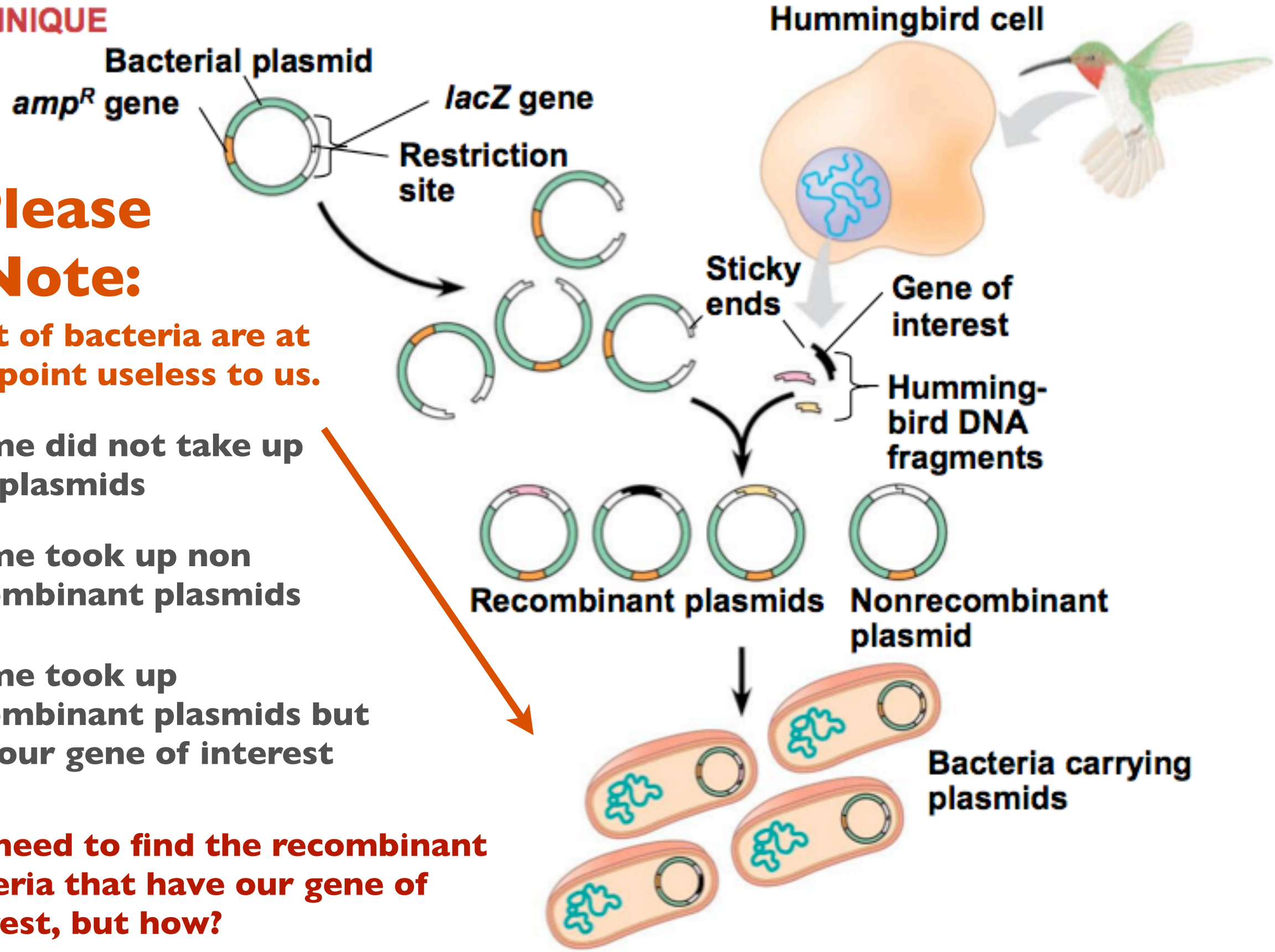
TECHNIQUE



4. Mix the plasmids (DNA) with bacteria and *encourage bacteria to take up the plasmids.

*lab techniques such as cold to hot water bath transfers.

TECHNIQUE



Please Note:

Most of bacteria are at this point useless to us.

-some did not take up any plasmids

-some took up non recombinant plasmids

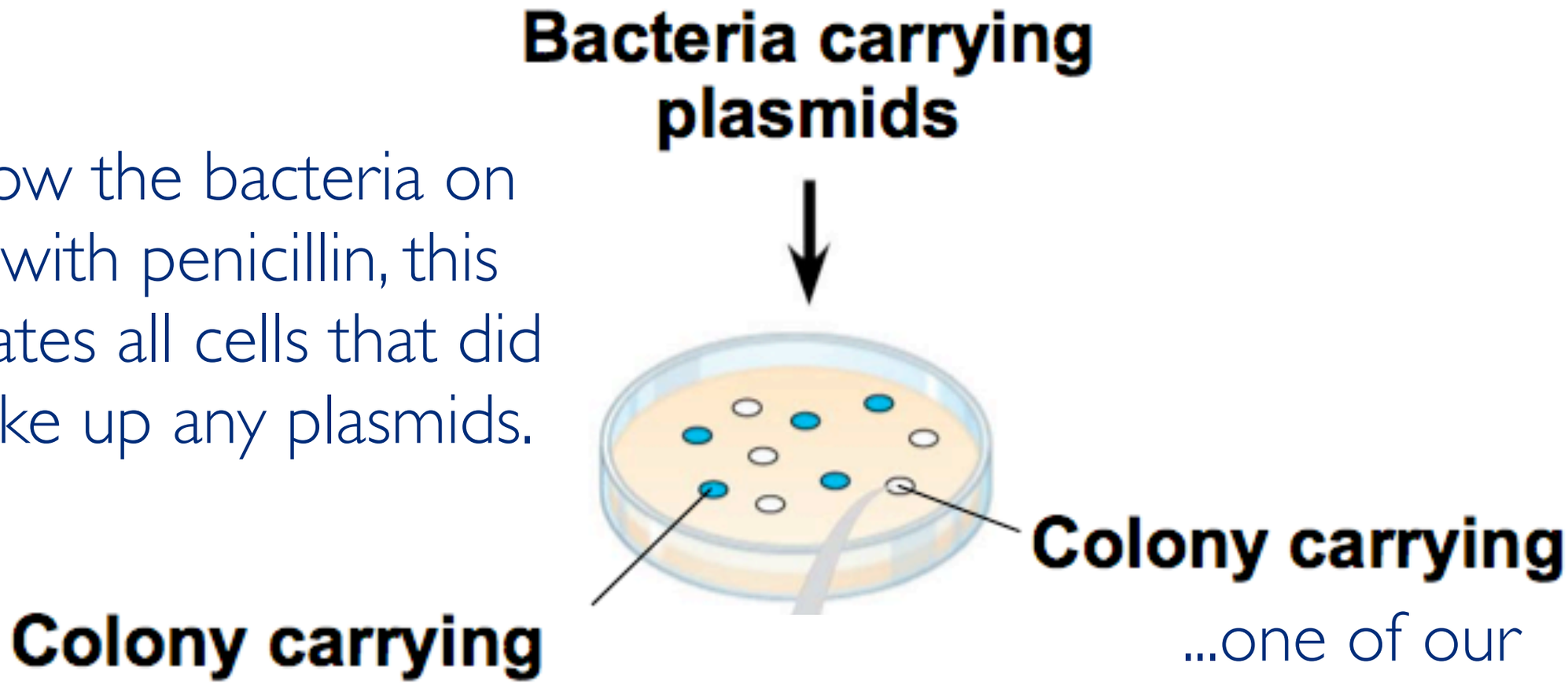
-some took up recombinant plasmids but not our gene of interest

-we need to find the recombinant bacteria that have our gene of interest, but how?

...the amp^R gene and the Lac Z gene will help us

CLONING EUKARYOTIC GENES

5. Grow the bacteria on agar with penicillin, this eliminates all cells that did not take up any plasmids.

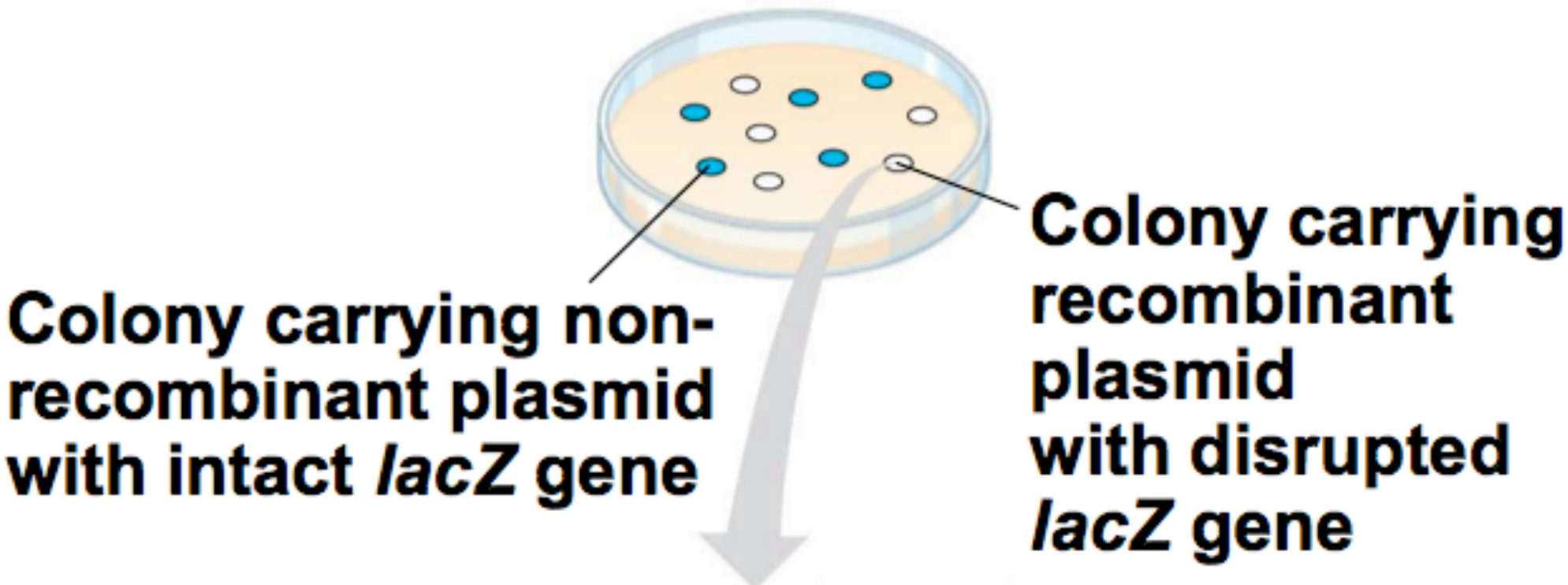


...one of our engineered plasmids with amp^R gene

...one of our engineered plasmids with amp^R gene

CLONING EUKARYOTIC GENES

5. Grow the bacteria on agar with X-gal. This will help to distinguish between (white) recombinant and (blue) non recombinant plasmids.



An intact Lac Z gene (non recombinant plasmid) can produce a functional enzyme beta-galactosidase that will hydrolyze X-gal and turn it blue.

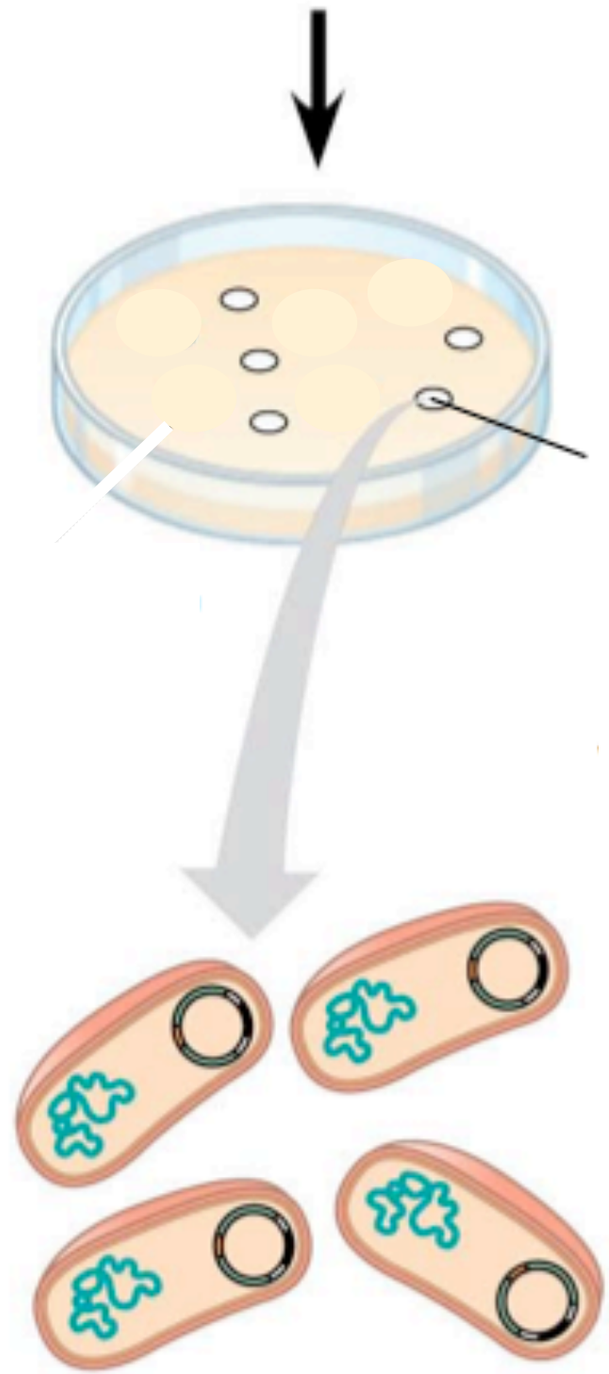
A disrupted Lac Z gene (recombinant plasmid) can NOT produce a functional enzyme beta-galactosidase and will NOT hydrolyze X-gal and remains white.

CLONING EUKARYOTIC GENES

At this point, we know the bacteria have our plasmids, we know that a piece of Hummingbird DNA is in the correct position

RESULTS

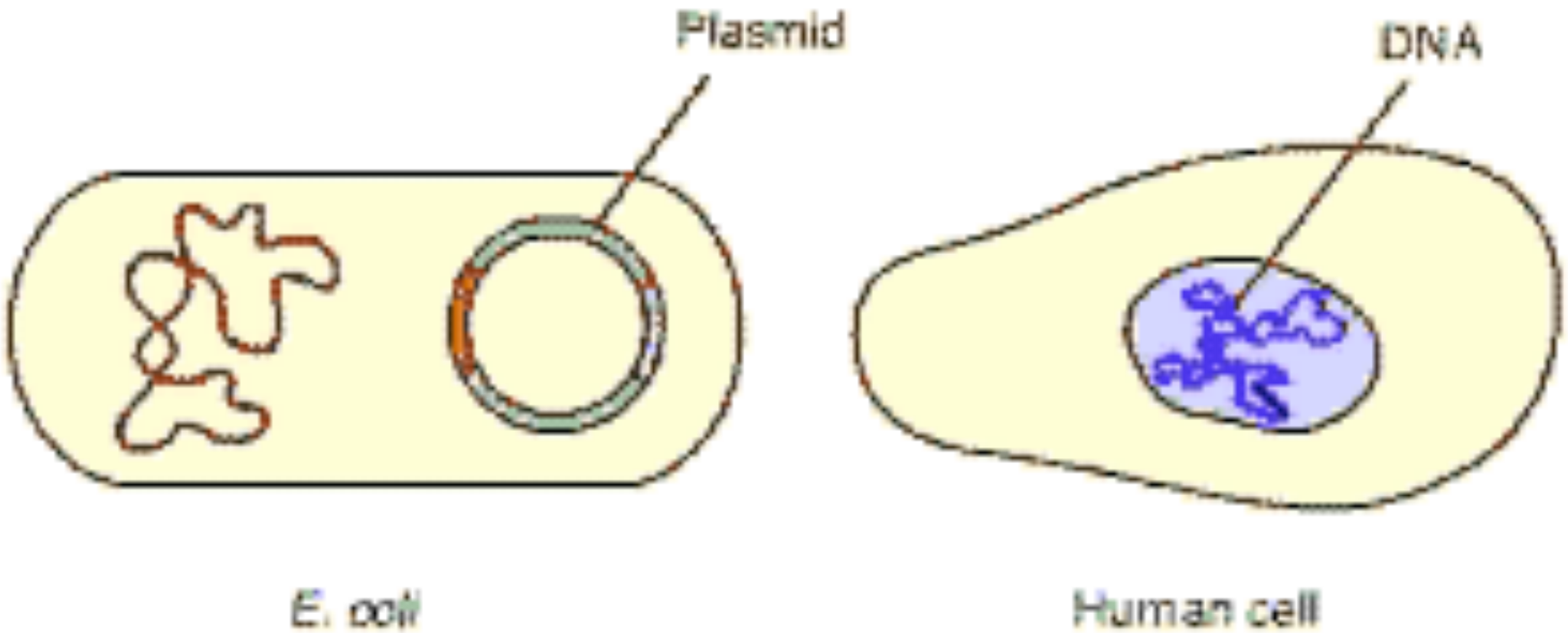
Most of these colonies are carrying other genes, or pieces of genes or even even noncoding pieces of DNA...not our single gene of interest!



Shortly, we will learn how to find the colony carrying our gene interest.

One of many bacterial clones

CLONING REVIEW



STORING CLONED GENES IN DNA LIBRARIES

- The cloning process just described was a "shotgun" approach because the entire genome was digested into fragments.

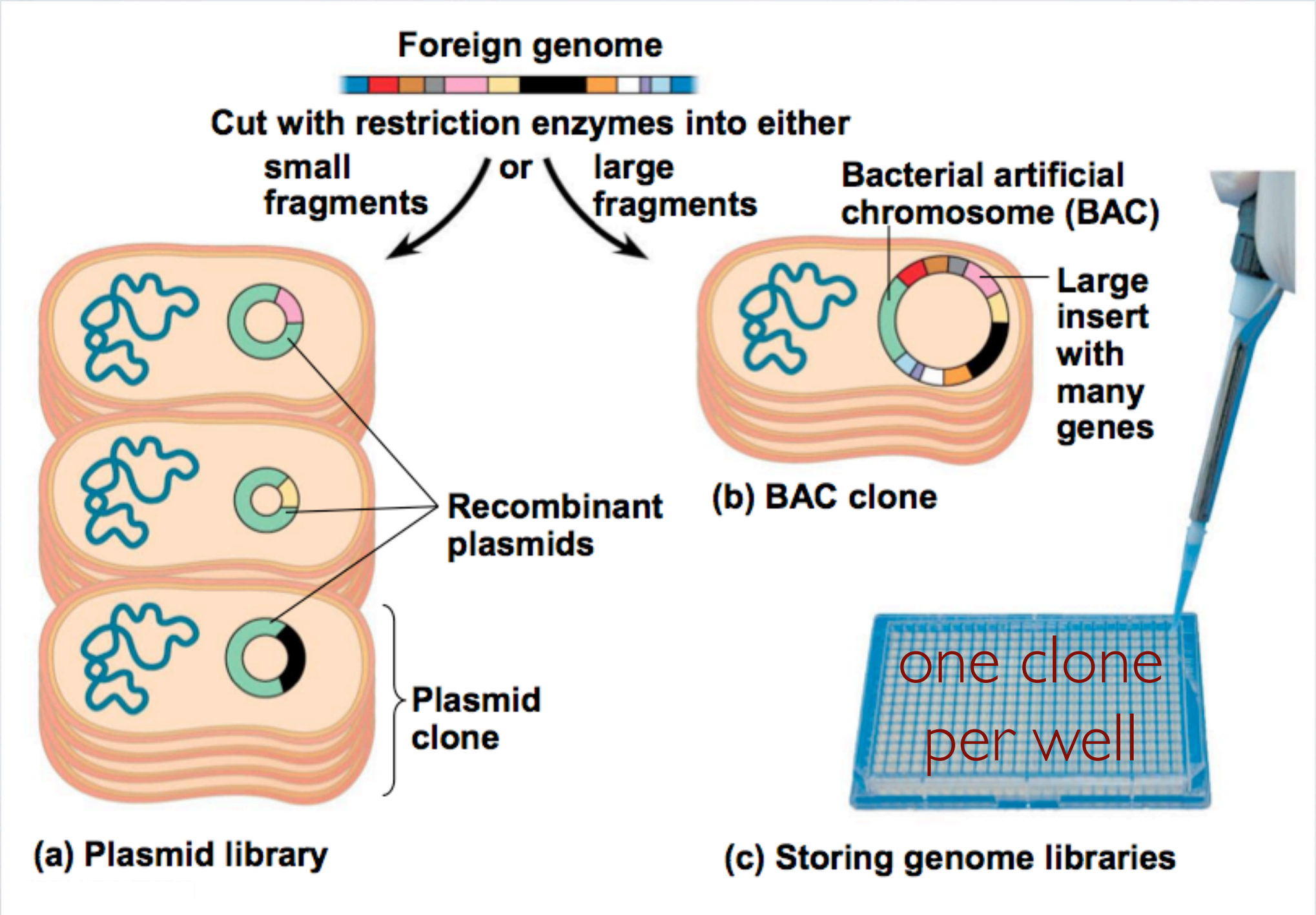
- At the conclusion of this approach we have hundreds or thousands of recombinant bacteria each carrying a particular segment of the original genome.

- The complete set recombinant bacteria (plasmid clones) are called **genomic libraries**.

- Each clone is like a book in a library with its own specific information.

DNA LIBRARIES

- Another type of vector commonly used to build genomic libraries are **bacterial artificial chromosomes (BAC)**.

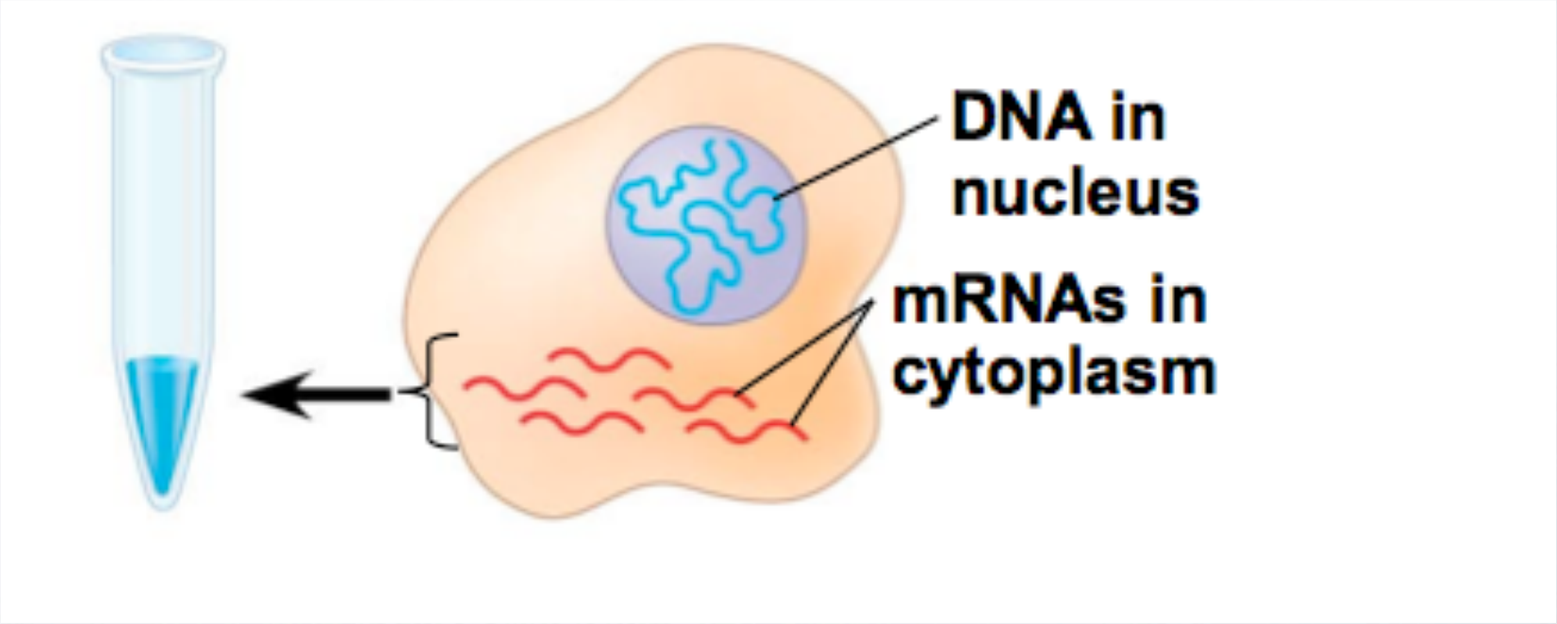


DNA LIBRARIES

- Researchers can also create DNA libraries using cDNA.

- A **complementary DNA (cDNA)** library is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a particular cell

- A **cDNA library** represents only part of the genome—only the subset of genes transcribed into mRNA in the original cells



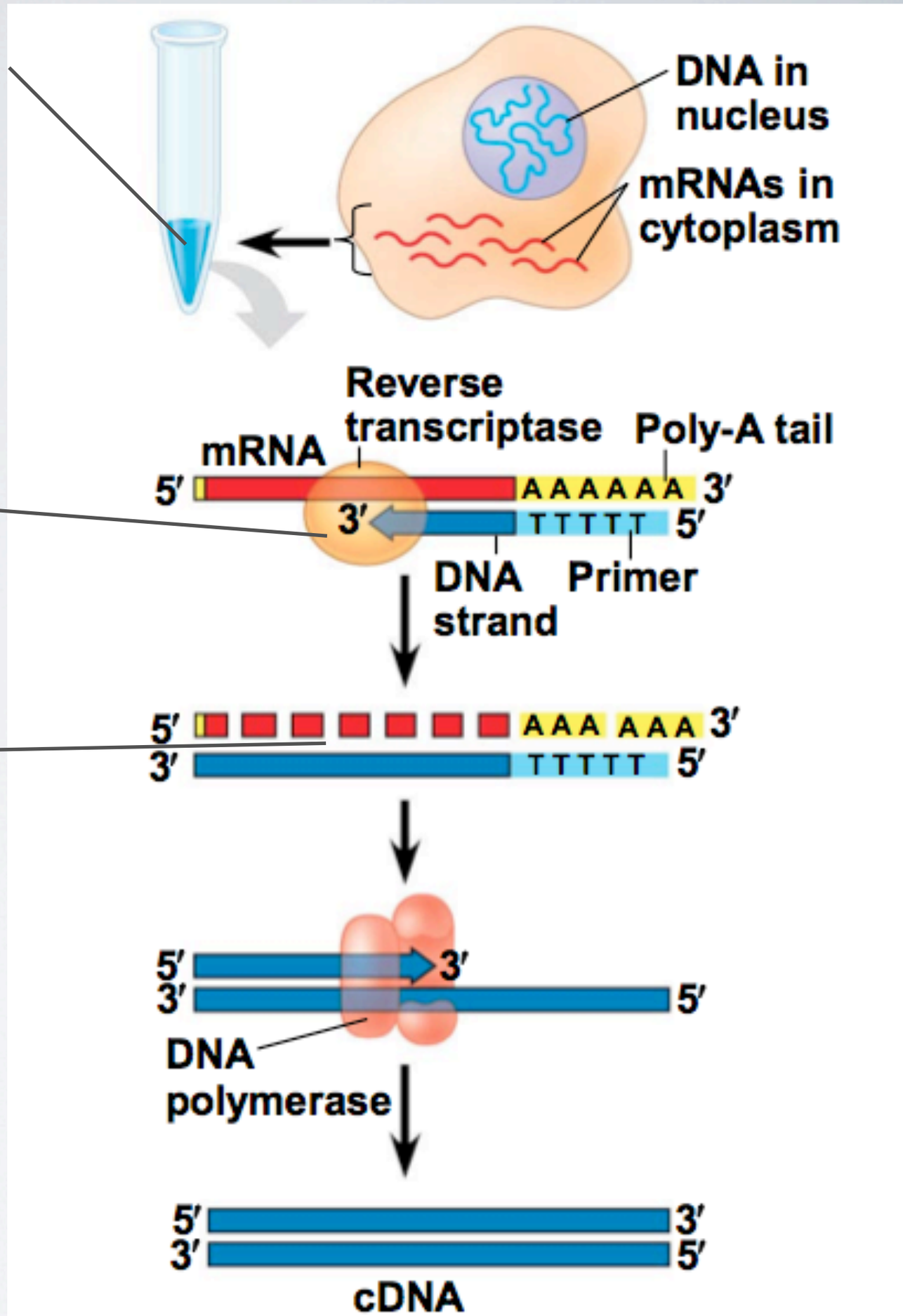
Reverse transcriptase is added to a test tube containing mRNA isolated from a certain type of cell.

Reverse transcriptase makes the first DNA strand using mRNA as a template.

mRNA is degraded by another enzyme.

DNA polymerase make the second strand

The result is a cDNA, which carries the coding sequence of the gene but no introns.



COMPARING DNA LIBRARIES

Genomic Libraries

-contains all the genes in a genome particular type of cell

cDNA Libraries

-contains only the genes expressed by a particular type of cell at a particular time

-contains regulatory sequences, introns and other noncoding regions of DNA

-contains only the gene itself

FINDING GENES OF INTEREST IN DNA LIBRARIES

- Now lets return to our gene cloning procedure where we created recombinant bacteria but did not know which one carried our gene of interest.

- A clone carrying the gene of interest can be identified with a **nucleic acid probe** having a sequence complementary to the gene.

- This process is called **nucleic acid hybridization**.

- We first need to know the gene sequence or at least some of the gene sequence our "gene of interest".

A probe can be synthesized that is complementary to the gene of interest

For example, if the desired gene is

5′ ··· CTCATCACCGGC··· 3′

– Then we would synthesize this probe

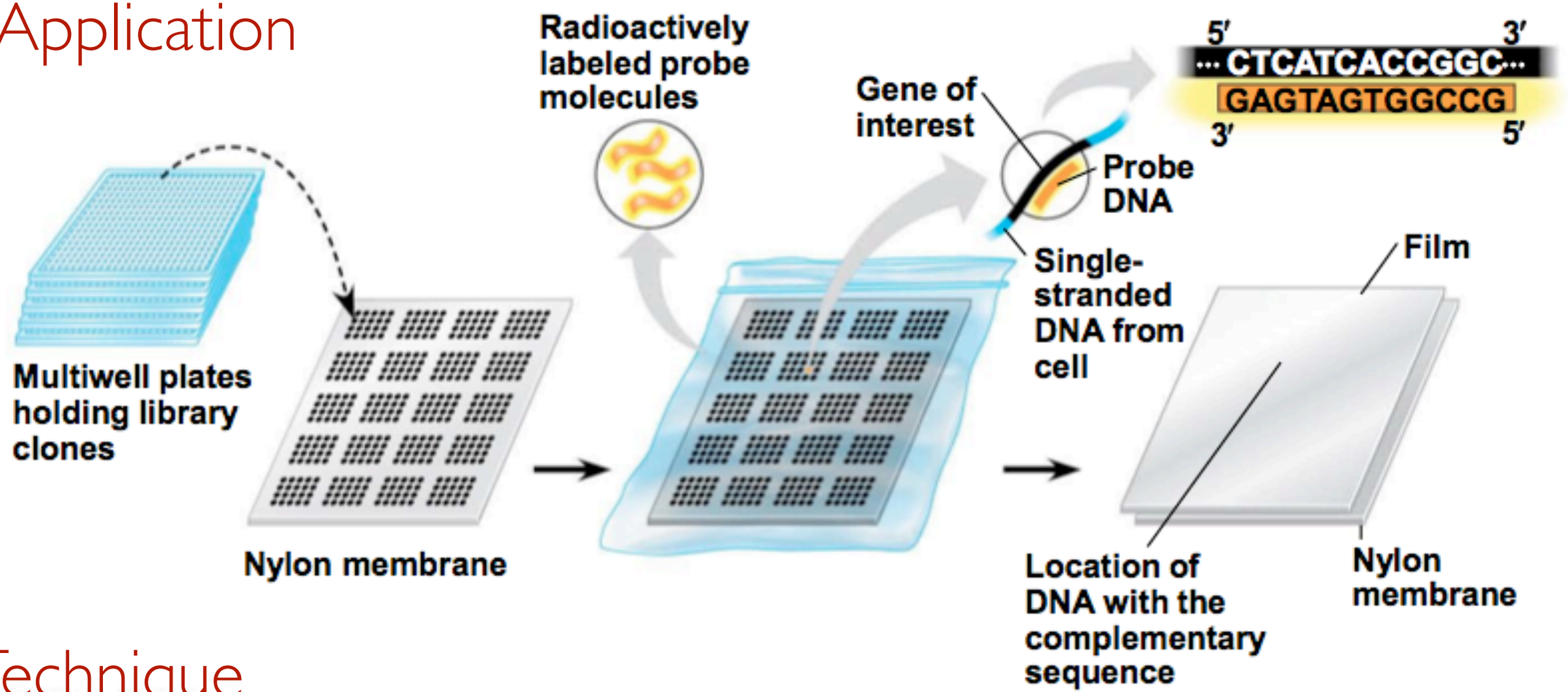
3′ GAGTAGTGGCCG 5′

The DNA probe can be used to screen a large number of clones simultaneously for the gene of interest.

