DISCOVERING DNA

Biology Practical—DNA extraction

INSTRUCTIONS FOR TEACHERS AND TECHNICIANS

It is expected that each practical part of this task can be completed in 20-30 minutes. Students should work individually, although they could work in pairs if necessary. The practical work may be carried out in a classroom or laboratory. Discussion and further research will depend on the time available. Extension activities could also be completed individually as homework exercises.

The following apparatus and materials are required.

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APPARATUS

(i) One disposable plastic test tube with cap
(ii) Three disposable pasteur pipettes
(iii) One pair of safety goggles
(iv) One plastic microcentrifuge tube
(v) 40cm length of cord or string
(vi) A test tube rack

For the plant DNA extraction, students must also have access to the following apparatus:

- A beaker
- A stick blender (this should be for lab use only)
- Fine mesh strainer (or filter paper and funnel)

MATERIALS

Each pair of students must be provided with the following materials:

(i) A beaker of ice cold water
(ii) A beaker of washing up liquid
(iii) A beaker of saturated salt solution (or table salt for plant DNA extraction)
(iv) Ice cold ethanol or isopropyl alcohol (IPA)

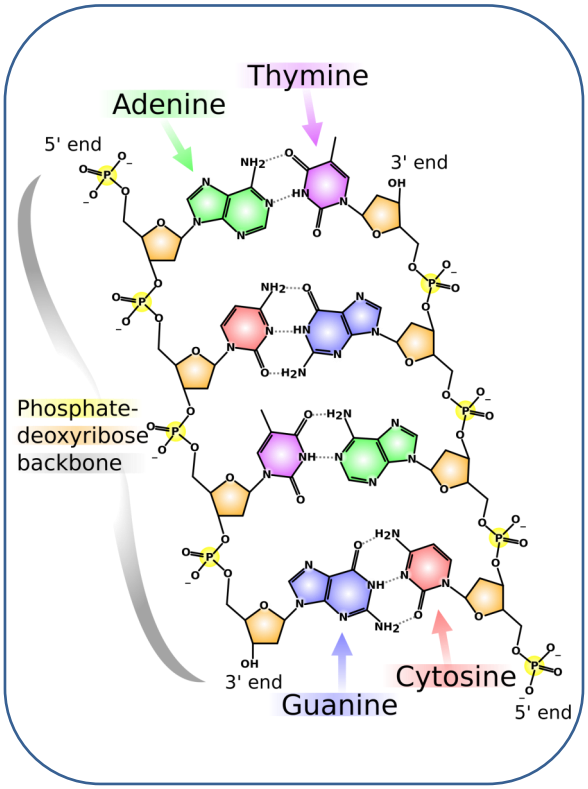
Students should wear goggles when dealing with ethanol or IPA, and these chemicals should be dispensed by the teacher for younger groups.

In the first practical activity students will extract their own DNA from cheek epithelial cells. If your school does not allow human biological samples to be used in the lab, then please carry out the second activity on DNA extraction from a plant sample.

Students should be asked to ensure that they are happy to give a saliva sample, and should not be made to take part if they do not wish to do so. Students who do not wish to take part may assist another group, or simply observe the class.

STRUCTURE OF DNA

In 1868, a biologist named Freidreich Meischer carried out research which indicated that the nucleus of cells contains a material which he called nuclein. It was not until much later, in the 1940s, that deoxyribonucleic acid (DNA) was recognised as the carrier of the genetic code. The DNA structure was determined by James Watson and Francis Crick in 1953. They found that DNA was a double helix consisting of two strands with opposite polarity. The Watson and Crick model is often described as the DNA ladder.



DNA is made of building blocks known as nucleotides. Each nucleotide is composed of three parts, a phosphate group, deoxyribose sugar, and one of the four nitrogenous bases, adenine, guanine, cytosine or thymine (abbreviated to A,G,C and T). The bases are always hydrogen bonded in complementary pairs, adenine with thymine and guanine with cytosine.

The two structures that make up the backbone of the DNA ladder are made of deoxyribose sugar units linked together by phosphodiester bonds. The carbohydrate backbone acts as a support for the rungs of the ladder, which are made of the nitrogenous bases. It is the order of these bases that determine which protein is encoded by each gene.

