DISCOVERING DNA

Biology Practical—DNA extraction

Topics covered:

- **Extracting DNA from cheek epithelial cells or plant cells**
- **DNA biotechnology and genetic modification**

INTRODUCTION

The purpose of this practical is to gain an understanding of DNA, and it's biotechnological applications. You will use a simple technical protocol to extract DNA from either saliva or plant cells. Household chemicals are used to complete this experiment.

APPARATUS

You are provided with the following 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, you will 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

You are 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)

You should wear **goggles** when dealing with ethanol or IPA. These chemicals may be dispensed by the teacher.

PROCEDURE

Work individually, unless your teacher instructs otherwise. You will need to share the stick blender for plant DNA extraction.

Experimental Protocol

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

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.

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.

Troubleshooting

- **There's no DNA in the test tube**
  - Regular gum chewers often have fewer dead epithelial cells within the oral cavity. This can account for the lack of DNA in the experiment.
- **There's very little DNA in the test tube**
  - This may also be due to gum chewing, or can be because the IPA was not cold enough. It may be possible to extract more DNA by adding a little more ice cold alcohol. Try keeping the IPA in the freezer.
- **It's difficult to transfer the DNA to the microcentrifuge tube**
  - The key here is patience. Gently squeeze the bulb of the disposable pipette, insert the pipette into the test tube and 'hover' over the clump of DNA. Release the bulb and the DNA will be taken into the pipette. You can now transfer it to the microcentrifuge tube. If you've missed, empty both the pipette and microcentrifuge tube and try again!

Extension activities

1 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.

2 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.

3 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!

4 Research an aspect of modern DNA technology, for example:

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

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