

HiPer[®] Multiplex PCR Teaching Kit

Product Code: HTBM023

Number of experiments that can be performed: 5

Duration of Experiment:

Protocol: 2 hours

Agarose Gel Electrophoresis: 45 minutes

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Control Multiplex PCR Product, DNA Ladder and all the PCR reagents at -20°C
 - Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)



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Aim: To learn the process of PCR amplifying multiple DNA targets in a single reaction tube using more than one pair of primers.

Introduction:

Multiplex PCR is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. As an extension to the practical use of PCR, this technique has the potential to produce considerable savings in time and effort within the laboratory without compromising on the utility of the experiment.

Principle:

Multiplex PCR is a widespread molecular biology technique for simultaneous amplification of two or more products in a single PCR experiment. This specialized PCR reaction employs different primer pairs in the same reaction for amplification of multiple target sequences in a single reaction mixture. This type of PCR often requires extensive optimization of annealing conditions compared to standard PCR systems using only two primers as the additional challenge of multiplex PCR is the varying hybridization kinetics of different primer pairs. Primers that bind with high efficiency could utilize more of the PCR reaction components, thereby reducing the yield of other PCR products. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis.

There is an increasing demand for multiplex PCR techniques in Pathogen Identification, High Throughput SNP Genotyping, Mutation Analysis, Gene Deletion Analysis, Template Quantification, Linkage Analysis, RNA Detection and Forensic Studies.

Multiplex PCR can be broadly divided into the following two categories:

Single template PCR reaction - This technique uses a single template which can be a genomic DNA along with several pairs of forward and reverse primers to amplify specific regions within a template.

Multiple template PCR reaction - This technique uses multiple templates and several primer sets in the same reaction tube. Presence of multiple primer may lead to cross hybridization with each other and the possibility of mis-priming with other templates

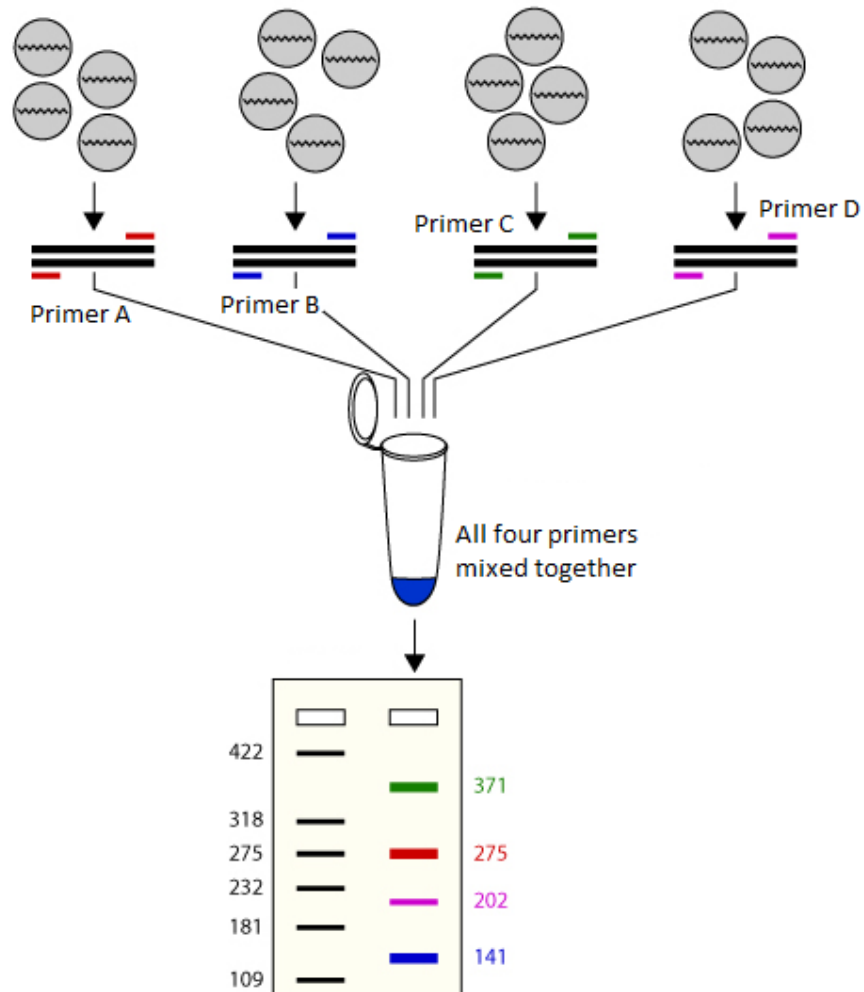


Fig 1: In Multiplex PCR multiple bands are simultaneously amplified according to the number of primers used

HiPer® Multiplex PCR Teaching Kit can be used to learn the process of multiplex PCR where a single template DNA is used along with two sets of primers, GAPDH and 18s. After the PCR two amplicons of different sizes are visible on the agarose gel as two different sets of primers are used.

