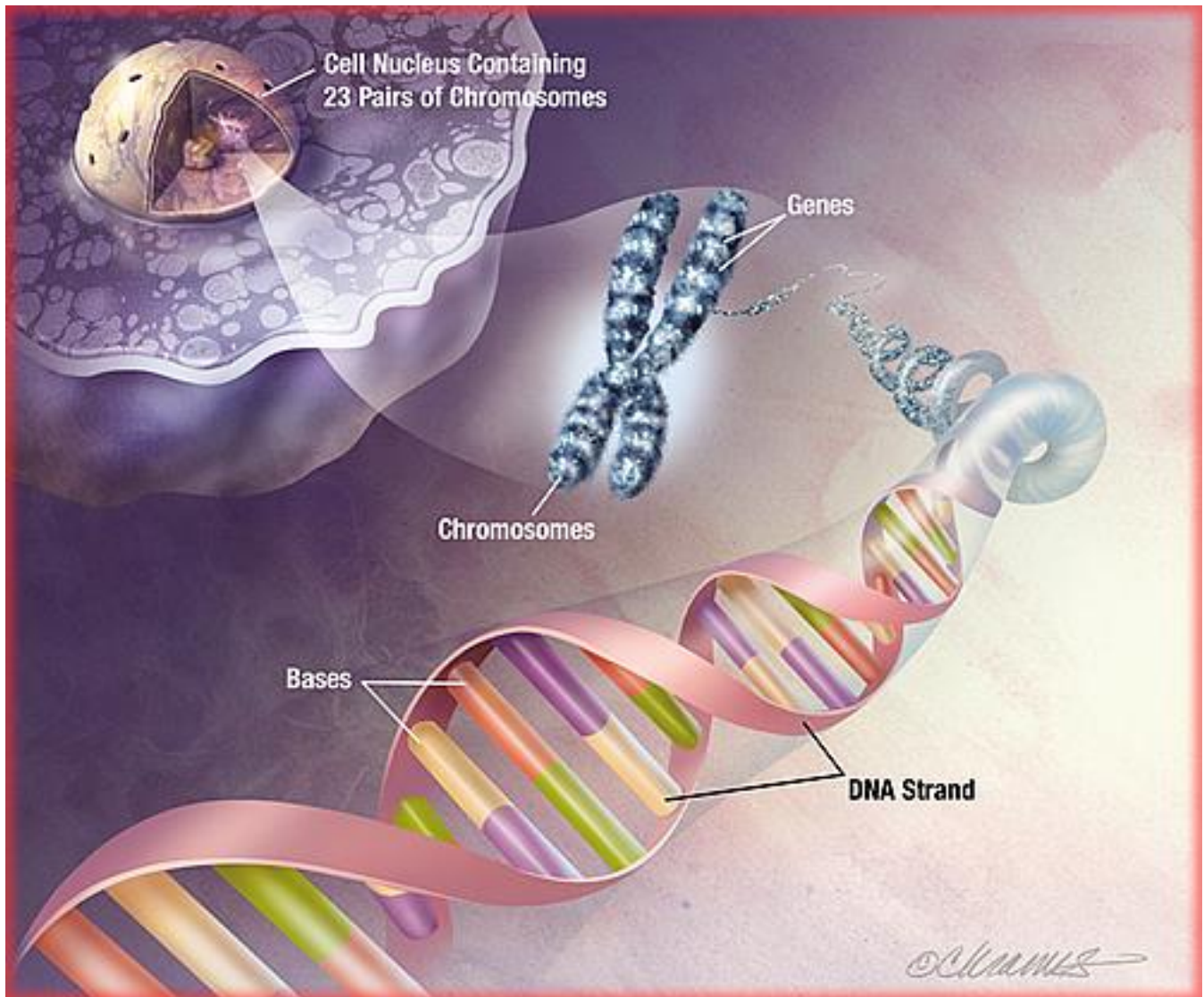


UNRAVELING GENES

**TAKING A LOOK AT
DEOXYRIBONUCLEIC ACID (DNA)**



**GENES STORE OUR DNA, THE CODE FOR OUR INHERITED
TRAITS.
IN THIS ACTIVITY, YOU CAN REMOVE DNA FROM CELLS AND
EXAMINE IT.**

UNRAVELING GENES

HIGHLIGHTS

ACTIVITY SUMMARY

Genes store our DNA (Deoxyribonucleic Acid), the code for our inherited traits. In this activity visitors can extract and examine DNA from a cellular solution of bovine thymus.

In this activity, visitors can:

- use micropipettes and chemicals to separate DNA from a cellular solution
- use a spooling method to remove DNA from a cellular solution and examine it

OBJECTIVES

After completing this activity in Spark!Lab, visitors will be able to:

- identify what DNA is, where it is found, and what it does
- identify at least one reason scientists extract and study DNA
- identify at least one application of DNA research



MATERIALS

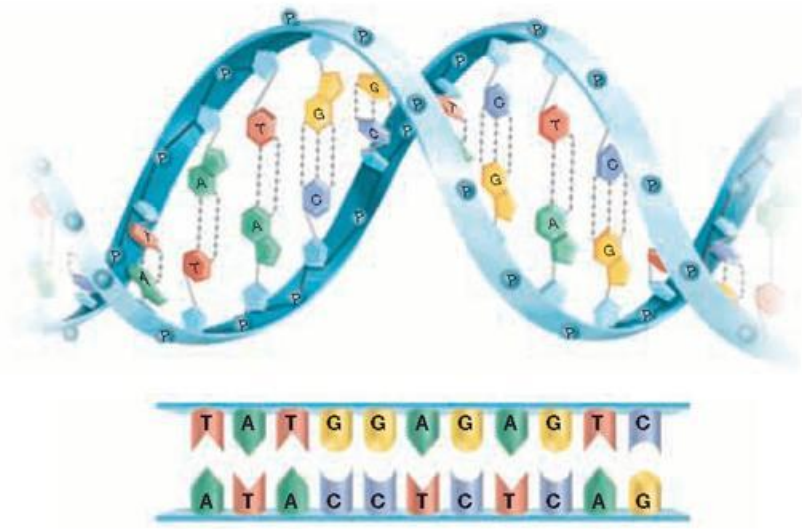
Materials for Lab Bench Experiment

All materials are stored under the lab bench counter, in the prep lab, or in the Spark!Lab storage room.