Once identified, the clone carrying the gene of interest can be cultured.

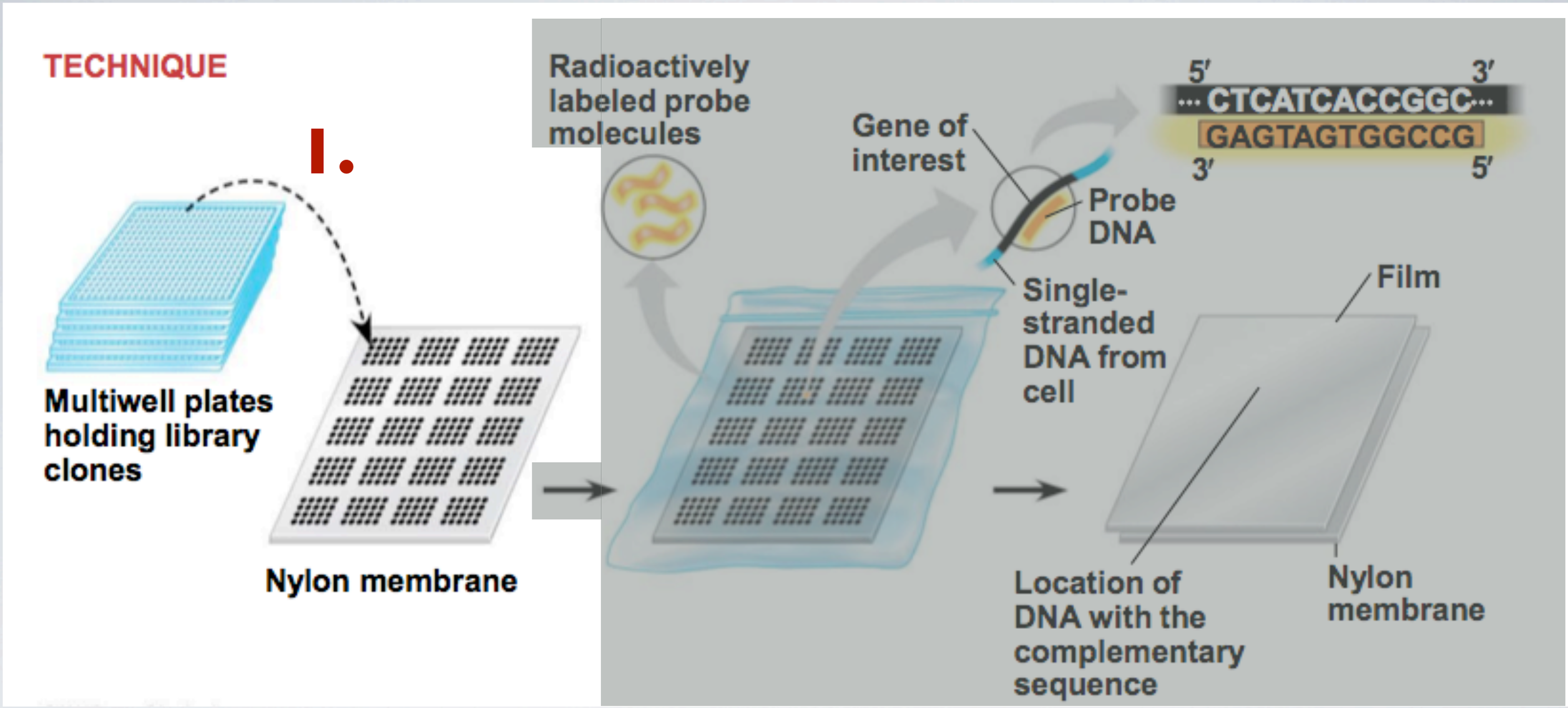
Hybridization with a complementary nucleic acid probe detects a specific DNA sequence within the mixture of DNA molecules. In this case, a collection of bacterial clones from a hummingbird genomic library is screened to identify clones that carry our gene of interest. Remember the clones are stored on multi-well plates with a each holding one clone.

Application



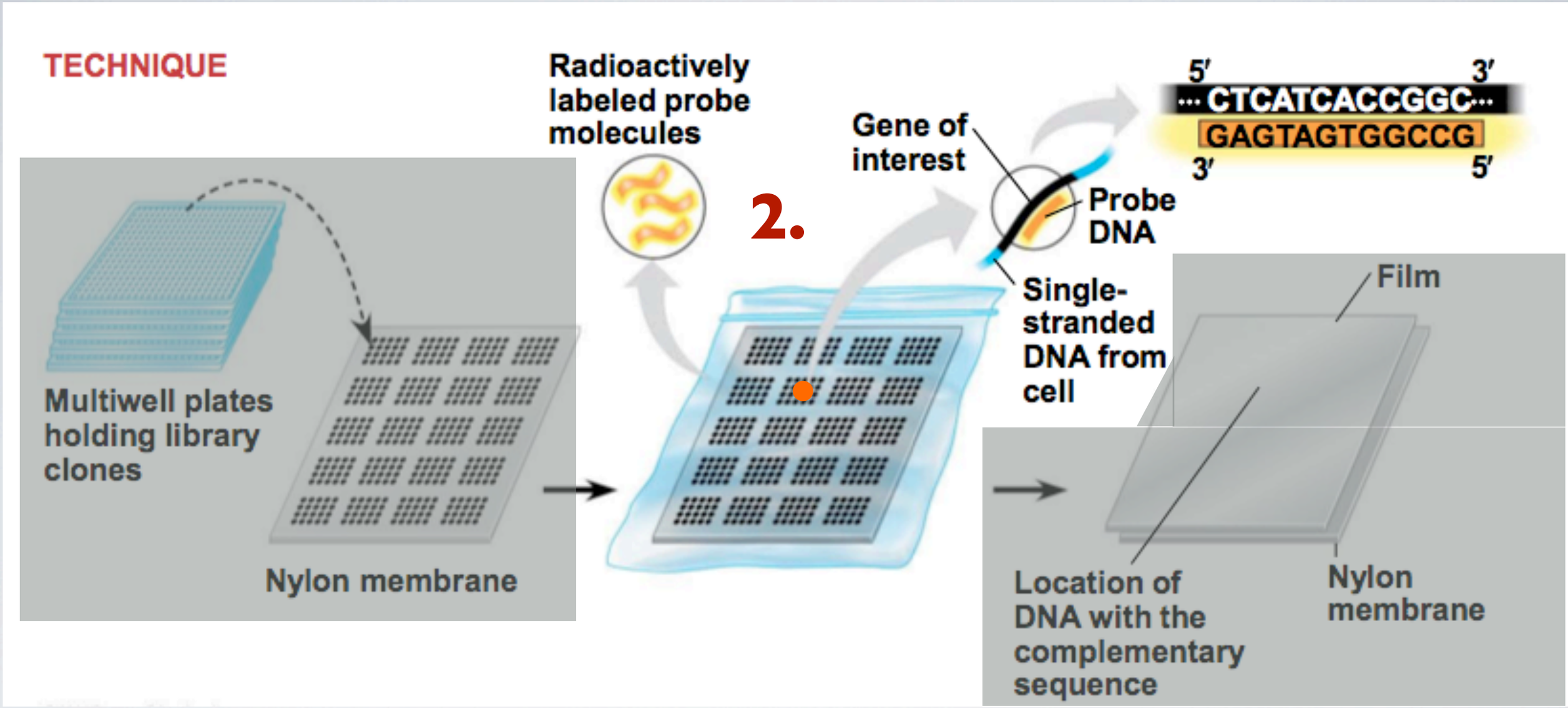
Technique

Cells from each clone are applied to a special nylon membrane. Each membrane has room for thousands of clones, so only a few are needed to hold all clones in the genomic library. These membranes are screened using the radioactive or fluorescent probes.



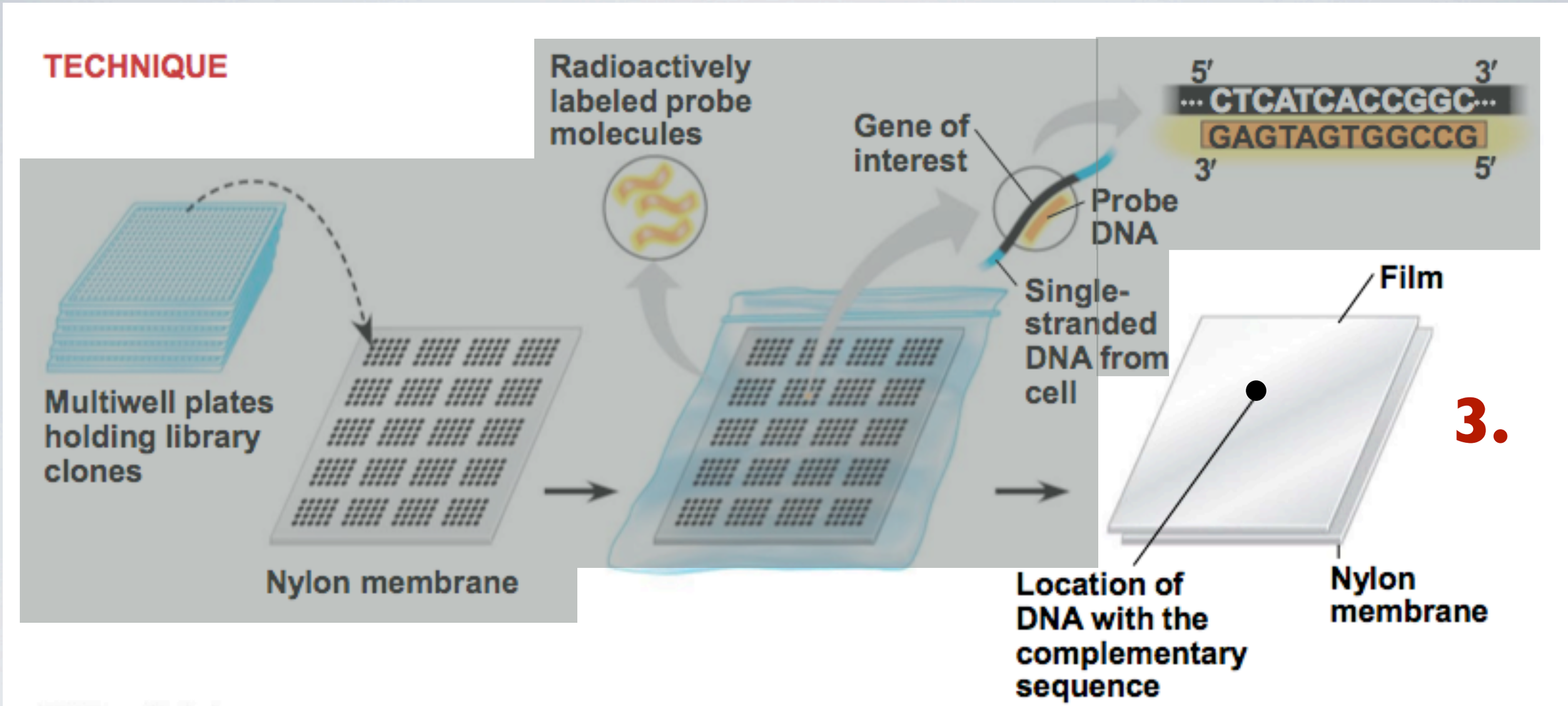
I.

Plate by plate, cells from each well, are transferred to a defined spot on the nylon membrane. The nylon membrane is treated in a way to break open the cells and denature their DNA; the resulting single stranded DNA molecules stick to the membrane.



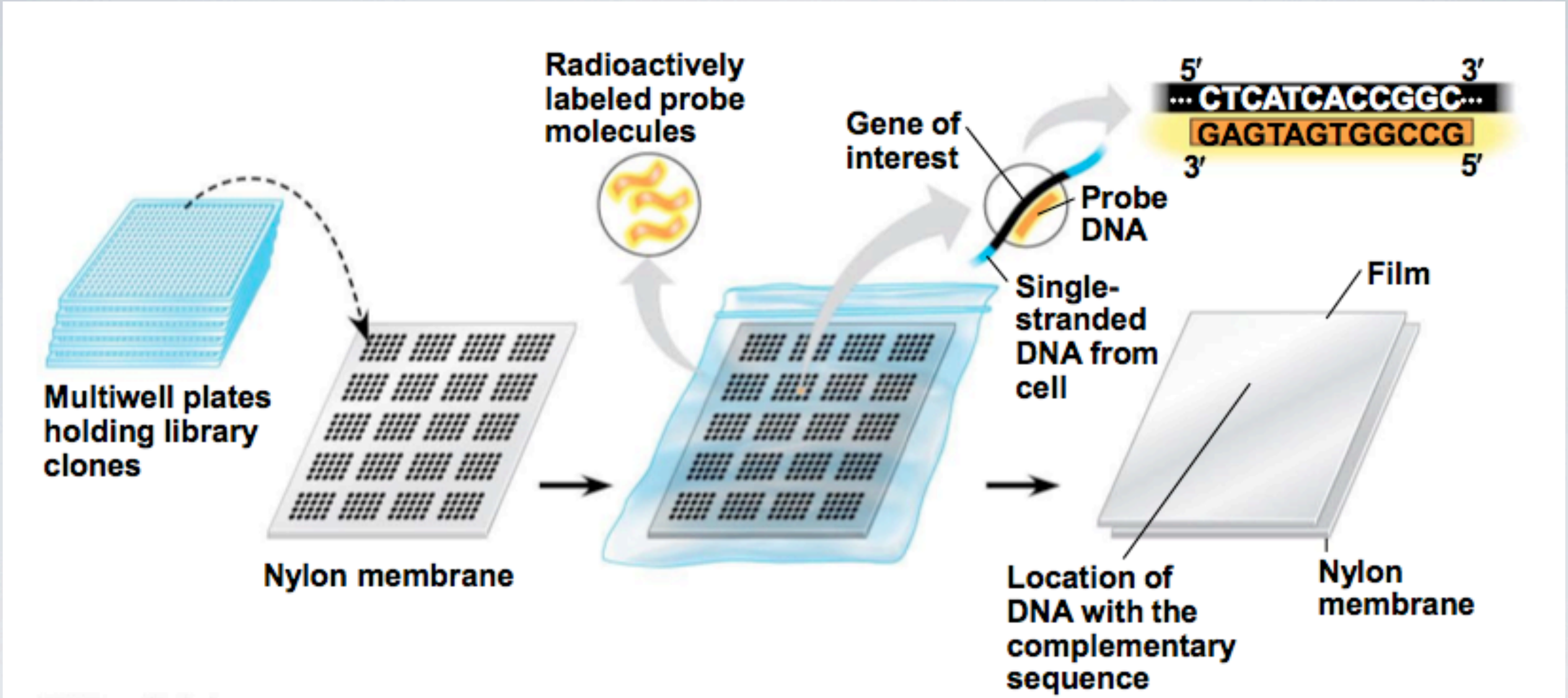
2.

The membrane is then incubated in a solution with the "labeled" probes complimentary to our gene of interest. The single strand of clone DNA sticks to the membrane, excess DNA and cell parts are rinsed out and if our gene is present our probe will bind to the gene.



3.

The membrane is laid under photographic film, allowing "labeled" areas to expose the film. The spots on the film correspond to the locations on the membrane of DNA that our probe hybridized to, each spot is traced back to the well containing the clone(s) that hold our gene of interest.



Results

By using probes with different nucleotide sequences in different experiments, researchers can screen the collection of bacterial clones for different genes.

BACTERIAL EXPRESSION SYSTEMS

What problems would you encounter after a plasmid carries a eukaryotic gene into a prokaryote?

Will the prokaryote express this gene?
Why would it? Why wouldn't it?

It should express the gene because the genetic code is universal!

BUT, we may have problems because gene expression in the two are different (prokaryotes do not use TATA boxes, repressors, activators etc) AND bacteria can carry out RNA processing (removing introns, adding caps and tails).

And lets not forget eukaryotes often require post translational modifications before the protein becomes functional, something foreign to bacteria.

BACTERIAL EXPRESSION SYSTEMS

SO, How can we overcome these problems?

- **First**, we can use engineered **expression vectors,** vectors that contain a highly active promoter just upstream of the restriction site where the eukaryotic gene is inserted

- **Second**, we can use cDNA which already has the introns removed, with the bases necessary to include the caps and tails.

- **Third**, we can use an entirely different approach all together...

EUKARYOTIC EXPRESSION SYSTEMS

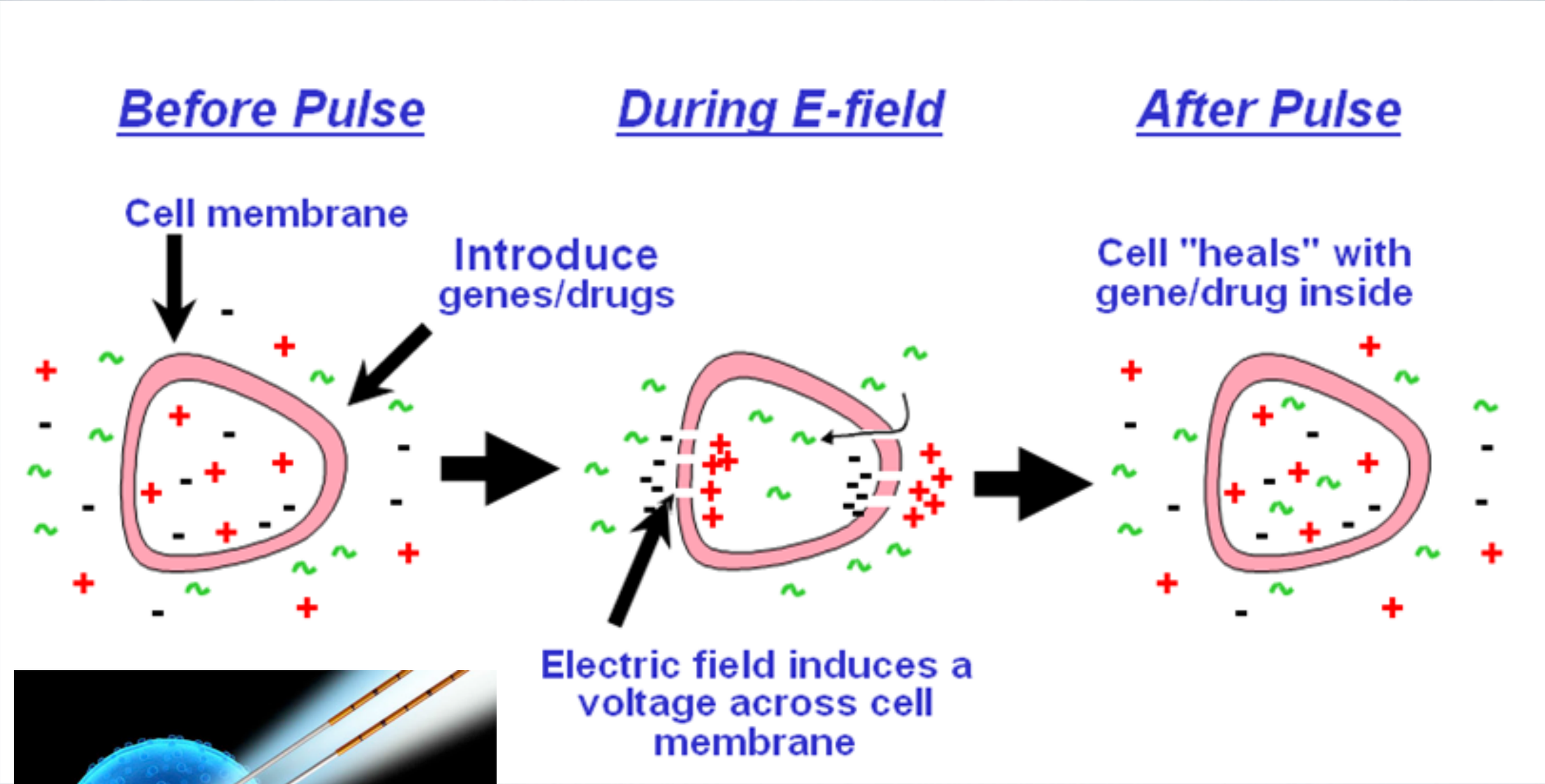
- To avoid prokaryotic/eukaryotic compatibility issues, molecular biologists simply use eukaryotic expression systems, in other other eukaryotic cells, such as *yeasts*.

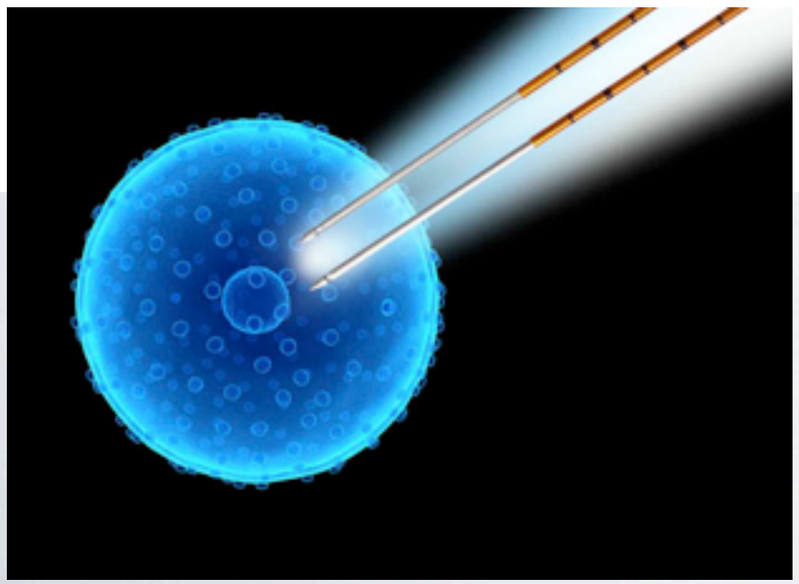
- Yeasts (single celled fungi), offer two distinct advantages.

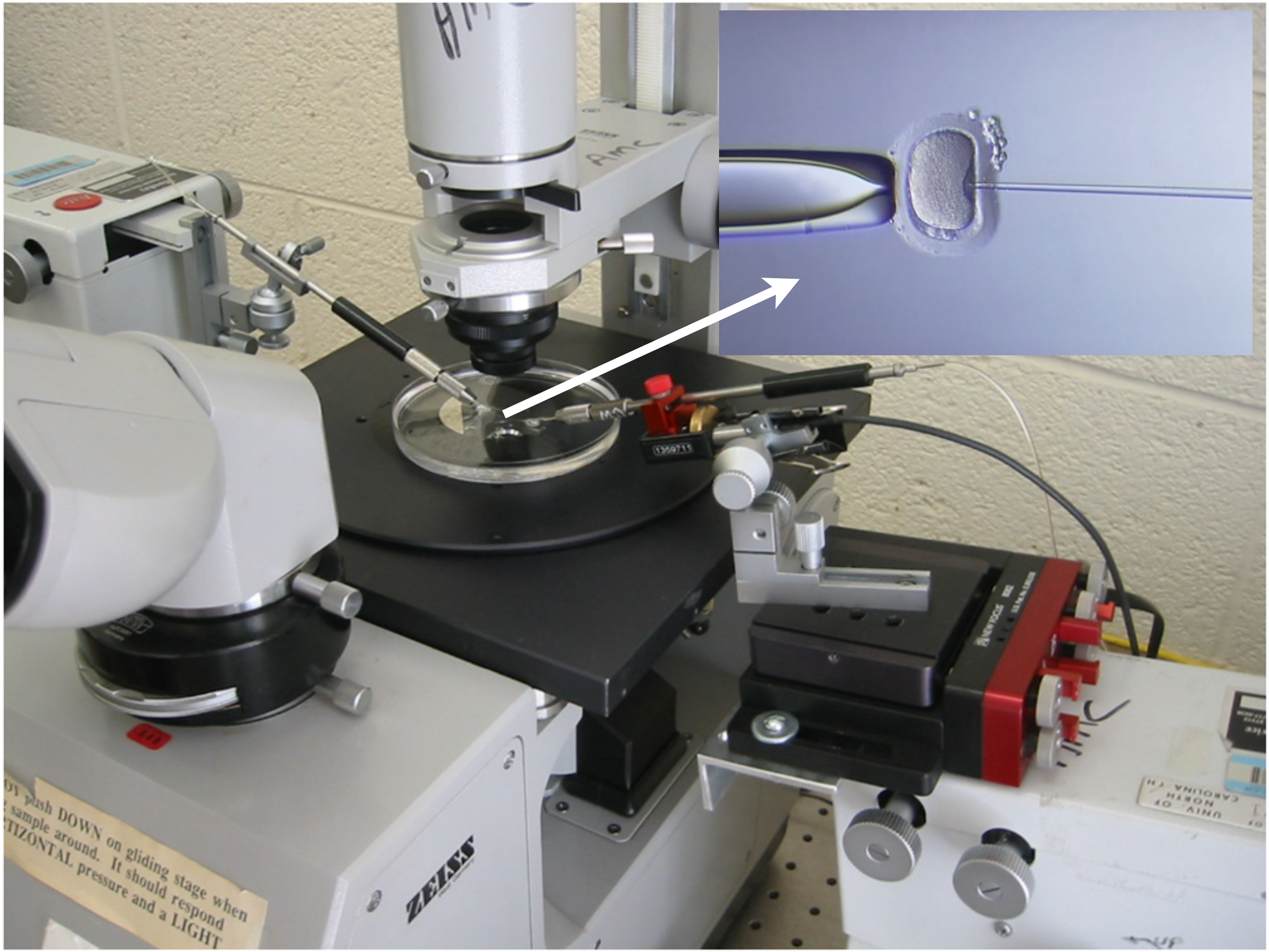
- First, yeasts are easy to grow, quick to reproduce and they contain plasmids (a rarity among eukaryotes).

- Second, yeasts being eukaryotes have the potential to carry out RNA processing and the potential to correctly modify the proteins after translation.

Note: Other methods have been developed to introduce recombinant DNA into eukaryotic cells. Such as electroporation, microinjection and others.






push DOWN on gliding stage when
sample around. It should respond
TIZONTAL pressure and a LIGHT
ZEISS

EVOLUTIONARY ANCESTRY SEEN THROUGH GENE EXPRESSION

- Given the differences between eukaryotes and prokaryotes it is quite remarkable that bacteria can express eukaryotic proteins at all (even if they are not post translationally modified).

- The countless examples of genes being moved from one species into another and then for those genes to be expressed is testament of our shared evolutionary ancestry.

- Consider the *Pax-6* gene...

Vertebrates

↓

Pax-6 gene

↓

controls gene expression of the single lens vertebrate eye

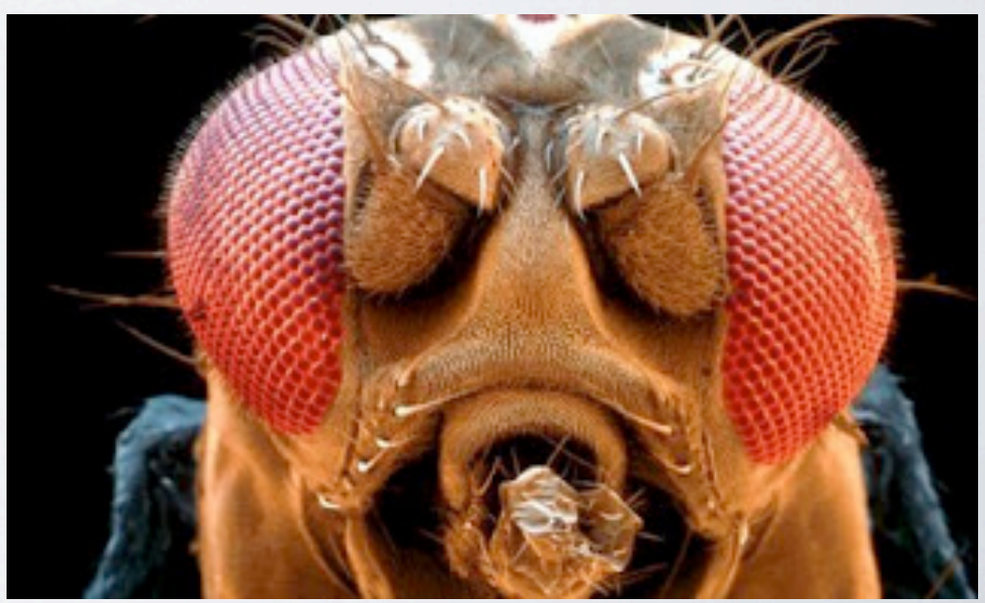
Insects

↓

Pax-6 gene

↓

controls gene expression of the compound insect eye



Experiment

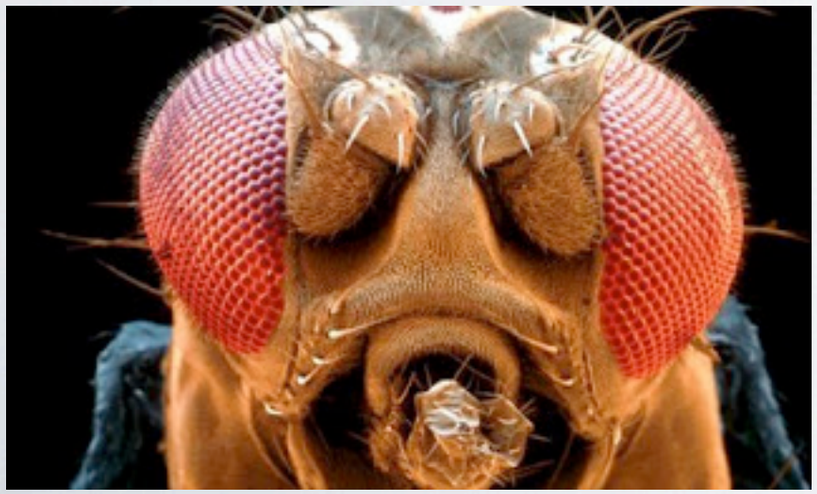
Remove Pax-6 gene from mouse

Remove Pax-6 gene from fly

Insert Pax-6 gene into fly

Insert Pax-6 gene into mouse

Results



Both versions of the Pax-6 gene can substitute for each other, evidence of their evolution from a gene in a common ancestor

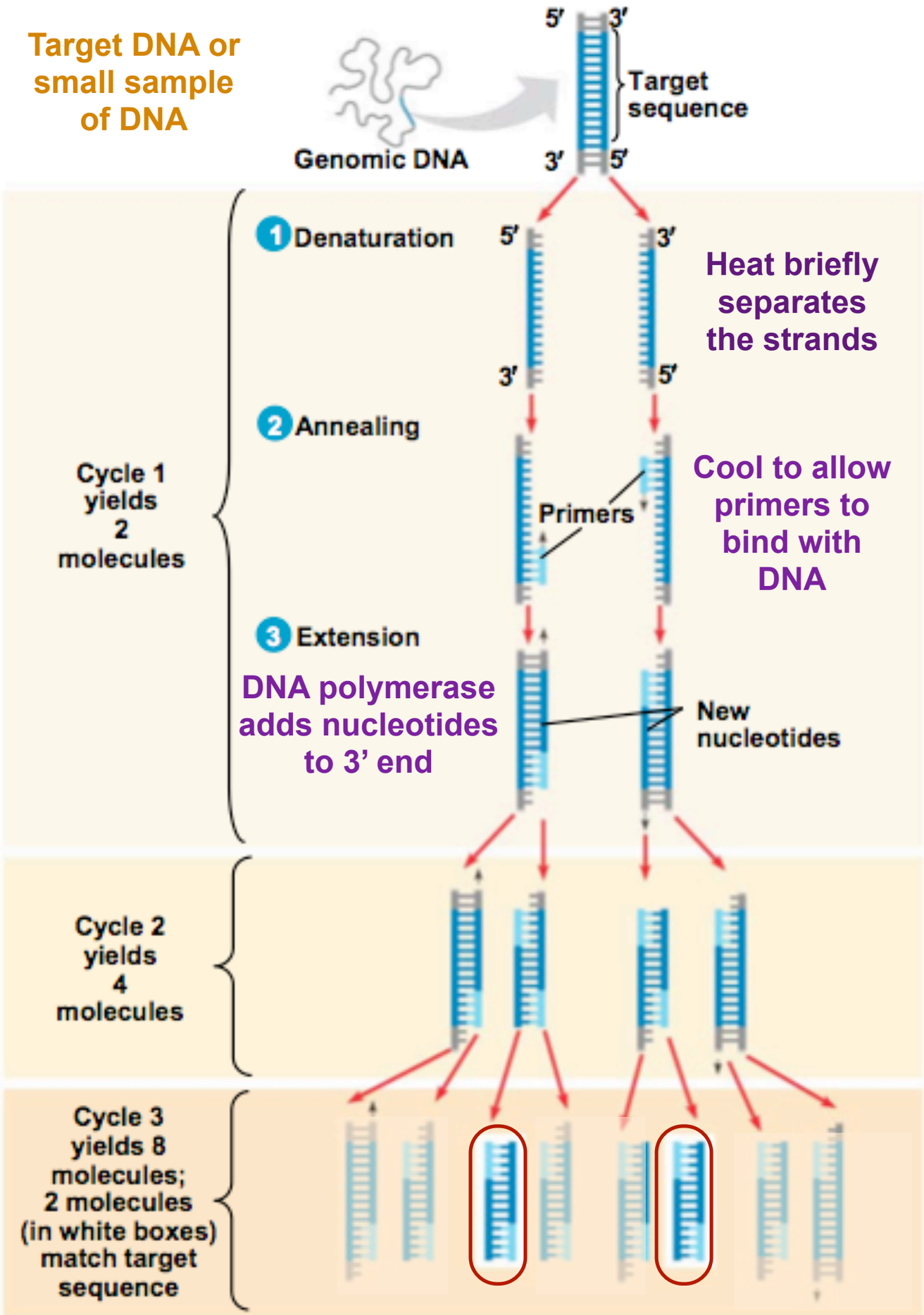
AMPLIFYING DNA *IN VITRO: PCR*

- DNA cloning remains the best method of preparing large quantities of a particular DNA sequence.

