Ver. 1.2

ClassRoom PCR Teaching Kit (KIT-7839-10)

PCR Amplification & Gel Electrophoresis

Objective

With this kit, students will be able to gain an understanding and hands-on experience in PCR & Agarose Gel Electrophoresis.

Storage Instruction

Upon arrival, open the kit and store the components according to the storage temperature listed in page 2.

Kit Content

No.	Product	KIT-7839-10	Storage	Box
1	Taq DNA Polymerase	10 µL	–20 °C	
2	10X Taq Buffer	100 µL	–20 °C	
3	25 mM $MgCl_2$	100 µL	–20 °C	
4	dNTP Mix, 10 mM each	30 µL	–20 °C	1
5	16S rRNA Forward Primer (10 µM)	30 µL	–20 °C	
6	16S rRNA Reverse Primer (10 µM)	30 µL	–20 °C	
7	Water, PCR Grade	1 mL	–20 °C	
8	DNA Template	60 µL	–20 °C	2
9	Control PCR Product	30 µL	–20 °C	
10	Floro$^+$Red Nucleic Acid Stain	60 µL	4 °C	
11	6X DNA Loading Dye	30 µL	4 °C	3
12	1 kb DNA Ladder	30 µL	4 °C	
13	50X TAE Buffer	2 x 60 mL	Room Temp	
14	PCR tubes, 0.2 mL	10 pcs	Room Temp	4
15	Agarose	7 grams	Room Temp	

Room Temp: 21 – 25 °C

Product Specification

	KIT-7839-10
Number of reactions	10 reactions
Duration	≤ 3 hours
Storage conditions	i) Room Temperature (21 – 25 °C) ii) 4 °C iii) –20 °C

Materials Supplied by Users

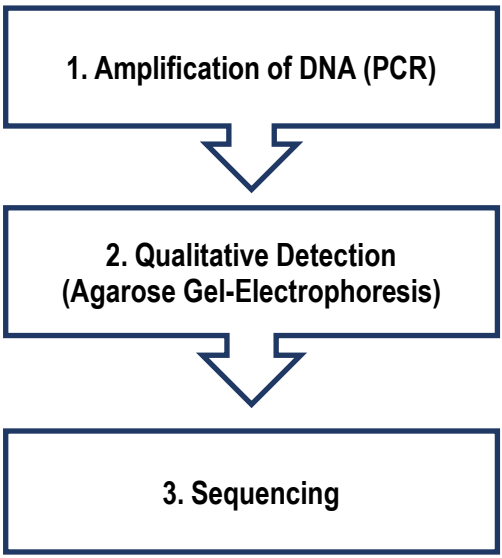
- ✓ Thermocycler
- ✓ Gel Electrophoresis System (inclusive of gel caster)
- ✓ UV Transilluminator

Precautions for Users

- ✓ Always wear a lab coat, disposable gloves and apply proper aseptic techniques to conduct molecular biology experiment.
- ✓ Read the entire protocol before starting the experiment.
- ✓ Store and prepare the solutions on ice while using.

Summary of the PCR Amplification & Gel-Electrophoresis Workflow

This teaching kit focuses on the amplification of DNA using PCR technique, followed by qualitative detection of the PCR product using agarose gel-electrophoresis, and finally sending the PCR product for Sanger Sequencing to obtain the sequences.



Introduction

Polymerase chain reaction (PCR) is a method for amplifying a specific target DNA region *in vitro* involving the sequential use of oligonucleotide primers and *Taq* polymerase (Brooker 1999). It is a technique developed by Kary Mullis in 1985. The goal of PCR is to make many copies of the target template DNA.

There are several reagents that are added to facilitate the synthesis of DNA (Brooker 1999). These include:

a) **Two oligonucleotide primers** - short pieces of single-stranded DNA which are complementary to sequences within the DNA fragment.

b) **Deoxynucleotides (dNTPs)** - single units of the bases A, T, G, and C, which are "building blocks" for new DNA strands.

c) ***Taq* polymerase** - a thermostable form of DNA polymerase enzyme that catalyses the synthesis of new DNA strands.

d) **Buffers** – To provide suitable chemical environment for the activity of DNA polymerase.

