

05 | Performing PCR



Aim To amplify the DNA barcode gene (cytochrome c oxidase subunit 1) for animals, using a PCR.

Activity outline An explanation of how PCR works is followed by details on the specific PCR that students will perform to amplify the ‘barcode’ from their DNA sample. Students then set up their PCR tubes and start the thermal cycling.

Age range Key stage 4 and above (14 years and older)

Timing

- 10 min - explanation of PCR
- 10 min - giving details of specific PCR
- 15 min - to set up reactions
- 15 min - to start thermocycling
- 10 min - looking ahead to next lesson

Venue This practical activity needs to be carried out in a science laboratory

Resources

- Student protocol: PCR – copying the DNA barcode
- **Presentation:** 05_P_Performing-PCR

Preparation

Before the session

For each pair of students, prior to the session:

- ☐ Prepare a PCR tube, labelled **MM**, containing 12.5 µl of the 2 x PCR Master mix.
 - ▲ *This tube contains the enzyme DNA polymerase and should be kept on ice until needed to preserve enzyme activity.*

- ☐ Prepare a microfuge tube, labelled **FOR**, containing 4 µl of the Forward primer.

The forward primer used was designed by Folmer et al. (1994). The sequence complementary to the cytochrome c oxidase subunit 1 gene is shown in blue colour, the additional sequence added to the primer to help with DNA sequencing is shown in light grey.

5' - TGTAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG - 3'

- ☐ Prepare a microfuge tube, labelled **REV**, containing 4 µl of the Reverse primer.

The reverse primer used was designed by Folmer et al. (1994). The sequence complementary to the cytochrome c oxidase subunit 1 gene is shown in blue colour, the additional sequence added to the primer to help with DNA sequencing is shown in light grey.

5' - CAGGAAACAGCTATGACTAACTTCAGGGTGACCAAAAATCA - 3'

- ☐ Prepare a microfuge tube, labelled **H₂O**, containing 10 µl of nuclease-free water.

Summary of aliquotting:

Label	Tube contents	No. of tubes	Aliquot	Used
MM	2 x PCR Master Mix	1 per pair + 1 extra	12.5 µl in PCR tube	12.5 µl
FOR	Forward primer	1 per pair + 1 extra	4.0 µl	2.0 µl
REV	Reverse primer	1 per pair + 1 extra	4.0 µl	2.0 µl
H₂O	Nuclease-free water	1 per pair + 1 extra	10.0 µl	3.5 µl

In addition, prior to the session:

- ☐ Ensure that the MiniPCR software is installed on the computers that the MiniPCR thermocyclers will be linked to and that they are heating and cooling as expected.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994) 'DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.' *Molecular Marine Biology and Biotechnology* 3 (5); pp 294-299.

Laboratory set up

Laboratory work stations

At the start of the session set up the laboratory work station for each pair of students, so that it contains:

- P20 micropipette
- Box of micropipette tips
- Waste container
- PCR tube rack
- 0.2 ml PCR tube of **MM** (12.5 µl 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 primer)
- Microfuge tube of **REV** (reverse primer)
- Microfuge tube of **H₂O** (nuclease-free water)
- Permanent marker pen

Class equipment

Set up the science laboratory to contain:

- Microcentrifuge
- 2 MiniPCR thermal cyclers
- Gloves

Disposal

The pipette tips, microfuge tubes and PCR tubes that come into contact with biological materials will be autoclaved to denature the DNA and enzymes before disposal. Used tips and microfuge tubes should be placed into a sealed plastic jar for autoclaving later.

Answers to 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?**

A negative control doesn't contain the template DNA, so there should not be any DNA amplified. It is used to detect contamination (for example from not changing pipette tips between adding different reagents to the PCR) or false positives by the appearance of amplified bands of DNA in this reaction when there should not be.

- B. What is a buffer?**

A buffer is a solution that maintains a stable pH.

- C. What is a nucleotide?**

Nucleotides are the monomers that join to form the DNA polymer. Each nucleotide is composed of a pentose sugar, phosphate group and nitrogenous base.

- D. What type of bonds form between complementary base pairs?**

Hydrogen bonds

- E. What type of bonds form between adjacent nucleotides as part of the sugar-phosphate backbone?**

Phosphodiester bonds, a type of covalent bond

- F. What are the 3 stages of a PCR and what happens in each stage?**

Denaturation – the hydrogen bonds holding the DNA strands together are broken at high temperature, so the double-stranded template becomes single-stranded.

Annealing – the single-stranded primers will form hydrogen bonds with complementary DNA sequences in the DNA template, making short double-stranded regions.

Extension – a thermostable DNA polymerase enzyme catalyses the addition of complementary nucleotides to the end of the short double-stranded region made by the primer, extending the DNA and, after many cycles amplifying the region of DNA bracketed by the primers.

- 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?**

A region of the cytochrome c oxidase subunit I is the gene amplified in this project and used for barcoding animals.

This gene is part of the DNA in the mitochondrion, rather than the nucleus. Since there are multiple copies of the mitochondrion per cell (and only one nucleus), it makes it easier to amplify mitochondrial DNA by PCR, when you have a small sample.

Cytochrome c oxidase subunit I produces a protein that is involved in electron transport during respiration.