Kit Contents:

The kit can be used to perform multiplex PCR using a single template and two primers.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			5 expts	
1	*TKC117	10X Assay Buffer	0.03ml	-20°C
2	*TKC401	Control Multiplex PCR Product	0.060 ml	-20°C
3	*TKC118	2.5 mM dNTP mix	0.03 ml	-20°C
4	*MBT049	100 bp DNA Ladder	0.030 ml	-20°C
5	*TKC397	GAPDH Forward Primer (10 nM)	0.008 ml	-20°C
6	*TKC398	GAPDH Reverse Primer (10 nM)	0.008 ml	-20°C
7	*TKC399	18s Forward Primer (10 nM)	0.022 ml	-20°C
8	TKC400	18s Reverse Primer (10 nM)	0.022 ml	-20°C
9	*TKC120	Taq DNA Polymerase	0.005 ml	-20°C
10	*TKC009	Template DNA	0.015 ml	-20°C
11	ML024	Molecular Biology Grade Water	0.3 ml	R T
12	*TKC119	25 mM MgCl ₂	0.03 ml	-20°C
13	MB002	Agarose	6 g	R T
14	ML016	50X TAE	60 ml	R T
15	ML015	6X Gel Loading Buffer	0.03 ml	2-8°C
16	MB161	Mineral oil (optional)	0.15 ml	R T
17	CG282	Polypropylene Tubes, 0.2 ml (PCR Tubes)	5 Nos.	R T

*** Always give a quick spin before opening the vial as the liquid material may stick to the wall or to the cap of the vial.**

Materials Required But Not Provided:

Glasswares: Measuring cylinder, Beaker

Reagents: Ethidium bromide (10 mg/ml), Distilled water

Other requirements: Thermocycler, Electrophoresis apparatus, UV Transilluminator, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Microwave/ Hotplate/ Burner, Crushed ice

Storage:

HiPer® Multiplex PCR Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store Control Multiplex PCR Product, 100 bp DNA Ladder and all PCR reagents at -20°C and 6X Gel Loading Buffer should be stored at 2-8°C. Other reagents can be stored at room temperature (15-25°C).

Important Instructions:

- Read the entire procedure carefully before starting the experiment.
- Keep all the solutions in the icebox during use.
- The 100 bp DNA ladder supplied in the kit is ready to use and can be directly loaded onto the agarose gel.

Procedure:

1) Preparation of master mix for PCR

To a PCR tube add all the following ingredients in order

Sr. No.	Ingredients for PCR	Volume in μl
1	Molecular Biology Grade Water	24.5 μ l
2	10X Assay Buffer	5 μ l
3	Template DNA	2 μ l
4	GAPDH Forward Primer (10 nM)	1 μ l
5	GAPDH Reverse Primer (10 nM)	1 μ l
6	18s Forward Primer (10 nM)	3 μ l
7	18s Reverse Primer (10 nM)	3 μ l
8	25 mM MgCl ₂	5 μ l
9	2.5 mM dNTP Mix	5 μ l
10	Taq DNA Polymerase	0.5 μ l
	Total volume	50 μl

2) Tap the tube for 1 – 2 seconds to mix the contents thoroughly.