To make copies of the DNA, this double-stranded template is denatured by heat treatment (**denaturation**) and the oligonucleotide primers are then bound to the template DNA as the temperature is lowered. The binding of the primers to the DNA is called **annealing**. Once the primers have annealed, *Taq* polymerase will catalyse the synthesis of complementary DNA strands by facilitating the binding and joining of complementary nucleotides that are free in solution (dNTPs). Thus, during this **extension** stage, the new synthesis effectively extends the primers creating a complementary, double-stranded molecule from a single-stranded template [**Figure 1**].

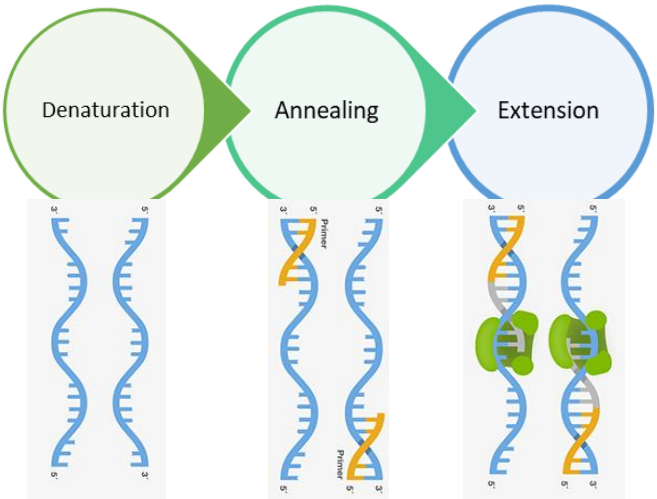


Figure 1 - The technique of polymerase chain reaction (PCR). During each cycle, oligonucleotides that are complementary to the targeted DNA sequence bind to the DNA and act as primers to synthesis this DNA region.

A thermocycler is used to carry out PCR. During every cycle, the thermocycler increases the temperature to denature the DNA strands and then lowers the temperature to allow annealing and DNA synthesis (extension) to take place. A typical PCR run consists of 20 to 30 cycles of replication and takes 1 – 2 hours to complete [**Figure 2**].

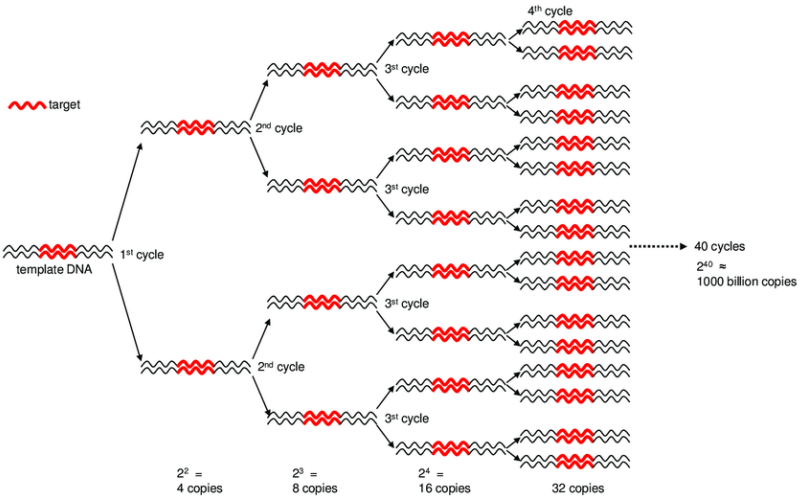


Figure 2 - The exponential amplification of DNA in PCR Amplification.

Once the PCR amplification is done, agarose gel electrophoresis is carried out to detect and analyse DNA qualitatively.