Human cells are diploid, containing 46 chromosomes arranged in 23 pairs. Chromosomes contain DNA, encoding all the genetic information which is inherited from the biological parents.

The human genome consists of around 6 billion base pairs. Of this vast amount of information, only around 2% of genes code for proteins, with the rest of the genome being noncoding RNA, regulatory sequences, introns and 'junk' DNA. Some of these noncoding sequences may have as yet undiscovered functions. In addition to genomic DNA, mitochondria contain their own DNA which replicates independently from cell chromosomal DNA.

All plant and animal cells contain DNA, but there are some interesting differences between the two which make plant genetics particularly interesting. As with mitochondria in animal cells, chloroplasts in plant cells contain their own DNA, which replicates independently of the plant genomic DNA.

Many plant species are capable of self-fertilization, something which is very rare among animals. This means that a single plant can be both mother and father to the next generation.

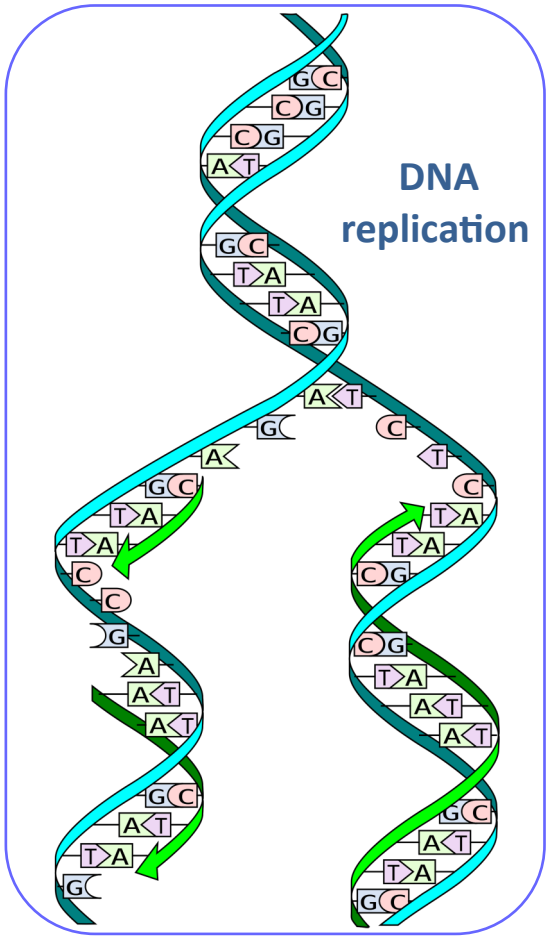
While animal cells are diploid, containing two copies of each chromosome, many plant species are polyploid, with multiple copies of each chromosome present within the cell nucleus. This is particularly common among the ferns and flowering plants, with between 30-70% of living plant species estimated to be polyploid. Many common crop species are induced to become polyploid. In other crops, polyploid varieties are favoured for their sterility. Many seedless fruits have no seeds because they are polyploid. These crops are propagated using asexual techniques such as grafting.

Madeleine Price Ball

DNA REPLICATION

DNA contains all the information required to copy itself, resulting in genetic information being passed on to the next generation of cells. DNA also provides the instructions to make cellular proteins of various functions. A large number of proteins and enzymes, including DNA polymerases, are involved in DNA synthesis.

The process of DNA replication starts when one double stranded DNA molecule separates into two strands. This is known us unzipping. Unzipping the DNA allows a protein called DNA polymerase to access the nucleotide sequence. DNA polymerase synthesises a new strand of DNA, using the original strand as a template for complimentary base pairing.



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DNA polymerases have the ability to 'edit' newly synthesised DNA for possible errors in base pairing, and to repair DNA that is damaged during the life of the cell, usually by exposure to carcinogens, X-rays and UV light.

The editing properties of DNA polymerase help to reduce errors , but cannot eliminate them entirely. Mutations do occur, and can be caused by radiation, viruses, transposons and mutagenic chemicals as well as errors in DNA replication.

There are many different types of mutation, including point mutations—the changing of one base for another—insertions and deletions, amplifications and translocations. The impact of the mutation depends on how many bases have been altered, and may be harmful or beneficial to the organism.