- 3 test tube racks
- 3 sets of 4 color coded micropipettes (12 in total)
- Micropipette tips
- 3 250 mL Erlenmeyer flasks containing EDTA solution
- 3 250 mL Erlenmeyer flasks containing SDS solution
- 3 250 mL Erlenmeyer flasks containing NaCL solution
- 3 250 mL Erlenmeyer flasks containing Ethyl Alcohol solution (in ice bath)
- Glass stirring rods
- Vortex machine (optional)

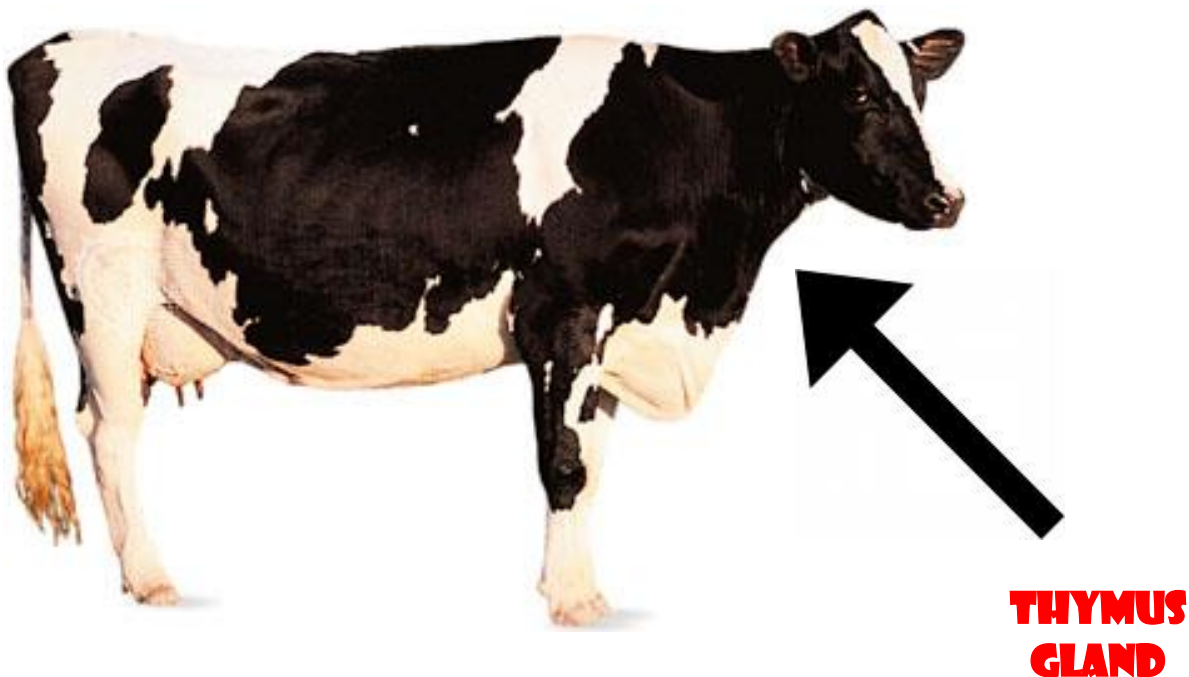
Materials found in the refrigerator or freezer:

- Test tubes with bovine thymus in a buffer solution
- 3 250 mL Erlenmeyer flasks containing Ethyl Alcohol solution (in ice bath)



WHAT IS DNA?

DNA is inside the cells of every living thing, including you. The information contained in your DNA is an important part of who you are because it includes characteristics such as eye color and blood type that you inherited from your parents. Long, twisting molecules of DNA inside cells wind tightly around proteins to form chromosomes. Each chromosome contains segments of DNA called genes. Genes tell every cell in your body what to do. They are the built-in assembly, instruction, and repair manuals for every living thing.