Agarose gel allows:

a) Analysis of PCR products after polymerase chain reaction to assess for target DNA amplification.

b) Estimation of the size of DNA molecules using a DNA marker or ladder which contains DNA fragments of various known sizes.

c) Rough estimation of DNA quantity and quality. Quantity is assessed using DNA ladder which contains specific amounts of DNA in different bands. Quality of DNA is assessed by observing the absence of smeared bands (or contaminating DNA bands).

(Molecular Station 2008)

Samples are loaded in wells at one end of the gel, and an electric field is applied across the gel. This electric field causes negatively charged DNA molecules to migrate from one side of the gel (negative) to the other (positive). The migration of molecules in response to an electric field is called electrophoresis (Brooker 1999). DNA sample is separated according to their molecular weights. Small DNA fragments migrate to the end of the gel faster than the larger ones. These separated bands can be visualized with nucleic acid stains. For example, Floro^{+}Red is a stain that binds to DNA and RNA and can be seen under ultraviolet (UV) light.

Once a single and intact band is observed, the PCR product can be sent for Sanger sequencing. Sequencing is done to obtain the DNA sequences of the target band. These sequences can later be interpreted using free software such as BLAST.

Protocols

PCR Amplification

PCR Reaction Mix

Preparation of PCR Reaction Mix

i) Thaw the PCR components at room temperature except *Taq* DNA polymerase on ice. Keep **ALL** the tubes on ice after thawing. Briefly vortex and briefly spin to bring down the contents.

ii) Prepare the PCR reaction mix according to table below:

No.	PCR components	Volume (µL)
1	Water, PCR Grade	32
2	10X *Taq* Buffer	5
3	25 mM $MgCl_2$	5
4	dNTP mix, 10 mM each	1
5	16S rRNA Forward Primer (10 µM)	0.75
6	16S rRNA Reverse Primer (10 µM)	0.75
7	*Taq* DNA Polymerase	0.5
8	DNA Template	5
	Total	50

Note: For negative control, add 5 µL of Water instead of DNA template

iii) Tap the tube for 5 seconds to mix the contents thoroughly and briefly spin down to bring down the contents.

iv) Place the tube on ice and proceed to PCR cycle condition.

PCR Cycle Condition

PCR Cycle Condition

i) Setup the PCR cycle condition on a thermal cycler according to table below:

Steps	Temperature	Time	No. of Cycle
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	30 sec	25
Annealing	54 °C	30 sec	
Extension	72 °C	90 sec	
Final extension	72 °C	5 min	1
Cooling	4 °C	∞	

ii) After setup, place the tubes into the thermal cycler and start the program.

iii) After the amplification is completed, place the tube containing PCR product on ice.

Gel Electrophoresis

Preparation for Agarose Gel

Prepare the following:

A) Preparation of 1X TAE buffer, 500 mL

i) Add 10 mL of 50X TAE buffer into 500 mL glass bottle containing 490 mL of distilled water.

ii) Mix well before use.

B) Preparation of 1% agarose gel, 50 mL

i) Weigh 0.5 g of agarose powder and mix it into a 100 mL glass beaker containing 50 mL 1X TAE buffer.

ii) Heat the mixture using a microwave or hot plate until the agarose powder has completely dissolved. Use spatula to stir the mixture occasionally.
CAUTION: Hot solution, handle with care.

iii) Allow the agarose gel solution to cool to 55 – 60 ^{o}C.

iv) Add 5 µL of Floro^{+}Red Nucleic Acid Stain to the gel solution and mix by swirling for 10 seconds.

v) Pour the solution into the gel caster.
Note: Remove bubbles if any as it affects DNA migration.

vi) Place the comb immediately and allow the gel to solidify for at least 30 minutes at room temperature.

Sample Loading

Loading the PCR Product

i) Remove the gel comb and place the solidified agarose gel into a gel tank.

