

# 05 | Performing PCR



## Equipment list

### Laboratory work station

- P20 micropipette
- Box of micropipette tips
- Waste container
- Permanent marker pen
- PCR tube rack
- 0.2 ml PCR tube of **MM** (12.5  $\mu$ l of 2 x PCR master mix)
- Microfuge tube rack
- Microfuge tube labelled with initials, containing DNA sample from previous session
- Microfuge tube of **FOR** (forward primers)
- Microfuge tube of **REV** (reverse primers)
- Microfuge tube of **H<sub>2</sub>O** (nuclease-free water)

### Class equipment

- 2 MiniPCR thermal cyclers
- Microcentrifuge
- 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.

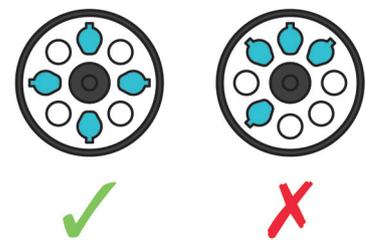
In this session you will be using biological materials (enzymes and DNA). You should wear gloves and work carefully to prevent contamination of the sample.

## 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.

The MiniPCR thermocycler is regularly tested for electrical safety. It will be linked to a computer to run. Care should be taken to position the thermocycler and computer away from the area in which the PCR tubes are being set up to avoid the possibility of getting the electrics wet.



## Reagents

Substance	Hazard	Precautions
<b>DNA samples from insects</b>	Biological materials: very low risk of contaminating living cells.	Use in a controlled way to prevent contamination, mirroring aseptic techniques Autoclave (heat to high temperature and pressure) materials that come into contact with DNA before disposal.
<b>Primers (FOR, REV)</b>	Biological materials: very low risk of contaminating living cells.	Use in a controlled way to prevent contamination, mirroring aseptic techniques Autoclave (heat to high temperature and pressure) materials that come into contact with DNA before disposal.
<b>PCR master mix (MM)</b>	Classified as non-hazardous. Contains biological materials: very low risk of contaminating living cells.	Use in a controlled way to prevent contamination, mirroring aseptic techniques Autoclave (heat to high temperature and pressure) materials that come into contact with DNA before disposal.

## Instructions | PCR

### 1. To set up the PCR with your invertebrate DNA:

- Label the 0.2 ml PCR tube of **MM** (12.5 µl of 2 x master mix), with your initials on the top and on the side.

*The PCR master mix contains the buffer, magnesium chloride, deoxyribonucleotide triphosphates (dNTPs), and thermostable DNA polymerase, which are needed for a PCR.*

**▲** *Sometimes the lid of the PCR thermocycler rubs the pen off the lid of the PCR tube, which is why you should also label the side of the tube.*

- Transfer 5.0 µl of your extracted DNA sample, in a microfuge labelled with your initials from last lesson, into the PCR tube using a P20 micropipette.

*This provides the 'template' to make lots more copies of the gene used as a barcode.*

- Using a new pipette tip, transfer 2.0 µl of forward primer, from the tube of **FOR**, into the PCR tube using a P20 micropipette.

- Using a new pipette tip, transfer 2.0 µl of reverse primer, from the tube of **REV**, into the PCR tube using a P20 micropipette.

*Primers are short stretches of single-stranded DNA that will bind to the complementary DNA sequence at either end of the gene used as a barcode. This allows DNA polymerase to bind and extend this region of the DNA.*

- Using a new pipette tip, transfer 3.5 µl of nuclease-free water, from the tube of **H<sub>2</sub>O**, into the PCR tube using a P20 micropipette.

*This is used to make the total volume of the reaction 25 µl.*

- Gently pipette the reaction up and down 3 or 4 times to mix.

### Summary of PCR contents for your invertebrate DNA



Label	Reagent	Volume in PCR
MM	2 x PCR Master mix	12.5 µl
Initials	DNA sample	5.0 µl
FOR	Forward primers	2.0 µl
REV	Reverse primers	2.0 µl
H <sub>2</sub> O	Nuclease-free water	3.5 µl
<b>Total volume</b>		<b>25.0 µl</b>

2. Within the class there should also be **one negative control** set up. This should contain everything except the DNA sample.

**To set up the PCR negative control:**

- Label the 0.2 ml PCR tube of **MM** (12.5  $\mu$ l of 2 x master mix) with **-ve** on the top and on the side.
- Using a new pipette tip, transfer 2.0  $\mu$ l of forward primer, from the tube of **FOR**, into the PCR tube using a P20 micropipette.
- Using a new pipette tip, transfer 2.0  $\mu$ l of reverse primer, from the tube of **REV**, into the PCR tube using a P20 micropipette.
- Using a new pipette tip, transfer 8.5  $\mu$ l of nuclease-free water, from the tube of **H<sub>2</sub>O**, into the PCR tube using a P20 micropipette.

*This is used to make the reaction volume the same 25  $\mu$ l as the experimental PCR.*

- Gently pipette the reaction up and down 3 or 4 times to mix.

**Summary of PCR contents for the negative control**



Label	Reagent	Volume in PCR
MM	2 x PCR Master mix	12.5 $\mu$ l
FOR	Forward primers	2.0 $\mu$ l
REV	Reverse primers	2.0 $\mu$ l
H <sub>2</sub> O	Nuclease-free water	8.5 $\mu$ l
<b>Total volume</b>		<b>25.0 <math>\mu</math>l</b>

3. Place the PCR tubes into the MiniPCR thermocycler. Click the lid of the machine closed.

4. Start the thermal cycling programme using the MiniPCR software. This will amplify the DNA and involves heating and cooling the reaction multiple times.

Initial denaturation	Denaturation	Annealing	Extension	Final extension
94.0 °C	95.0 °C	50.0 °C	72.0 °C	72.0 °C
60 s	30 s	30 s	30 s	30 s
<b>Number of cycles: 35</b>				

Heated lid

5. The programme will take about 2 hours to run. When it is complete PCR tubes should be stored in the fridge until you are ready to do gel electrophoresis.

## Questions

- A. The negative control reaction contains all of the same reagents as the experimental PCR, except for the DNA. Why do you think this needs to be included?
  
  
  
  
  
  
  
  
  
  
- B. What is a buffer?
  
  
  
  
  
  
  
  
  
  
- C. What is a nucleotide?
  
  
  
  
  
  
  
  
  
  
- D. What type of bonds form between complementary base pairs?
  
  
  
  
  
  
  
  
  
  
- E. What type of bonds form between adjacent nucleotides as part of the sugar-phosphate backbone?
  
  
  
  
  
  
  
  
  
  
- F. What are the 3 stages of a PCR and what happens in each stage?
  
  
  
  
  
  
  
  
  
  
- G. A region of which gene is amplified in this PCR and used as the barcode for animals? Where is this gene found? What does this gene encode?