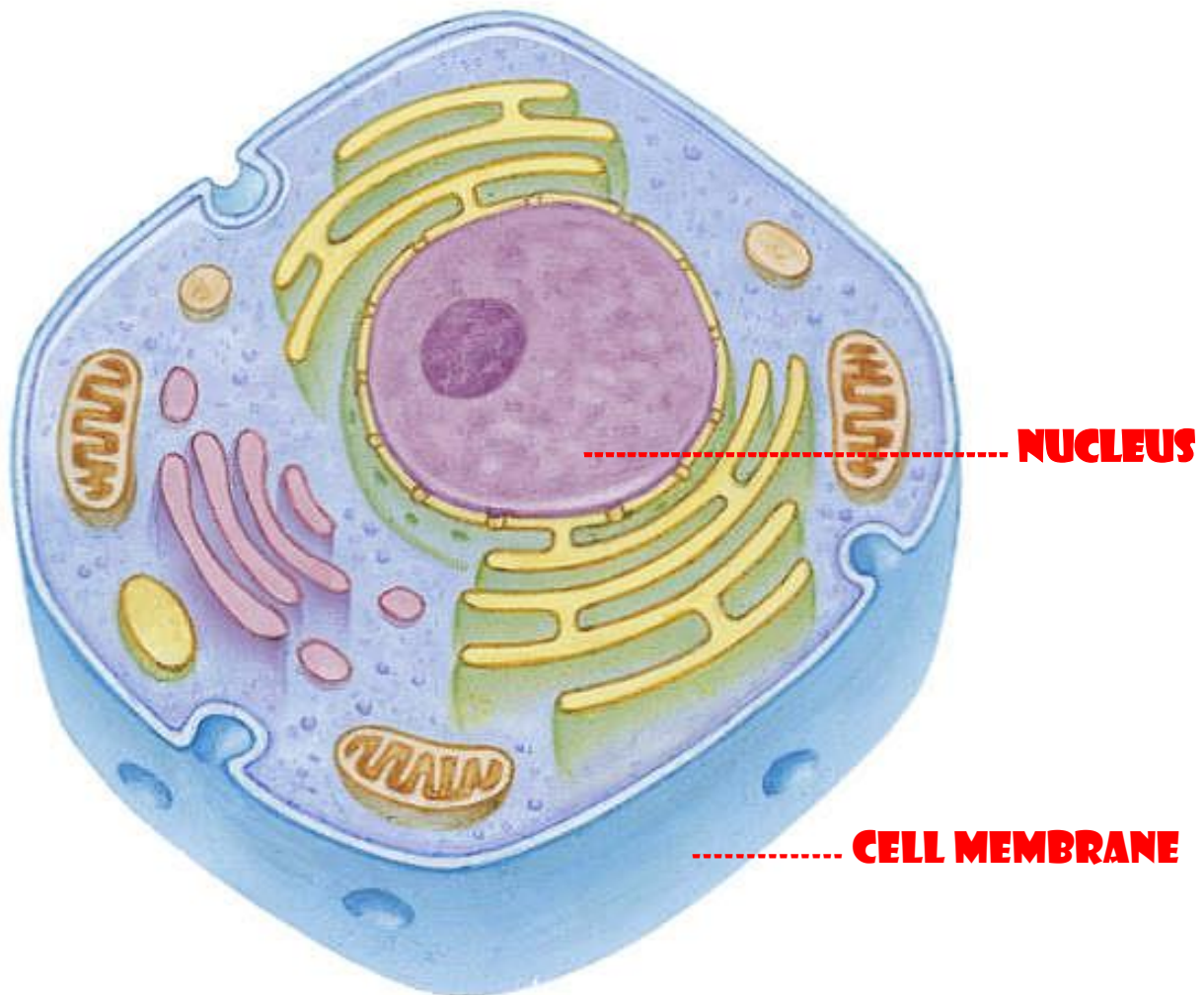
THYMUS

In humans and other mammals, the thymus gland helps make white blood cells, which fight disease. In young animals, the thymus—a gland found at the base of the neck—produces many white blood cells which are good sources of DNA. In this experiment we use calf thymus.

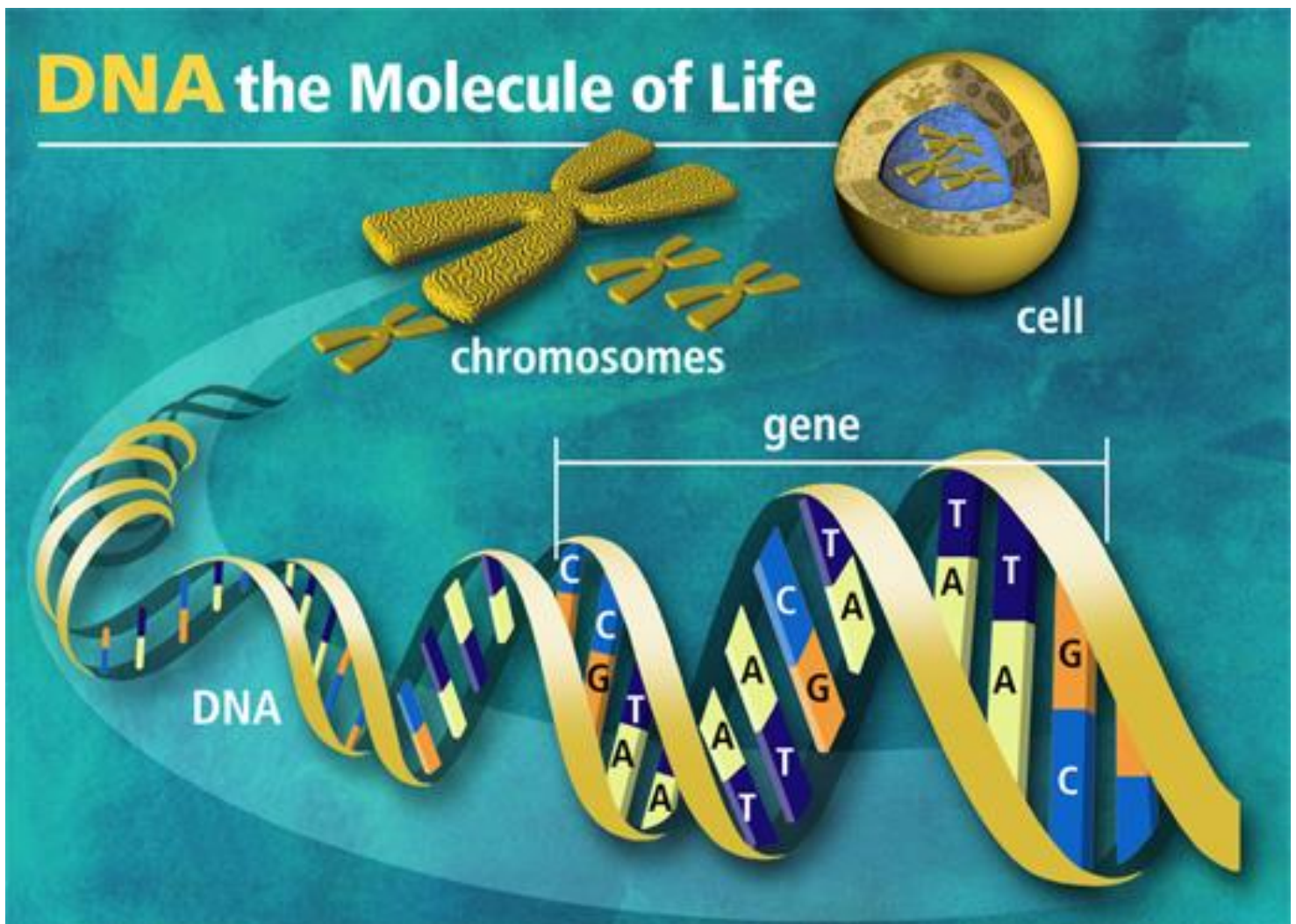
HOW DO YOU GET DNA OUT OF A CELL?

