HiPer® Random Amplification of Polymorphic DNA (RAPD) Teaching Kit

Product Code: HTBM031

Number of experiments that can be performed: 5

Duration of Experiment:
Protocol: 3.5 hours
Agarose Gel Electrophoresis: 2 hours

Storage Instructions:

- The kit is stable for 12 months from the date of receipt
- Store DNA samples, 100 bp 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 understand the concept of DNA fingerprinting by Random Amplification of Polymorphic DNA (RAPD) in context of bacterial strain identification.

Introduction:

Random Amplified Polymorphic DNA (RAPD) is a method of producing a biochemical fingerprint of a particular species. Relationships between species may be determined by comparing their unique fingerprint information. RAPD-PCR is means of creating a biochemical fingerprint of an organism and is used to analyze the genetic diversity of an individual by using random primers. In this method random primer sequences are added each to an individual sample of DNA which is then subjected to PCR. The resulting amplified DNA markers are random polymorphic segments with varying band sizes which can be analysed after performing gel electrophoresis.

Principle:

Genetic analysis of organisms at the molecular level is a very important and widely practiced scientific tool. Several techniques have developed to identify each individual or type of individual in a species unambiguously. One important PCR-based genetic analysis is random amplified polymorphic DNA analysis (RAPD). RAPD uses small (10 nucleotide length), nonspecific primers to amplify seemingly random regions of genomic DNA. If primers with arbitrary sequences (random primers) are used for PCR amplification, DNA segments to be amplified will be selected at random which will thus provide a truly random sample of DNA markers. In RAPD no knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. The principle is that, the single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome. The assumption is made that a given DNA sequence (complementary to that of the primer) will occur in the genome, on opposite DNA strands, in opposite orientation within a distance that is readily amplifiable by PCR.

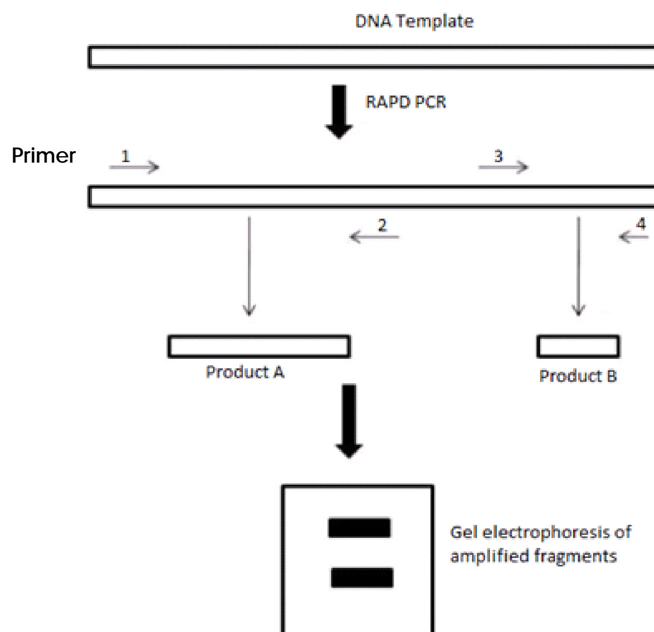


Fig 1: Schematic representation of a RAPD PCR

As with most PCR techniques, RAPD requires very little starting material for analysis and is relatively insensitive to template integrity. Slight modifications to the basic PCR method are made for RAPD. First, the primers are approximately 10 bases in length compared to the 17- to 23-base primer length of normal PCR. Because primers are shorter, the annealing temperature is reduced to less than 40°C. In RAPD analysis, for PCR to occur: a) the primers must anneal in a particular orientation (such that they point towards each other) and, b) they must anneal within a reasonable distance of one another. Successful primer pairs produce different banding profiles of PCR products between individuals, strains, or species when analyzed by gel electrophoresis.
The HiPer® Random Amplification of Polymorphic DNA (RAPD) Teaching Kit is designed for understanding the concept of DNA fingerprinting by RAPD PCR in context of bacterial strain typing.

Kit Contents:

The kit can be used to perform amplify a particular template DNA using PCR.

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	TKC384	10X Assay Buffer	0.06 ml	-20^{0}C
2	TKC118	dNTP Mix	0.045 ml	-20^{0}C
3	TKC385	Taq Polymerase	0.015 ml	-20^{0}C
4	TKC386	Random Primer	0.030 ml	-20^{0}C
5	ML024	Molecular Biology Grade Water	0.5 ml	RT
6.	TKC390	E. coli K12 Genomic DNA	0.010 ml	-20^{0}C
7.	TKC387	E. coli 0103:K:h8 Genomic DNA	0.010 ml	-20^{0}C
8.	TKC388	Bacillus cereus Genomic DNA	0.010 ml	-20^{0}C
9.	TKC389	Bacillus subtilis Genomic DNA	0.010 ml	-20^{0}C
10.	TKC391	Test Genomic DNA	0.010 ml	-20^{0}C
11.	MBT049	100 bp DNA Ladder	0.060 ml	-20^{0}C
12.	MB073	Ultra Resolution Agarose	6 g	RT
13.	ML016	50X TAE	60 ml	RT
14.	ML015	6X Gel Loading Buffer	0.1 ml	2-8 ^{0}C
15.	CG282	Polypropylene Tubes, 0.2 ml (PCR Tubes)	30 No	RT
16.	MB161	Mineral oil (optional)	0.3 ml	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)
Other requirements: Thermocycler, Electrophoresis apparatus, UV Transilluminator, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Microwave/ Hotplate/ Burner, Crushed ice, Gloves

Storage:

HiPer® Random Amplification of Polymorphic DNA (RAPD) Teaching Kit is stable for 12 months from the date of receipt without showing any reduction in performance. On receipt, store all the DNAs, 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.
- Carry out the entire experiment wearing gloves to avoid any contamination.
- Use fresh autoclaved tips for every sample to avoid contamination.