- However, in cases where the source of DNA is scanty or impure (ex. DNA from fossils or crime scenes) then the polymerase chain reaction (PCR) is a faster, more selective and overall better choice.

- PCR through automated machines can make billions of copies of the DNA sequence within hours.

- In fact, PCR is increasingly being used to make enough DNA fragments to insert into a vector, thus skipping entirely the steps of making and screening a DNA library.



Application

Any specific sequence or segment of DNA can be copied many times *in vitro*.

Technique

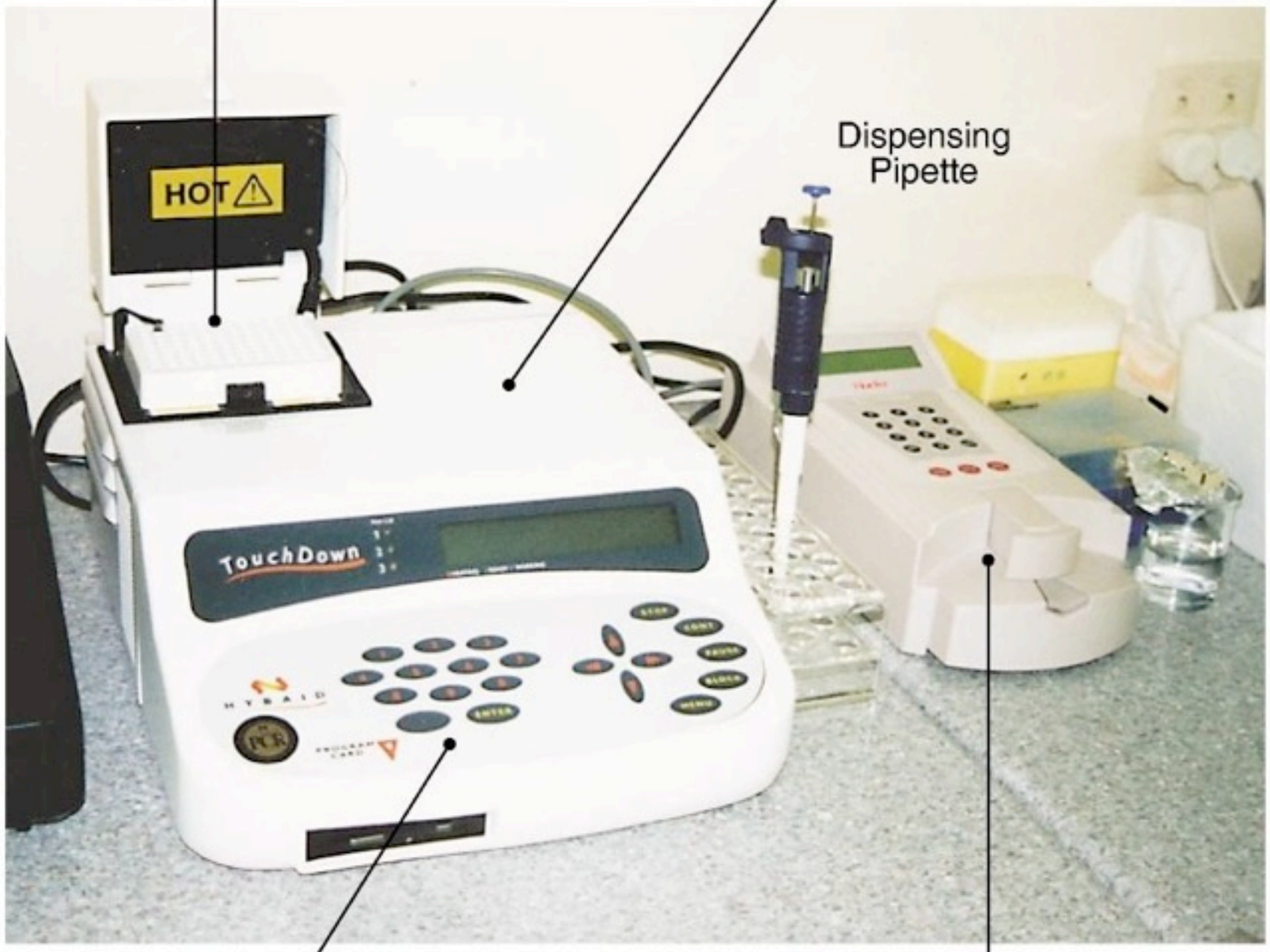
PCR requires: the target DNA, a heat resistant DNA polymerase, millions of single nucleotides (all 4), two long primers (longer = more specificity). One complementary primer binds to one end of the target DNA and the second primer binds to the other end.

Results

After 3 rounds, 2 of the 8 molecules match the target sequence. After 30 rounds over 1 billion will match the target sequence! (exponential growth)

Loading Tray
Prepared samples in tiny PCR tubes are placed in the loading tray and the lid is closed.

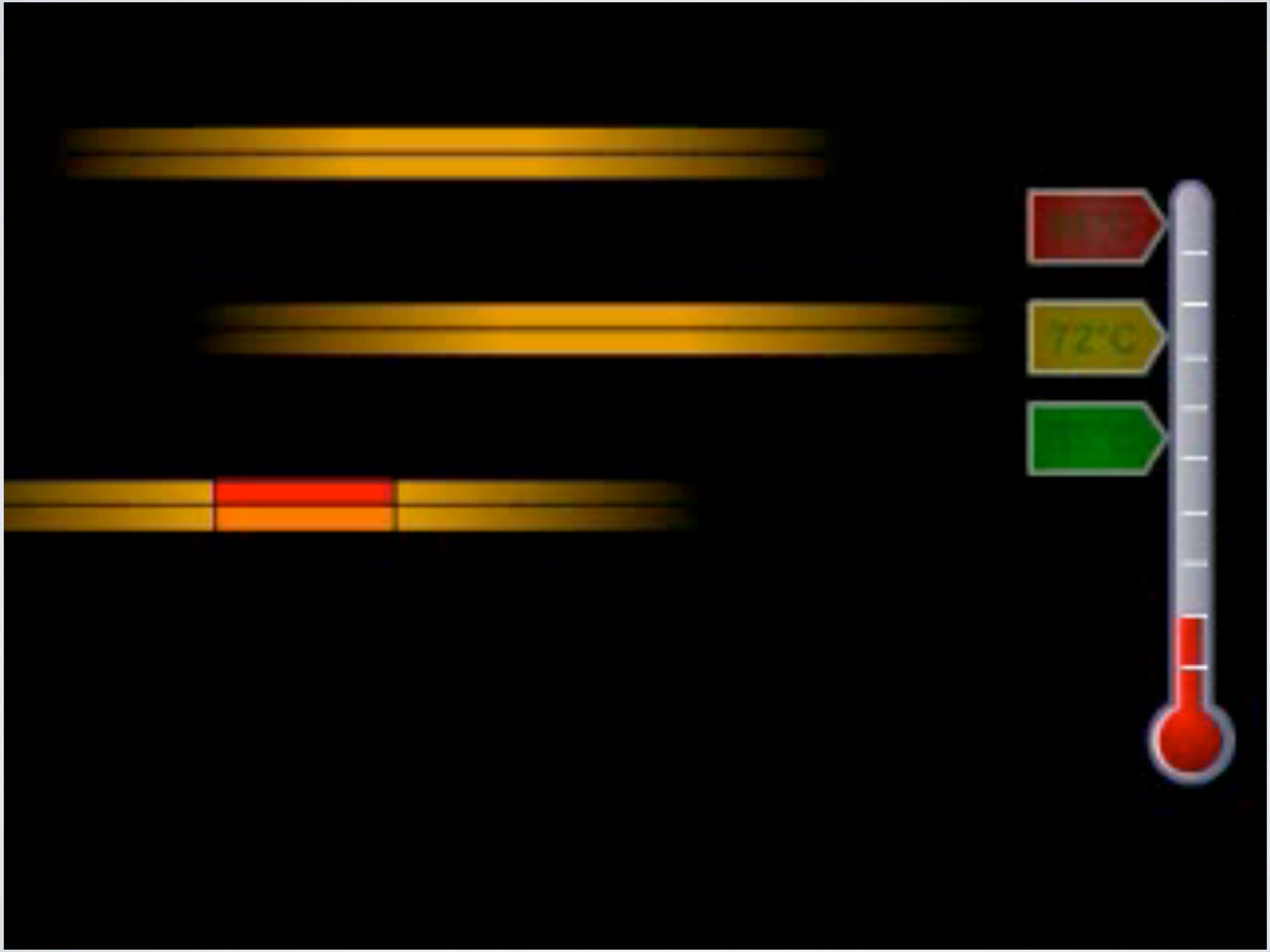
Temperature Control
Inside the machine are heating and refrigeration mechanisms to rapidly change the temperature



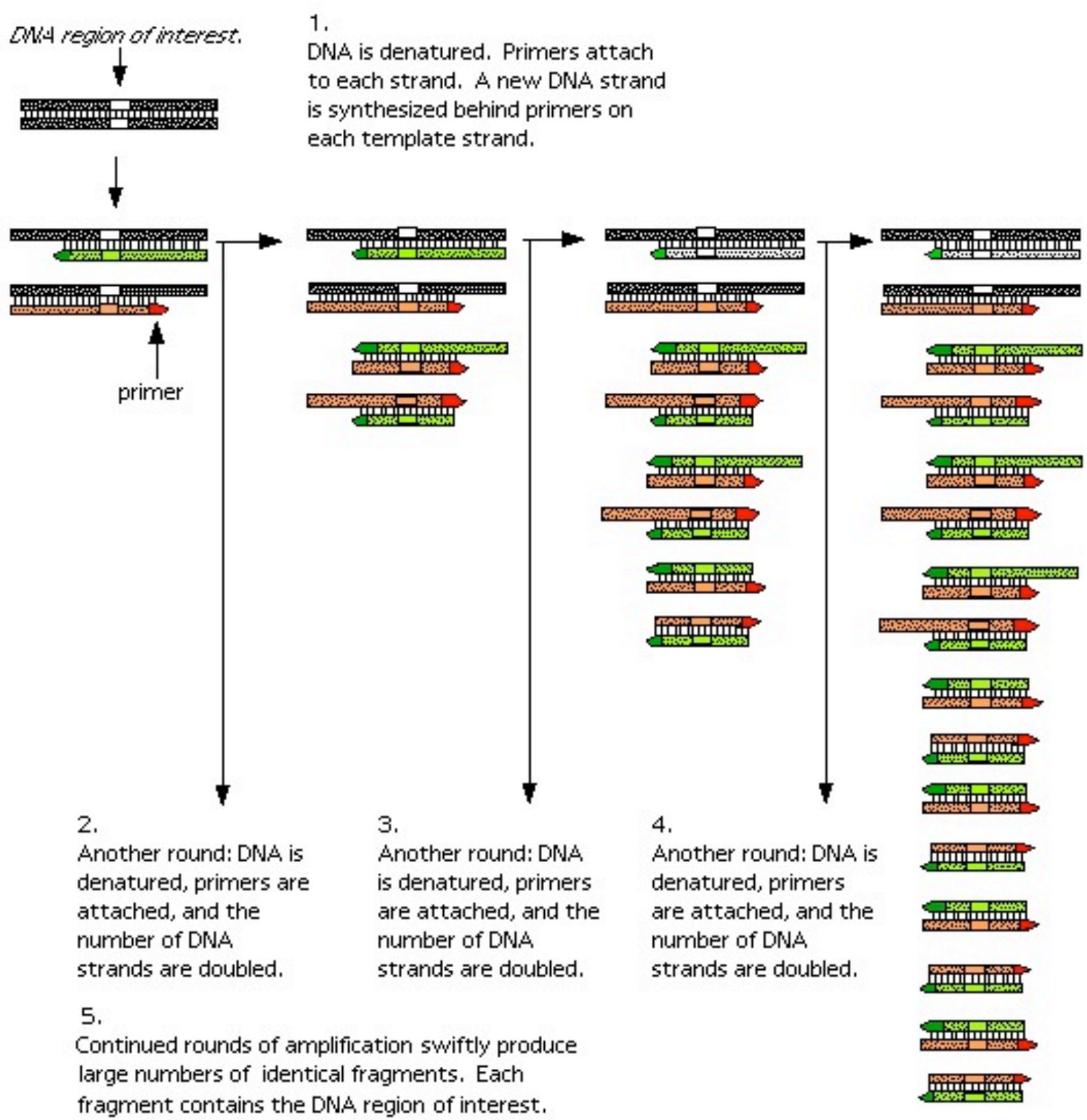
Controls
The control panel allows a number of different PCR programmes to be stored in the machines memory. To carry out a PCR run, it usually means just starting one of the stored programmes.

DNA Quantitation
The amount of DNA in a sample can be determined by placing a known volume in this quantitation machine. For many genetic engineering processes, a minimum amount of DNA is required.

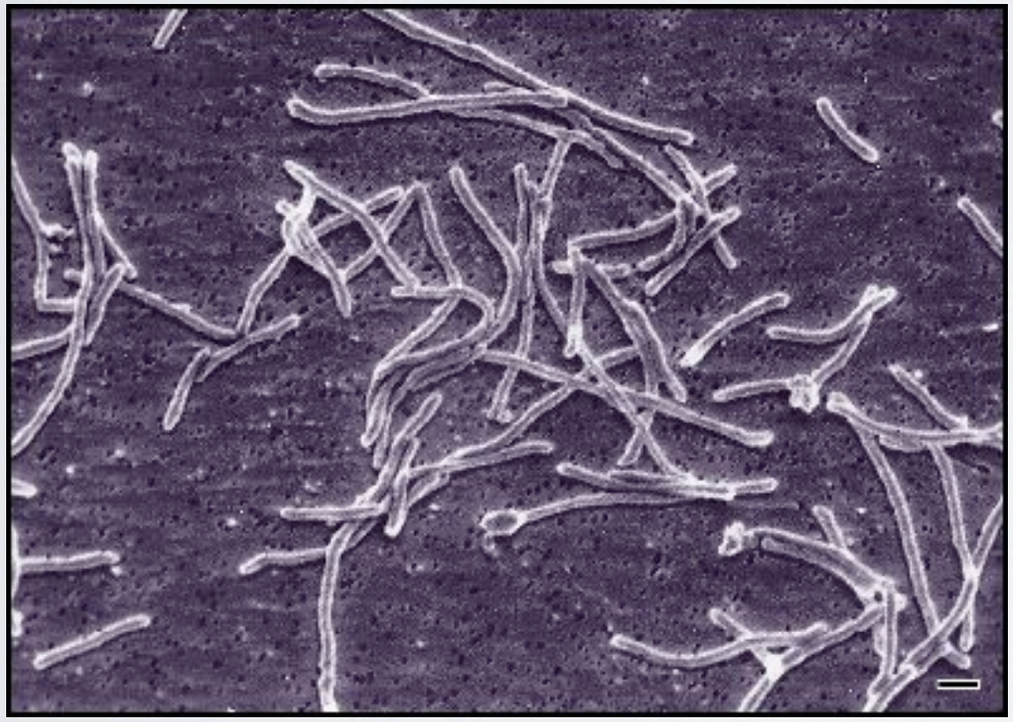
PCR VIDEO



POLYMERASE CHAIN REACTION



- The key breakthrough for automated PCR was the discovery of the unusual heat-stable DNA polymerase called Taq polymerase.
- *Thermus aquaticus* a thermophilic bacterium was discovered in Yellowstone National Park in 1969 .
- Seven years later, in 1976, taq polymerase was isolated.



The commercial use of enzymes from *T. aquaticus* has not been without controversy. After Dr. Brock's studies, samples of the organism were deposited in the American Type Culture Collection, a public repository. Other scientists, including those at Cetus, obtained it from there. As the commercial potential of Taq polymerase became apparent in the 1990s,[12] the National Park Service labeled its use as the "Great Taq Rip-off".[13] Researchers working in National Parks are now required to sign "benefits sharing" agreements that would send a portion of later profits back to the Park Service


The search for ever more heat-stable
DNA polymerases continues.

BIOTECHNOLOGY

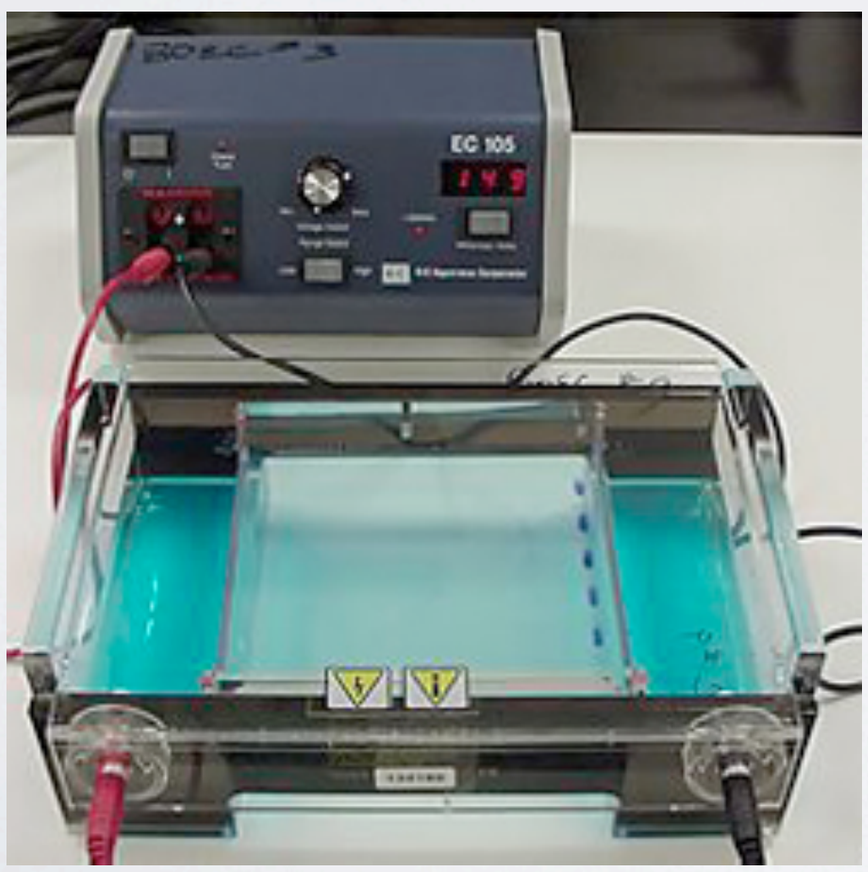
II. Main Idea

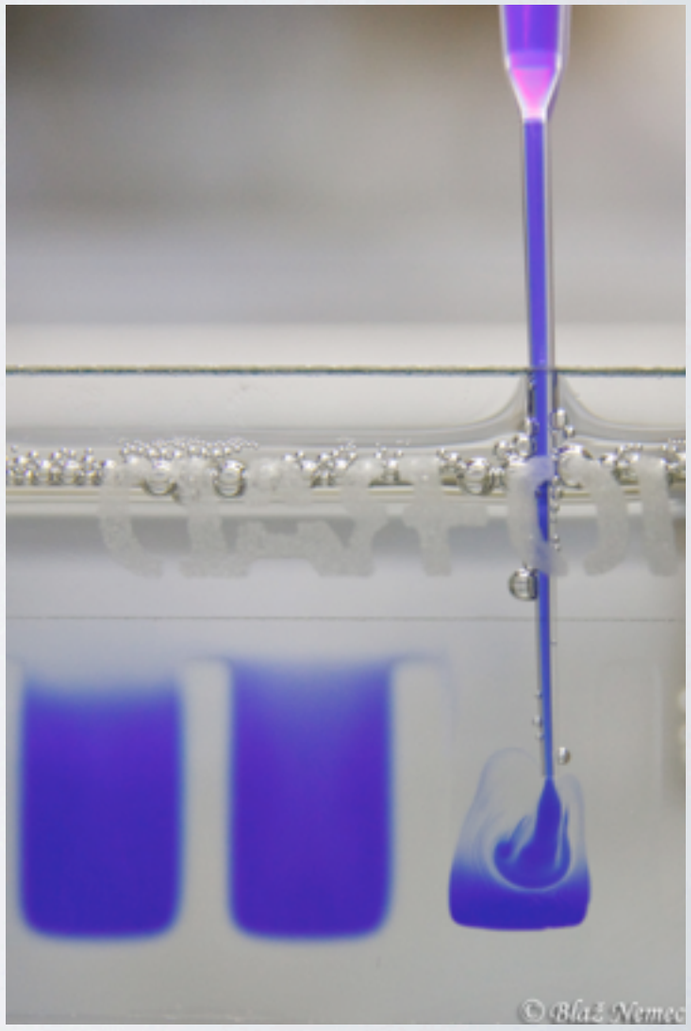
Obtaining large amounts of specific DNA either by cloning or PCR has allowed us to study the sequence, expression and function(s)of a gene.

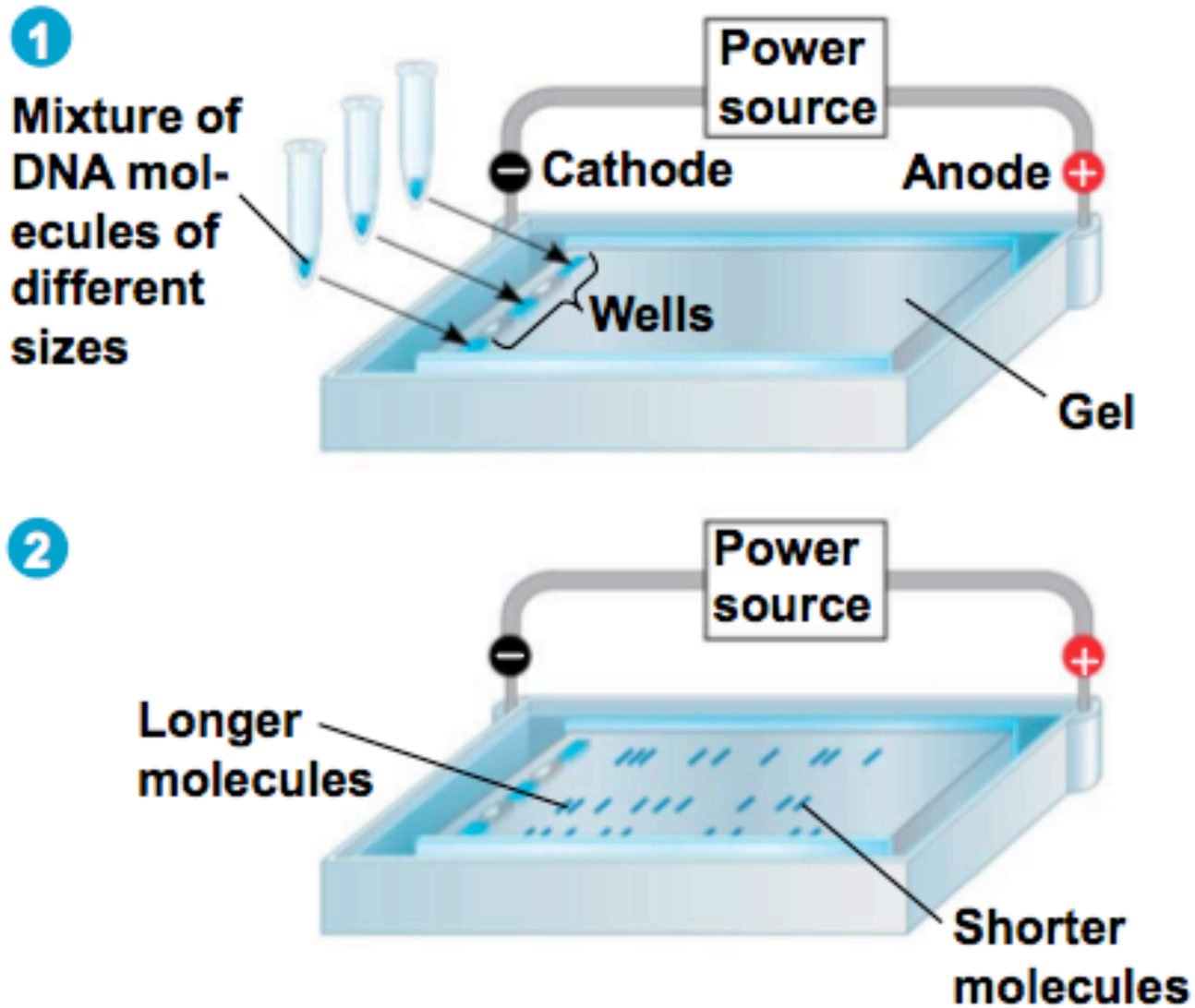
GEL ELECTROPHORESIS

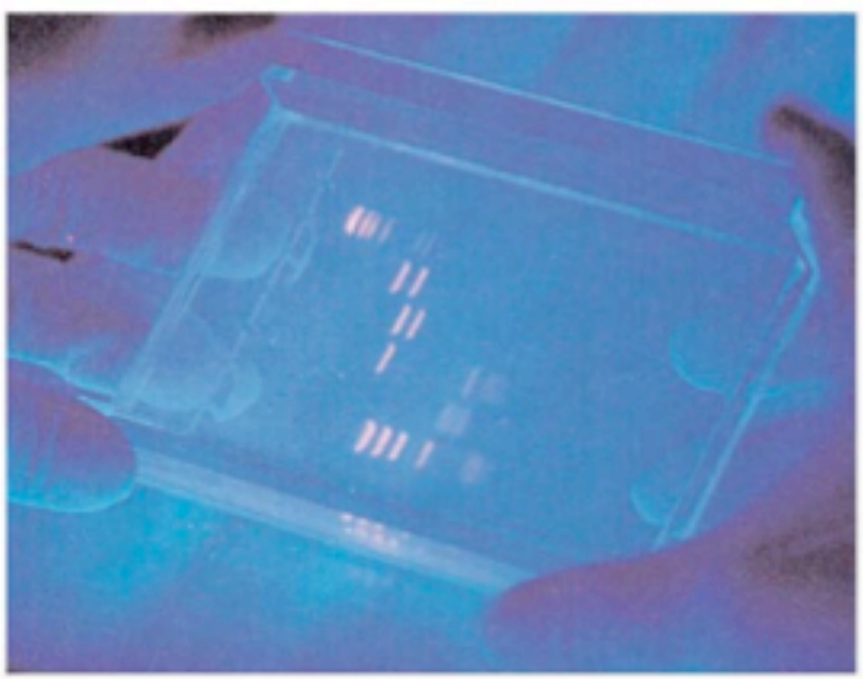
- Many approaches for studying DNA involve **gel electrophoresis**.

- A gel made of agarose, a polysaccharide, acts as a molecular filter to separate nucleic acids or proteins on the basis of size, electrical charge or other physical property.







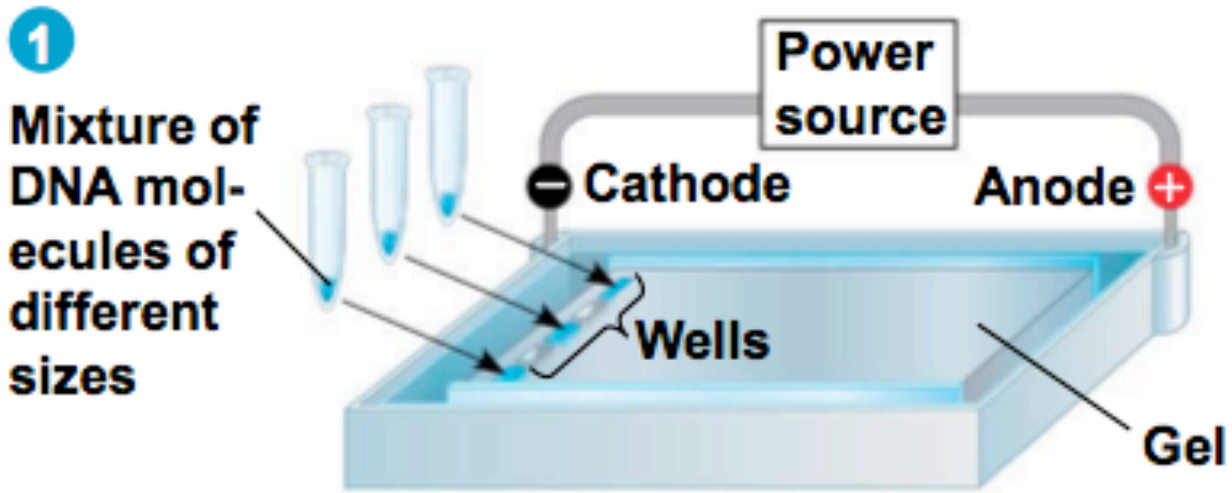


Application

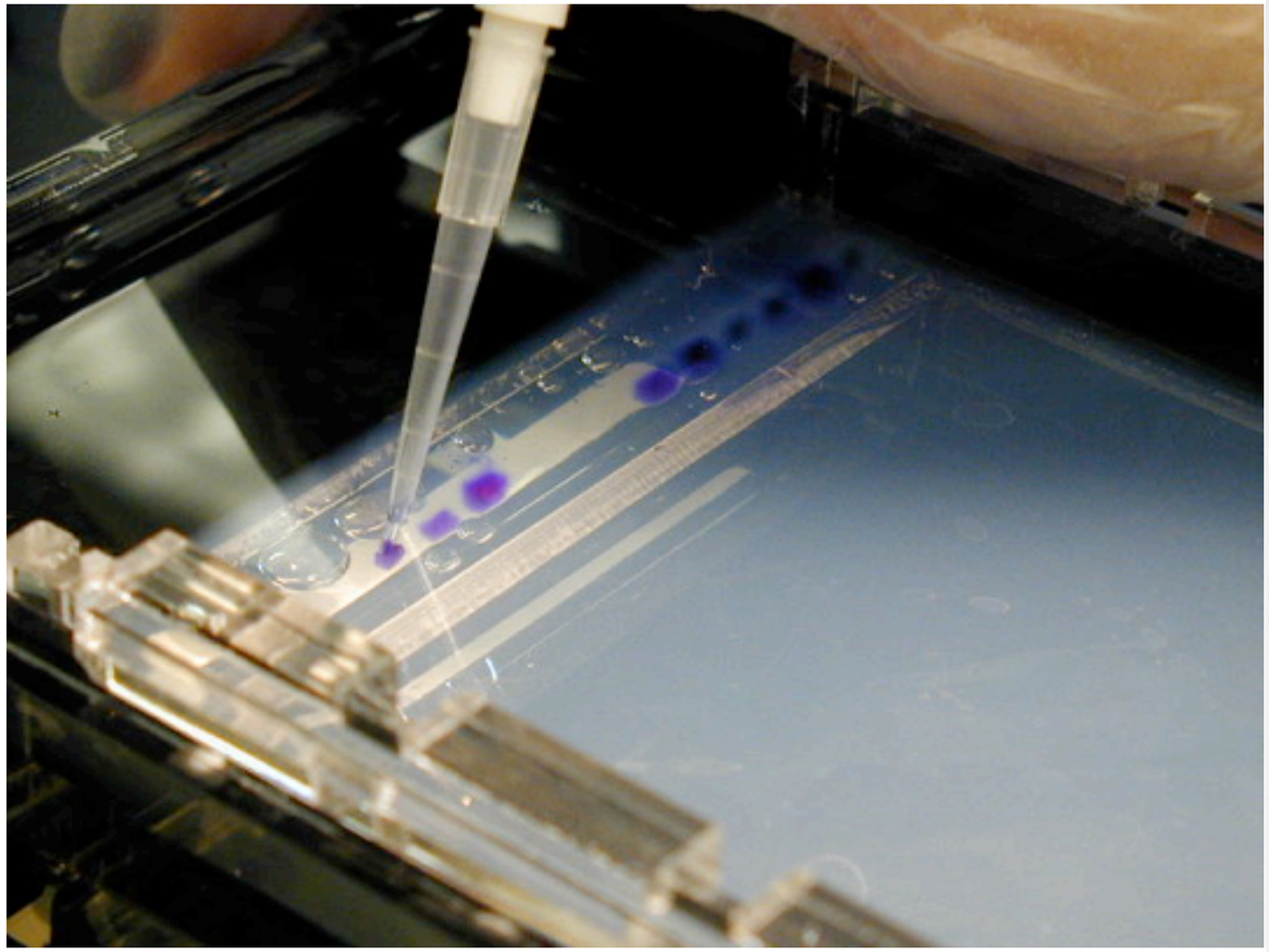
Used for separating nucleic acids or proteins when you have multiple fragments. For instance, fragments analysis or cloned genes and genomic DNA.