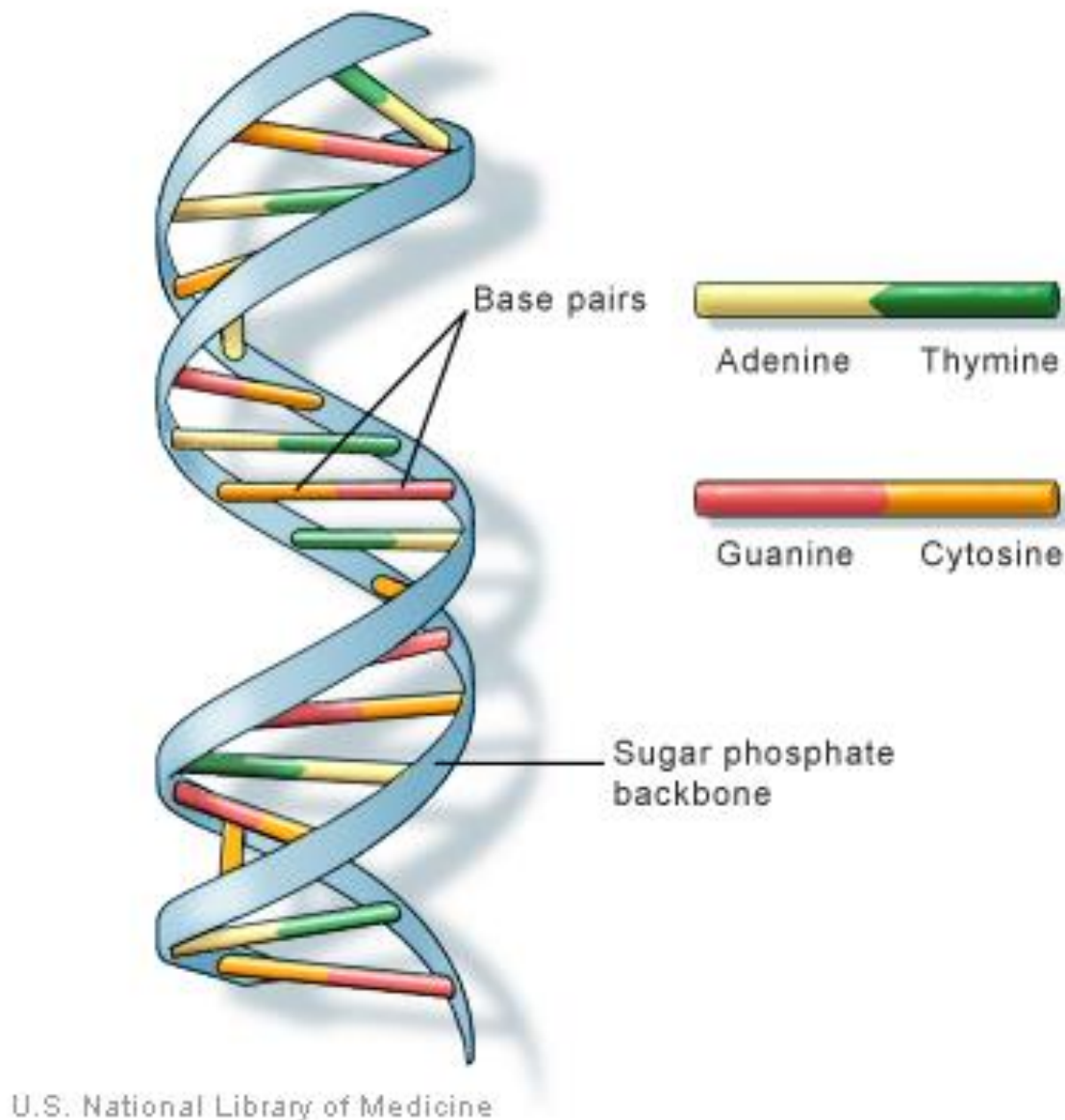
This experiment shows one way to remove DNA from cells. Chemicals are used to separate the DNA from the thousands of other materials in the cells. The DNA is taken out of solution by “spooling” it--twirling it onto a glass rod.

CROSS SECTION OF A CELL



THE NUCLEUS OF A CELL CONTAINS CHROMOSOMES, WHICH ARE MADE UP OF TIGHTLY WOUND STRANDS OF DNA.





DNA consists of two long, twisted chains made up of nucleotides. Each nucleotide contains one base, one phosphate molecule, and the sugar molecule deoxyribose.

The bases in DNA nucleotides are

- Adenine
- Thymine
- Cytosine
- Guanine

WHY DO SCIENTISTS EXTRACT DNA?

Extracted DNA is used in many ways:

- to locate a gene that causes an inherited characteristic, such as a hereditary disease
- to determine a child's biological parent
- to match suspects with evidence, such as bloodstains, from the scene of a crime
- to make a clone
- to replace genes in an organism with genes from another (gene replacement therapy)



EXTRACTING DNA STEP BY STEP

DNA is located in the nuclei of cells.

1. TAKE A LOOK AT YOUR CELL SOLUTION

Examine a test tube of the cellular solution containing the DNA that you will be extracting.

The cellular solution should be a cloudy white liquid, with a small clump of cells at the bottom of the test tube.

The cellular solution contains a high number of white blood cells that have already been separated from thymus tissue.

2. PRESERVE THE DNA

First, you have to protect the DNA strands from breaking as you remove them from the cells.

Using the pipette with pink tape, add 1.0 milliliter (mL) of ethylenediaminetetraacetic acid (EDTA) solution.

EDTA solution protects the DNA from other chemicals in the solution that might break apart the DNA. Specifically, it binds with calcium and magnesium ions that can degrade the DNA.

3. BREAK OPEN THE CELLS

To extract the DNA, you must remove the cell membrane and the nuclear membrane. These membranes of the nuclei are made of fat and protein.

Using the pipette with blue tape, add 0.3 mL of sodium dodecyl sulfate (SDS) solution. Cap the test tube and mix the SDS with the DNA sample by tapping the side of the test tube with your finger.

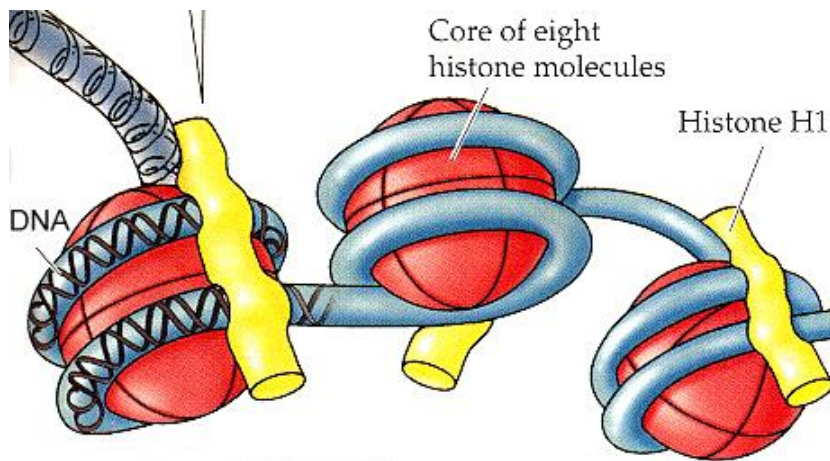


SDS is a detergent which breaks open the cells by breaking down the fats in the membrane.

4. UNRAVEL THE DNA

The next step is to clean the proteins from the DNA to make it flexible enough to spool.

Using the pipette with red tape, add 0.25 mL of sodium chloride (NaCl – table salt) solution. Cap the test tube and mix the NaCl with the DNA sample by tapping the test tube with your finger.



The DNA is wound around clusters of proteins called histones. The salt solution denatures the histones, which release the DNA.