Many genetic diseases are caused by DNA mutations. Some are new mutations, and others are inherited from the parents. A single gene disorder is the result of just one mutated gene, and over 4000 human diseases are caused by these types of gene defects. These diseases include Cystic Fibrosis, Huntington's disease, Phenylketonuria and Duchenne muscular dystrophy. Newborn babies have a blood sample taken around one week after birth, which is screened for the most common of these genetic conditions.

Modern biotechnological techniques can be used to study DNA in great detail. These techniques can also be used to manipulate DNA. Many scientists believe that genetic engineering may be the key to curing genetic diseases. In order to manipulate DNA, it must first be extracted from the organism. When cells are lysed (broken open), DNA from the chromosomes is released and can be isolated and purified. any tissue or body fluid (except urine) can be used as a source of DNA. The most common sources are hair, cheek cells, blood and saliva.

The following experiments will allow you to extract your own DNA and DNA from a plant source.

Experimental Protocol (Saliva)

Half fill your test tube with cold water. Gently chew on the inside of your cheek for 30 seconds. Pour the water from your test tube into your mouth and swirl like mouthwash for another 30 seconds. Carefully dribble the water back into your test tube and put the lid on.

Chewing on the inside of the cheek dislodges dead epithelial cells. It is from these cells that the students will extract their DNA. These cells are constantly replaced by the body, so removing them does no harm. Swirling the water creates a cell suspension which is necessary for DNA extraction. During the 'dribbling' step, it is likely that students will have some spillage. Keep some paper towels on hand to clean up any mess, and ensure the towels are disposed of quickly.

Fill a clean pipette with 1ml of washing up liquid. Carefully dispense the washing up liquid into your test tube. Cap the tube. Gently invert the tube five times—do not shake!

Washing up liquid causes the cell and nuclear membranes to break down and allows chromosomes to float freely in the cell suspension. Once the chromosomes are free of the nucleus, it is possible to unwind the compressed DNA and therefore make it visible to the naked eye.

Fill a clean pipette with salt solution. Add five drops to your test tube. Gently invert the tube five times—do not shake!

Adding salt solution removes proteins and histones which are associated with the DNA, this allows the DNA to unwrap from the chromosome to a double helix. The DNA molecule carries a strong negative charge. Salt neutralises this negative charge and allows the DNA to precipitate when alcohol is added to the mixture. Without the salt, the DNA would stay in solution.

Put on your safety goggles. Add 3-4ml of isopropyl alcohol to your test tube. Cap your tube and gently invert. Place the tube in a test tube rack and leave for five minutes. **It's important that you don't disturb your tube during this time.**

Carefully examine your test tube, disturbing the contents as little as possible. You should see a silvery stringy substance where the water and alcohol layers meet. This is your own DNA!

Ice cold isopropyl alcohol and ethanol cause the DNA to precipitate out of solution. This renders the DNA visible to the naked eye, and allows it to be removed from the test tube.

SAFETY: Isopropyl alcohol and ethanol are both skin and eye irritants. Ensure that students wear goggles whilst handling these chemicals. If these chemicals are spilled on the skin wash thoroughly in clean water. These chemicals are highly flammable—keep away from naked flames and heat sources.

Use a clean pipette to extract your DNA precipitate from your test tube. Carefully add the DNA to a clean microcentrifuge tube. Loop a piece of string over the hinge of the tube before you close it to make a bracelet or necklace.

The DNA should be easy to extract from the test tube. It is safe for pupils to take the microcentrifuge tube away with them as a reminder of the lesson—the contents are not harmful to health. Pupils should be reminded that shaking can disrupt the precipitated DNA.

Experimental Protocol (Plant Cells)

Make up the DNA extraction mix in a beaker. You'll need 3g of salt, 10ml washing up liquid and 100ml of water. Stir gently to dissolve the salt, but try not to make too many bubbles. Weigh 100g of your plant material (split peas or chopped onion are good choices). Add the plant material to the beaker of salty water and use a stick blender to blend for approximately 10 seconds. Filter the 'soup' through mesh strainer or filter paper and collect the liquid in a beaker

Adding the water creates a cell suspension which is necessary for DNA extraction. The salt also neutralises the strong negative charge on the DNA, allowing it to precipitate when alcohol is added to the mixture. Without the salt the DNA would stay in solution. Washing up liquid causes the cell and nuclear membranes to break down into micelles, allowing the chromosomes to float freely in the cell suspension. Blending the mixture breaks down cell walls. Filtering ensures that the tough cell wall is separated from the rest of the plant material.