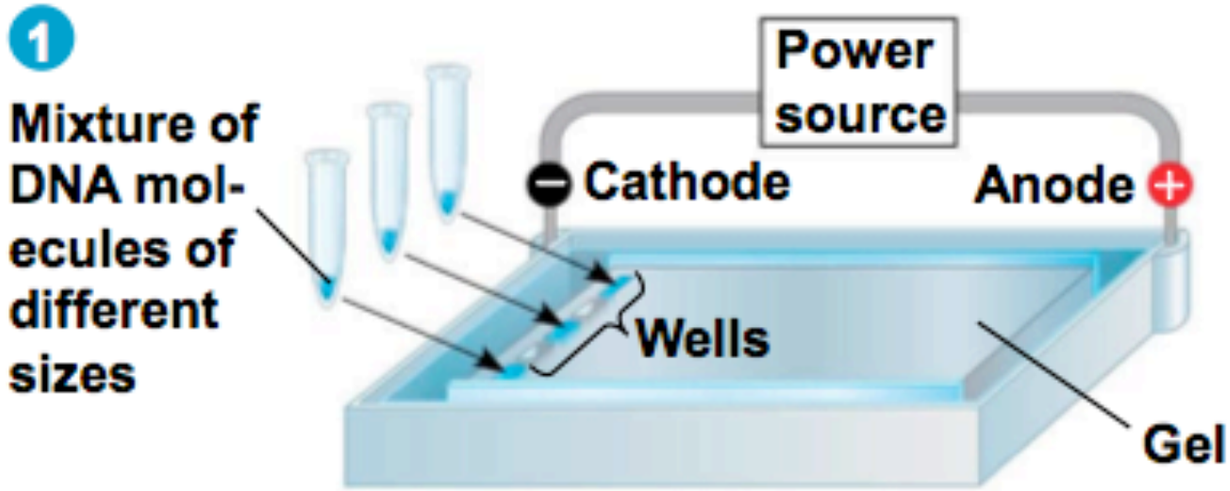
Technique

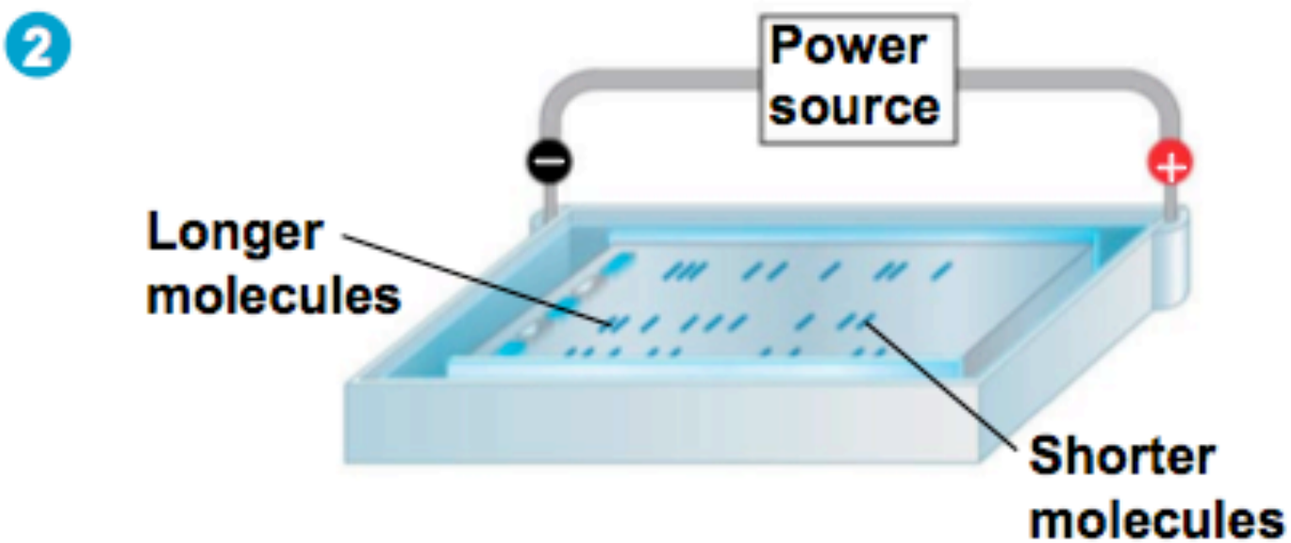
Separates molecules based on the basis of their rate of movement through the agarose gel in an electrical field. The distance the molecule travels is inversely proportional to its length. A mixture of DNA molecules, usually fragments produced by restriction enzyme digestion or PCR amplification is separated into bands. Each band contains thousands of molecules of the same length.

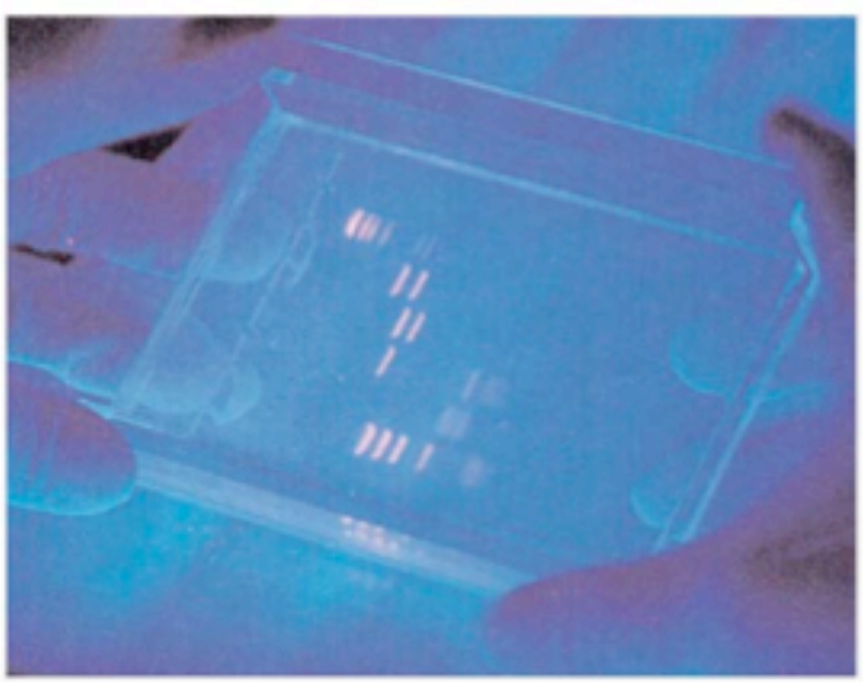


Each sample, a mixture of DNA molecules, is placed in a separate well near one end of a thin slab of agarose gel. The gel is placed into a plastic support and immersed in an aqueous buffered solution in a tray with electrodes at each end.



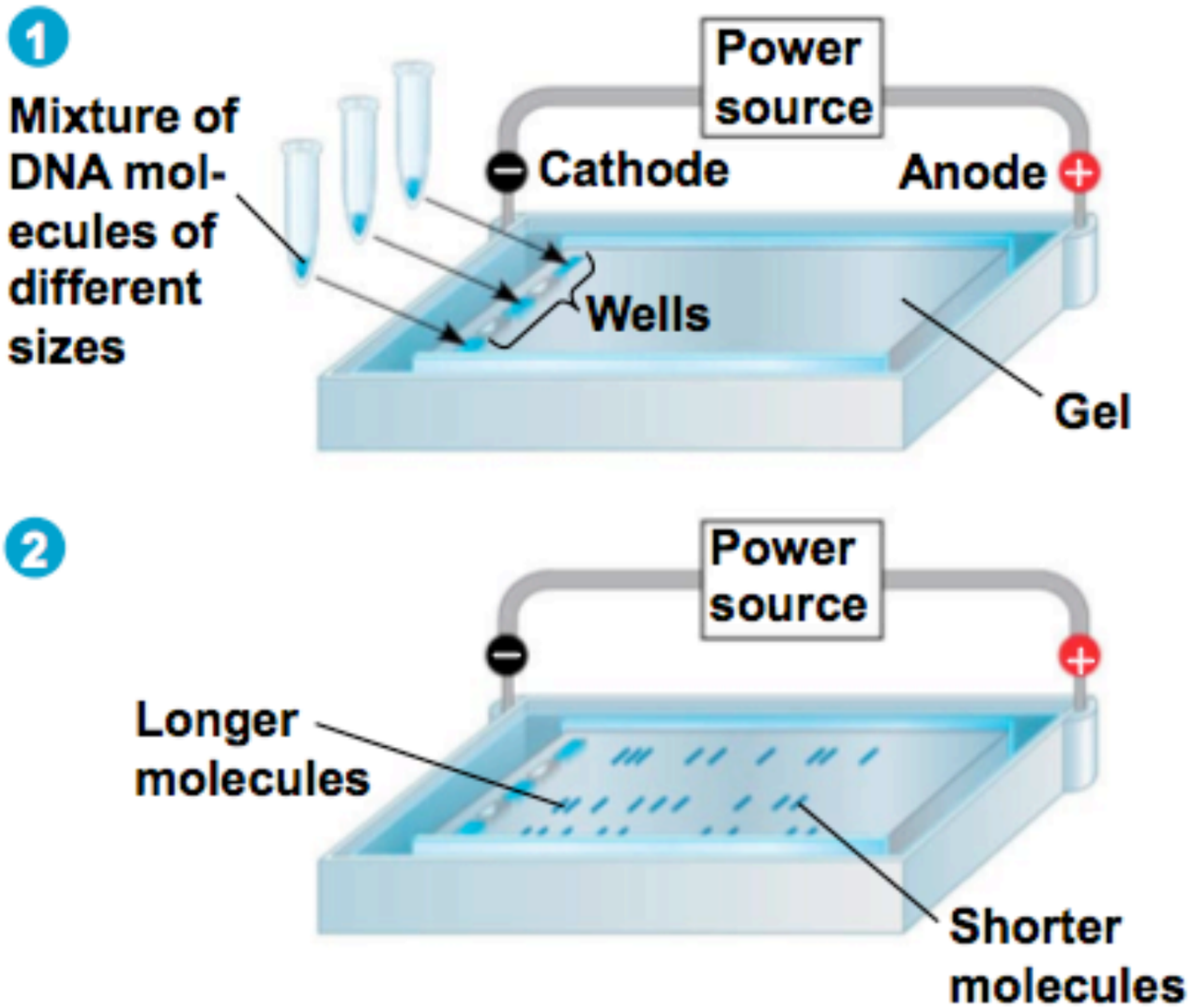


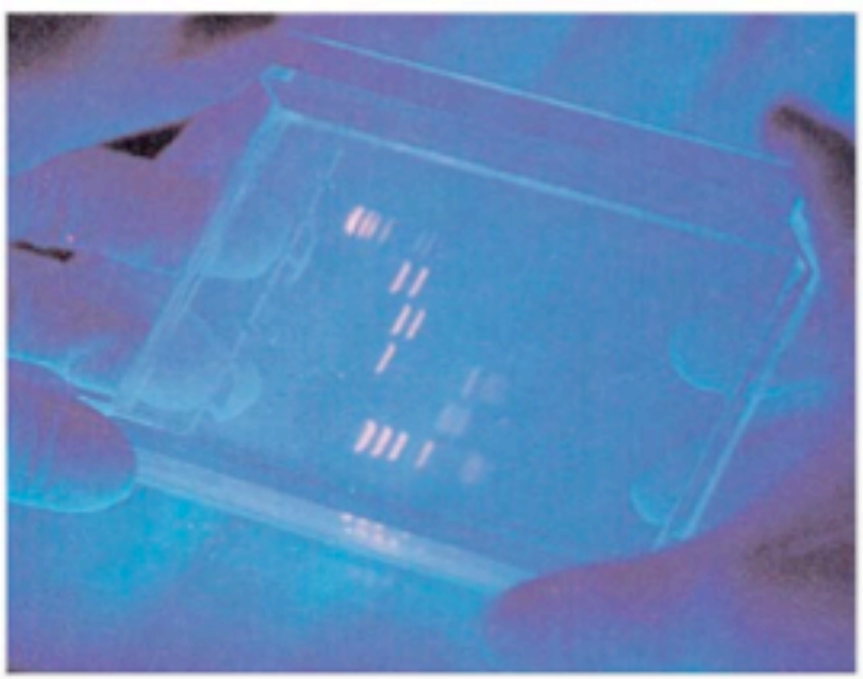




When the current is turned on, the negatively charged DNA molecules move toward the positive electrode, with shorter molecules moving faster than longer ones.

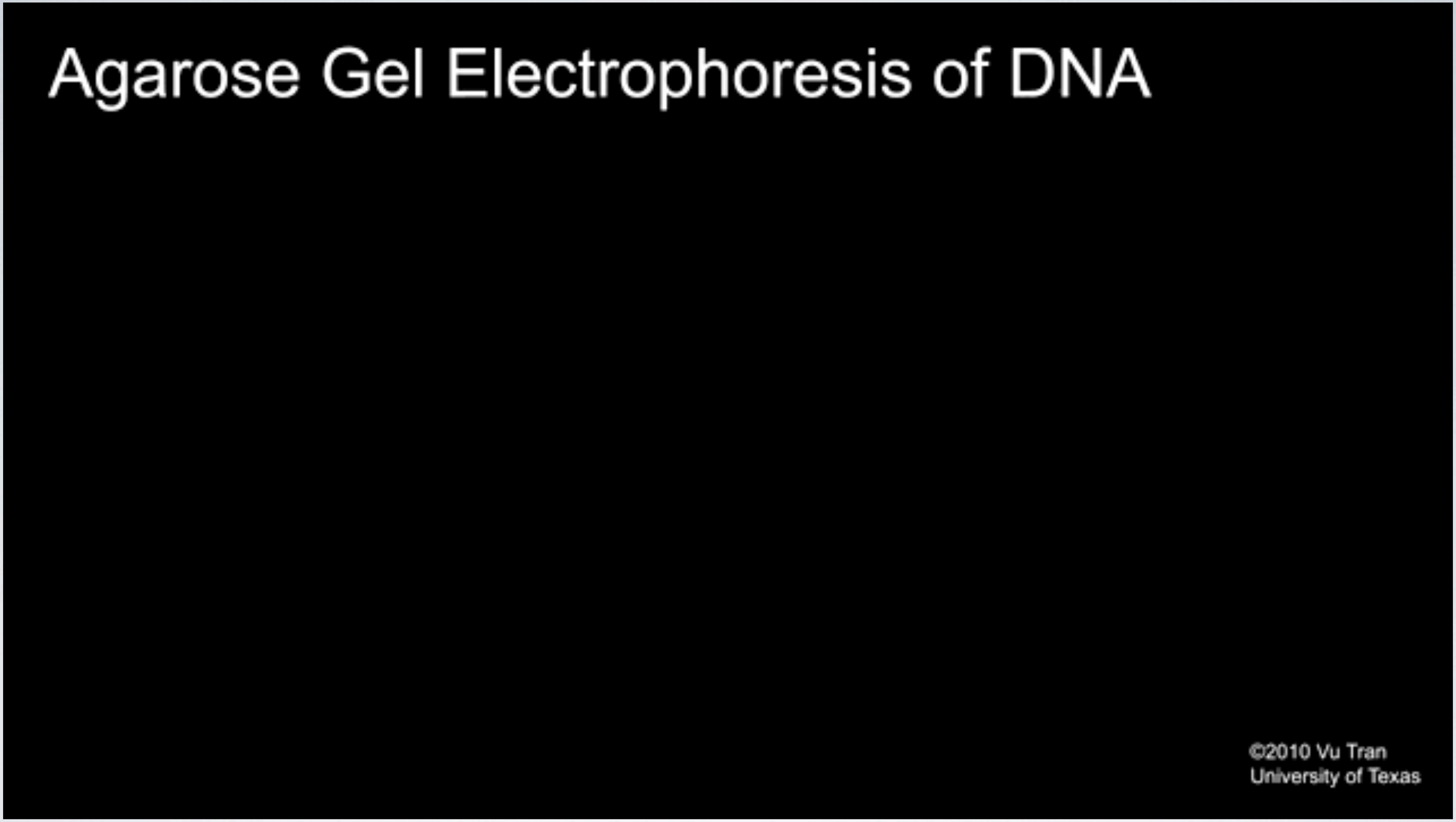
Bands are shown to the left but at this time in the procedure, in the actual gel you would not see any bands.





Results

After the current is turned off, a DNA binding dye (ethidium bromide) is added. This dye fluoresces pink under UV light, revealing the separated bands to which it binds. In the gel to the left the pink bands correspond to DNA fragments of different lengths. If all the samples were initially cut with the same restriction enzyme, then the different bands indicate that they came from different sources.



GEL ELECTROPHORESIS VIDEO

RESTRICTION FRAGMENT ANALYSIS

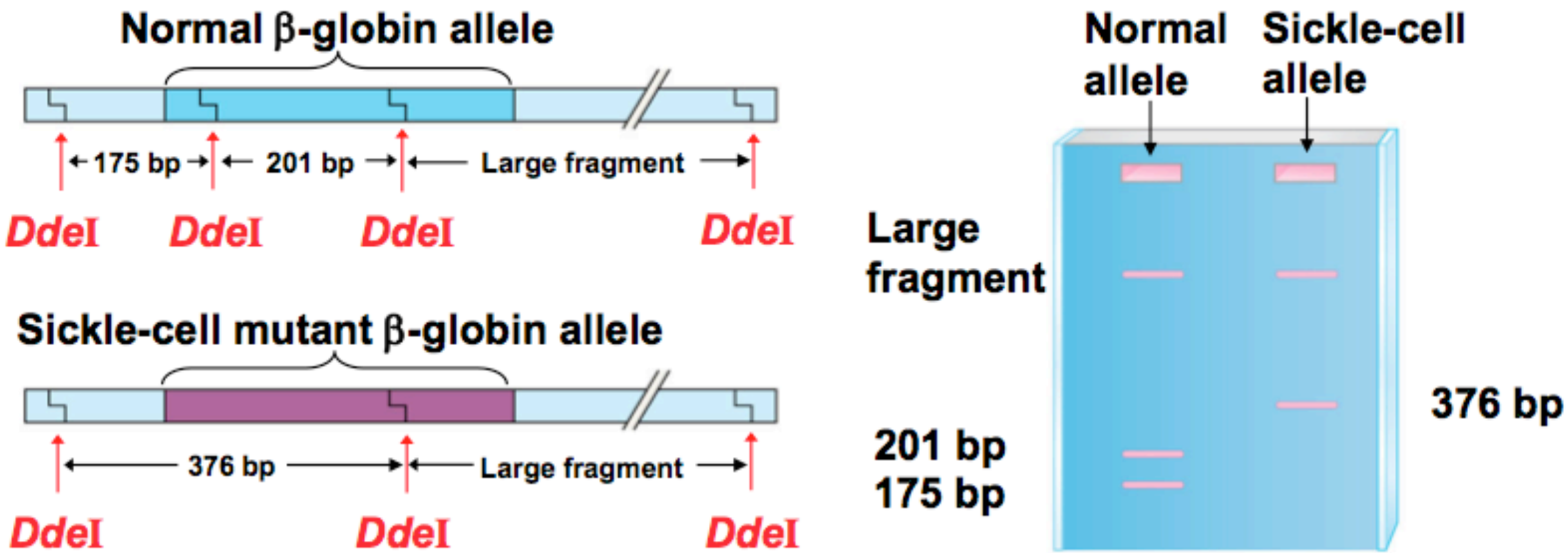
- In restriction fragment analysis, DNA fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis

- Restriction fragment analysis can be used to compare two different DNA molecules, such as two alleles for a gene if the nucleotide difference alters a restriction site.

- Variations in DNA sequence are called polymorphisms

- Sequence changes that alter restriction sites are called **RFLPs** (**restriction fragment length polymorphisms**)

Normally there will be a third well/column with engineered pieces of DNA fragments each with a known size. This way you can predict the relative size of your fragments by comparison to the "knowns".



(a) *Dde*I restriction sites in normal and sickle-cell alleles of the β-globin gene

(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

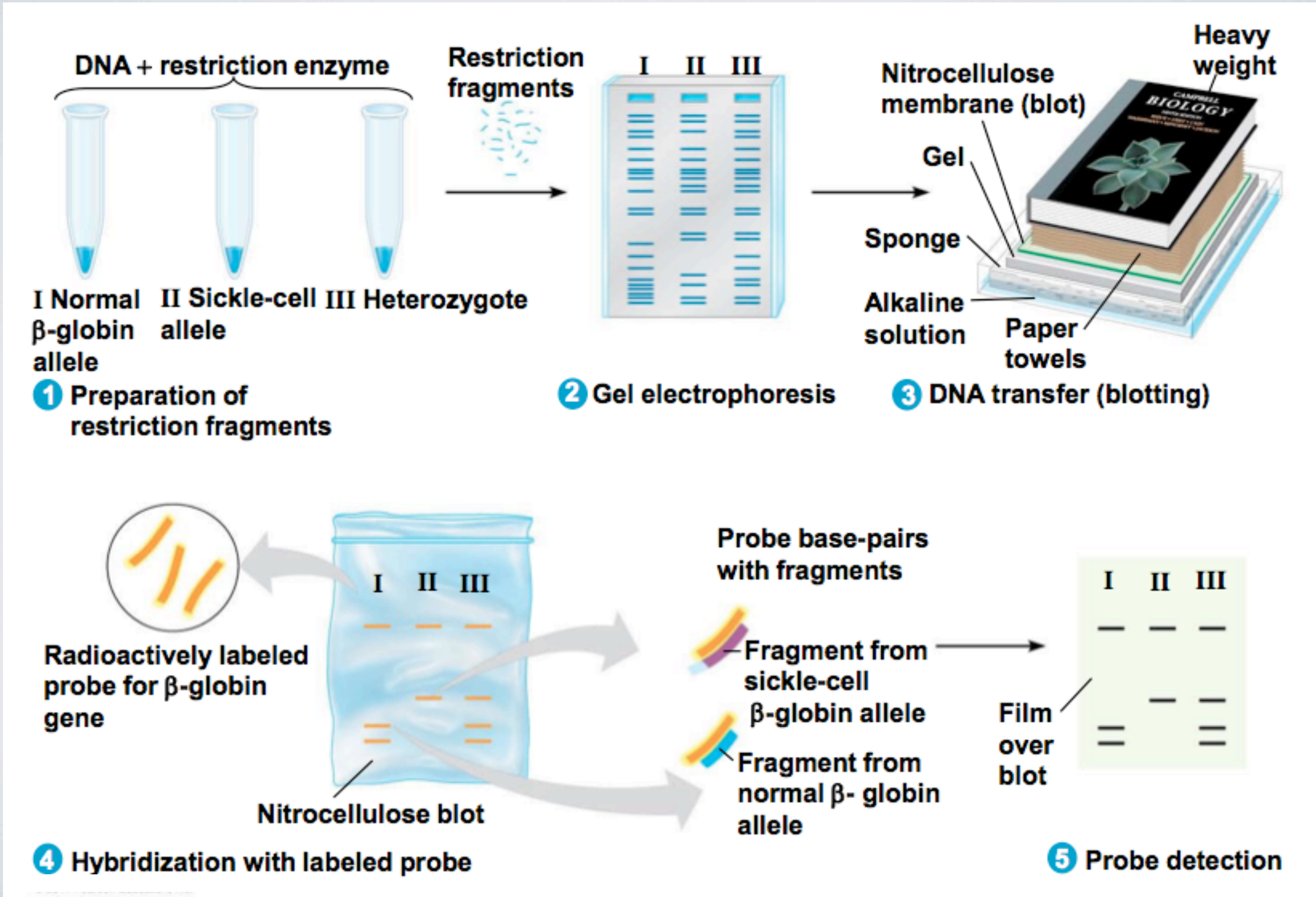
SOUTHERN BLOTTING

- A technique called **Southern blotting** combines gel electrophoresis of DNA fragments with nucleic acid hybridization

- Specific DNA fragments can be identified by Southern blotting, using labeled probes that hybridize to the DNA immobilized on a "blot" of gel

Application

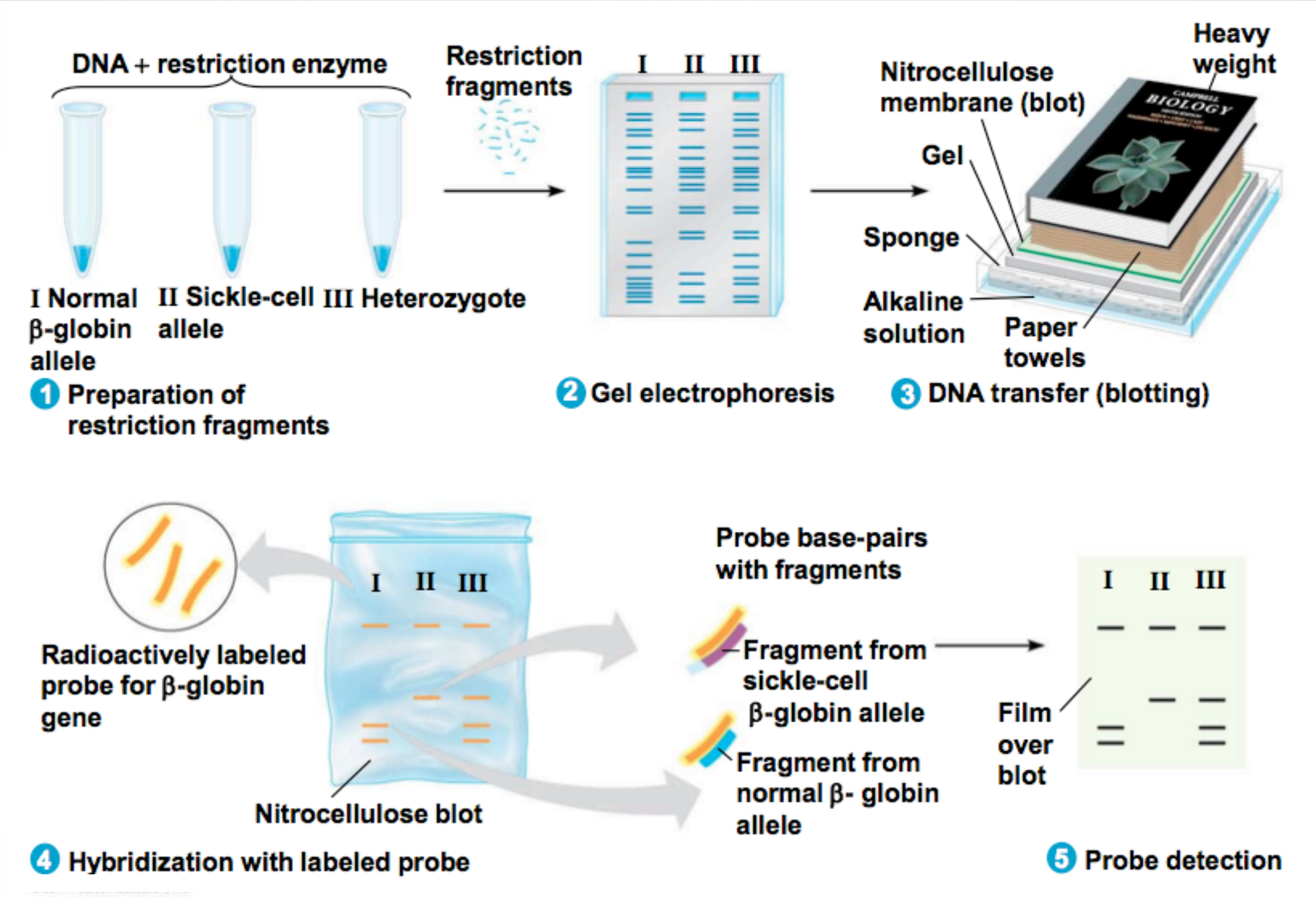
Detect specific nucleotide sequences within a complex DNA sample, in particular this procedure can be used to compare restriction fragments produced from different DNA samples of genomic DNA.

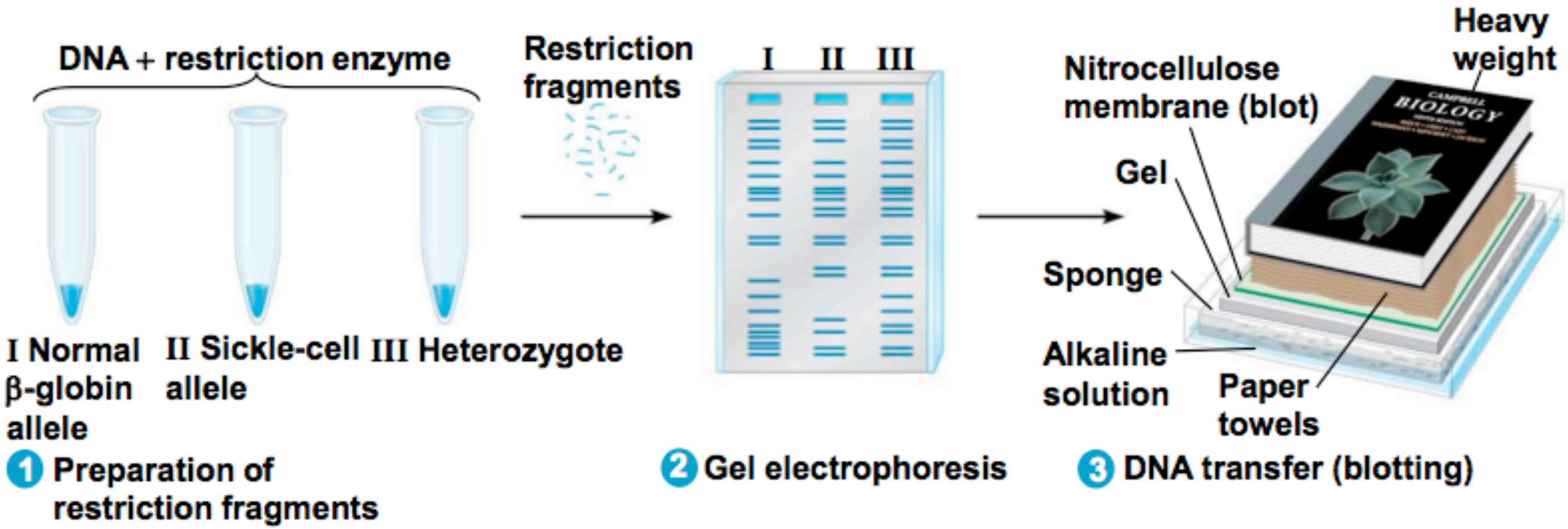


Technique

Here we compare DNA from 3 individuals:

AA (2 normal beta-globin allele)
aa (2 sickle cell alleles)
Aa (one of each)





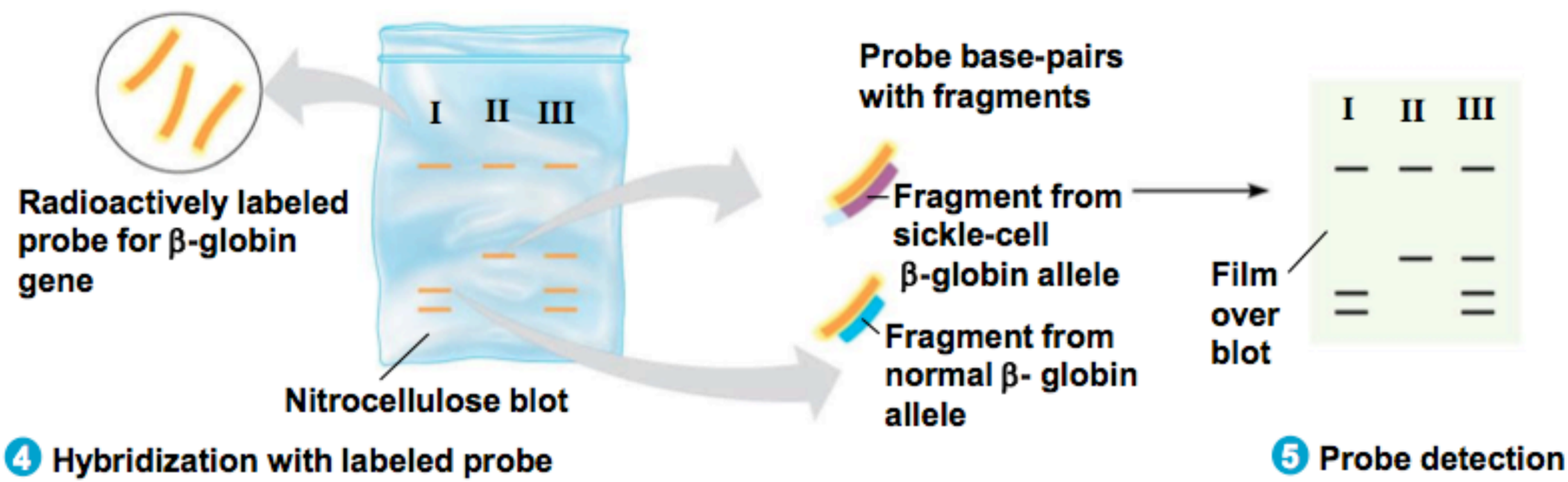
1. Each DNA sample is mixed with same restriction enzyme, in this case DdeI. Digestion of each sample yields a mixture of thousands of restriction fragments

2. Fragments are separated by electrophoresis, forming particular banding patterns. In reality there would many more fragments.

3. With gel arranged as shown above, capillary action pulls the alkaline solution upward through the gel, denaturing and and transferring the DNA to a nitrocellulose membrane. This makes a blot just like the gel.