Procedure:

1) Preparation of master mix for PCR
   Prepare a PCR master mix by adding the following reagents:

Sr. No.	Ingredients for PCR	Volume in µl
1	Molecular Biology Grade Water	76 µl
2	10X Assay Buffer	10 µl
3	2.5mM dNTP each	7.5 µl
4	Random Primer	5 µl
5	Taq DNA Polymerase	2.5 µl

2) Tap the tube for 1 – 2 seconds to mix the contents thoroughly.
   NOTE: All the above reagents should be added on ice.

3) Aliquot 20 µl of the above PCR master mix to each of the five different PCR (placed on ice) and label them as 1, 2, 3, 4 and 5.

4) Add 1 µl of E. coli K12 Genomic DNA to PCR tube labeled 1. Similarly add 1 µl each of genomic DNA of E.coli 0103:K:h8, Bacillus cereus, Bacillus subtilis and Test Genomic DNA to the tubes labeled 2,3, 4 and 5, respectively. Mix the contents gently.

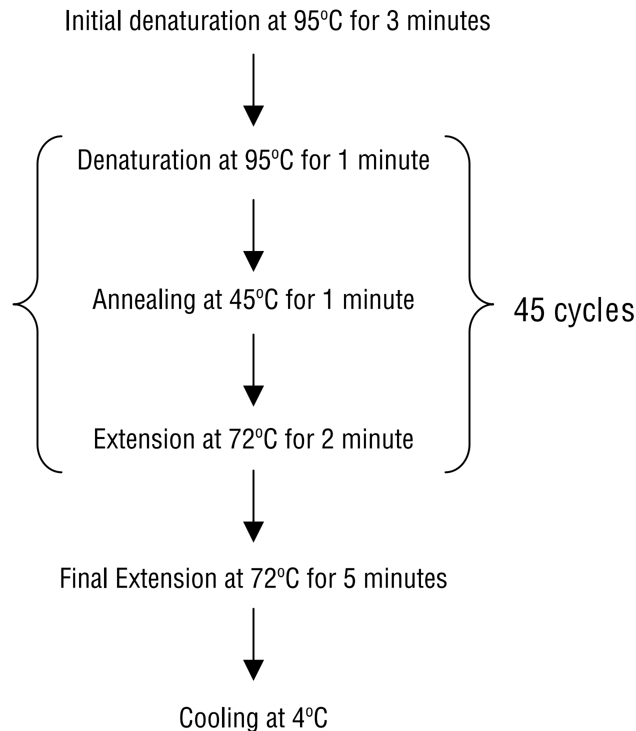
5) Add 25 µl of mineral oil in the PCR tubes to avoid evaporation of the contents.

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

6) 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 45 cycles using the following reaction conditions.



Following PCR amplification, add 2 µl of 6X Gel loading buffer to each of the PCR tubes and mix thoroughly. Let stand for some time for the two layers to separate (if mineral oil is used) and perform agarose gel electrophoresis as described below.

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 of 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.

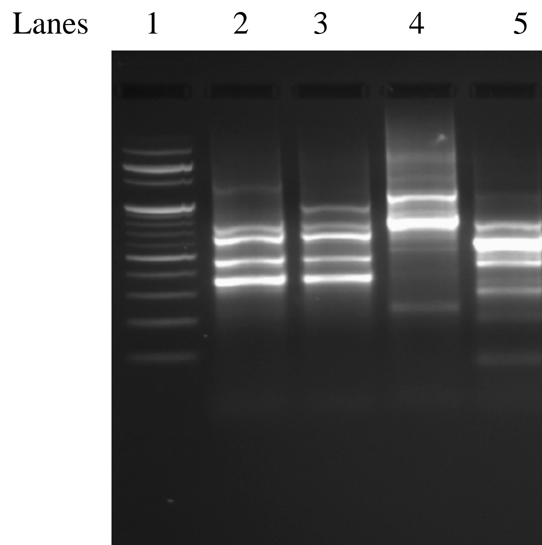
Loading of the DNA samples: Load 5 µl of ready to use DNA ladder into the first and last well. Load 15 µl of PCR sample (added with dye)
Note: Care should be taken while pipetting out the PCR product from the tube so as to avoid the mineral oil layer.

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

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.

Observation and Result:

After completion of the PCR, perform agarose gel electrophoresis. Compare the RAPD patterns of the four different bacterial genomic DNA



Lane 1: 100 bp DNA Ladder
Lane 2: RAPD Pattern of E.coli K12 Genomic DNA
Lane 3: RAPD Pattern of E.coli 0103:K:h8 Genomic DNA
Lane 4: RAPD Pattern of Bacillus cereus Genomic DNA
Lane 5: RAPD Pattern of Bacillus subtilis Genomic DNA

Interpretation:

The RAPD pattern i.e. number and size of the amplified PCR product varies among different bacterial strains. This happens due to the variation in their genomic DNA sequences and annealing sites of the random primer. By doing this experiment one can study the RAPD fingerprints for differentiating bacteria at genus and species level. The RAPD patterns of E. coli and Bacillus show significant differences but the RAPD patterns within the species of same genus i.e. E.coli K12, E.coli 0103:K:h8 show less differences. From all the RAPD patterns one can check with which reference sample the test sample matches.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	No or poor amplification yield	Template or dNTPs may be degraded, enzymes may have been inactive	Store the kit at -20ºC and avoid repeated freeze thaw. Also keep all the materials in ice while performing the experiment
		Thermoycler 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
2	Smearing of the product	DNA degraded	Work in sterile conditions to avoid contamination. Avoid vigorous mixing of the DNA samples

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