Pour some of the mixture into a test tube until the tube is one third full.
Using your clean pipette, add some protease to the tube—a few drops will do. Gently invert the tube three times—do not shake!

Protease is necessary to remove proteins which are contained within the plant cell and associated with the DNA. There are a variety of household substances which can be used as proteases—meat tenderizer, pineapple juice and contact lens solution are all good options.

Put on your safety goggles. Add 3-4ml of isopropyl alcohol to your test tube. Cap your tube and gently invert. Place the tube in a test tube rack and leave for five minutes. **It's important that you don't disturb your tube during this time.**

Carefully examine your test tube, disturbing the contents as little as possible. Use a stirring rod to gently agitate the liquid for a few seconds. You should see a silvery stringy substance where the water and alcohol layers meet. This is your plant DNA!

Ice cold isopropyl alcohol and ethanol cause the DNA to precipitate out of the solution. Adding the salt earlier neutralised the charge on the DNA, and the strands aggregate together. This renders the DNA visible to the naked eye, and allows it to be removed from the test tube.

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Genetic engineering

Definition: the science of changing the structure of the genes of a living thing in order to make it healthier, stronger or more useful to humans.

Genes may be added, removed or mutated in an attempt to change the nature of the organism. An organism that is generated through genetic engineering is considered to be a genetically modified organism (GMO). The first GMOs were bacteria in 1973, GM mice were generated in 1974. Insulin producing bacteria were the first GMOs to be commercialised in 1982, and genetically modified food has been on sale since 1994.

Genetic engineering techniques are regularly applied in various fields. GM cell lines and GM animals are regularly used in scientific research, while pharmaceuticals such as insulin and human growth hormone are now produced from GM bacteria. In agriculture many crop plants have been engineered to possess desirable traits such as resistance to pests, herbicides or harsh environmental conditions. Plants have also been modified for use in producing biofuels.

Humulin

Humulin is the brand name for a group of biosynthetic human insulin products, developed at Genentech in 1978. Humulin is synthesised in a laboratory strain of *Escherichia coli* bacteria which have been genetically altered with recombinant DNA to produce human insulin. Before the development of biosynthetic human insulin, the only available drug for medical use was purified animal-sourced insulin. The vast majority of insulin currently used worldwide is biosynthetic human insulin or its analogues. More recently, scientists have developed a technique to allow insulin to be produced by a plant. This is not yet commercialised, but is expected to reduce production costs.

Cloning

Definition: a cell, group of cells, or organism that is descended from and genetically identical to a single common ancestor, such as a bacterial colony whose members arose from a single original cell.

Cloning is the process of producing genetically identical individuals. Cloning occurs naturally is many species, including most plants and some insects. There is a lot of ethical debate as to whether 'artificial' cloning should be used for human gain, however it has been common practice in horticulture for hundreds of years.

Dolly

Dolly was the first mammal to have been successfully cloned from an adult cell. She was formed by taking a cell from the udder of her biological mother, and inserting it into a sheep ovum. The resulting embryo was then placed inside a female sheep, who underwent a normal pregnancy. Dolly was publicly significant because her development and birth proved that it was possible to take genetic material from an adult cell, programmed to expressed only a distinct subset of its genes, and reprogramme it to grow an entirely new organism. Cloning Dolly had a low success rate per fertilized egg; she was born after 277 eggs were used to create 29 embryos, producing three lambs at birth, of which only Dolly survived.

Genetic testing

Definition: a genetic test examines the genetic information to determine if that person has or will develop a certain disease or could pass a disease to his or her offspring

Genetic testing (or DNA-based testing) is a sophisticated way of testing for genetic disorders by examining the DNA molecule itself. Genetic testing is used for several reasons, including to identify genetic carriers of inherited diseases, genealogical DNA testing, presymptomatic testing for adult onset disorders such as Huntington's disease or Alzheimers and forensic identification. Several hundred genetic tests are currently in use, and more are being developed every year. As genetic testing may open up ethical or psychological issues, it is often accompanied by counselling.