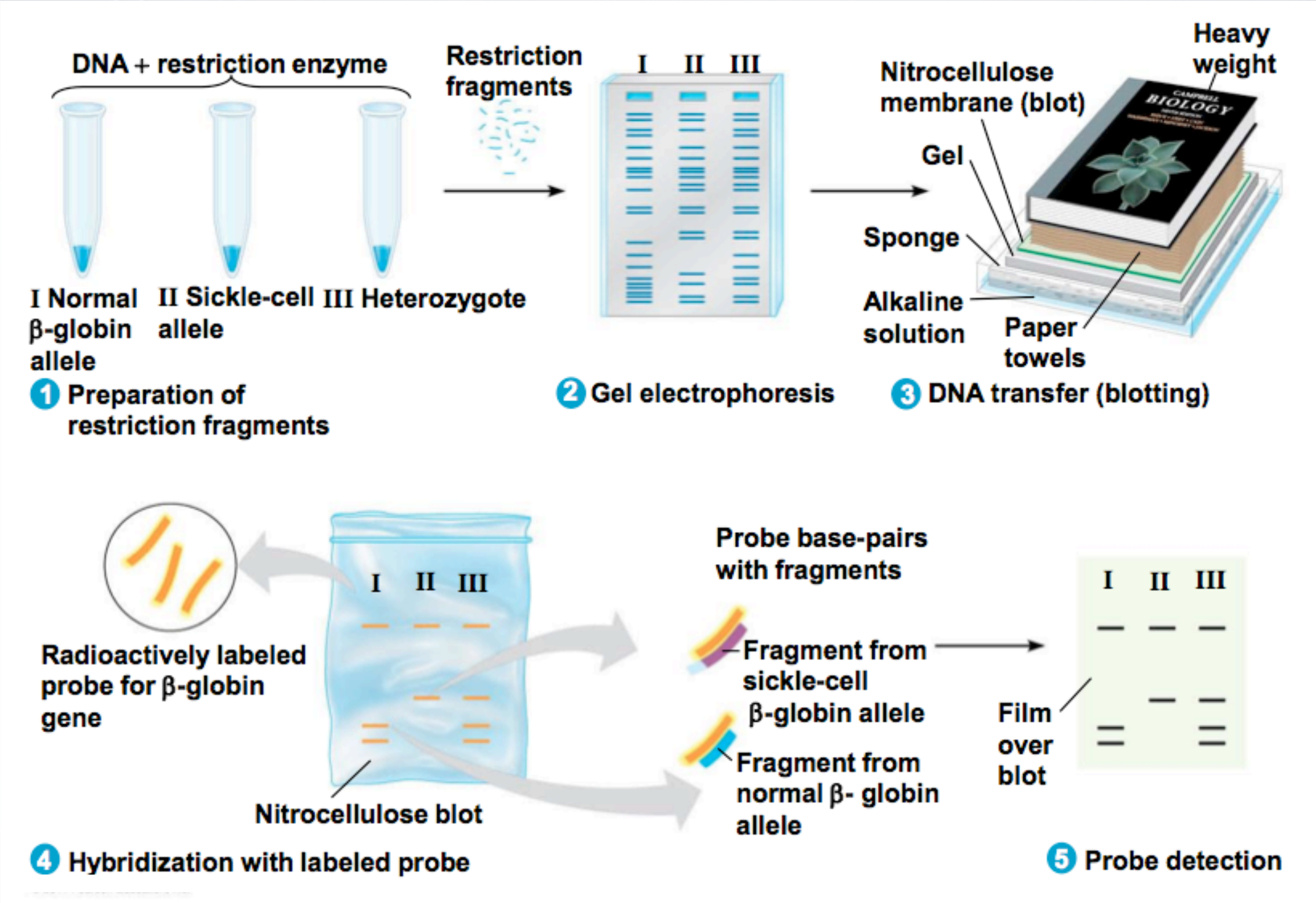
4. The nitrocellulose blot is exposed to a solution containing a labeled probe. The single-stranded DNA complementary to the beta-globin gene. Probe molecules attach by base pairing to any restriction fragments containing a part of the beta-globin gene. (bands would not yet be visible)

5. A sheet of photographic film is laid over the blot. The radioactivity in the bound probe exposes the film to form an image corresponding to those bands containing DNA that base -paired with the probe.

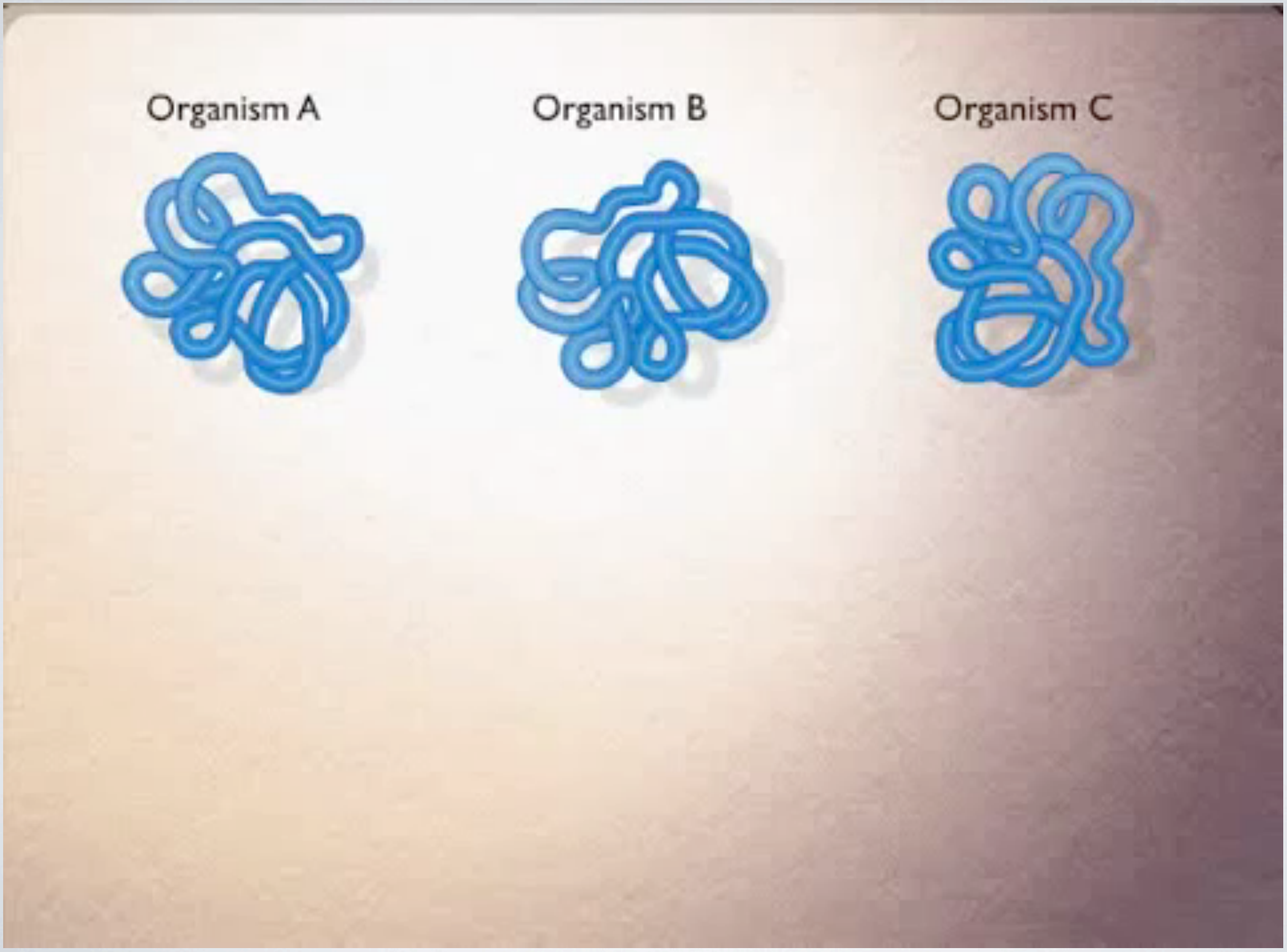


Results

Band patterns are clearly different, the patterns for individuals I and II resemble those seen (4 slides earlier) for the normal and mutant allele respectively. The band pattern for individual III is a combination of I and II thus it is the heterozygote.



SOUTHERN BLOTTING VIDEO



DNA SEQUENCING

- Once a gene is cloned, its complete nucleotide sequence can be determined.

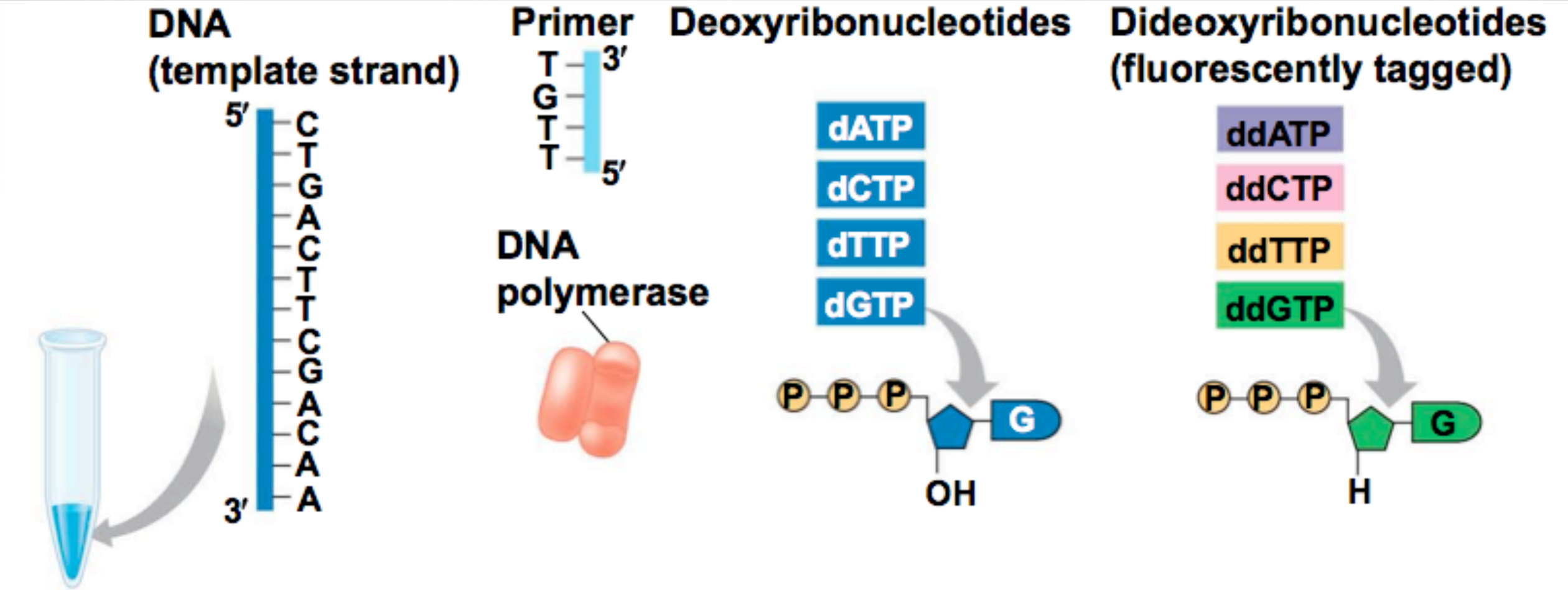
- The first generation of automated sequencing was based upon a technique called *dideoxyribonucleotide chain termination method*.

- Today's second and third generation automation is faster and less expensive.

- Knowing a genes sequence has many applications.

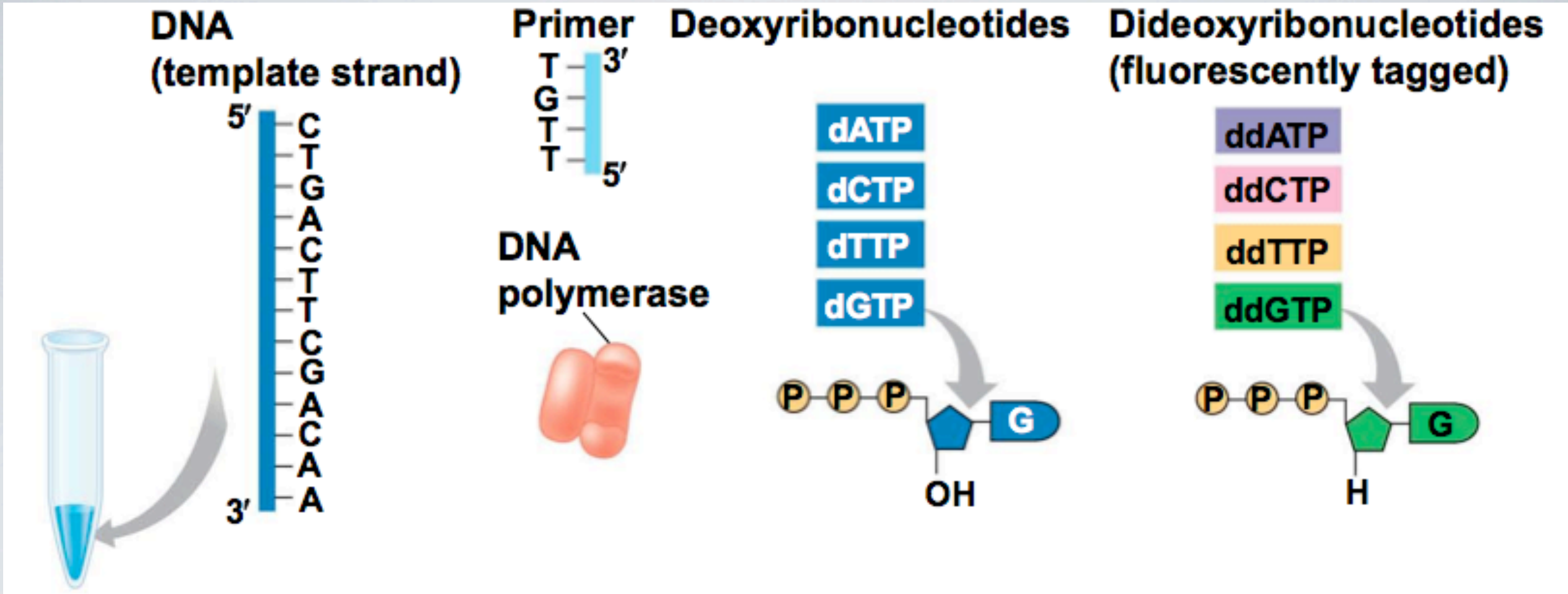
Application

This method can rapidly sequence any cloned DNA up to 800-1000 base pairs in length.

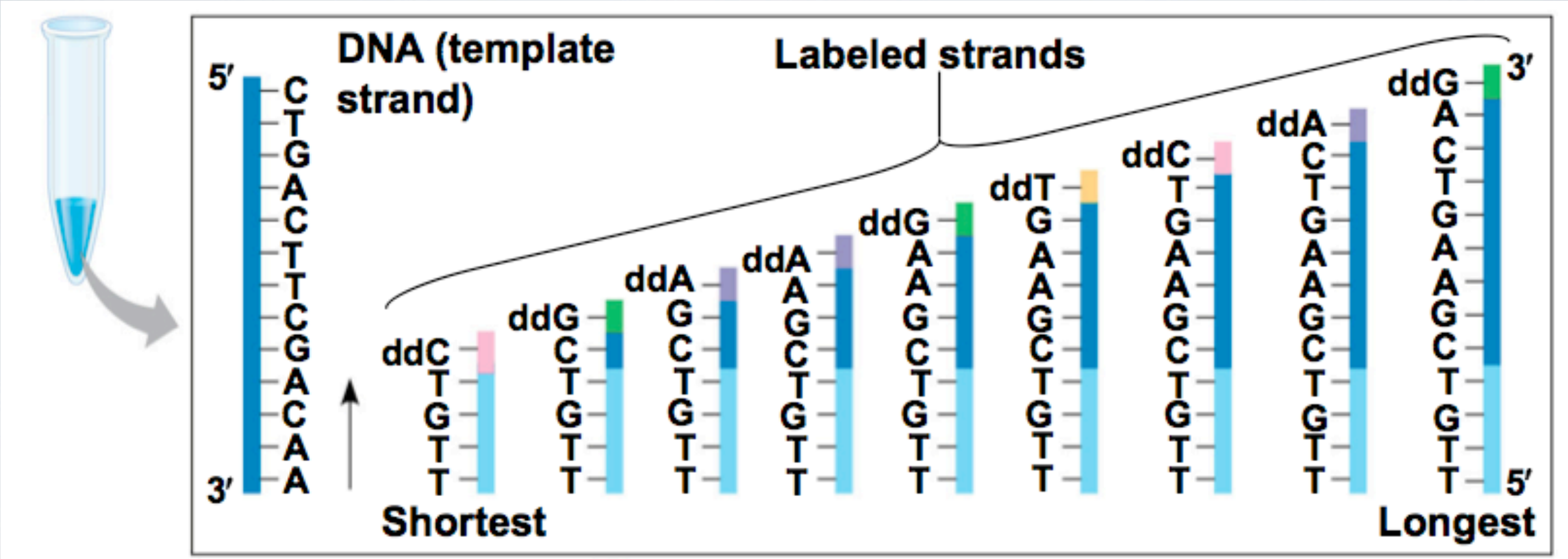


Technique

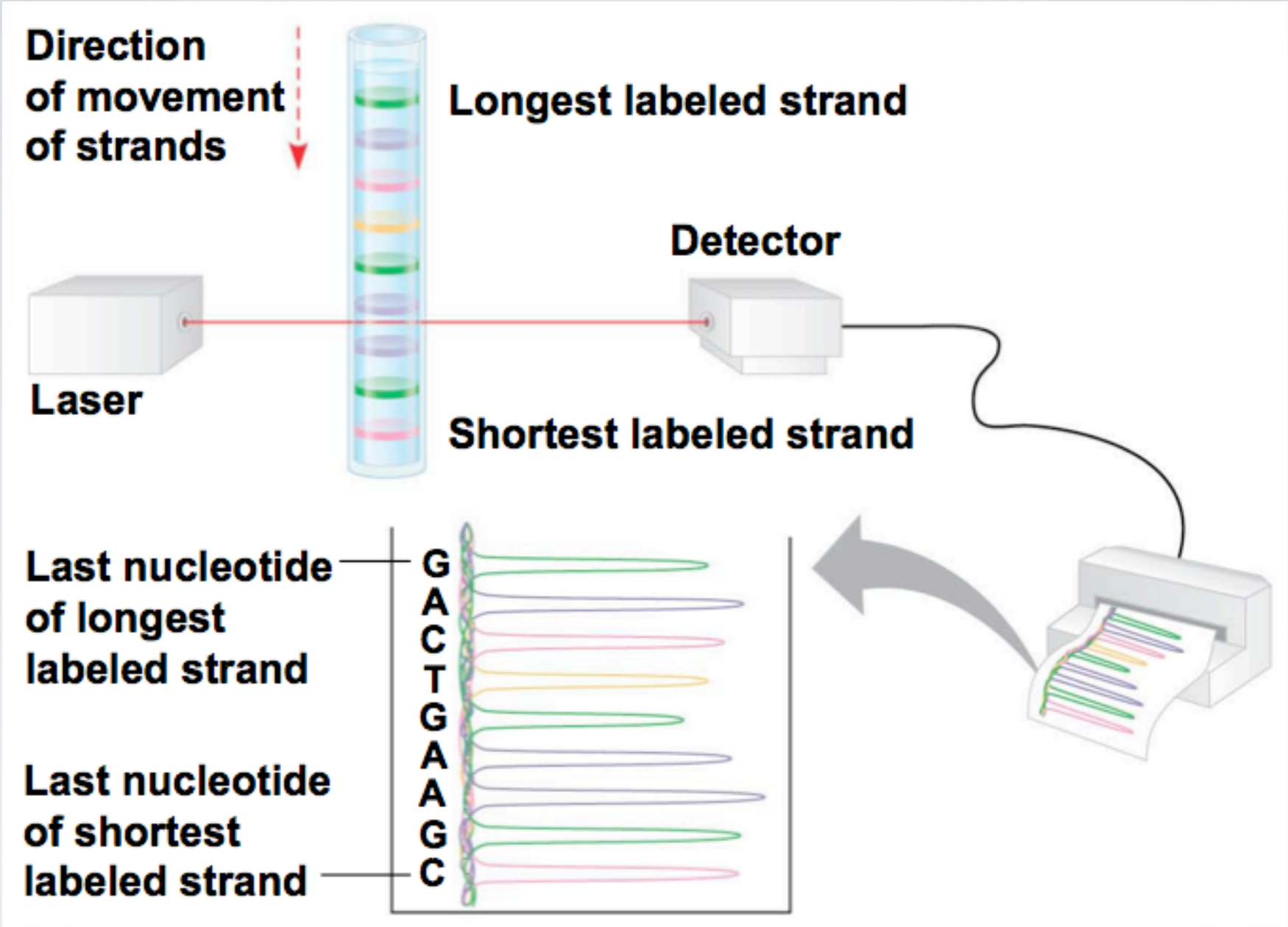
This method synthesizes a set of DNA strands complimentary to the original DNA fragment. Each strand starts with same primer and ends with a modified nucleotide (ddNTP). Incorporation of the ddNTP terminates a growing strand because it lacks the 3' -OH group (site for next nucleotide attachment). In the set of strands synthesized, each nucleotide position along the original sequence is represented by strands ending at that point with the complimentary ddNTP. Because each type of ddNTP is tagged with a distinct fluorescent label, the identity of the ending nucleotides of the new strands, and ultimately the entire original sequence, can be determined.



1. The fragment of DNA to be sequenced is denatured into single strands and incubated in a test tube with the ingredients for DNA synthesis: a primer to base pair at the known 3' end of the template strand, DNA polymerase, the four dNTP's, and the four tagged ddNTP's each with a specific fluorescent molecule.

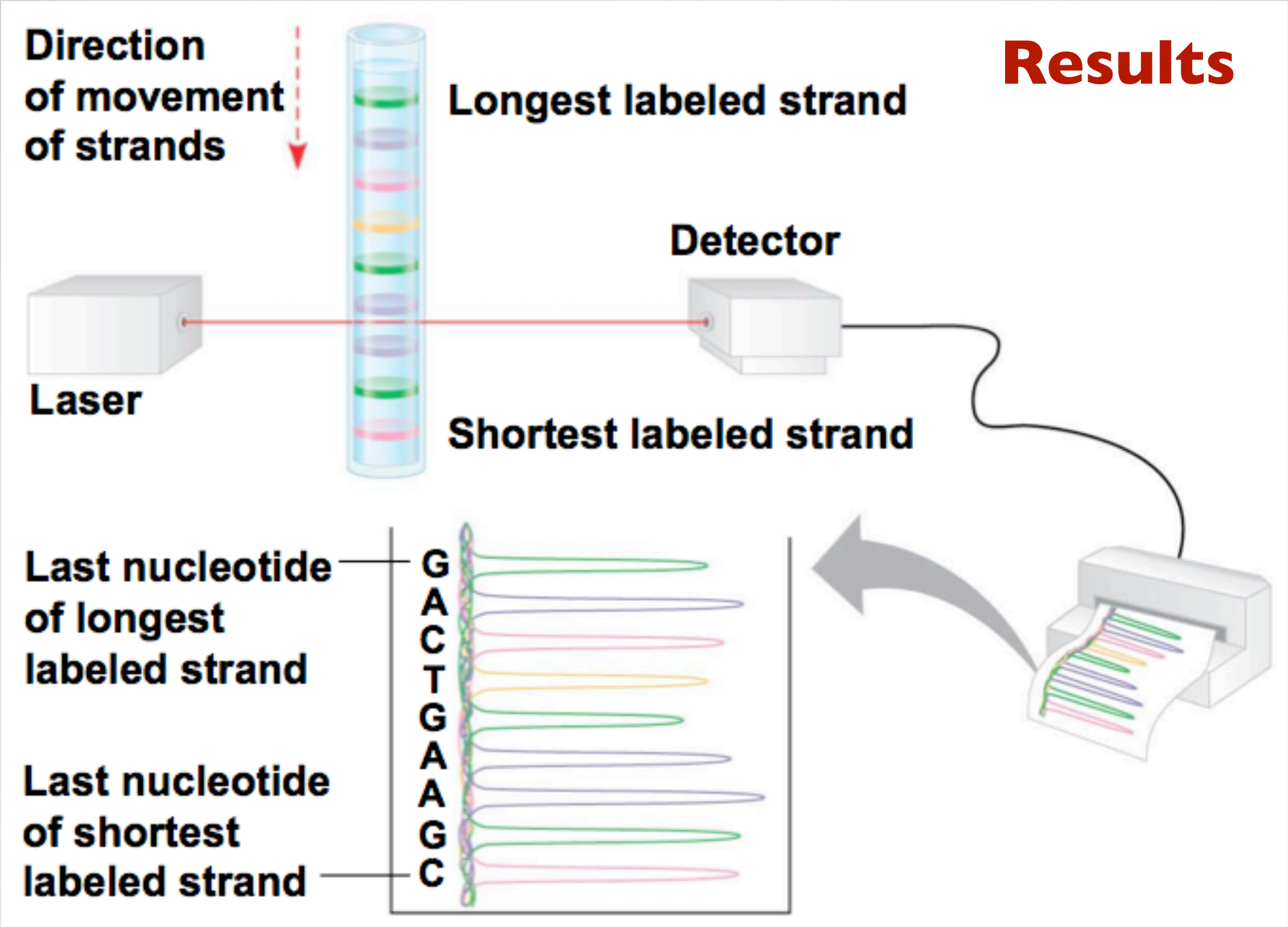


2. **Synthesis of each new strand starts at the 3' end of the primer and continues until a ddNTP is used, at random, instead of the normal equivalent dNTP. This prevents further elongation of the strand. Eventually, a set of labeled strands of various lengths is generated, with the color of the tag representing the last nucleotide in the sequence.**



3. The labeled strands i the mixture are separated by passage through a polyacrylamide gel, with shorter strands moving through more quickly. For DNA sequencing the gel is formed in a tube rather than a slab. The small size of the tube allows a florescence detector to sense the color of each fluorescent tag as the strands come through. Strands differing in length by as little as one nucleotide can be distinguished from each other.

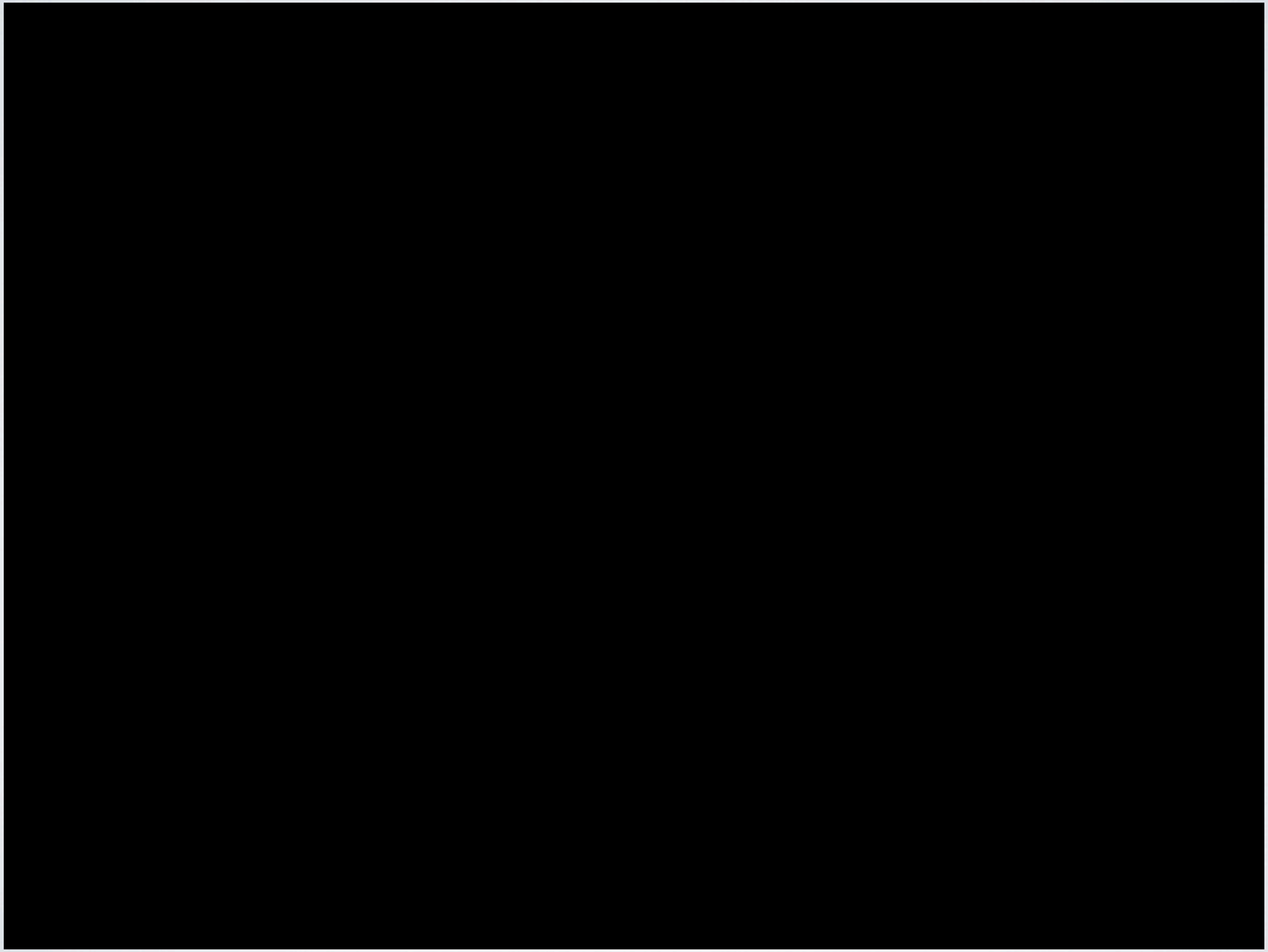
Results



The color of the fluorescent tag on each strand indicates the identity of the nucleotide at its end. The results can be printed out as a spectrogram, and the sequence, which is complementary to the template, can then be read from bottom (shortest strand). Notice that the sequence here begins after the primer.

DNA SEQUENCING: 3RD GENERATION

IBM'S DNA TRANSISTOR



DNA SEQUENCING: 3RD GENERATION

PACIFIC BIOSCIENCES "SMART"

ANALYZING GENE EXPRESSION

- Nucleic acid probes can hybridize with mRNAs transcribed from a gene

- Probes can be used to identify where or when a gene is transcribed in an organism

- Scientists can study the expression of single genes, to see how the gene expression changes through development.

- Scientists can also study the expression of multiple genes, to see how the genes act together to produce and maintain an organism.

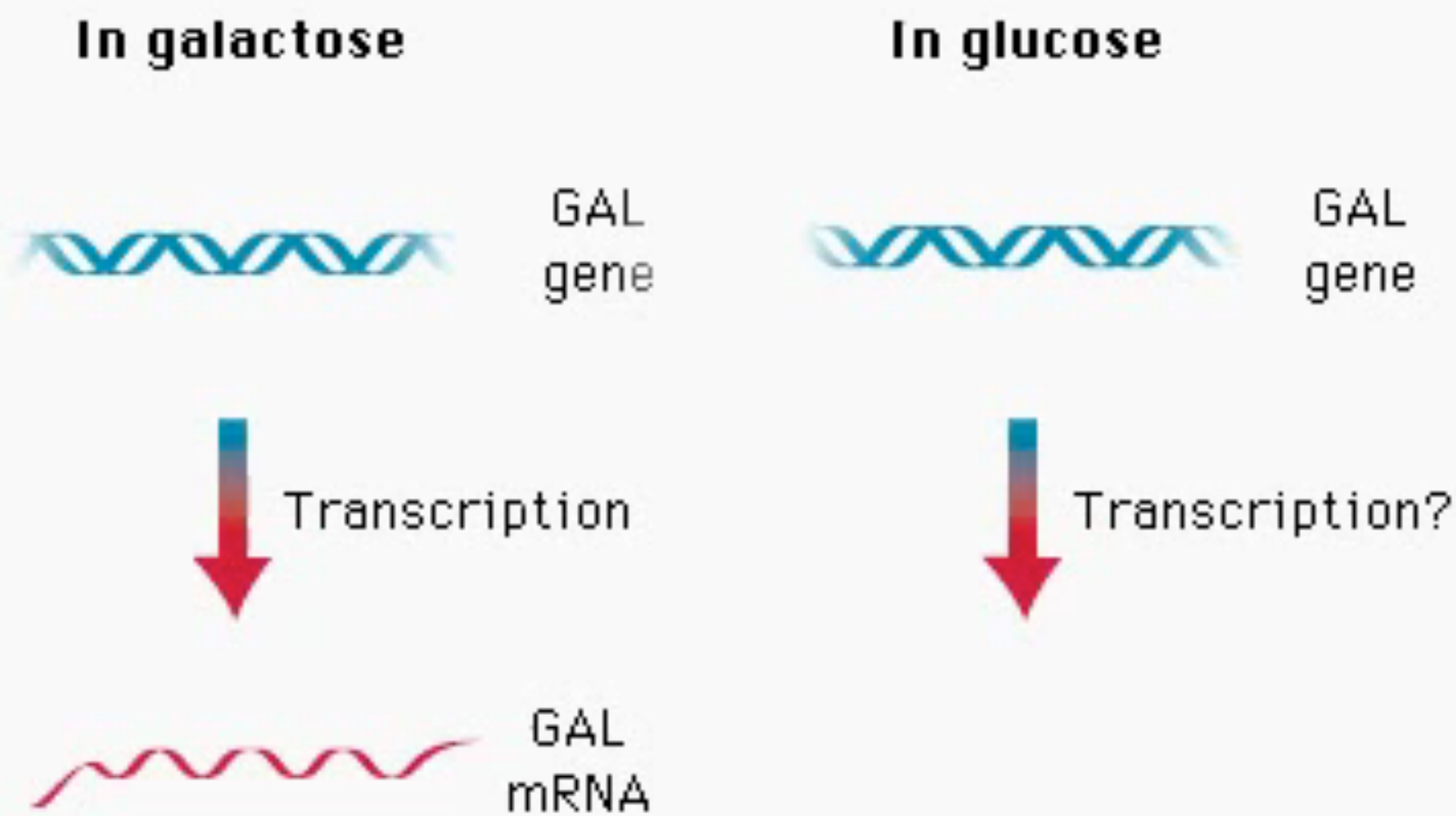
SINGLE GENE EXPRESSION

- **Northern blotting** combines gel electrophoresis of mRNA samples from embryos at different stages followed by hybridization with a specific gene probe on a membrane.

