



Polymerase chain reaction

Components

	NAME	DESCRIPTION	AUDIENCE
	<i>Polymerase chain reaction</i> teacher guide	This guide describes two activities that introduce students to polymerase chain reaction (PCR). It includes teacher notes and paper PCR templates to use in the classroom activity (game).	teachers
	<i>PCR challenge</i> presentation/game	This presentation allows students to practice PCR in a classroom game. It is preceded by background information on PCR and an overview of the game.	teachers
	<i>Rules of the challenge</i> procedure sheet	These instructions guide students through the <i>PCR challenge</i> game.	students
	<i>PCR and melanoma mutations</i> workbook	This workbook accompanies the classroom game, <i>PCR challenge</i> . It includes background information about melanoma and PCR, and review questions.	students
	<i>Searching for mutations with polymerase chain reaction</i> workbook	This workbook is used when visiting the LotteryWest Biodiscovery Centre at the Harry Perkins Institute of Medical Research. It guides students through procedures for PCR and gel electrophoresis in the laboratory.	students

Purpose

Students develop an understanding of a common technique in biotechnology, polymerase chain reaction (PCR), which is used to amplify DNA segments.

Outcomes

Students understand that:

- PCR is a laboratory-based technique that allows scientists to amplify specific segments of DNA;
- PCR has widespread application in areas of science such as forensics, disease diagnosis and genetic research;
- components of PCR (DNA template, primers, *Taq* polymerase, nucleotides, buffers, $MgCl_2$ and thermocycler) have specific roles in the reaction;
- procedural steps of PCR, including temperature fluctuations, relate to specific stages in the amplification process: denaturing, annealing and elongation, and properties of DNA;
- PCR results for the associated laboratory activity are interpreted via gel electrophoresis;
- PCR products can be sequenced (order of nucleotides determined) with sequencing technology to identify genetic mutations, including mutations associated with melanoma.

Activity summary

ACTIVITY	POSSIBLE STRATEGY
Show the presentation, <i>PCR challenge</i> , to the class. Discuss background information and the game procedure.	class discussion
Students race the clock in the classroom game. Which team can complete three complete PCR cycles using paper, scissors and tape? Using the timer embedded in the <i>PCR challenge</i> presentation, students produce as many DNA segments as possible in the designated time frame.	class works in teams
After playing <i>PCR Challenge</i> , students complete questions in the workbook, <i>PCR and melanoma mutations</i> .	students work individually or as a team
If students can attend the LotteryWest Biodiscovery Centre, at the Harry Perkins Institute of Medical Research, they may participate in a SPICE-developed PCR laboratory activity.	visit to LotteryWest Biodiscovery Centre

This resource includes two separate PCR activities:

- a paper-based classroom game (*PCR challenge*); and
- a PCR laboratory procedure for schools that can visit the LotteryWest Biodiscovery Centre at the Harry Perkins Institute of Medical Research in Perth (or have their own thermocycler).

PCR challenge

Preparation

The game mimics processes that occur within the thermocycler during PCR. The game and workbook use a melanoma context that's used throughout the SPICE resource package, *Gene expression*.

Appendix 1 of this guide contains full instructions for preparing the PCR templates (included in Appendix 2):

- Photocopy primers onto coloured paper.
- Cut them up in advance, or students may do this before they play the game.

Introducing the game

We suggest students play the game in teams. Their aim is to make as many DNA segments as they can during three PCR cycles. The PCR challenge presentation includes a timer that displays time and temperature for each stage of PCR. It may be used to add a competitive element to the game.

The procedure sheet, *Rules of the challenge*, contains a printable summary of the PCR challenge procedure. You may not need this if you use the presentation.

Notes for teachers that accompany *PCR challenge* follow on the next page.

PCR laboratory activity

Together with the LotteryWest Biodiscovery Centre at the Harry Perkins Institute of Medical Research, SPICE has developed a PCR laboratory activity, Searching for mutations with polymerase chain reaction. The PCR laboratory activity is set within a melanoma context, used throughout the SPICE resource package, *Gene expression*.

The aim of the PCR laboratory activity is to use PCR to produce sufficient DNA from melanoma samples for genetic sequencing. Genetic sequencing is not part of the activity. Gel electrophoresis is used to visualise PCR products.

Students will need to attend the LotteryWest Biodiscovery Centre for a full-day workshop to participate in this activity. During the workshops students:

- combine DNA samples, from normal cells and melanoma cells, with reagents and primers;
- load DNA samples into a PCR thermocycler and produce billions of copies of the target sequence (a segment of the BRAF gene);
- prepare an agarose gel;
- load PCR products and control samples onto agarose gel and apply an electrical current to separate DNA fragments;
- visualise DNA fragments via gel electrophoresis; and
- complete an accompanying worksheet that considers potential sequencing results.

