HiGenoMB™ Unzipping Genes

HIMEDIA®

Product Information

HiPer® Single Nucleotide Polymorphism (SNP) Teaching Kit

Product Code: HTBM034

Number of experiments that can be performed: 10

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 2X PCR Master Mix, Normal DNA, SNP DNA, 50 bp DNA Ladder, Primer mix for SNP and Molecular Biology Grade Water 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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For life is precious

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Aim:

To detect genetic disorder through the study of Single nucleotide polymorphism

Introduction:

Single nucleotide polymorphism (SNP) is a variation in the DNA sequence at a precise position among individuals which lead to genetic variation among people. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. Most commonly, these variations are found in the DNA between genes. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function. Researchers have found that SNPs can also be used to track the inheritance of disease genes within families.

Principle:

A single nucleotide polymorphism, or SNP is an alteration at a single position in a DNA sequence among individuals. If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP. If a SNP occurs within a gene, then the gene is described as having more than one allele. In these cases, SNPs may lead to variations in the amino acid sequence which are often associated with genetic disorders and other related diseases. SNPs occur normally throughout a person's DNA and function as biological markers which help scientists to detect genes that are associated with a particular genetic disorder. SNPs can also be used to track the inheritance of disease genes within families. Some of these genetic differences, however, have proven to be very important in the study of human health. Although a particular SNP may not cause a disorder, some SNPs are associated with certain diseases. Detection of those diseases is essential for molecular characterization, diagnosis, prevention and treatment of these diseases is carried out by the technique called SNP. Development of SNP-specific primers that amplify a specific fragment in PCR when a specific SNP is present helps in disease detection. SNP detection consists of two DNA templates, one wild-type and the other template with SNP are used for PCR amplification individually along with 2 pairs of primers, one pair specific to wild-type and the other, specific to SNP-type primer.

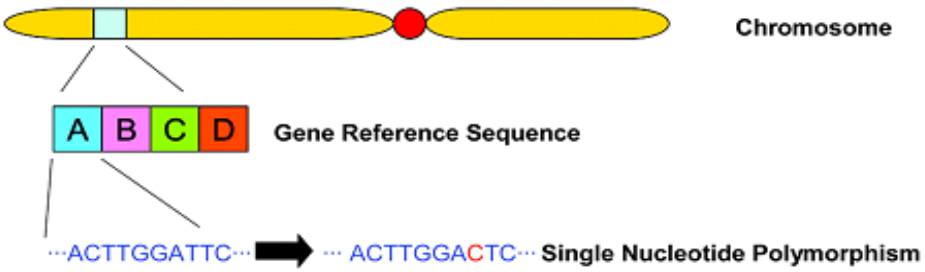


Fig 1: In Single Nucleotide Polymorphism (SNP) Thymine is replaced by Cytosine in a certain point of DNA

HiPer® Single Nucleotide Polymorphism (SNP) Teaching Kit is designed for the detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) which is a SNP based disorder in human.

Kit Contents:

The kit can be used to 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
			10 expts	
1	MBT061	2X PCR Master Mix	0.6 ml	-20°C
2	*TKC415	SNP DNA	0.060 ml	-20°C
3	*TKC416	Normal DNA	0.060 ml	-20°C
4	*ML065	Molecular Biology Grade Water for PCR	1 ml	-20°C
5	*TKC417	Primer Mix for SNP	0.030 ml	-20°C
6	*MBT084	50 bp DNA Ladder	0.040 ml	-20°C
7	ML015	6X Gel Loading Buffer	0.050 ml	-20°C
8	MB002	Agarose	12 g	R T
9	ML016	50X TAE	120 ml	R T
10	CG282	Polypropylene Tubes, 0.2 ml (PCR Tubes)	20 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® Single Nucleotide Polymorphism (SNP) Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store 2X PCR Master Mix, SNP DNA, Normal DNA, 50bp DNA Ladder, MB Grade water and Primer Mix for SNP at -20ºC and 6X Gel Loading Buffer should be stored at 2-8ºC. Other reagents can be stored at room temperature (15-25ºC).