Note: Ensure the gel is placed in the correct orientation: The gel wells at cathode (black) & DNA migrates towards anode (red).

ii) Pour 1X TAE buffer into the gel tank until the agarose gel is fully submerged with buffer.
[Standard level: Approximately 0.5 – 0.8 cm above the gel surface]

iii) Load the PCR product as below:

Gel Well No.	Components	Amount (µL)	6X Loading Dye (µL)	Water (µL)	**Total Amount (µL)**
1	1 kb DNA Ladder	2 µL	-	-	**2 µL**
2	Control PCR Product	2 µL	1 µL	3 µL	**6 µL**
3	Negative control	2 µL	1 µL	3 µL	**6 µL**
4	PCR product	2 µL	1 µL	3 µL	**6 µL**

Note: Avoid bubbles from forming and do not poke the bottom of the wells during loading.

Gel Electrophoresis

Performing Gel Electrophoresis

i) Place lid onto the gel tank, connect the power supply to the gel tank according to the convention: anode (Red) and cathode (black).

ii) Run the electrophoresis at 100 volts, until the dye front (bromophenol blue) migrated 2/3 of the gel length.

iii) Once the gel electrophoresis has completed, view the gel under a UV Transilluminator.

DNA Sequencing	**[OPTIONAL] DNA Sequencing** i) Send the remaining PCR Product to Apical Scientific Sdn. Bhd. for **DNA Sequencing+ PLUS Service [SS1201]** to obtain the sequence of the target region. ii) Go to https://base-asia.com/standard-sequencing/ for more details on how to order sequencing services. iii) Choose primer 785F and 907R (universal primer) when placing your order for DNA sequencing service.

Expected Results

Below is the expected gel image result (**Figure 3)** that can be observed under the UV Transilluminator.

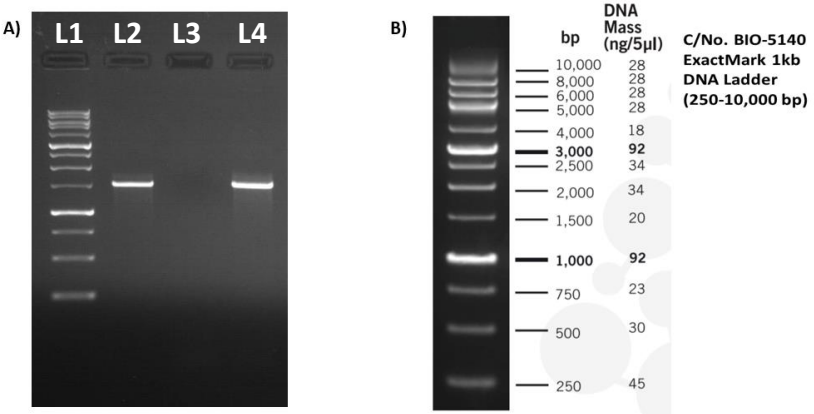


Figure 3 – Gel image that shows a successful PCR amplification with a target band at 1500bp.

A) Condition: 1% TAE agarose gel, 10 cm gel for 60 min.
Lane 1: 1 kb DNA Ladder
Lane 2: Control PCR Product
Lane 3: Negative control &
Lane 4: Amplified PCR Product

B) 1kb DNA Ladder sizing

Troubleshooting Guidelines

Problem	Possible Causes	Recommendation
Low or No Amplification	Insufficient quantity	Examine the quantity of input DNA
	Incorrect annealing temperature	Check the annealing temperature as per the protocol
	Missing reaction component	Repeat reaction setup
	Incorrect thermocycler programming	Check program, verify time and temperatures
Multiple or Non-Specific Products	Excess primer	Please follow the concentration and suggested primer volume in the protocol
	DNA contamination	• Use positive displacement pipettes or non-aerosol tips. • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	Please use the suggested template volume in the protocol

Problem	Possible Causes	Recommendation
DNA smearing on Agarose Gel	Too much template was added	Please follow the concentration and suggested template volume in the protocol
	Template contained an exonuclease or was degraded	Use fresh DNA template
	Impurities	• Repeat reaction setup • Use fresh components

Please contact us at https://base-asia.com/contact/ for more information.