5. ISOLATE THE DNA FROM THE REST OF THE CELLULAR MATERIAL

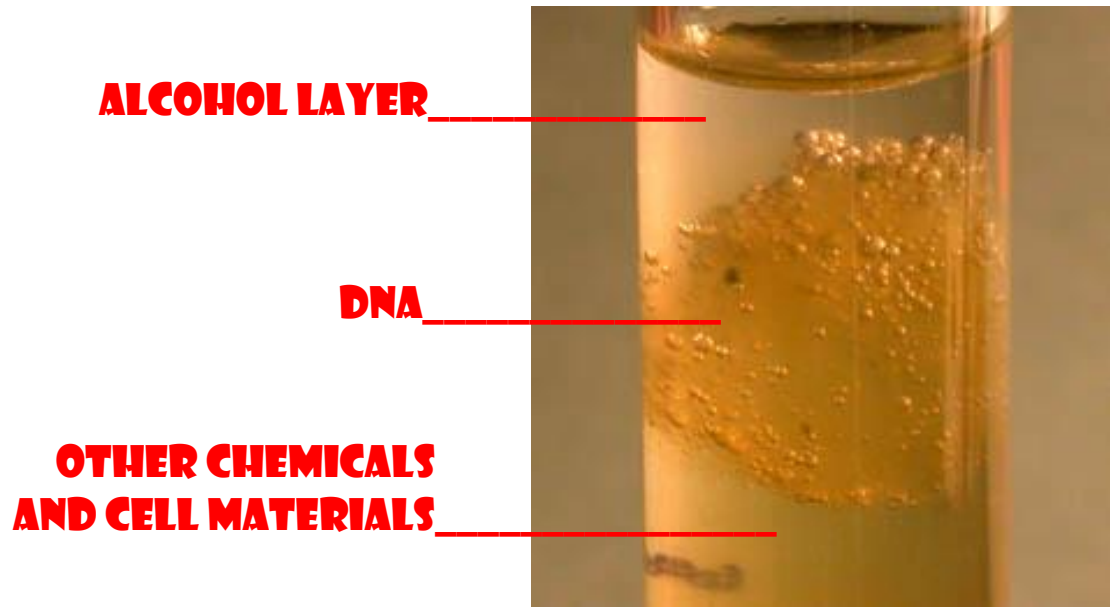
The DNA is now out of the nuclei and unraveled, but you cannot see it because it is still dissolved in the solution. To extract it, you need to add a chemical that will pull the DNA out of the solution while everything else dissolves.



Gently tilt the test tube to a 45 degree angle. Using the pipette with yellow tape, **slowly** add 1.0 mL of very cold ethyl alcohol (C_2H_5OH) solution. Since the alcohol solution is less dense, it will float on top of the cell solution layer. The ethyl alcohol attracts the DNA to this top layer.

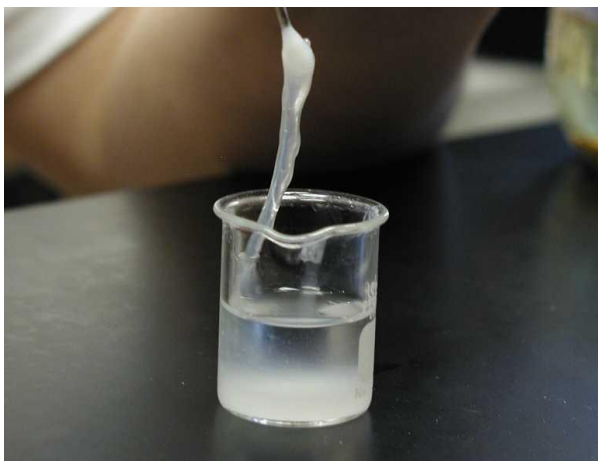
DNA is not soluble in the cold alcohol and precipitates out of solution.

The alcohol sits on top of the rest of the liquid in the test tube. Where the two layers meet, you should see a white, cloudy layer of DNA.



6. "SPOOL" THE DNA

To spool the DNA, use the glass stirring rod. Gently lower the tip of the glass stirring rod into the layer of DNA. The mass of DNA will adhere to the glass rod.

