

04 | DNA extraction

01

Buzz about barcoding

02

Barcoding basics

03

Pipetting for electrophoresis

04

DNA extraction

05

Performing PCR

06

DNA sequencing

07

Becoming a scientist

08

Bioinformatics: DNA identity

09

Bioinformatics: family tree

10

Science communication

Equipment list

Laboratory work station

- P200 micropipette
- Box of micropipette tips
- Waste container
- Permanent marker pen
- White cutting tile and scalpel
- Plastic pestle
- Piece of Parafilm™
- Piece of aluminium foil
- Heatproof beaker
- Stopwatch
- Microfuge tube rack
- Microfuge tube of **CHEL** (200 µl of 10% Chelex)

Class equipment

- Microfuge tubes
- Microcentrifuge
- Kettle
- Freezer (-20°C)
- Gloves



Health and safety

All students working in a science lab are expected to follow good laboratory practice, including: not eating or drinking in the lab, tying back long hair, keeping lab benches clear of clutter, clearing up spills immediately, handling materials and equipment with care, and washing hands with soap after completing lab work.

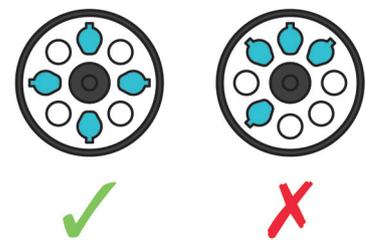
In this session you will be using biological materials (insects). You should wear gloves and work carefully to prevent contamination of the sample.

Equipment

Care should be taken when using a micropipette to eject tips downwards into a waste disposal container.

The microcentrifuge is regularly tested for electrical safety. To help maintain the microfuge in good working order, make sure that you balance the rotor before starting.

A kettle boils water, which presents a scalding hazard. Pour carefully and avoid contact with scalding water.



Reagents

Substance	Hazard	Precautions
10% Chelex (CHEL)	Food safety standard, but can stain skin.	You will use gloves to prevent contamination of the sample with your DNA, not because of Chelex.

Instructions | DNA extraction

1. Make sure that you have recorded the information about your sample (see session 2) before starting DNA extraction.
-

2. Find the microfuge tube containing 200 μ l of Chelex in your microfuge tube rack.
-

3. Write your initials on the top and the side of the tube using a permanent marker pen.
-

4. Put gloves on to avoid contaminating the sample with your DNA.
-

5. Place your sample on a white cutting tile. Using a scalpel to cut away from you, carefully remove a small piece of tissue to extract DNA from (this needs to be a couple of millimetres, eg; half an ant or a leg / antenna from a bee).

◆ **Top tip:** *The DNA extraction works better with a small amount of sample, don't add too much.*

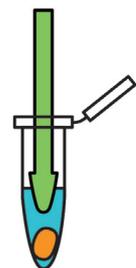
6. Carefully transfer the section of invertebrate sample into the labelled microfuge tube of Chelex.

Chelex beads bind the magnesium ions (Mg^{2+}) that are often cofactors for nucleases. Since nucleases are enzymes that catalyse the breakdown of DNA, removing the cofactors that they need will reduce the breakdown of DNA.

7. Crush the sample thoroughly, grinding for at least 2 minutes using the plastic pestle in the microfuge tube.

This disrupts chitin and connective tissue, producing small clumps of cells that are more easily lysed to extract DNA.

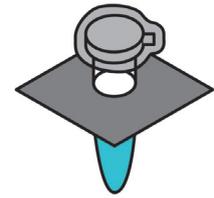
◆ **Top tip:** *A really thorough grinding will separate the cells of the tissues and give better DNA extraction.*



8. To incubate the microfuge tube at 90-100°C for 15 minutes:

- Close the microfuge tube and seal it with Parafilm™.
- Take the piece of aluminium foil and fold it into quarters, then carefully pierce a small hole through the middle of the foil using a pencil.

- Pour boiling water from a kettle into a heatproof beaker.
- Push the microfuge tube containing your insect sample and Chelex through the hole in the aluminium foil and place the foil and microfuge tube carefully on the top of the boiling water in the heatproof beaker.



The aluminium foil will keep the tube afloat and the Parafilm™ will stop water accidentally getting into the tube and potentially contaminating your sample.

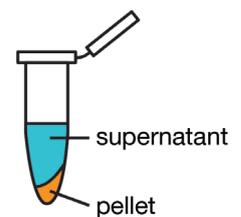
- Set the stopwatch for 15 minutes and leave your sample for this incubation period.

During this time the cells in your sample will lyse (burst), releasing their contents, including DNA, into the solution.

- 9. Carefully remove your tube from the beaker after the incubation, pat it dry with a paper towel and remove the Parafilm™.

- 10. Centrifuge at maximum speed (7,000 rpm) for 1 minute. Take care to balance the rotor by distributing the tubes evenly.

This will separate the suspension into a pellet and supernatant. The pellet contains the Chelex and cell debris at the bottom of the tube, the supernatant (liquid) contains the extracted DNA.



- 11. Place a new microcentrifuge tube into your microfuge tube rack and label it with your initials.

- 12. Set a P200 micropipette to 50 µl. Carefully insert a new tip into the supernatant and aspirate (suck up) the supernatant, taking care not to disturb the pellet. Dispense the 50 µl of supernatant into the microfuge tube labelled with your initials.

◆ **Top tip:** *Make sure that you don't transfer any Chelex as this will stop the PCR from working. If you do disturb the Chelex pellet, simply spin the microfuge tube again before removing the supernatant.*

It is important not to disturb the pellet as the Chelex beads bind magnesium ions (Mg²⁺). Whilst this is important to prevent the breakdown of DNA during extraction, the DNA polymerase enzyme that you will use in the next step of PCR needs magnesium ions to function. So if any of the Chelex beads from the pellet get taken into the PCR it will bind to the magnesium needed by the DNA polymerase enzyme and stop the PCR from working.

- 13. Store your labelled microcentrifuge tube at -20°C until you are ready to use it in a PCR.