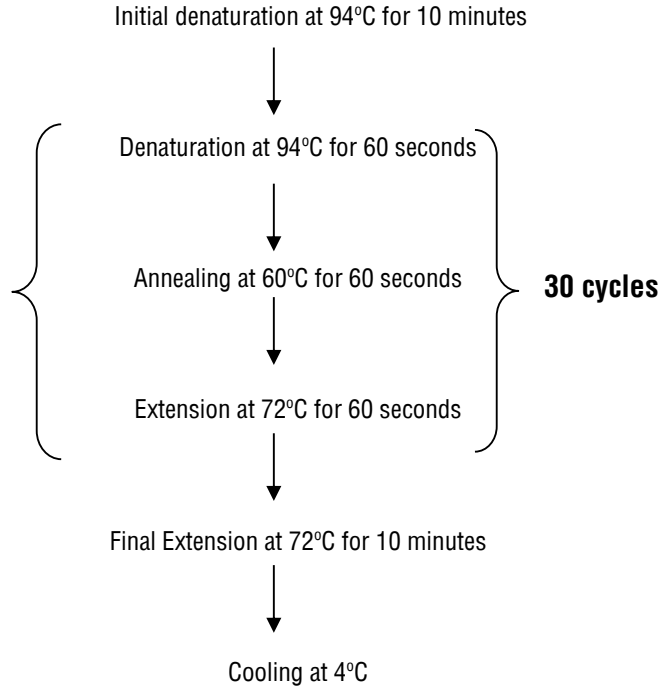
3) Add 25 μ l of mineral oil in the tube to avoid evaporation of the contents.

NOTE: It is not essential to add mineral oil if the thermocycler is equipped with a heating lid.

4) Place the tube in the thermocycler block and set the program to get DNA amplification.

PCR Amplification Cycle:

Carry out the amplification in a thermocycler for 30 cycles using the following reaction conditions.



Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 2% agarose gel, add 1g agarose to 50ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate, swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool down to about 55-60°C. Add 0.5µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

Loading of the DNA samples: Load 5 µl of ready to use DNA ladder into the first well. Add 2 µl of 6X Gel loading buffer to 10 µl of PCR product. Load the PCR samples into the following wells.

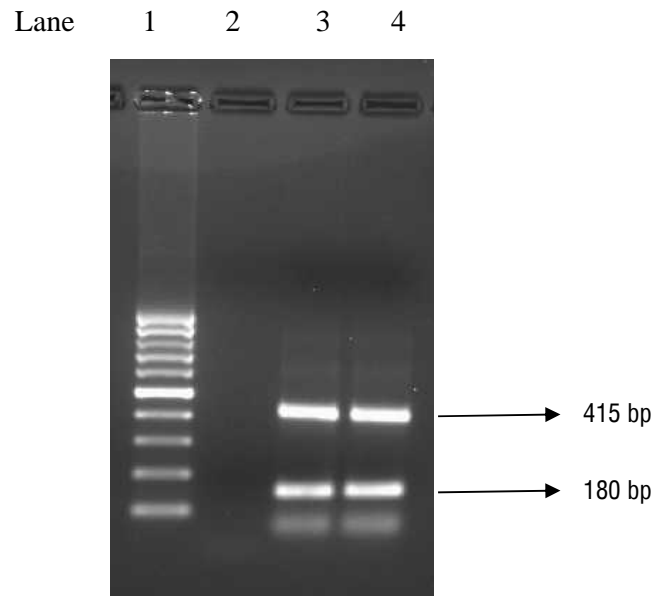
Note: Care should be taken while pipetting out the PCR product from the tube so as to avoid the mineral oil layer.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black-Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

* Molecular biology grade water is recommended (Product code: ML024).

Observation and Result:

After completion of the PCR, perform agarose gel electrophoresis. Compare the amplified product with the ladder and determine its size.



Lane 1: 100 bp DNA Ladder

Lane 2: Negative Control

Lane 3: Control Multiplex PCR Product

Lane 4: Test Multiplex PCR Product

Interpretation:

After performing agarose gel electrophoresis, one can check the simultaneous amplification of two PCR products. The amplicon size for GAPDH primers is 416 bp and that for 18s primers is 182 bp. The optimized conditions result in the multiple amplification of two PCR products of desired size.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Non –specific/ spurious bands observed	Template DNA or dNTPs concentration inappropriate	Take the same amount of template DNA and dNTPs as specified in the procedure
		Template DNA is degraded	Minimize damage to template DNA by avoiding vortexing or vigorous mixing
2	No or poor amplification yield	Template or dNTPs may be degraded, enzymes may have been inactive	Store the kit at -20°C and avoid repeated freeze thaw. Also keep all the materials in ice while performing the experiment
		Thermocycler operation or program improper	Ensure proper functioning of Thermocycler. Run positive control with every reaction
		Inadequate mixing of the reaction tube	Mix the reaction mixture using a micropipette, avoid air bubble
3	Smearing of the product	DNA degraded	Work in sterile conditions to avoid contamination. Avoid vigorous mixing of the DNA samples
4	Primer-Dimer observed	Concentration of primers and dNTPs may be inappropriate	Use recommended concentration of primers and dNTPs

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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