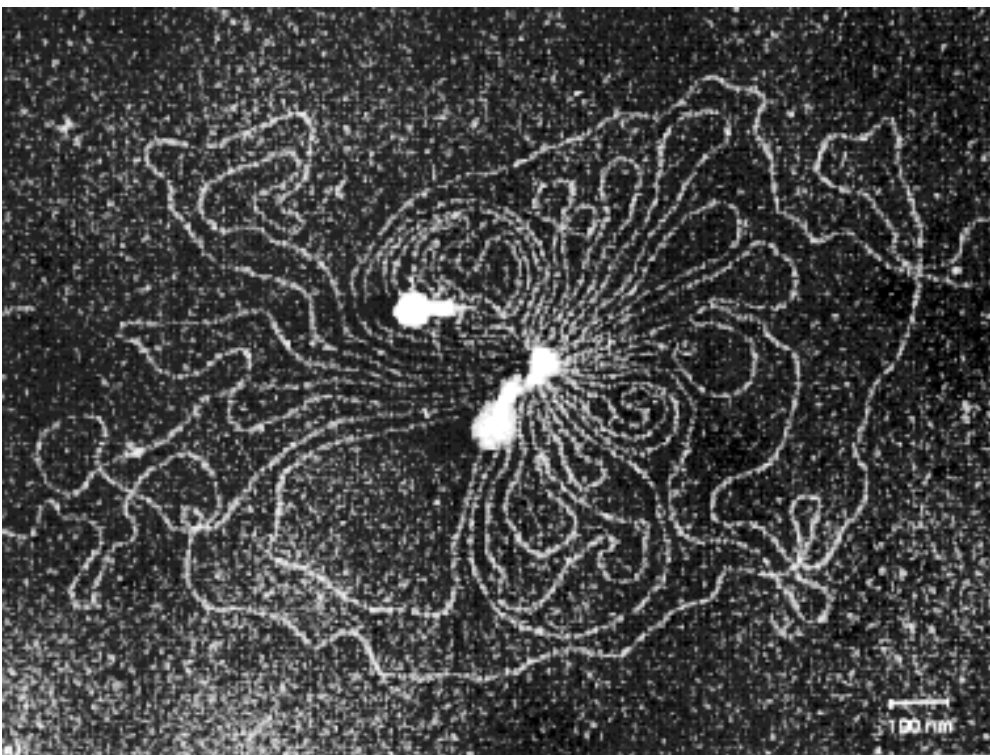
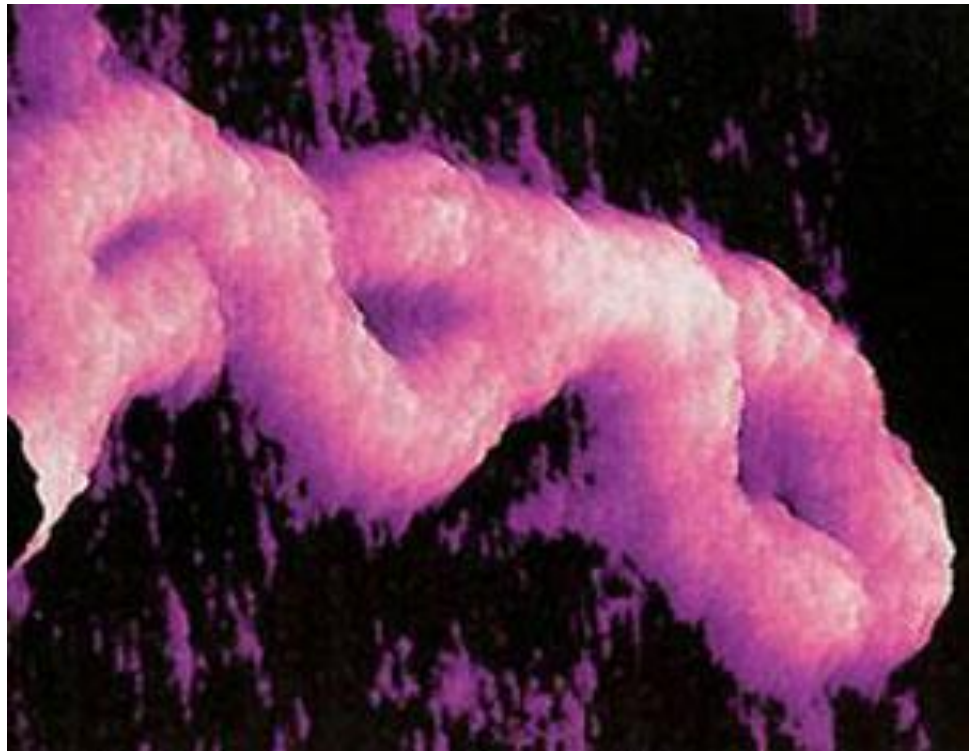


Slowly twist the rod and then lift it straight out of the test tube. Be careful to avoid touching the glass rod and DNA to the sides of the test tube as you remove it.

The DNA will look white and stringy – a bit like mucus.

*Scanning electron
micrograph of stained
DNA*

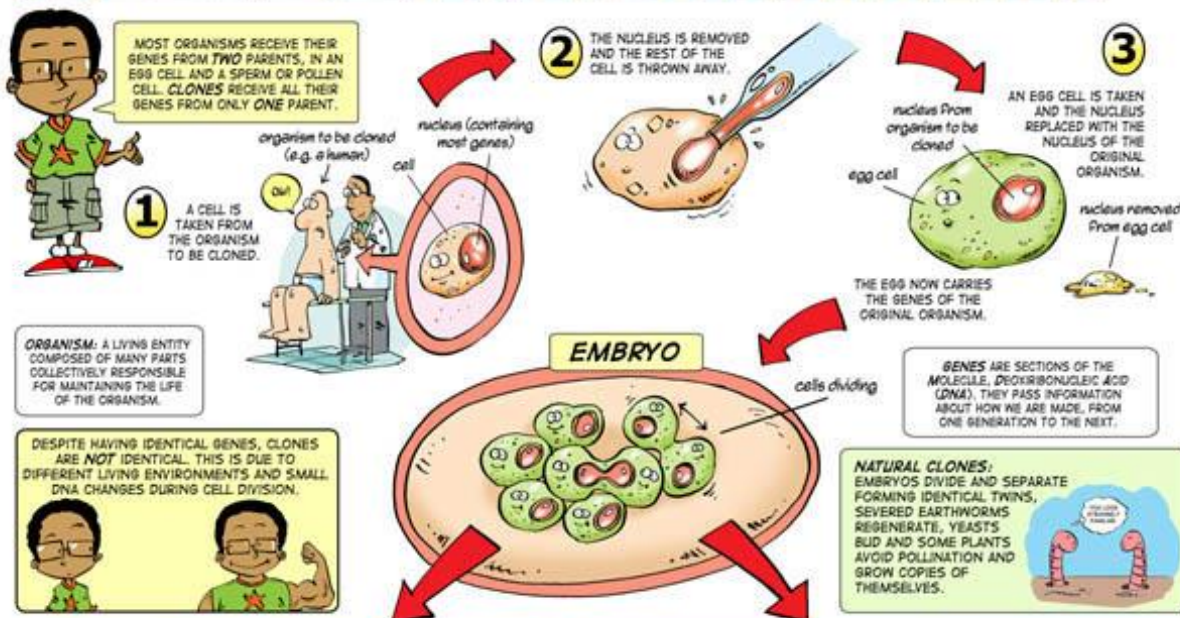
Magnified 850,000x



*Stereoscopic dark field
micrograph of unshadowed
and unstained filaments of
DNA*

Magnified 120,000x

BIOTECHNOLOGY: CLONING



normal cell

stem cell

I CAN GROW INTO ANY CELL I LIKE

CELLS CAN BE TAKEN FROM THIS EMBRYO. THEY ARE CALLED **EMBRYONIC STEM CELLS**. THEY CAN BE GROWN INTO ANY OF THE BODY'S MANY CELL TYPES.

STEM CELLS CAN POTENTIALLY BE USED TO REPAIR ORGANS SUCH AS THE LIVER, SPINAL CORD AND BRAIN. THEY CARRY THE ORGANISM'S OWN GENES AND ARE thus LESS LIKELY TO BE REJECTED.

ADULT STEM CELLS ARE FOUND IN FULLY DEVELOPED ORGANISMS (FOR EXAMPLE IN THE BONE MARROW BLOOD AND UMBILICAL CORD). THESE CAN BE STORED FOR LATER TREATMENTS.

CLONING

IF THE EMBRYO IS PLACED IN A NATURAL ENVIRONMENT (LIKE A WOMB), IT WILL GROW AND DEVELOP AS NORMAL.