- Scientists again expose a film to the membrane and the identification of mRNA (a band on the film) at a particular developmental stage suggests protein function at that stage

- Faster and more sensitive techniques are supplanting this once mainstay approach.

NORTHERN BLOT VIDEO



SINGLE GENE EXPRESSION

- **Reverse transcriptase-polymerase chain reaction (RT-PCR)** is quicker and more sensitive because it requires less mRNA than Northern blotting

- Once again, mRNA is isolated from different embryological stages

- Reverse transcriptase is added to mRNA to make cDNA, which serves as a template for PCR amplification of the gene of interest

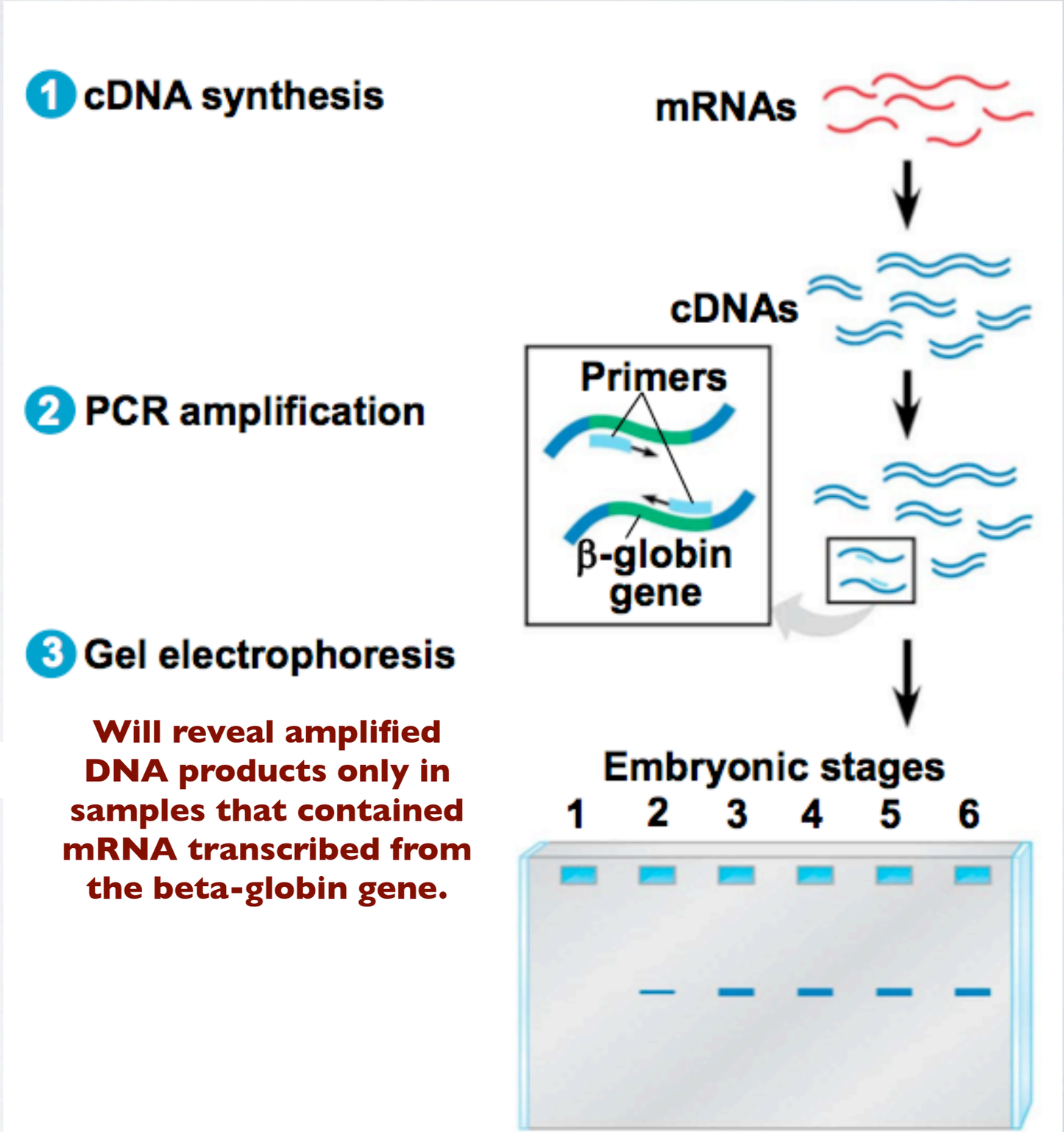
- The products are run on a gel and the mRNA of interest identified according to the stage in development

Application

RT-PCR uses the enzyme reverse transcriptase (RT) in combination with PCR and gel electrophoresis. RT-PCR can be used to compare gene expression between samples-for instance, in different embryonic stages, in different tissues, or in the same cell under different conditions.

Technique

In this example, samples containing mRNA's from six embryonic stages of hummingbird were processed. (The mRNA from only one stage is shown.)



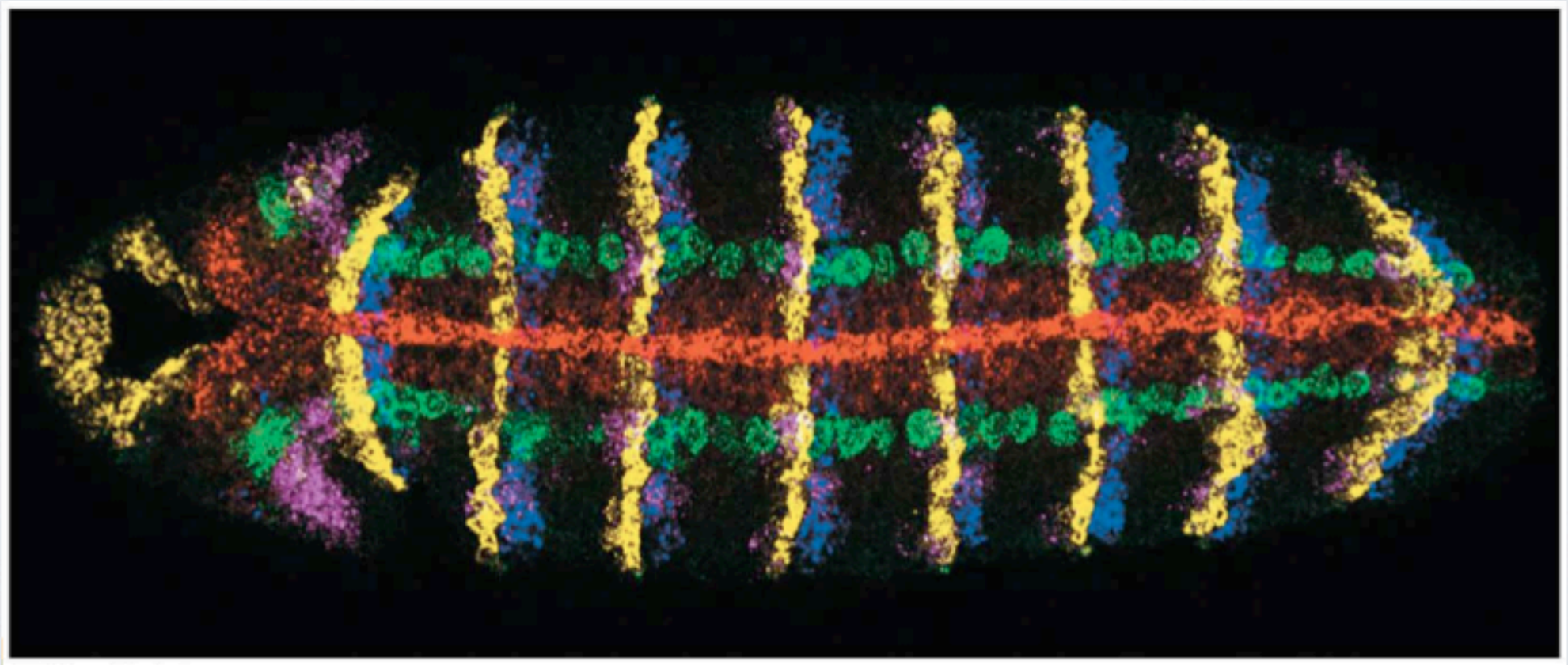
Results

The mRNA for this gene first is expressed at stage 2 and continues to be expressed through stage 6. The size of the amplified fragment (shown by its position on the gel) depends on the distance between the primers that were used.

SINGLE GENE EXPRESSION

- An alternative way to determine which cells or tissues are expressing certain genes is, ***in situ* hybridization.**

- ***In situ* hybridization** uses fluorescent dyes attached to probes to identify the location of specific mRNAs in place in the intact organism



MULTIPLE GENE EXPRESSION

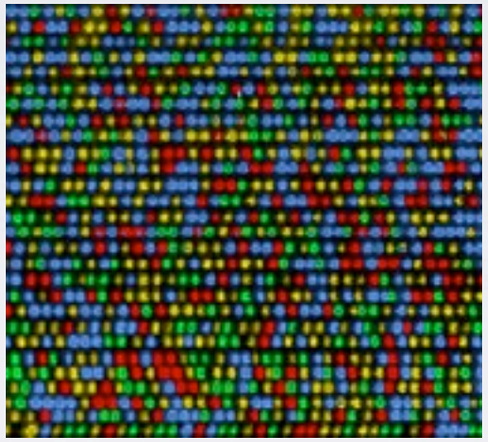
- Researchers use gene sequences as probes to investigate which genes are transcribed in different stages of development or different tissues.

- Scientists look for groups of genes that are expressed in a coordinated manner, with the aim of identifying networks of genes expression over the entire genome.

- Automation makes possible the ability to look at thousands of genes and their expression at one time, through **DNA microarray assays**.

MULTIPLE GENE EXPRESSION

- **DNA microarray assays** compare patterns of gene expression in different tissues, at different times, or under different conditions.



- Also called "DNA chips"

- 90% of a nematodes genes have been assayed during every stage of its life, nearly 60% of those genes are expressed differently at different times.

- The data supports the model in developmental biology that development involves a complex program of gene expresssion.

- This illustrates the ability of microarrays to reveal general profiles of gene expression over the lifetime of an organism.

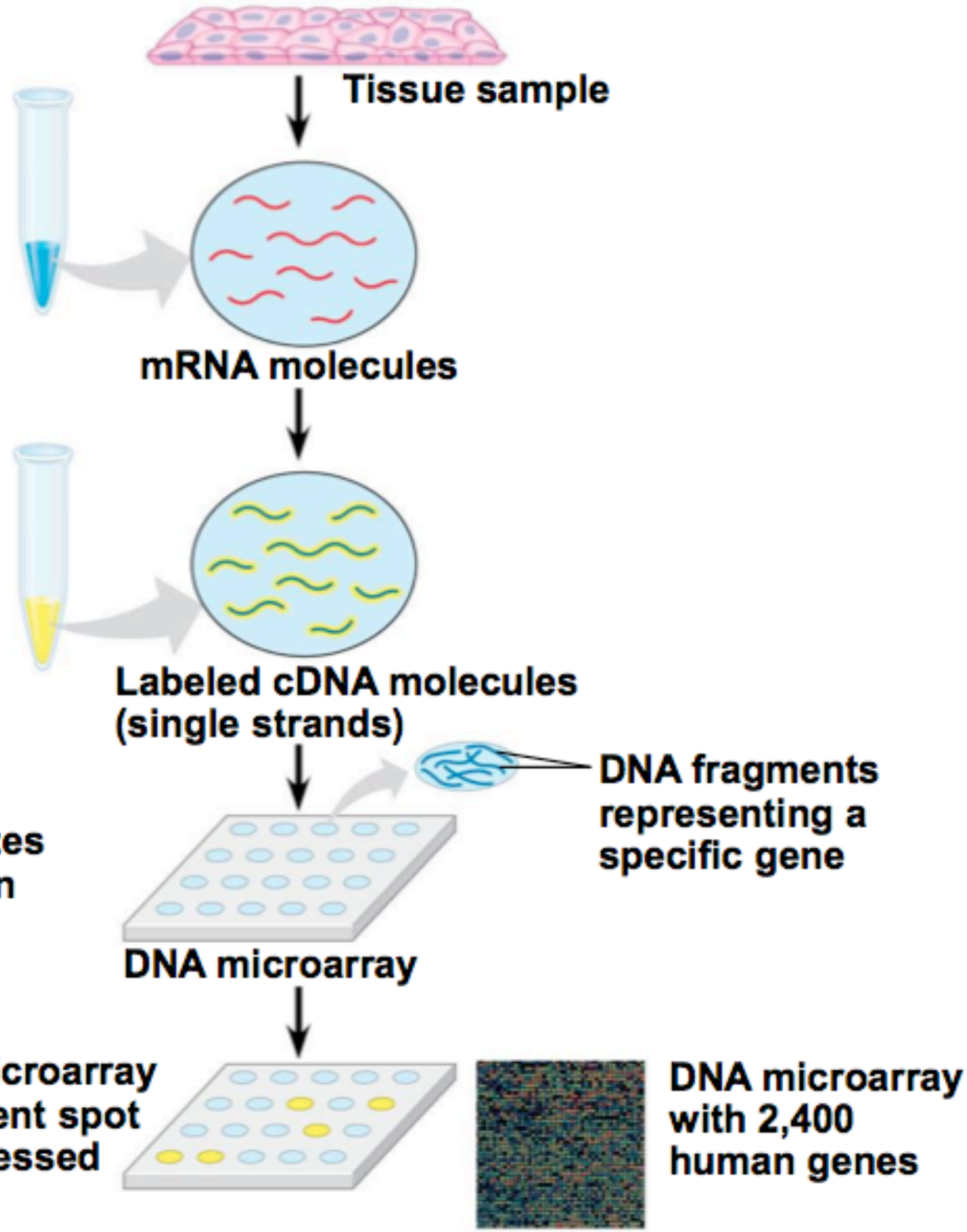
TECHNIQUE

1. Isolate mRNA.

2. Make cDNA by reverse transcription, using fluorescently labeled nucleotides.

3. Apply the cDNA mixture to a microarray, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.

4. Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot (yellow) represents a gene expressed in the tissue sample.



The Colors of a Microarray

GREEN represents **Control DNA**, where either DNA or cDNA derived from normal tissue is hybridized to the target DNA.

RED represents **Sample DNA**, where either DNA or cDNA is derived from diseased tissue hybridized to the target DNA.

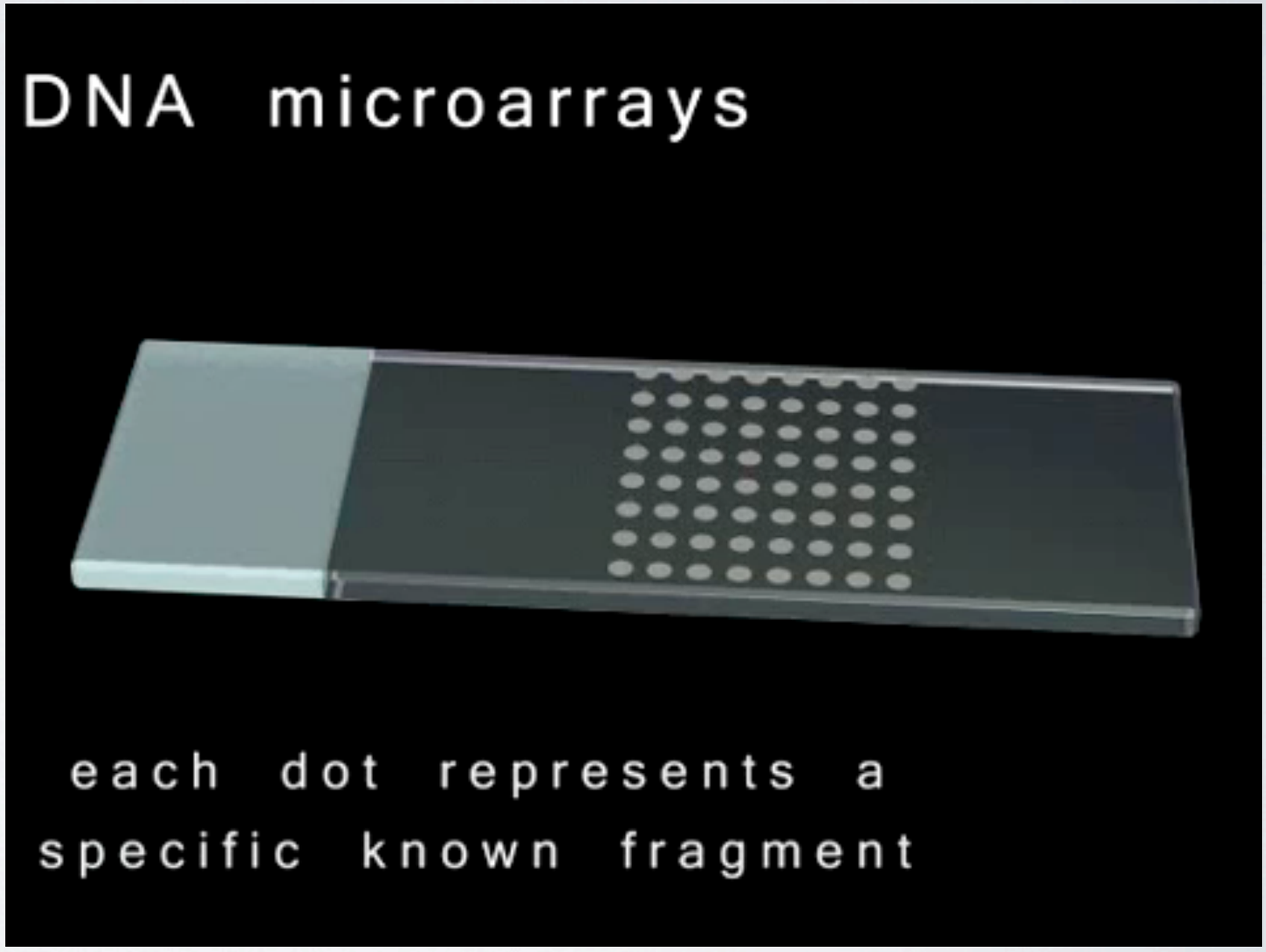
YELLOW represents **a combination of Control and Sample DNA**, where both hybridized equally to the target DNA.

BLACK represents areas where **neither the Control nor Sample DNA** hybridized to the target DNA.

Each spot on an array is associated with a particular gene. Each color in an array represents either healthy (control) or diseased (sample) tissue. Depending on the type of array used, the location and intensity of a color will tell us whether the gene, or mutation, is present in either the control and/or sample DNA. It will also provide an estimate of the expression level of the gene(s) in the sample and control DNA.

- In addition to better understanding gene interactions and functions, microarray assays can also...Contribute to our understanding of disease and thus suggest new diagnostic and therapeutic treatments.

- For example, comparing gene expression in breast cancer cells and normal cells has already revealed information that has lead to more effective treatments.



DETERMINING GENE FUNCTION

- The most common way (slowly being replaced) to determine gene function involved disabling a gene and then observing the consequences in the cell or organism, this approach is called ***in vitro* mutagenesis**.

- A cloned gene was mutated in such way that when introduced into the host cell it would "knock out" the other normal gene resulting in 2 mutated genes.

- If the introduced mutation alters or destroys the function of the gene product, the phenotype of the mutant cell may help reveal the function of the normal missing protein.

DETERMINING GENE FUNCTION

- The newer method (becoming more common) **RNA interference (RNAi)**.

- Here double stranded synthetic RNA probes of a specific gene(s) degrades mRNA or blocks translation.

- Once again the phenotypes are analyzed to deduce the functions of those genes.

- However, in humans ethical considerations prohibit knocking out genes to determine function, so alternative approach is necessary.

DETERMINING GENE FUNCTION IN HUMANS

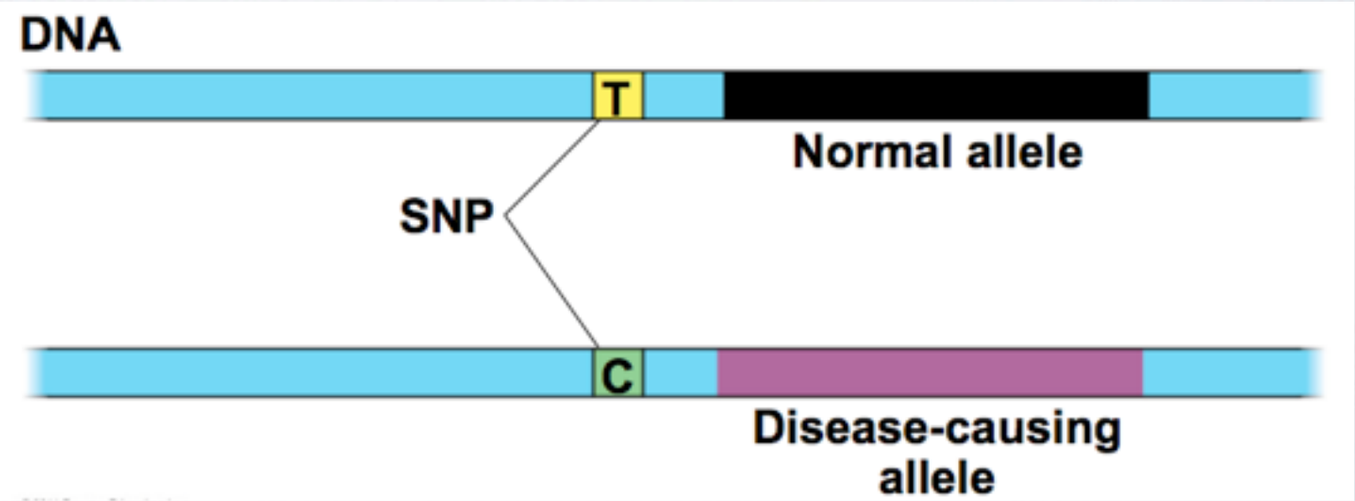
- The alternative method (used to study human genes) analyzes genomes of a large group of people with certain phenotypic condition or disease and then look for similarities between the diseased group and from the non-diseased group this is called **genome-wide association studies**.

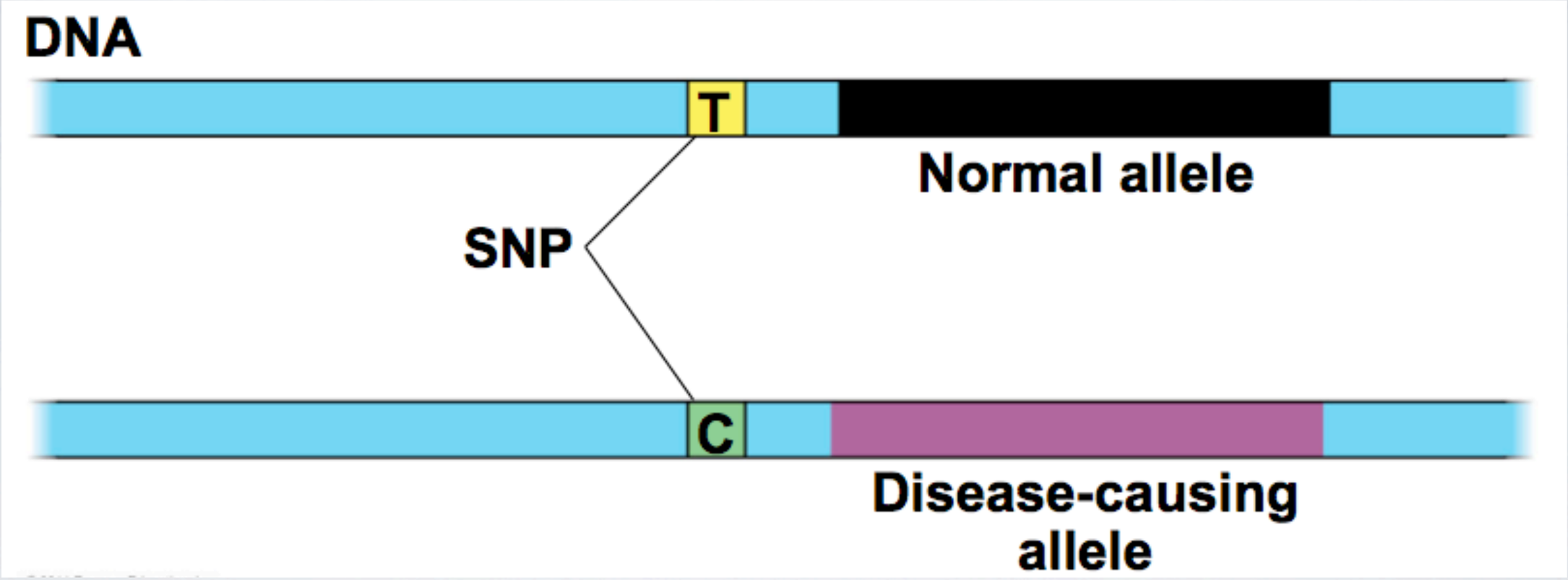
- Here researchers look for ***genetic markers***, DNA sequences that vary in the population, such variation may be the basis for different alleles.

- In fact, just like coding sequences may vary so to might non-coding sequences these small nucleotide differences in people at a specific locus is called ***polymorphisms***.

DETERMINING GENE FUNCTION IN HUMANS

- The useful genetic markers are single base-pair variations in the genomes of the human population.
- A single base site where 1% or more of the population varies is called a **single nucleotide polymorphism (SNP)** pronounced "snip"
- A few million SNP's occur in the human genome, about once every 100-300 base pairs.





- SNP's can be detected by microarray analysis or by PCR.
- In most cases the SNP is found in non-coding regions of DNA and do not contribute to the disease themselves
- Instead certain SNP's have been found very close to disease causing alleles (making crossing over unlikely and likely being inherited with the allele) making them a great "genetic marker" for disease causing alleles.

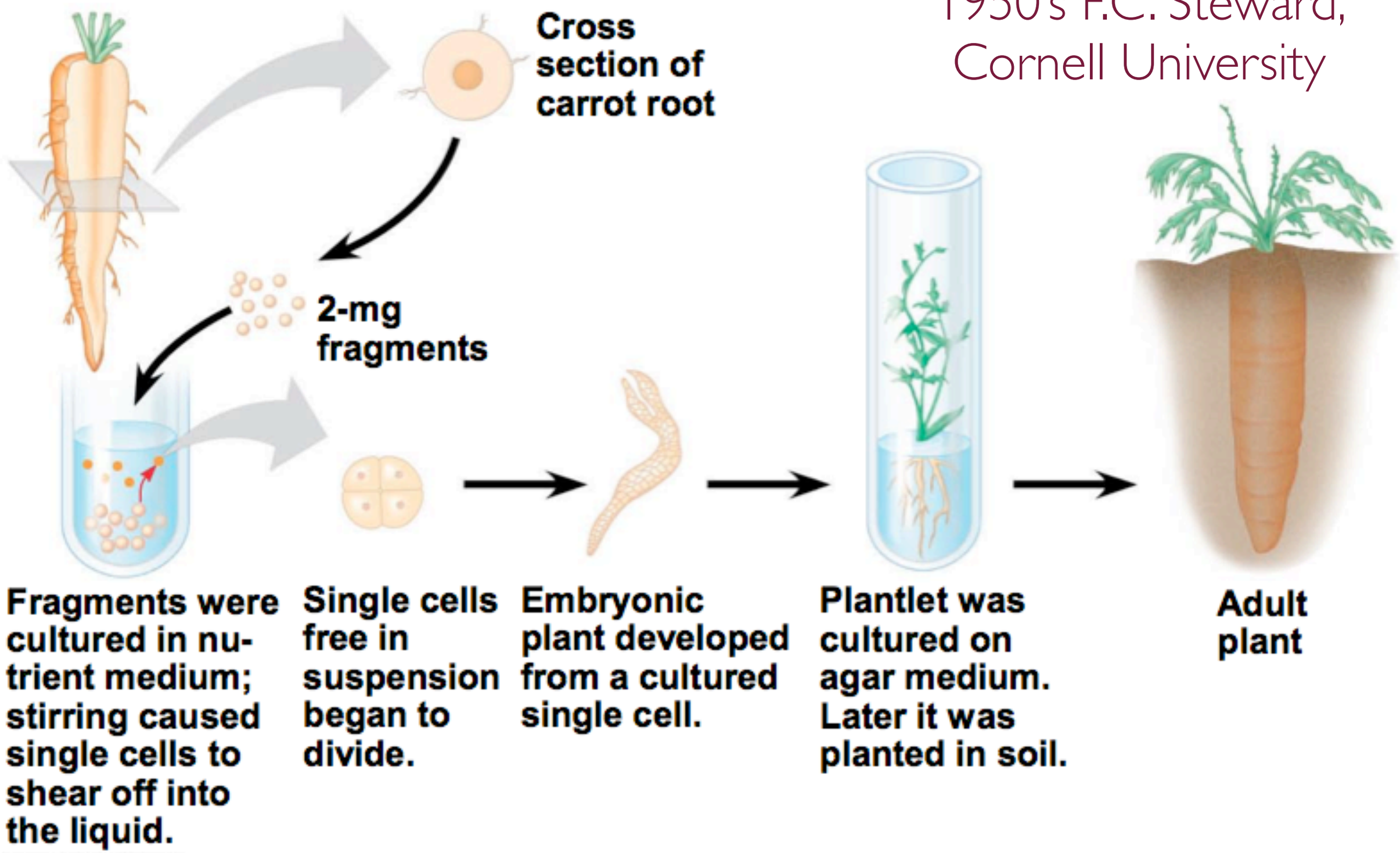
BIOTECHNOLOGY

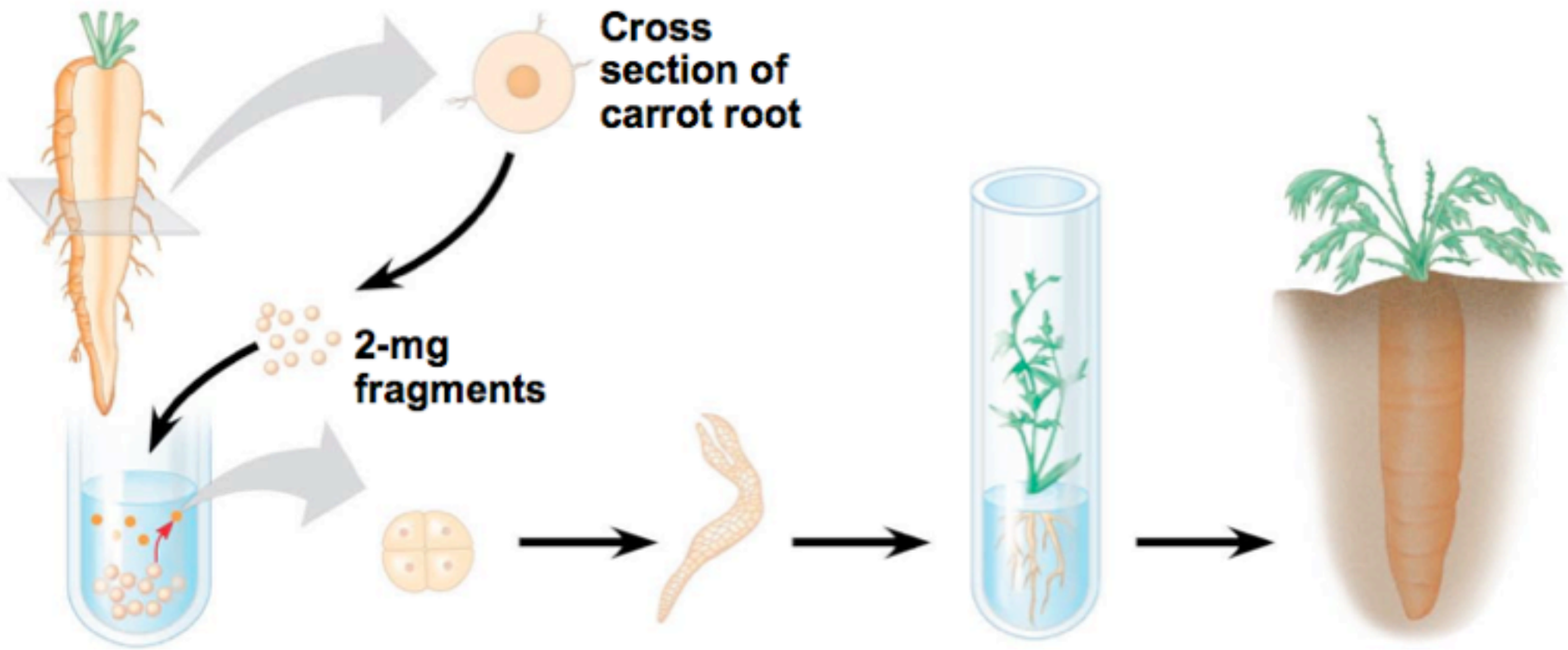
III. Main Idea

Cloning organisms has many potential applications, one of which is the production of stem cells.