Cystic fibrosis

Cystic fibrosis (CF) is a genetic disorder that affects the lungs, pancreas, liver and intestine. It is characterised by abnormal transport of chloride and sodium across an epithelium, leading to thick, viscous secretions. Difficulty breathing, poor growth and frequent lung infections are the most serious symptoms. CF is caused by a gene mutation, and is most common among Caucasians. One in 25 people of European descent is a carrier of the mutated gene. Shortly after birth infants are tested for a wide variety of genetic conditions, including CF. This allows appropriate treatments to be administered before symptoms worsen. Adults can also be tested to find out if they are genetic carriers of the condition, and so may pass it on to their children.

DNA fingerprinting

Definition: the analysis of DNA from samples of body tissues or fluids in order to identify individuals

DNA fingerprinting is widely used by forensic scientists to assist in the identification of unknown individuals. This technique can be useful for criminal investigation and parental testing. Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different to distinguish between individuals, unless they are identical twins. DNA profiling uses repetitive sequences that are highly variable, called variable number tandem repeats (VNTRs). VNTRs are very similar between closely related humans, but so variable that unrelated individuals are extremely unlikely to have the same VNTRs. DNA is often collected from individuals upon arrest, and the sequences are stored in a national DNA database.

Colette Aram

Colette Aram was 16 years old when she was abducted and murdered in 1983. The case was difficult to investigate, with few leads for the police to follow up. Colette's murder was the first case to be featured on BBC Crimewatch when it aired in 1984. Despite a good public response to the programme, police were unable to catch the killer. By 2008, following advances in DNA technology, police were able to develop a DNA profile of the suspect. In June 2008 a young man, Jean-Paul Hutchinson was arrested on a motoring offence. His DNA was collected and found to be a near identical match to the killer of Colette Aram. Jean-Paul was only 20 years old, so had not been born at the time of Colette's murder. Jean-Paul's father Paul Stewart Hutchinson was arrested and entered a guilty plea at trial in 2009. He was sentenced to life in prison, and committed suicide in his cell in 2010.

Extension activities

Carry out your own research into the history of DNA, from the work of Freidreich Miescher to Watson and Crick. You could present your findings to the class, create biographies of the prominent scientists involved, produce poster displays or timelines to explain the history of DNA or hold a debate on which scientists should be credited as the discoverers of the structure of DNA.

Use a modelling kit (such as Molymod) to produce an accurate model of the structure of DNA. Or, if time allows, create a model using art supplies such as card, straws, string, modelling clay and polystyrene. Make models as accurate as possible, and label them to show each part of the DNA molecule.

Create a DNA bracelet using nucleotide base sequences and coloured beads.
Visit **www.ncbi.nlm.nih.gov/nuccore,** a DNA sequence database. Choose the **advanced** search option, in the drop down menu select **organism,** then type in the animal you'd like to use as your DNA source. [Note: not all animals will have DNA sequences available in the database.] Once you have run your search, click on the first DNA sequence result. On the next screen, under the title, click **FASTA**. You will see the DNA sequence expressed as a list of single bases.

Next you need to build your bracelet. Decide on four bead colours for your four bases. Select around 20-25 bases from your DNA sequence in the database. Take two lengths of string and tie them together. Start threading your beads onto the first string according to your DNA sequence. Then you'll need to work out the complementary sequence for the second string. Remember A pairs with T and C pairs with G. Once your two strings are complete tie a knot and wear with pride!

Research an aspect of modern DNA technology, for example:

- **Cloning**
- **Genetic engineering**
- **Screening for genetic diseases**
- **Genetic fingerprinting**
- **The Human Genome Project and DNA sequencing**

Use your findings to produce a balanced article clearly stating the benefits and problems with your chosen technology.

Educational resources from the Linnean Society of London

For more information contact:

The Education Officer
The Linnean Society of London
Burlington House, Piccadilly
London W1J 0BF

T: +44 (0)20 7434 4479
E: education@linnean.org

The LINNEAN SOCIETY of London

A Forum for Natural History

www.linnean.org

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