FUTHI IS A CALF CLONED FROM A DAIRY COW IN SOUTH AFRICA. IT IS EXPECTED THAT FUTHI WILL BE A GOOD MILK PRODUCER, JUST LIKE HER OLDER TWIN.

HOW?

CLONING CAN BE USED TO INCREASE POPULATION NUMBERS OF ENDANGERED SPECIES; THE COELACANTH IS ONE SUCH POSSIBILITY.

HOWEVER, THE LACK OF GENETIC DIVERSITY MAKES THE SPECIES VULNERABLE TO ENVIRONMENTAL CHANGES LIKE NITROGEN LOSS.

CLONING IS A VERY CONTROVERSIAL ISSUE.

ARE YOU DESTROYING LIFE IF TAKING STEM CELLS RESULTS IN THE EMBRYO DYING?

DOES A CLONE HAVE ITS OWN SOUL?

CLONING REQUIRES LOTS OF EGGS. WILL WOMEN BE **EXPLOITED** FOR THEIR EGGS FOR HUMAN CLONING?

EGGS BROUGHT THE CASH

INFERTILE COUPLES MAY SOON BE ABLE TO CLONE THEMSELVES.

WHO WILL DECIDE WHO IS CLONED - ONLY THE VERY RICH?

SHOULD THERE BE A GLOBAL BAN ON CLONING? REPRODUCTIVE, OR THERAPEUTIC TOO?

NO CLONING ZONE



SCIENCE BACKGROUND

Key Science Concepts

- Science process skills: identification, problem solving, critical thinking
- Cell and DNA properties
- Genetic code
- DNA replication
- Protein synthesis
- Chromosomes
- DNA extraction
- DNA fingerprinting techniques
- Genetic engineering

Cell and DNA Properties

All living things are made up of cells. A simple organism like a bacterium consists of a single cell, but a complex organism like a human being may consist of 50 to 75 trillion cells.

Cells are not all identical in shape, size, or function. A typical animal cell consists of three main parts: the cell membrane, the cytoplasm, and the nucleus. The cell membrane is a permeable boundary between the interior and exterior of the cell. The cytoplasm is a semi fluid substance inside the cell which contains various cellular structures, or organelles. The nucleus is an organelle inside the cytoplasm which is responsible for heredity control and in which most of the DNA molecules in the cell are found. Nuclei are found in the cytoplasm of all animals, fungi, plants, and protistan (eukaryotes). Simple cells like bacteria and blue-green algae (prokaryotes), along with some specialized ones like red blood cells in animals, do not

contain a true nucleus, yet contain free floating hereditary material (DNA).

DNA is responsible for carrying heritable traits. Heritable traits are transferred from parents to offspring and include functional and structural characteristics.

DNA is a nucleic acid formed of simple building blocks called nucleotides. Nucleotides consist of three distinct chemical subunits: a five-carbon sugar (deoxyribose), acidic phosphate (PO_4), and a nitrogen-rich base which is either adenine (A), thymine (T), guanine (G), or cytosine (C). In a DNA molecule, the basic unit is repeated in a double helix structure made from two chains of nucleotides linked between the bases by hydrogen bonds.

The four bases (A, T, G, and C) vary greatly in relative amounts from one species to another, but the relative amounts are always the same among all members of the same species. In addition, the amount of adenine present is always equal to the amount of thymine, and the amount of guanine is always equal to the amount of cytosine ($\text{A}=\text{T}$ and $\text{C}=\text{G}$). This is known as the principle of base pairing. The pairing of A only with T and C only with G is the feature of DNA that allows it to serve as a template not only for its own replication, but also for the synthesis of proteins.

Genetic Code

The long string of genetic information describes the whole life of an organism, along with its shape and character. The instructions coded in the double helix are very similar to digitally stored computer information. Modern technology is digital because it uses the digits 0 and 1 (the binary system) to encode information. DNA is similar in that it uses the bases A, T, C, and G in an almost infinite number

of permutations and combinations to encode information on a chemical basis.

Every single cell, no matter its size or function, contains a complete copy of the DNA for the entire organism. Such a complete specification is known as the genome.

DNA Replication

The base-pairing feature of DNA suggests that each strand of DNA serves as a template for directing the synthesis of a complementary strand. The result is two identical double-stranded DNA molecules with one parental strand and one daughter strand. The replication of a chromosomal DNA molecule does not begin at one end of the molecule and continue uninterrupted all the way to the other end. Scattered throughout the molecule are particular base sequences that serve as initiation and termination points. Therefore, different portions of a DNA molecule are replicated separately and within a reasonable amount of time. Replication of a portion of DNA molecule flanked by an initiation point and termination point begins with the action of the gyrase enzyme that moves along the parental DNA, uncoiling and denaturing (separating into single strands) the double helix. Uncoiling and denaturation exposes the bases in each parental strand and enables the bases to direct the order in which deoxyribonucleotides are added by the DNA polymerase enzyme to the strand being synthesized. Other enzymes work at joining all of the short pieces of new DNA into an intact new strand. Still other enzymes act like editors, checking the base pairs formed, removing incorrect bases, and replacing those parts with correct bases.

Protein Synthesis

Protein synthesis occurs in the cytoplasm of the cell, from instructions from DNA. DNA is divided into genes and the bases in each gene are arranged in precise order. The cell uses a genetic code that reads one codon (three bases) at a time. Each codon specifies an amino acid; the sequence of codons specifies the amino acids that make a particular protein. Protein synthesis occurs in two stages: transcription and translation.

During transcription, the two strands of DNA separate, exposing a section of the DNA molecule that makes up one gene. The bases on this DNA strand act as a template to make messenger RNA (mRNA). RNA nucleotides line up to match complementary bases on the DNA strand. This copy of the DNA base sequence then passes from the nucleus into the cytoplasm.

During translation, the mRNA strand attaches itself to a ribosome. The ribosome reads the mRNA bases one codon at a time and links the amino acids, brought by the transfer RNA (tRNA), into the correct order to form a protein molecule.

Chromosomes

DNA is split into a relatively small number of separate strands called chromosomes. Chromosomes are the structural carriers of hereditary characteristics, found in the cell nucleus. The number of chromosomes is characteristic of each species; a human has 46, a cabbage 18, a mosquito 6, a dog 78, and a goldfish 94. In sexually reproducing species, chromosomes generally occur in pairs. In mitosis, or ordinary cell division, each individual chromosome is duplicated and each daughter cell receives all chromosomes, a set exactly like its parent's. In Meiosis,

the process by which sex cells (ovum and sperm) are formed, each daughter cell receives half the number of chromosomes, one of each pair. A fertilized egg contains two sets of chromosomes, one set from each parent. Chromosomes are made of protein and nucleic acid. They represent the linear arrangement of genes, the units of inheritance.