CLONING PLANTS

1950's F.C. Steward, Cornell University





Plant cloning is used extensively today in agriculture!

Found that differentiated cells taken from root, grown in culture could develop into a normal adult plant!

These results showed that cell differentiation does not necessarily involve irreversible changes in the DNA.

Further...in plants some cells can "dedifferentiate" and "re-differentiate" into another cell type...these cells are totipotent!

CLONING ANIMALS

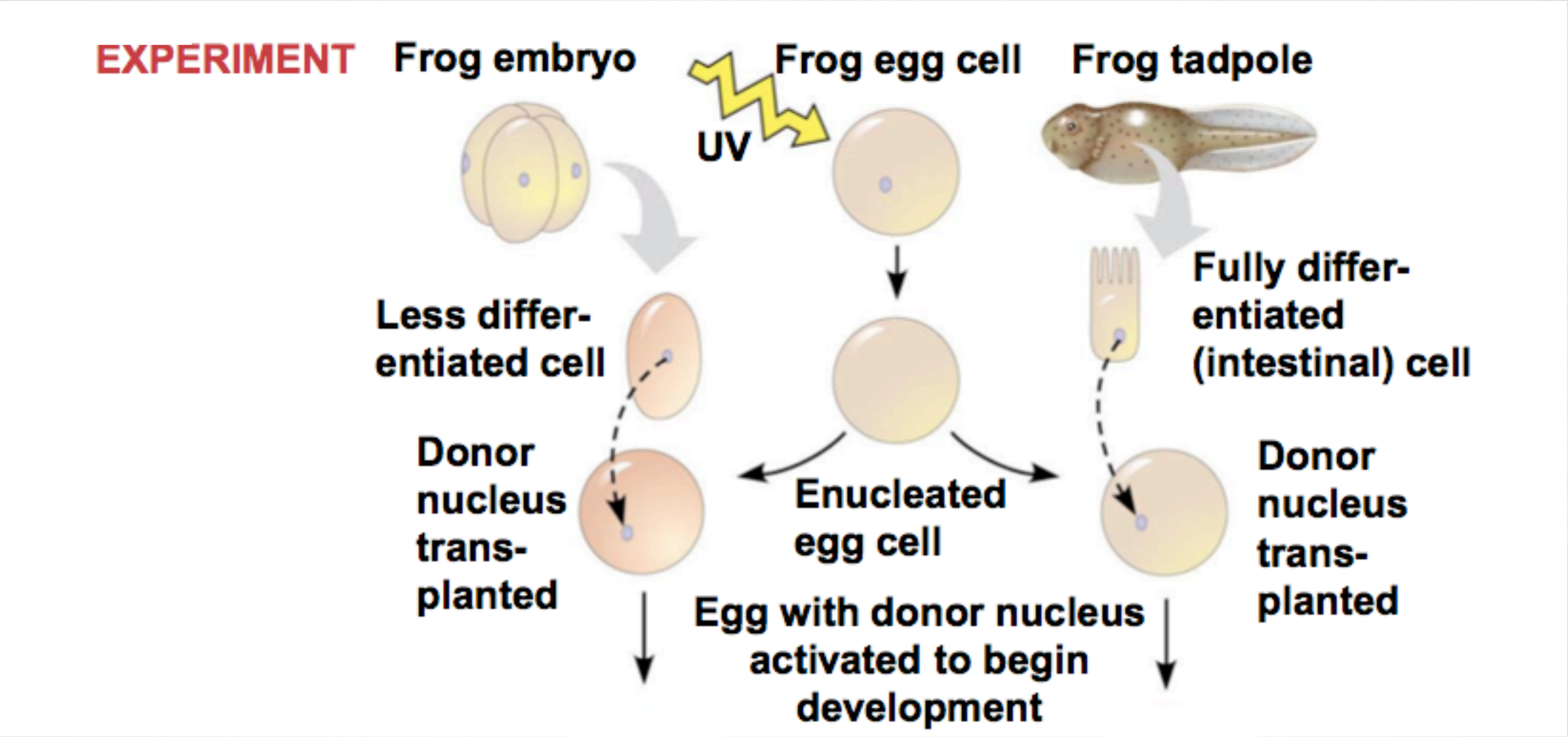
- Differentiated animal cells do not generally divide in culture.

- Differentiated animals cells will develop into other cell types or complete organisms.

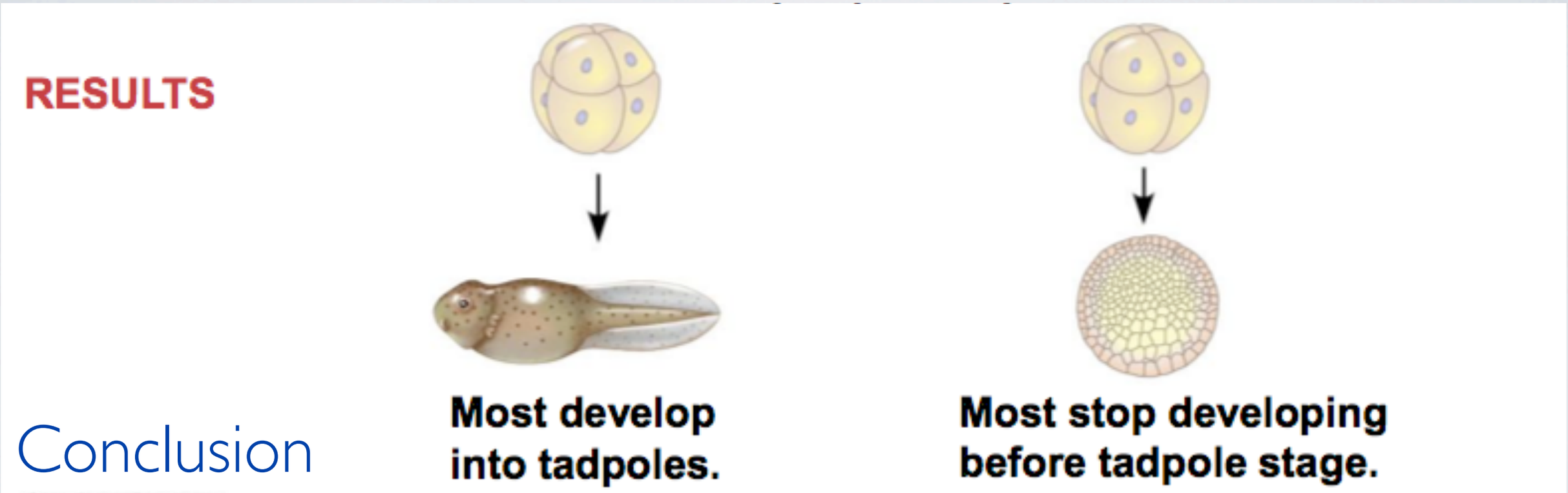
- The search for totipotent animal cells requires a different approach, called **nuclear transplantation**.

- *Nuclear transplantation*, removes the nucleus from an egg and replaces it with the nucleus of a differentiated cell.

John Gurdon and colleagues at Oxford University, destroyed the nuclei of frog eggs by exposing the eggs to UV light. They then transplanted the nuclei from cells of frog embryos and tadpoles into the enucleated eggs.



When the transplanted nuclei came from an early embryo, whose cells were relatively undifferentiated, most of the recipient eggs developed into tadpoles. BUt when the nuclei was came from fully differentiated intestinal cells of a tadpole, fewer than 2% of the eggs developed into into tadpoles, and most stopped developing at a much earlier stage.



Conclusion

The nucleus from a differentiated frog cell can direct the development of a tadpole. However, its ability to do so decreases as the donor cell becomes more differentiated, presumably because of changes in the nucleus.

REPRODUCTIVE CLONING OF MAMMALS

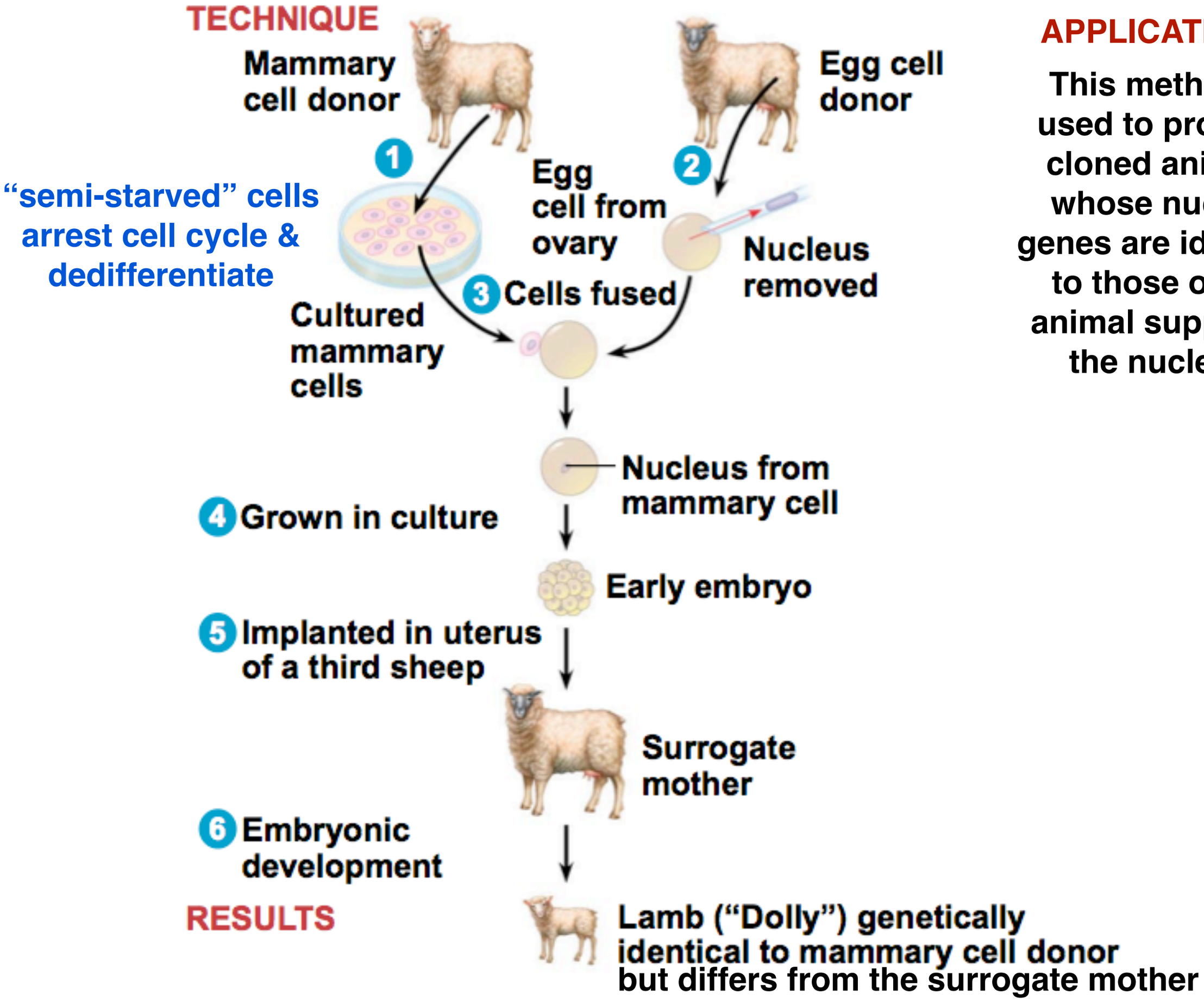
- Researchers now wondered whether nuclei of differentiated cells **could** be reprogrammed and act like as a donor nucleus.

- The answer came in1997, when scientists from Roslin Institute in Scotland made the headlines.

- They announced the birth of "Dolly" a lamb cloned from an adult sheep by nuclear transplantation from a differentiated cell.

- They were able to dedifferentiate the donor nuclei by culturing the cells in a nutrient poor medium and then fusing these cells with enucleated sheep eggs.

TECHNIQUE



APPLICATION

This method is used to produce cloned animals whose nuclear genes are identical to those of the animal supplying the nucleus.

REPRODUCTIVE CLONING OF MAMMALS

- Speculation arose regarding the success of Dolly, since she did suffer a seemingly high number of health complications and was consequently euthanized at age 6.

- However since 1997 many other mammals have been successfully cloned including: mice, cats, cows, horses, pigs, dogs and monkeys.

- The goal is usually reproductive cloning, that is simply making new individuals.

- Our knowledge of cloning continues to grow.

- CC (forCarbonCopy) was the first cat cloned; however, CC differed somewhat from her female “parent”

- Cloned animals do not always look or behave exactly the same

- Illustrating the importance of environmental influences and random phenomena in development.

Speculation abounds about cloning humans!

Researchers are getting closer, but the prospect raises unprecedented ethical issues.

PROBLEMS: IN CLONING ANIMALS

- Most clones like, "Dolly" exhibit defects of some sort or another, recent research has started to uncover reasons for these issues.

- The biggest problem in cloning animals relates to the epigenetic changes in chromatin.

- The methylation and acetylation patterns found on DNA must be reversed in the donors genes or the donors genes must be appropriately repressed or expressed *early* in development.

Remember DNA methylation/acetylation regulate gene expression, in fact we are finding much of cloning success resides in the ability to artificially manipulate these epigenetic patterns.

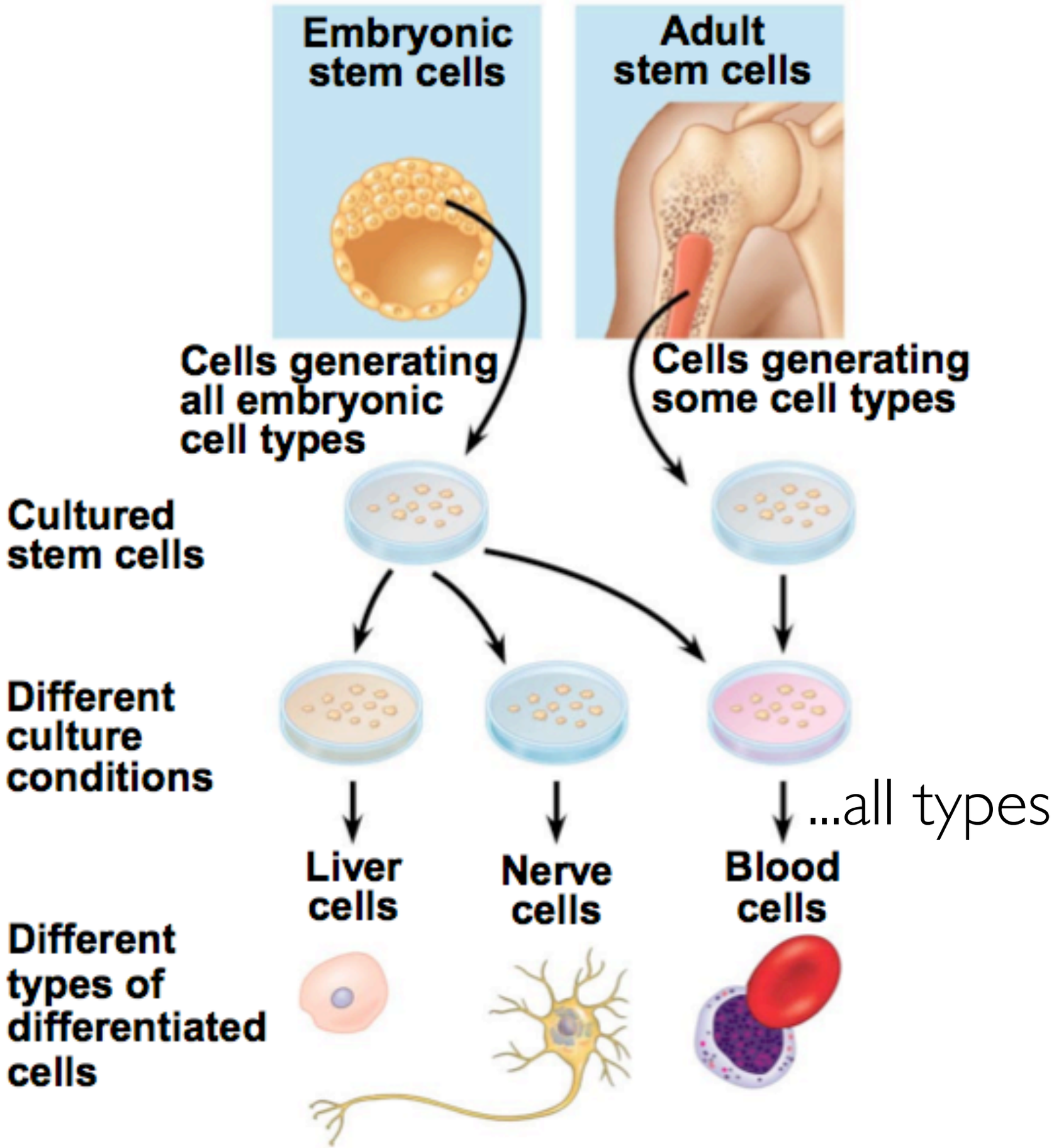
ANIMAL STEM CELLS

- A major goal of cloning human embryos is not reproduction, but production, the production of *stem cells* for treating human diseases.

- A **stem cell** is a relatively unspecialized cell that can both reproduce itself indefinitely and, under certain conditions, differentiate into specialized cells of one or more types.

- Early animal embryos (blastocyst stage) contain stem cells called **embryonic stem cells**, they are capable of producing any cell type.

- In contrast, **adult stem cells**, serve to replace non-reproducing specialized cells, including many different cell types BUT not ALL cell types.


Embryonic stem cells
Adult stem cells
Cells generating all embryonic cell types
Cells generating some cell types
Cultured stem cells
Different culture conditions
...all types
Liver cells
Nerve cells
Blood cells
Different types of differentiated cells

ANIMAL STEM CELLS

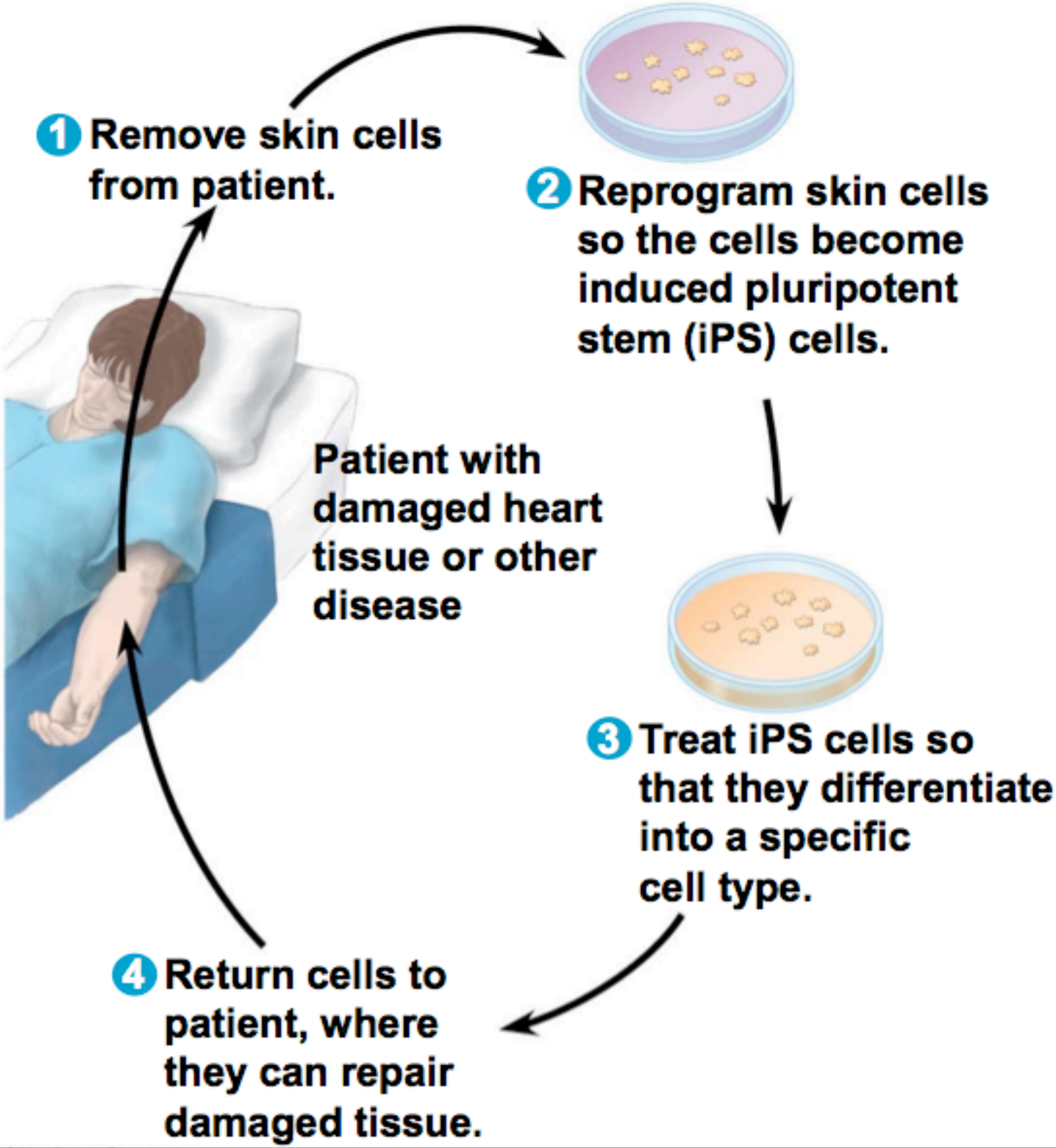
- Researchers continue more and more *adult stem cells*.
- *Adult stem cells* have been found in the brain, skin, hair, eyes, dental pulp and bone marrow.
- Scientists are getting better at finding, isolating and culturing these *adult stem cells*, but none are more versatile or promising than the *embryonic stem cells*.
- The ultimate goal behind stem cell research is to supply new operative cells to damaged or diseased organs.

ANIMAL STEM CELLS

- *Adult stem cells* are limited to certain tissue types, *embryonic stem cells* hold more promise because they are **pluripotent** (they can develop into many types of cells).
- The only way thus far to acquire ES cells is through embryos, which raises political and ethical issues.
- ES cells are currently obtained through embryos donated by patients undergoing infertility treatments.
- Scientists would like to clone the human embryos and use the embryos as a source of stem cells.
- A donor nucleus could from a diseased person, implanted into stem cell and used for therapeutic purposes.

ANIMAL STEM CELLS

- In 2007 researchers were able to reprogram differentiated cells to act like ES cells, they used retroviruses to introduce 4 stem cell regulatory master genes.
- Initially it look like the debate would be less imperative because these **induced pluripotent stem cells (iPS)** could do everything that ES cells could do.
- Recently however, differences between the iPS and ES cells are coming to light particularly with respect to gene expression and cell division.



Potential Treatments:

Cancers
Diabetes
Heart Disease
Alzheimer's
Parkinson's
Huntington's
Sickle Cell
Spinal Cord Injuries

BIOTECHNOLOGY

IV. Main Idea

The practical applications of DNA technology affect our lives in many areas such as medicine, forensics, agriculture and environmental science.

MEDICAL APPLICATIONS

- DNA technology has allowed us to identify the genes that play a role direct role in disease.

- It has even helped our understanding of "nongenetic" diseases and the indirect roles that certain genes play.

- Furthermore by comparing diseased and normal tissue we can identify gene expression patterns that could help to develop targets for prevention or therapy.

DIAGNOSIS

- PCR and gene probes are now used to diagnose and track down pathogens such as HIV.

- PCR and gene probes can also diagnose genetic disorders such as sickle cell, Huntington's, cystic fibrosis, hemophilia and Duchenne muscular dystrophy.

- Southern blotting is used to determine carriers of these genetic disorders.

- SNP's associated with increased risk of cancers, Alzheimer's or heart disease can be identified so that patients better understand their personal health risks.

TREATMENT

- Genome wide association studies have looked at gene expression patterns in cancers and has consequently affected the treatment protocols selected by patients and doctors.

- Many envision a future of "personalized medicine" where your treatment is dependent upon you specific genetic profile.

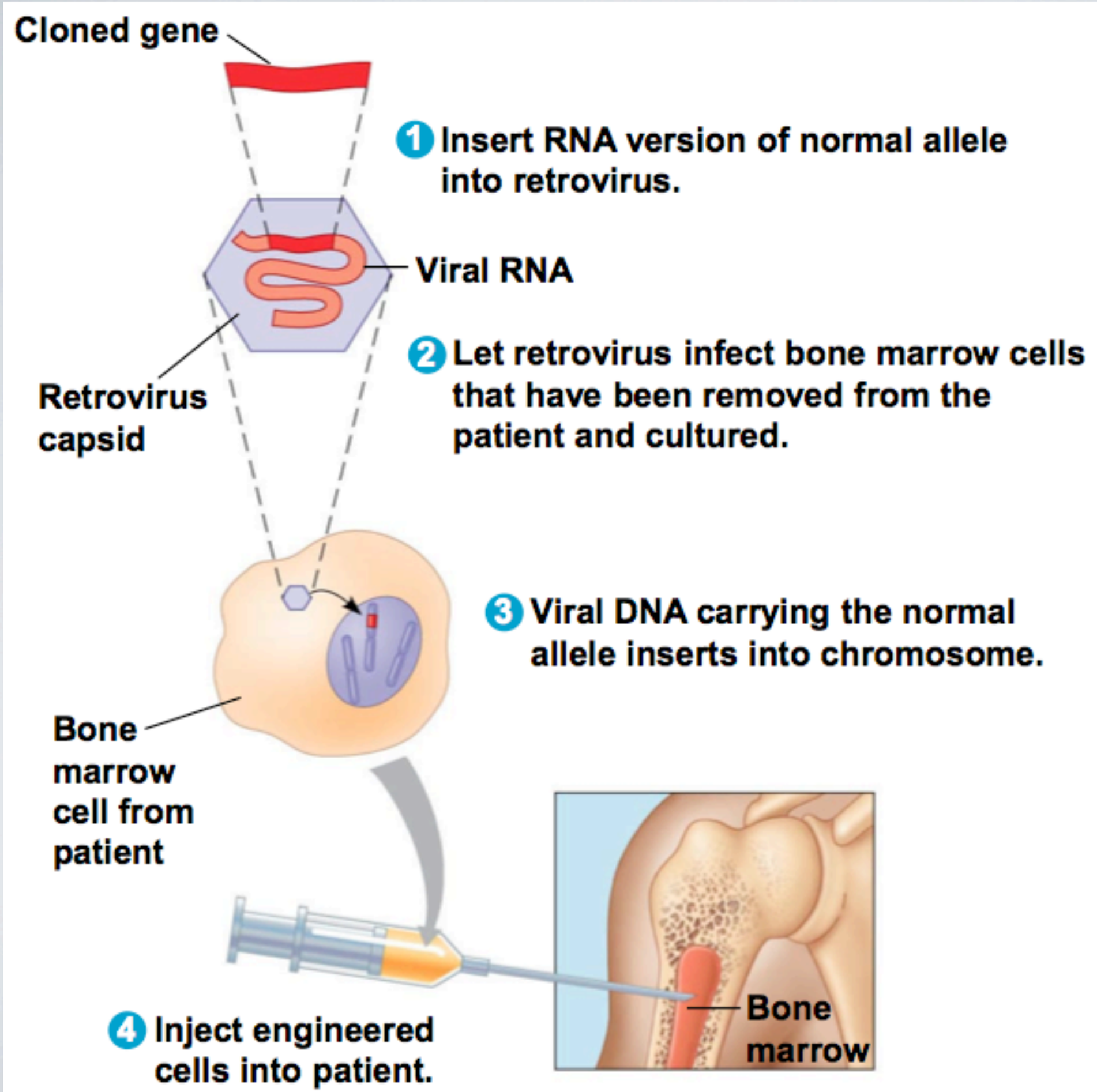
- This era of "personalized medicine" will likely become reality when complete genome sequencing technology becomes rapid (hours) and inexpensive (<1000$ per person).

HUMAN GENE THERAPY

- **Gene Therapy**- injecting genes into an afflicted individual for therapeutic purposes-holds great promise.

- For gene therapy in somatic cells to become permanent the cells that receive the "normal copy" must be the ones that replicate throughout life.

- Bone marrow cells, which include stem cells are prime candidates.



France, 2000
a trial begins with ten kids suffering from a blood disease (SCID), the results were mixed as 9/10 improved after 2 years but 3 subsequently developed leukemia, one of which died.

HUMAN GENE THERAPY

- Since the France 2000 trial, two other diseases have been treated using this method: one that causes blindness and the other a degeneration of the nervous system.

- Both were successful but had limited participants so researchers remain cautiously optimistic.

- Keep in mind, human gene therapy comes with its own set of technical and ethical issues.

PHARMACEUTICAL PRODUCTS

- Determining the sequence and structure of proteins crucial for tumor cell survival has lead to identification and production of small molecules that combat cancers by blocking the functions of these proteins.

- Recall the drug "Gleevec" used to treat chronic myelogenous leukemia, those treated in the early stages show almost complete and sustained remission.

- *Pharmaceutical products* are proteins that can be made on a large scale, using cells or whole organisms.

PROTEIN PRODUCTION IN CELLS

- DNA cloning and gene expression systems have engineered cells to secrete the "farmed protein" as it is being made, which drastically cuts time and cost of production.

- Today insulin created this way is used to manage diabetes in over 2 million people in the U.S.

- Other examples include human growth hormone (HGH) and tissue plasminogen activator (TPA), given to heart attack patients to reduces risk of subsequent heart attacks.

PROTEIN PRODUCTION BY "PHARM" ANIMALS

- By introducing a gene from one animal into another animal (often different species) that **transgenic** animal can act as a pharmaceutical factory for the protein of interest.

- The human gene for antithrombin was inserted into a goat and the engineered goats now secrete antithrombin in their milk.

- Details are still being worked out as sometimes the transgenic animal produces human proteins that are slightly different, so allergic reactions and contamination must be addressed.

FORENSICS & GENETIC PROFILES

- DNA left at a crime scene can help determine the guilt or innocence of a suspect.

- Body fluids or tissue can often be found at crime scenes, if enough is found DNA analysis can be run to determine the samples unique genetic profile.

- Early on fluid and tissue samples would need to be fresh and be left in relatively large amounts, then by the late 1980's the sample could be older and contained roughly 1000 cells, today all we need to collect is roughly 20 cells!

FORENSICS & GENETIC PROFILES

- In 1988 the FBI began started applying DNA technology to forensics (then called "DNA fingerprinting", today called "genetic profiling").

- They began by using RFLP's and southern blotting to identify similarities and differences in the samples.

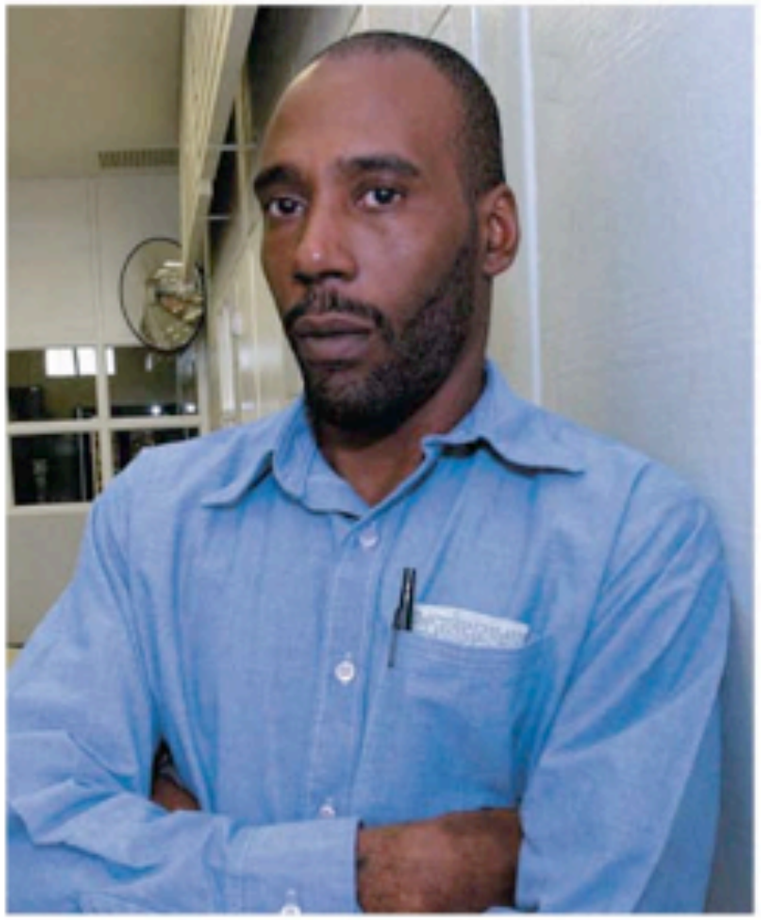
- Today we use **short tandem repeats (STRs)** tandemly repetitive units of two to five base sequences in specific regions of the genome.

- The number of repeats from person to person is highly variable (polymorphic) and even between the same persons two alleles.