Important Instructions:

- Read the entire procedure carefully before starting the experiment.
- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Centrifuge the components briefly once thawed.
- The 50bp 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**

Take two PCR tubes, label them and add the following ingredients in mentioned order:

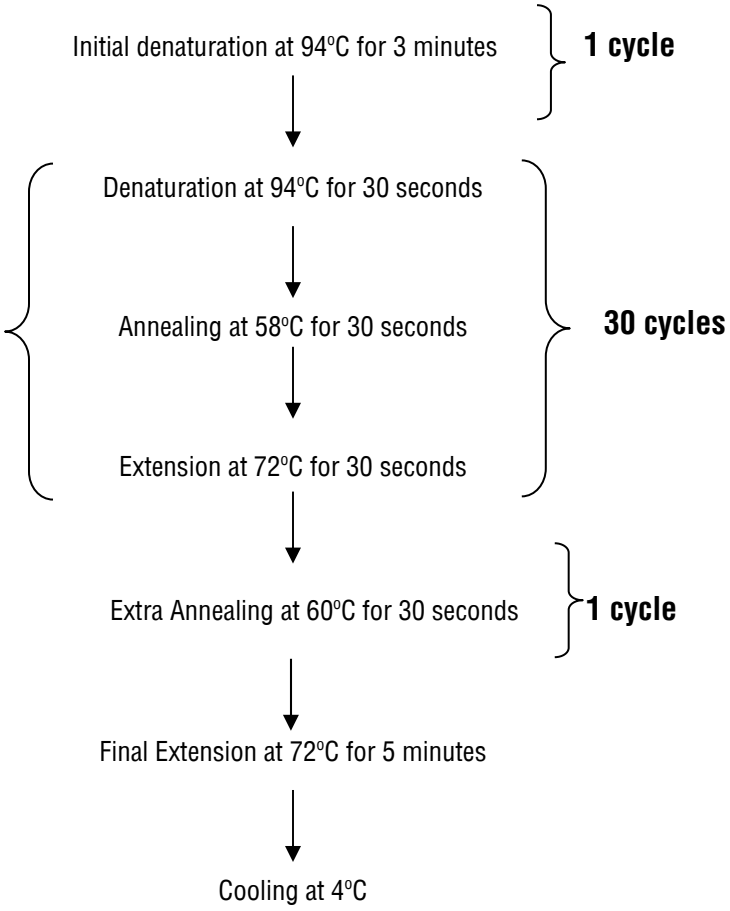
	Test Sample 1	**Test Sample 2**
Molecular Biology Grade Water	19 µl	19 µl
2X PCR Master mix	25 µl	25 µl
SNP DNA	5 µl	-
Normal DNA	-	5 µl
Primer Mix for SNP	1 µl	1 µl
Total volume	**50µl**	**50µl**

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

2) Place the tubes 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 1 g agarose to 50ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate by swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure).

NOTE: Preparation of 2% gel will take more time than the regular 0.8% gel. Care should be taken while melting. Continuous boiling is not recommended during the preparation. Make sure that melted agarose solution appear clear and transparent devoid of any suspended particles of agarose.

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 3 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. Mix well by pipetting and load the PCR samples into the following wells.

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 obtained from normal DNA with the SNP DNA.

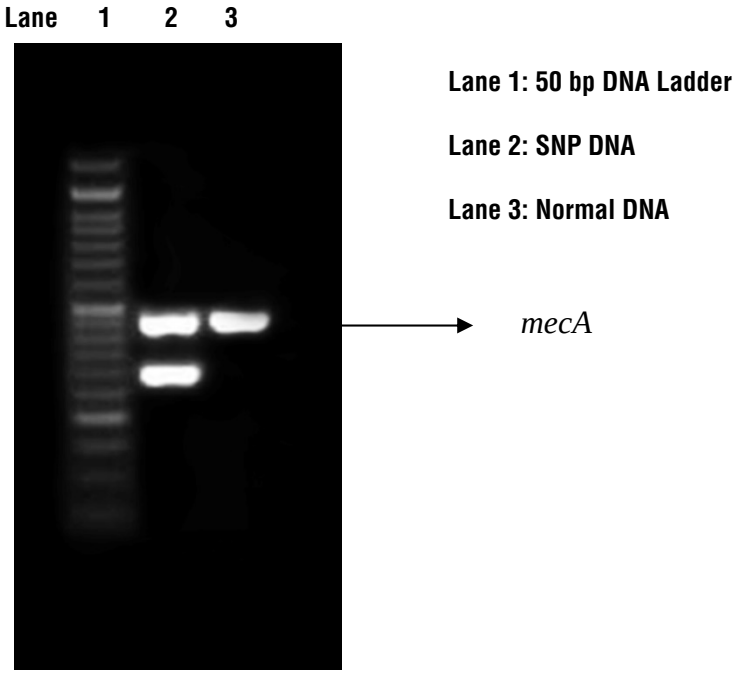


Fig 2: Gel image of amplified normal & SNP DNA

Interpretation:

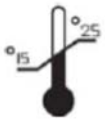
In this PCR reaction two targets are amplified simultaneously, one is specific for *Staphylococcus* genus and the other for methicillin-resistant staphylococci (*mec*A gene). The target specific for *Staphylococcus* genus amplifies in both SNP and normal DNA but the MRSA specific *mec*A gene is amplified only in the SNP DNA which is extracted from a MRSA strain.

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 damaged	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

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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