DNA Extraction

The DNA sample we get in this experiment comes from the bovine thymus. The thymus produces white blood cells. The human thymus gland is located in the chest and takes part in the immune response.

Ethylenediaminetetraacetic acid (EDTA) is a preservative and protects the DNA from being destroyed by magnesium (Mg) and/or calcium (Ca). Mg and Ca are nutrients needed for DNA replication.

Sodium Dodecyl Sulfate (SDS) is a biological detergent. It is added to dissolve the lipid layer of the cell membrane and the nuclear membrane.

Sodium Chloride (NaCl) is a salt solution. It is added to remove protein clinging to the DNA.

The 95% ethyl alcohol (C_2H_5OH) is added to the DNA solution to precipitate the DNA.

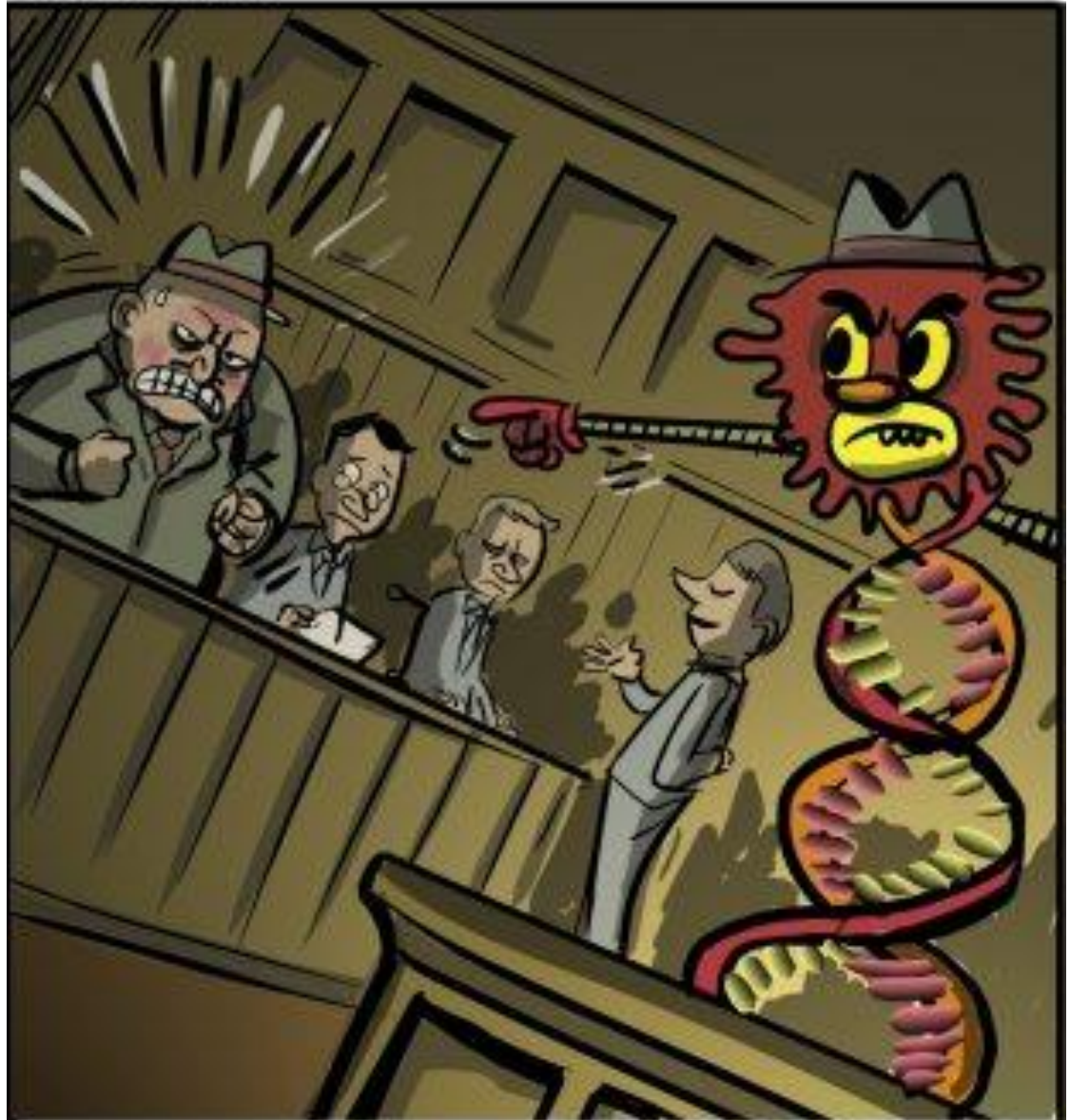
DNA Fingerprinting

DNA fingerprinting is a method of identification that compares fragments of DNA. DNA fingerprinting takes advantage of the fact that, with the exception of identical twins, the genetic material of each person is identical.

A DNA fingerprint is constructed by first extracting a DNA sample from body tissue or fluid such as hair follicles, blood or saliva. The sample is then segmented using enzymes, and the segments are arranged by size using a process called electrophoresis. The segments are marked with probes and exposed on X-ray film, where they form a characteristic pattern of black bars—the DNA fingerprint. If the DNA fingerprints produced from two different samples match, the two samples probably came from the same person.

Genetic Engineering

Genetic engineering is used to manipulate the DNA of a cell in order to change hereditary traits or produce biological products. Techniques include the use of hybridomas (hybrids of rapidly multiplying cancer cells and cells that make small amounts of a desired antibody) to make monoclonal antibodies (an antibody mass-produced from a single clone), gene splicing or recombinant DNA technique (in which the DNA of a desired gene is inserted into the DNA of a bacterium, which then reproduces itself, yielding more of the desired gene), and polymerase chain reaction (which makes perfect copies of DNA fragments and is used in DNA fingerprinting). Genetically engineered products include bacteria designed to efficiently break down oil slicks and industrial waste products, drugs (human and bovine growth hormones, human insulin, interferon), and plants that are resistant to disease and insects or that yield fruits or vegetables with desired qualities. Because genetic engineering involves techniques used to obtain patents on human genes and to create patentable living organisms, it has raised many legal and ethical issues.



Yes, he knew the risks when he got into the life but Luigi never thought he'd be convicted on evidence from his own DNA.