SHORT TANDEM REPEATS (STR)

- One person might have TAGG repeated 28 times at one locus and 20 times at the same locus on the other homolog.
- Now imagine comparing these genotypes of two individuals one might be 28,20 while the other has an equally unique 17,17 genotype.
- PCR, labeled primers and electrophoresis is all that is required to establish these profiles.
- Forensic scientists test for roughly a dozen STR markers (much less is actually needed) when comparing fluid/tissue samples, the chance that two people share the same STRs is astronomically small.

(a) This photo shows Washington just before his release in 2001, after 17 years in prison.



- The **Innocence Project**, a nonprofit organization dedicated to overturning wrongful convictions, uses STR analysis from archived samples from crime scenes to revisit old cases.

As of 2010, more than 250 people have been released from prison as a result of forensic and legal work.

Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley	17,19	13,16	12,12

(b) These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

GENETIC PROFILING

- Genetic profiles are useful in other areas besides forensics.
- Genetic profiles are used to establish paternity.
- Genetic profiles can be used for historical purposes, for example we that Thomas Jefferson or one of his close male relatives fathered at least one of the children of his slave Sally Hemings.
- Genetic profiles are used to identify victims in mass casualties, over 3000 victims were identified from the remains of the World Trade Center attack in 2001.

GENETIC PROFILING-RELIABILITY

- The more genetic markers you analyze, the more accurate the results.
- Forensics today use about 12 markers and with that number the odds that two people share exactly the same profile is somewhere between 10 billion and several trillion.
- The exact probability requires that we know the frequencies of each marker in various ethnic groups and the general population.
- Despite the possibility of human error, flawed evidence and insufficient data compelling genetic evidence is accepted by legal experts and scientists.

ENVIRONMENTAL CLEANUP

- Scientists can transfer genes for remarkable metabolic capabilities into other microorganisms that then can be used to treat environmental problems.
- Some bacteria can extract heavy metals such as copper, lead or nickel and incorporate them into compounds that we ca easily recover.
- Microbes may become important in mining metals as well as the clean-up of toxic mine waste.
- Microbes are being engineered to clean-up dangerous chlorinated hydrocarbons.
- Microbes are used regularly in waste water treatment facilities.

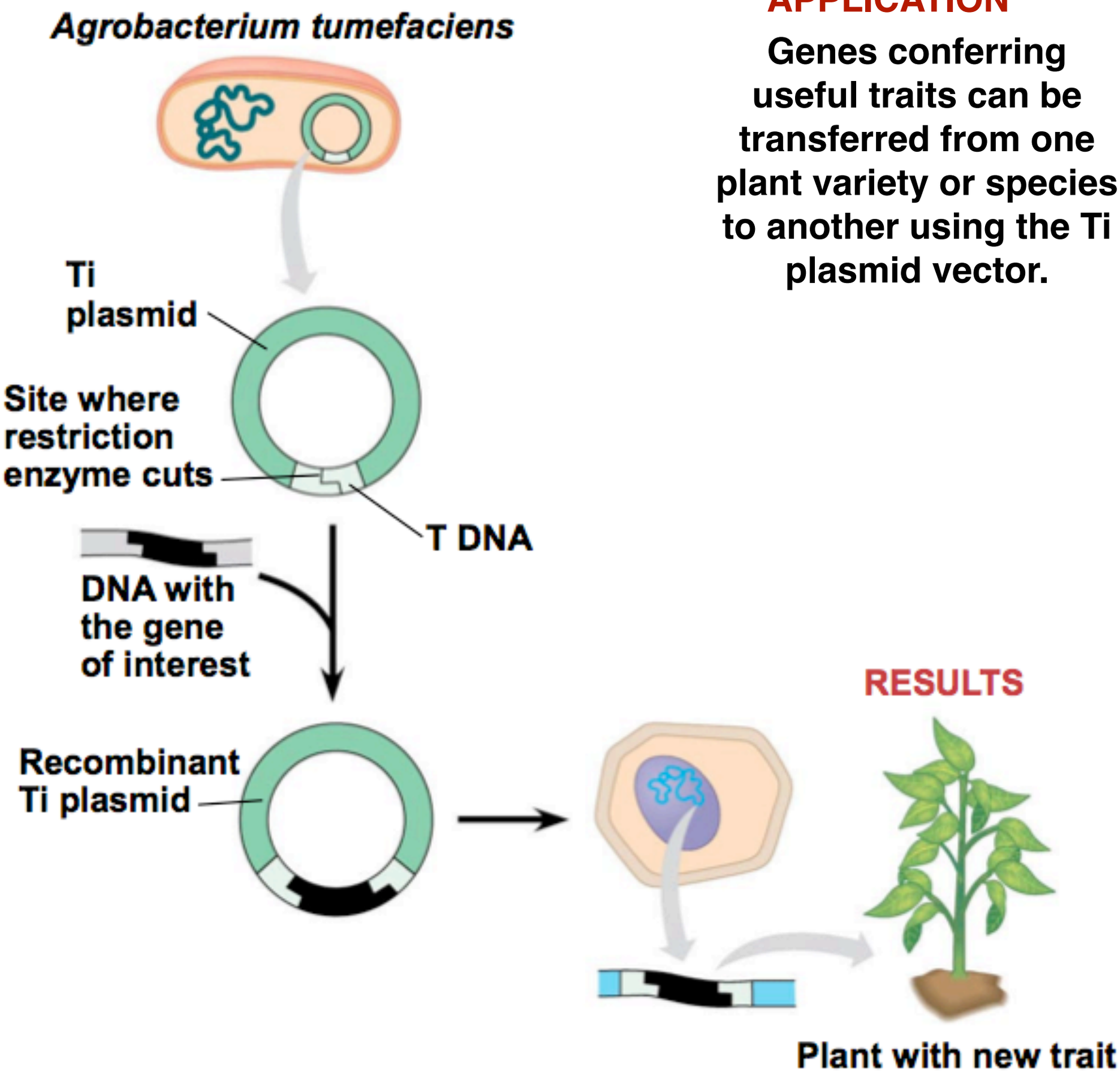
AGRICULTURAL APPLICATIONS

- For thousands of years people have employed selective breeding of livestock (animal husbandry) and crops.
- Today DNA technology looks to speed this up this selective process by creating transgenic organisms.
- The goal is essentially the same as traditionally breeding, create desirable traits in the livestock or crop.
- We want sheep with better wool, cows that grow faster, pigs with leaner meat, crops that pest resistant, crops that need little water or crops that can grow in salty soil.

AGRICULTURAL APPLICATIONS

- The most commonly used vector in plants is the **Ti plasmid** from the soil bacterium *Agrobacterium tumefaciens*.
- The vectors are engineered to carry genes of interest while at the same time not causing disease (which wild type does).
- Genetic engineering is replacing traditional breeding by inserting useful genes/traits into the plants.
- The most common traits being engineered today involve pest resistance, herbicide resistance, delayed ripening, improved nutritional content and salinity resistance.

TECHNIQUE



APPLICATION

Genes conferring useful traits can be transferred from one plant variety or species to another using the Ti plasmid vector.

RESULTS

Transformed cells carrying the gene of interest can regenerate complete plants that exhibit the new trait.

SAFETY & ETHICS

- The earliest and greatest concerns with recombinant technology was the possibility that hazardous new pathogens might be created.
- Today, strict global regulations and guidelines are in place.
- FIRST- Strict lab procedures are in place to protect researchers and the accidental escape of microbes from the lab.
- SECOND- Recombinant strains are often crippled so that they can not live outside of the artificial conditions created by the lab.
- THIRD- Some experiments or types of experiments have been banned altogether.

SAFETY & ETHICS

- Today the greatest concerns revolve around **genetically modified organisms (GMOs)** used as food.
- Today, some GMO animals exist but most GMOs are crops.
- Most GMO crops are grown in the U.S., Brazil and Argentina. Together they make up 80% of worldwide GMO crops
- In the U.S. most soybean, corn and canola crops are genetically modified.
- **Also, in the U.S. GM foods are NOT required to be labeled, and this is a growing point of contention for many people.**

SAFETY & ETHICS

- The GM revolution has been met with strong opposition in Europe, citizens have expressed health and environmental concerns regarding these plants.

- In 2000, 130 countries signed the Biosafety Protocol (which the U.S. under George Bush failed to sign) went affect.

- The Biosafety Protocol requires exporters to identify GM organisms present in food shipments and allows importing countries whether they pose health or environmental risks.

- Since 2000, GM crops grown in Europe have failed in local markets and they have on occasion refused U.S. imports of GM organisms, leading to trade disputes.

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SAFETY & ETHICS

- Additional concerns around GM crops involve the production of "superweed" and allergic reactions.
- If a gene conferring herbicide resistant in the crop plant makes its way to a weed we could have very difficult problem to, control.
- We know that pollen transfer can and will occur and that the division between plant species is not near as distinct as that between animals which increases the plausibility even more.
- Also, they is evidence that GM crops contain slightly altered molecules, which can and have caused severe allergic reactions.

SAFETY & ETHICS

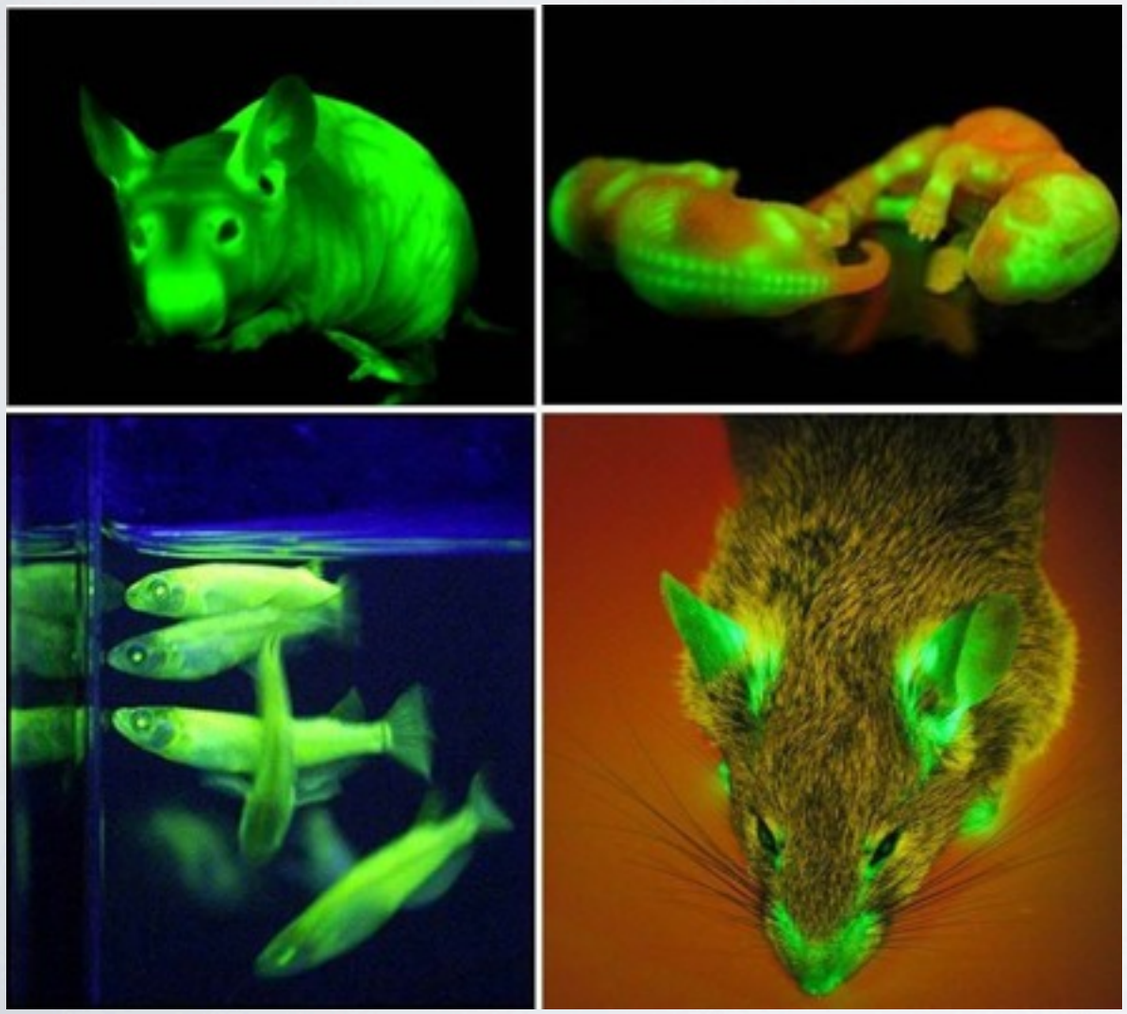
- Today governments and regulatory agencies are struggling with the facilitation of biotechnology.

- In the U.S. biotech applications and products are evaluated through the Food & Drug Administration, Environmental Protection Agency, Department of Agriculture and The National Institutes of Health.

- Unfortunately many of the people running these regulatory agencies have worked for or have close ties to the very companies that are producing the GM products.

- As result, non-profit organizations and watch groups along with social media play an increasingly important role our health, safety and decision making.

HERE WE GO...




Carrier
3:22 PM
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MyGenome
Health
Show All
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breast
Hereditary Breast and Ovarian Cancer
Genetic Disease | Cancers
Hereditary breast and ovarian cancer (HBOC) is a hereditary cancer syndrome. Two genes, BRCA1 and BRCA2, account for the majority of known genetic causes of this syndrome. Individuals with HBOC syndrome have a significantly increased risk of breast, ovarian, pancreatic, prostate and male breast cancers. Early ages of onset, bilateral disease and multiple primary cancers are also characteristic of HBOC syndrome.
Causal Variants
BRCA1
BRCA2
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Fucosidosis
Hereditary Breast and Ovarian Cancer
Hereditary Spherocytosis
Krabbe Disease
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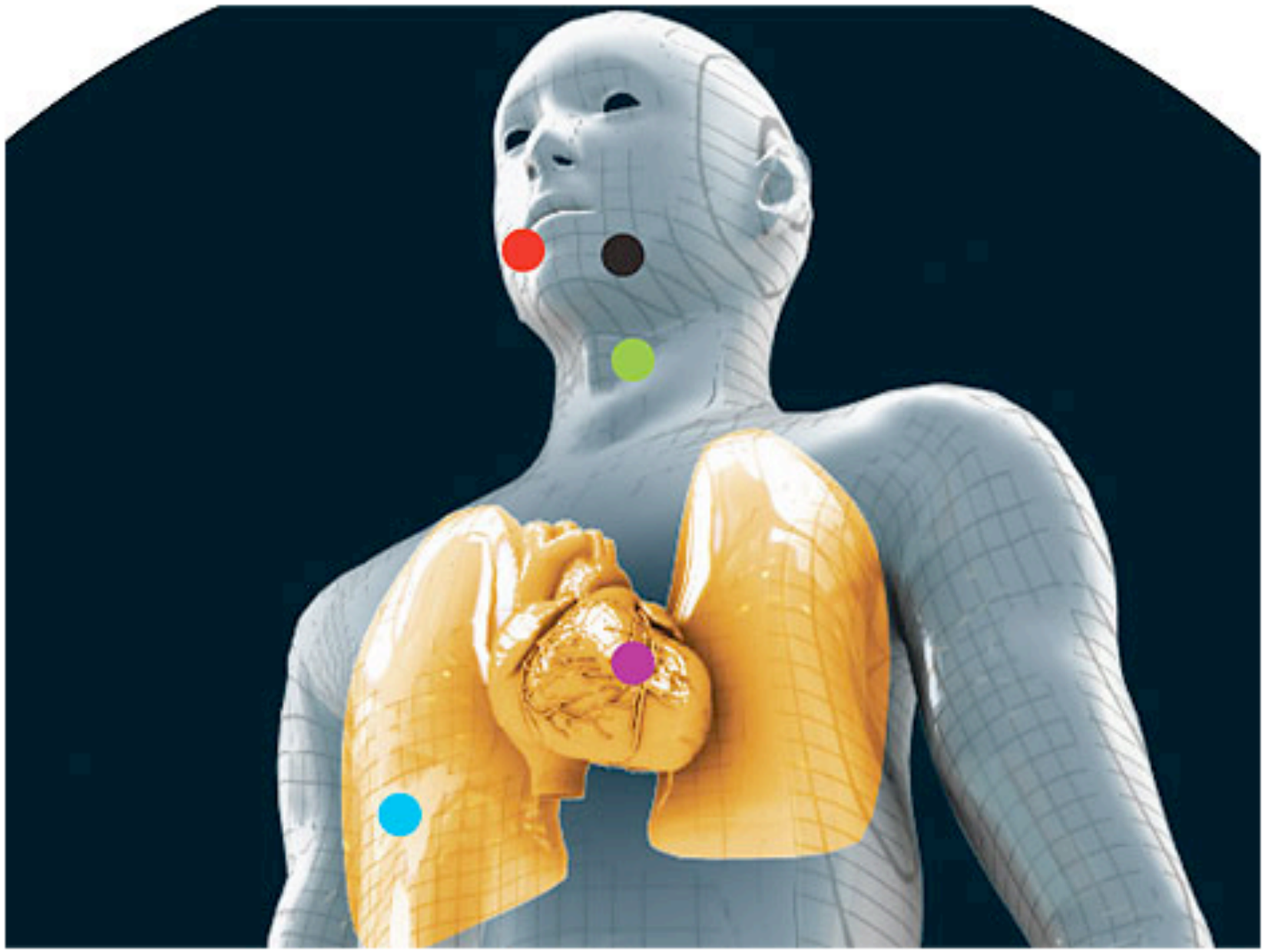
HERE WE GO...



* 1. Decay-Fighting Microbes

Bacteria living on teeth convert sugar into lactic acid, which erodes enamel and causes tooth decay. Florida-based company ONI BioPharma has engineered a new bacterial strain, called SMaRT, that cannot produce lactic acid—plus, it releases an antibiotic that kills the natural decay-causing strain. Dentists will only need to swab SMaRT, now in clinical trials, onto teeth once to keep them healthy for a lifetime.

* 2. Artificial Lymph Nodes

Scientists from Japan's RIKEN Institute have developed artificial versions of lymph nodes, organs that produce immune cells for fighting infections. Though they could one day replace diseased nodes, the artificial ones may initially be used as customized immune boosters. Doctors could fill the nodes with cells specifically geared to treat certain conditions, such as cancer or HIV.

* 3. Asthma Sensor

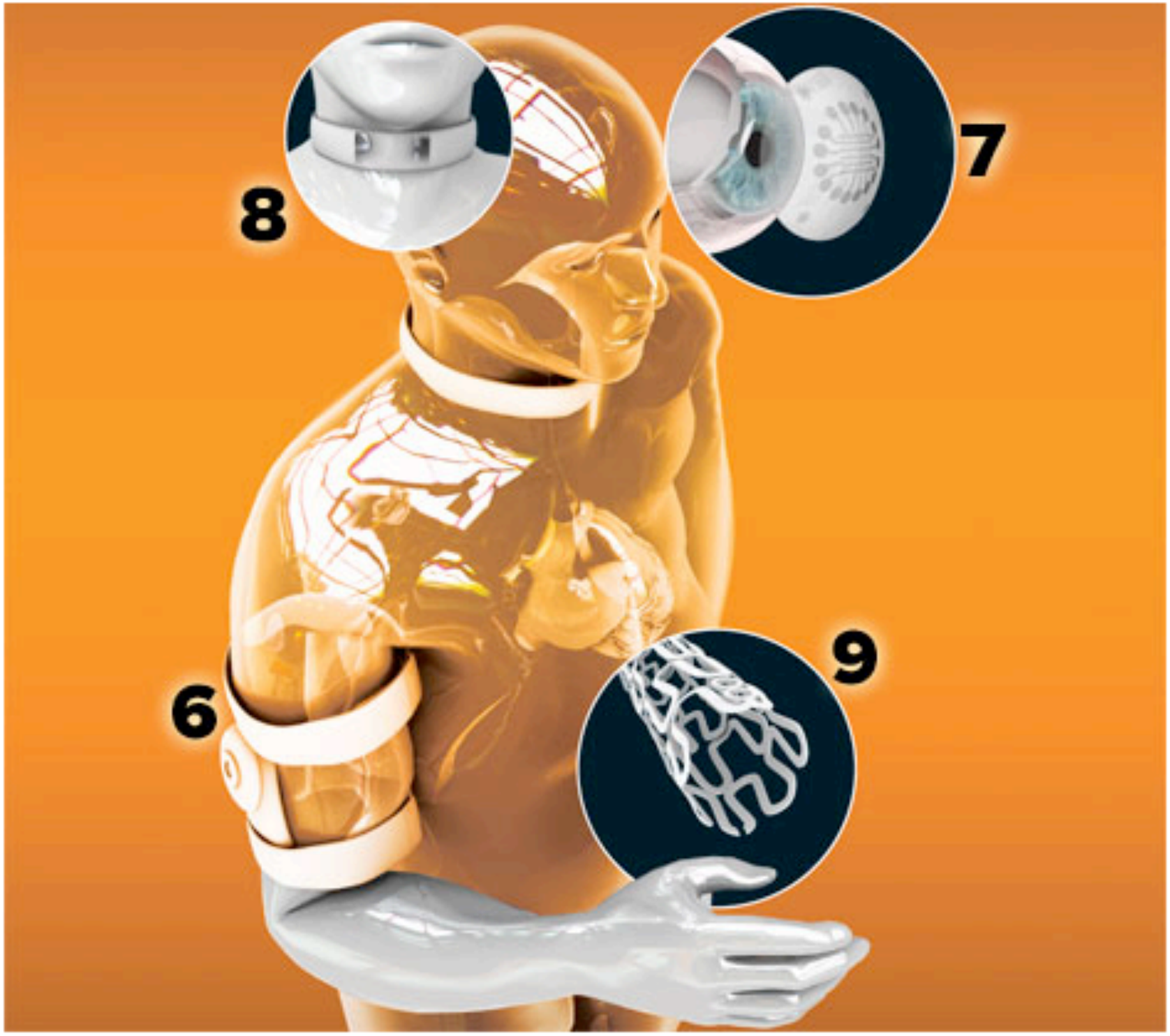
Asthma accounts for a quarter of all emergency room visits in the U.S., but a sensor developed at the University of Pittsburgh may finally cause that number to plummet. Inside the handheld device, a polymer-coated carbon nanotube —100,000 times thinner than a human hair—analyzes breath for minute amounts of nitric oxide, a gas that lungs produce prior to asthma attacks.

* 4. Cancer Spit Test

Forget biopsies—a device designed by researchers at the University of California-Los Angeles detects oral cancer from a single drop of saliva. Proteins that are associated with cancer cells react with dyes on the sensor, emitting fluorescent light that can be detected with a microscope. Engineer Chih-Ming Ho notes that the same principle could be applied to make saliva-based diagnostic tests for many diseases.

* 5. Biological Pacemaker

Electronic pacemakers save lives, but use hardware that eventually wears out. Now, researchers at several universities are developing a batteryless alternative: pacemaker genes expressed in stem cells that are injected into damaged regions of the heart. Better suited for physical exertion, biological pacemakers have been shown to bring slow canine hearts back up to speed without complications.



6. Prosthetic Feedback

One challenge of prosthetic limbs is that they're difficult to monitor. "You and I sense where our limbs are spatially without having to look at them, whereas amputees don't," says Stanford University graduate student Karlin Bark. Skin is sensitive to being stretched—it can detect even small changes in direction and intensity—so Bark is developing a device that stretches an amputee's skin near the prosthesis in ways that provide feedback about the limb's position and movement.

7. Smart Contact Lens

Glaucoma, the second-leading cause of blindness, develops when pressure builds inside the eye and damages retinal cells. Contact lenses developed at the University of California-Davis contain conductive wires that continuously monitor pressure and fluid flow within the eyes of at-risk people. The lenses then relay information to a small device worn by the patient; the device wirelessly transmits it to a computer. This constant data flow will help doctors better understand the causes of the disease. Future lenses may also automatically dispense drugs in response to pressure changes.

8. Speech Restorer

For people who have lost the ability to talk, a new "phonetic speech engine" from Illinois-based Ambient Corporation provides an audible voice. Developed in conjunction with Texas Instruments, the Audeo uses electrodes to detect neuronal signals traveling from the brain to the vocal cords. Patients imagine slowly sounding out words; then the quarter-size device (located in a neck brace) wirelessly transmits those impulses to a computer or cellphone, which produces speech.

9. Absorbable Heart Stent

Stents open arteries that have become narrowed or blocked because of coronary artery disease. Drug-eluting stents release medication that keeps the artery from narrowing again. The bio-absorbable version made by Abbott Laboratories in Illinois goes one step further: Unlike metal stents, it does its job and disappears. After six months the stent begins to dissolve, and after two years it's completely gone, leaving behind a healthy artery.

10. Muscle Stimulator

In the time it takes for broken bones to heal, nearby muscles often atrophy from lack of use. Israeli company StimuHeal solves that problem with the MyoSpare, a battery-operated device that uses electrical stimulators—small enough to be worn underneath casts—to exercise muscles and keep them strong during recovery.

11. Nerve Regenerator

Nerve fibers can't grow along injured spinal cords because scar tissue gets in the way. A nanogel developed at Northwestern University eliminates that impediment. Injected as a liquid, the nanogel self-assembles into a scaffold of nanofibers. Peptides expressed in the fibers instruct stem cells that would normally form scar tissue to produce cells that encourage nerve development. The scaffold, meanwhile, supports the growth of new axons up and down the spinal cord.

12. Stabilizing Insoles

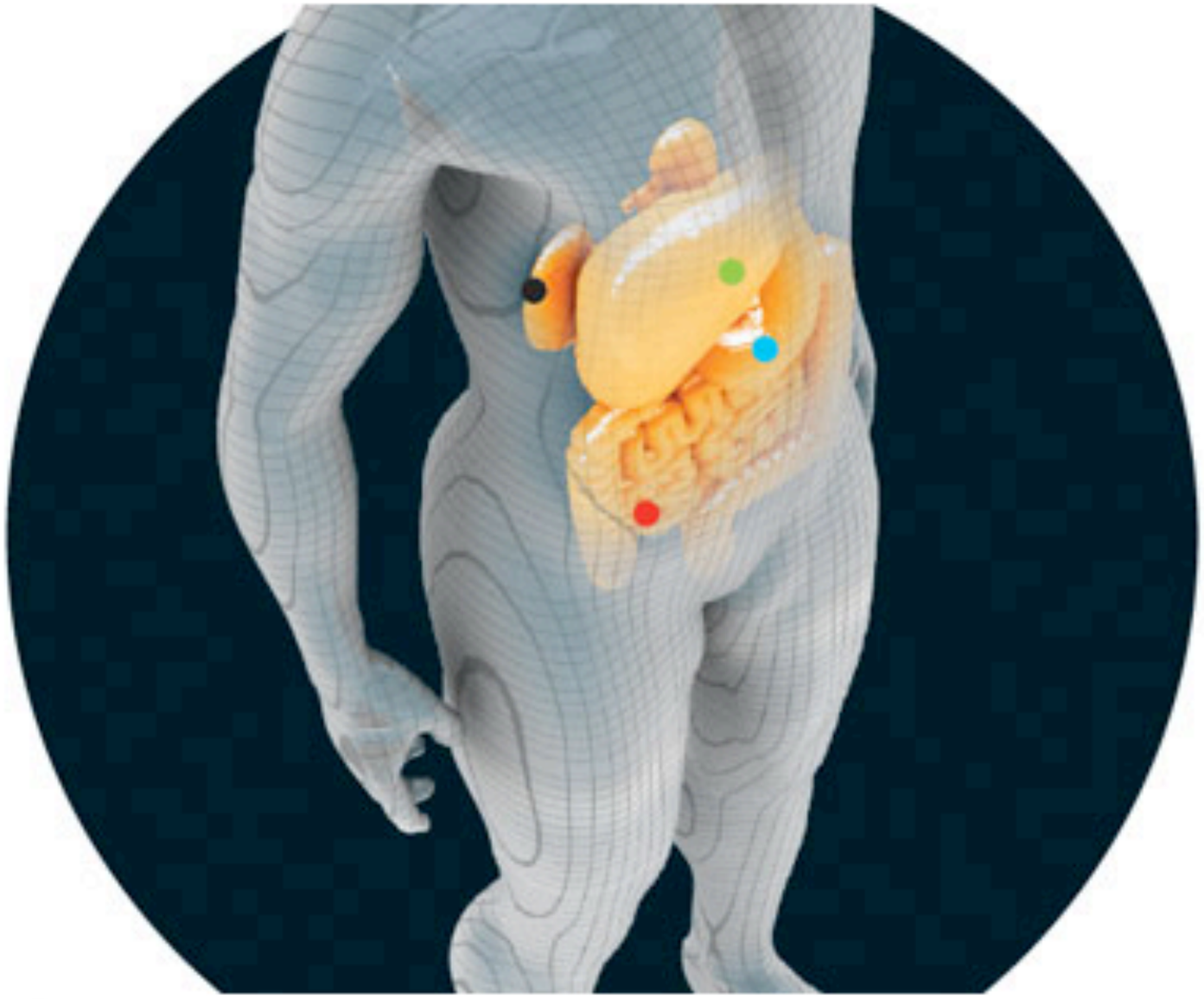
When Erez Lieberman's grandmother suffered a dangerous fall, he wanted to ensure it never happened again. "But it wasn't till a few years later at NASA that I found a way to channel that into something tangible," says the MIT graduate student. Using technology developed to monitor the balance of astronauts who have just returned from space, Lieberman's iShoe analyzes the pressure distribution of the feet. Doctors can use the insole to diagnose balance problems in elderly patients before falls occur.

13. Smart Pill

California-based Proteus Biomedical has engineered sensors that track medication use by recording the exact time drugs are ingested. Sand-grain-size microchips emit high-frequency electrical currents that are logged by Band-Aid-like receivers on the skin. The receivers also monitor heart rate and respiration and wirelessly transmit the data to a computer. "To really improve pharmaceuticals, we need to do what is now common in every other industry—embed digital technology into existing products and network them," says David O'Reilly, senior vice president of corporate development.

14. Autonomous Wheelchair

MIT researchers have developed an autonomous wheelchair that can take people where they ask to go. The chair learns about its environment by listening as a patient identifies locations—such as "this is my room" or "we're in the kitchen"—and builds maps using Wi-Fi, which works well indoors (unlike GPS). The current model, which is now being tested, may one day be equipped with cameras, laser rangefinders and a collision- avoidance system.



* 15. Gastrointestinal Liner

Obesity is associated with type II diabetes, which over time wears out the pancreas. A gastrointestinal liner developed by Massachusetts-based GI Dynamics may restore the obese to a healthy weight by preventing food from contacting the intestinal wall. The Endobarrier is routed endoscopically through the mouth—unlike a gastric bypass, no surgery is necessary—and lines the first 2 ft of the small intestine, where the most calories are absorbed (nutrients are still absorbed farther down the intestine).

* 16. Liver Scanner

How healthy is your liver? Until recently, answering that question often required a painful biopsy. French company EchoSens has developed a machine that scans the organ for damage in just 5 minutes. Studies have shown that damaged livers become stiffer and less elastic, so the scanner, called the Fibroscan, measures the organ's elasticity using ultrasound.

* 17. Nanoscale Adhesive

Gecko feet are covered with nano-size hairs that exploit intermolecular forces, allowing the lizards to stick firmly to surfaces. By replicating this nanoscale topography, MIT scientists have developed an adhesive that can seal wounds or patch a hole caused by a stomach ulcer. The adhesive is elastic, waterproof and made of material that breaks down as the injury heals.

* 18. Portable Dialysis

More than 15 million adult Americans suffer from diseases of the kidneys, which often impair the ability of the organs to remove toxins from the blood. Standard dialysis involves three long sessions at a hospital per week. But an artificial kidney developed by Los Angeles-based Xcorporeal can clean blood around the clock. The machine is fully automated, battery-operated, waterproof and, at less than 5 pounds, portable.

19. Walking Simulator

Stroke victims are being tricked into recovering more quickly with a virtual-reality rehabilitation program developed at the University of Portsmouth in Britain. As patients walk on a treadmill, they see moving images that fool their brains into thinking they are walking slower than they are. As a result, patients not only walk faster and farther, but experience less pain while doing so.

20. Rocket-Powered Arm

Adding strength to prosthetic limbs has typically required bulky battery packs. Vanderbilt University scientist Michael Goldfarb came up with an alternative power source: rocket propellant. Goldfarb's prosthetic arm can lift 20 pounds —three to four times more than current prosthetics—thanks to a pencil-size version of the mono-propellant rocket-motor system used to maneuver the space shuttle in orbit. Hydrogen peroxide powers the arm for 18 hours of normal activity.

POST SCRIPT

- We expect science to proceed with humility and caution. As citizens we must be educated and aware of the issues that confront us. We have to include these issues in our social dialogue. It will be more important than ever to question the sources of information and the bias they carry. Regardless of the intent, many of these biotech advancements they will likely become huge money makers. We have seen the oil industry invest a large amount of money on a misinformation campaign intended to misguide the public regarding carbon emissions and global climate change. Climate change is not something to debate, science supports hypotheses through data and right now the data and 99% of PhD's who study climate science tell us the earth is warming to dangerous levels and our actions are contributing to it. One thing is for certain, the advancements made in biotechnology will come fast and the world we know today will be very different than the one in ten years, let's make it a better than it is today for my kids and yours. Understand the issues, stay informed, discuss issues, use knowledge to guide your decisions and vote for those who do the same.