Contact the Community Education Manager at the Harry Perkins Institute of Medical Institute, Perth Western Australia for class bookings. <https://www.perkins.org.au/biodiscovery-centre/>

SLIDE	NOTES
1	<p>What is polymerase chain reaction?</p> <p>Polymerase chain reaction (PCR), a biotechnological technique developed in 1985, revolutionised how DNA is used in biological and medical research. Prior to the development of PCR, replication of DNA was complex, taking weeks to complete.</p>
2	<p>Is there horse in my beef burger?</p> <p>In 2013, routine testing by the Irish Food Safety Authority revealed horsemeat in beef burgers. The PCR technique was used to identify the type of meat. DNA from burger samples was amplified (copied) using PCR, and samples separated by size using agarose gel electrophoresis. By comparing these DNA samples with those of known size (cow, pig, horse), horsemeat was detected.</p>
3	<p>PCR and forensic science</p> <p>Criminals often leave clues to their identity, in the form of DNA, at crime scenes. But recovered samples often contain only small amounts, such as a hair sample, blood or saliva.</p>
4	<p>PCR ingredients</p> <p>Chemical reagents used in PCR include: buffer, MgCl₂ and sterile water.</p>
5	<p>Primers</p> <p>Primers are essential for PCR. They specify the segment of DNA to be amplified, and provide an attachment site for DNA polymerase.</p> <p>Once primers are attached, elongation can begin. DNA polymerase bind to the primers and begin adding nucleotides.</p>
6	<p>Taq polymerase</p> <p>Copying DNA requires DNA polymerase. <i>Taq</i> polymerase is commonly used in PCR. It comes from the bacterium <i>Thermus aquaticus</i>, a bacterium that lives in hot springs and hydrothermal vents and tolerates extreme temperatures. PCR requires application of high temperatures (>90°C) to separate strands of DNA. <i>Taq</i> polymerase remains stable at temperatures above 95°C.</p>
7	<p>Nucleotides</p> <p>Nucleotides are the building blocks of new DNA strands. DNA polymerase adds nucleotides during PCR elongation.</p>
8	<p>PCR equipment</p> <p>Primers, <i>Taq</i> polymerase, and nucleotides are combined with other PCR reagents and added to DNA samples. The mix is placed into a thermocycler, a machine that heats and cools DNA repeatedly, amplifying DNA.</p> <p>Thermocycler temperature settings are pre-programmed and vary according to the DNA segment to be amplified; type of polymerase; and length and composition of primers.</p>
9	<p>PCR stage 1: denature</p> <p>This activity simulates the three stages of PCR. Each stage of PCR is temperature dependent. The first stage occurs at 95°C. At this temperature double-stranded DNA separates into two single-stranded segments. This is called denaturation.</p>
10	<p>PCR stage 2: anneal</p> <p>The second stage, annealing, occurs at 60°C. At this temperature primers anneal or bind to the single-stranded DNA templates.</p>
11	<p>PCR stage 3: elongate</p> <p>The third stage, elongation, occurs at 72°C. At this temperature the enzyme <i>Taq</i> polymerase attaches to primers and adds nucleotides, building a new DNA strand.</p>
12 – 13	<p>PCR challenge / How to play</p> <p>PCR uses temperature variation to amplify DNA. This activity guides students through each PCR stage, and the result of each temperature cycle on DNA.</p> <p>Students take on the role of a thermocycler, and at each stage of the process mimic conditions that occur within the thermocycler.</p>
14	<p>PCR kit</p> <p>You'll need to demonstrate each part of the kit.</p> <p>Students' challenge is to make as many copies (maximum possible: 16) of the double-stranded DNA template as they can in three cycles of PCR.</p>

SLIDE	NOTES
15 – 18	<p>PCR cycle 1</p> <p>Step students through the first PCR cycle, using timings displayed on screen.</p> <ul style="list-style-type: none"> • Denature This slide prompts students to recreate the first thermocycler stage, denaturation. Allow students 60 seconds to complete this stage. • Anneal This slide prompts students to recreate the second thermocycler stage, annealing. Allow students 120 seconds to complete this stage. • Elongate This slide prompts students to recreate the third thermocycler stage, elongation. Allow students 180 seconds to complete this stage.
19 – 21	<p>PCR cycle 2</p> <p>Students have completed one PCR cycle and created a new double-stranded DNA segment, an exact copy of the DNA template.</p> <p>Students use this copy to begin the next PCR cycle, cycle 2.</p> <p>Students follow temperature and time prompts that appear on screen to complete cycle 2.</p> <p>The timer is designed to allow sufficient time for students to complete each PCR stage. The timer can be set to run automatically or manually.</p> <p>Select automatic to make the timer run through all three stages of PCR without stopping. It allows 45 seconds to denature, 90 seconds to anneal, and 180 seconds to elongate.</p> <p>If automatic isn't selected the timer must be manually advanced, using the 'D', 'A' and 'E' buttons above the counter.</p> <p>Additional time can be added if required by selecting the +30 button in the circle.</p> <p>Select mute to mute the timer (which beeps in the last 10 seconds).</p>
22 – 24	<p>PCR cycle 3</p> <p>Students have completed two PCR cycles and have created 4 double-stranded DNA segments, exact copies of the original DNA template.</p> <p>Students use one of the copies built during cycle 2 to begin cycle 3.</p> <p>Students follow temperature and time prompts that appear on screen to complete cycle 3.</p> <p>At the end of cycle 3, ask students to count the number of new DNA strands created.</p> <p><i>Did anyone reach the target of 16 new single-stranded segments of DNA?</i></p>

Technical requirements

The teacher guide, procedure sheet and workbooks require Adobe Reader (version 5 or later), which is a free download from www.adobe.com. The procedure sheet is also available in Microsoft Word format.

The presentation requires a modern browser (eg Internet Explorer 9 or later, Google Chrome, Safari 4.0+, Opera or Firefox) on computer or mobile device. It can be placed on a web or file-server and run either locally or remotely in a web browser. Javascript should be enabled for best results.

Acknowledgements

Practising PCR is based on two resources:

- *The PCR Dash* a classroom game
Developed by the College of Agricultural Sciences at Oregon State University. <http://agsci.oregonstate.edu/aquatic-bt/sites/default/files/PDFs/FSIID13.pdf>
- *Sequencing a genome: Inside the Washington University Genome sequencing unit*
Activity supplement: Paper PCR (DNA amplification), developed by the faculty of Arts and Sciences at Washington University in St. Louis. <http://www.nslc.wustl.edu/elgin/genomics/gsc/PaperPCR.pdf>

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Image credits

PCR and melanoma mutations (workbook)

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Associated SPICE resources

Gene expression 2: Polymerase chain reaction may be used in conjunction with related SPICE resources to address the broader topic of gene expression and regulation.

DESCRIPTION	LEARNING PURPOSE
<p><i>Gene expression (overview)</i></p> <p>This learning pathway shows how a number of SPICE resources can be combined to teach the topic: gene expression and regulation.</p> <p>All resources use a human disease context, melanoma, which helps students relate to advances in biotechnology and our understanding of molecular genetics.</p>	
<p><i>Gene expression 1: Melanoma risk factors</i></p> <p>Students use an interactive learning object to investigate risk factors associated with melanoma developing.</p>	Engage
<p><i>Gene expression 2: Polymerase chain reaction</i></p> <p>Students simulate polymerase chain reaction in the classroom.</p>	Explore
<p><i>BioDiscovery activity (optional)</i></p> <p>Students attend the LotteryWest Biodiscovery Centre at the Harry Perkins Institute of Medical Research to participate in a SPICE-developed PCR laboratory activity. See <i>Gene expression (overview)</i> for details.</p>	Explore
<p><i>Gene expression 3: Measuring gene expression</i></p> <p>Students measure gene expression via a microarray simulation conducted in the school laboratory.</p>	Explore
<p><i>Gene expression 4: Regulating gene expression</i></p> <p>An animation explains how gene expression is regulated by complex molecular interactions. These processes are important in increasing organism adaptability, flexibility and complexity.</p>	Explain
<p><i>Gene expression 5: Personalised medicine</i></p> <p>Students explore an interactive story to discover how increased understanding of molecular biology and advances in biotechnology have led to development of personalised medical treatments for melanoma patients.</p>	Elaborate

Appendix 1: PCR templates

For *PCR challenge* each pair needs:

- a double strand of DNA
- 10 forward primers
- 10 reverse primers
- 96 nucleotides of each type
- a blank piece of A3 paper
- 2 *Taq* polymerase labels

Preparing the PCR kit

1. Enlarge all templates to A3 size.
2. Photocopy, on pink paper (for each pair), the enlarged forward primer block.
3. Photocopy, on green paper (for each pair), the enlarged reverse primer block.
4. Photocopy, on white paper (for each pair), the enlarged DNA template, *Taq* polymerase badges and nucleotide templates.
5. Cut out forward primers, so each pair of students has 10 pink strips.



6. Cut out reverse primers, so each pair of students has 10 green strips.



7. Cut out nucleotides individually, so each pair of students has 96 of each type.
8. Place these together in an envelope.
9. Cut out the *Taq* polymerase labels and make into badges.
10. Provide students with the DNA template, primers, *Taq* polymerase badge, A3 paper and nucleotides — the PCR kit.

Appendix 2: PCR challenge templates

Forward primer template

5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A
5'	T	T	A	C	G	G	A

Reverse primer template

C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'
C	C	A	T	C	G	G	5'

DNA
template

3'	C	C	G	5'
C	C	G	C	G
G	C	A	T	A
A	T	A	T	A
T	A	T	A	A
G	C	C	C	C
G	C	C	C	C
C	C	G	C	C
C	C	G	C	C
C	C	A	T	A
A	T	A	T	A
T	A	T	A	A
T	A	T	A	A
5'	3'	A	A	5'

