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Product Name	Cat. No.	Pack Size
PCR teaching Kit	**# BB-MTK010S**	**5 Reactions**

Objectives:

- To perform PCR amplification of specific target sequence from template DNA.
- To analyzed the amplified product by Agarose gel electrophoresis.

Principle:

PCR-polymerase chain reaction is an *in vitro* method of enzymatic synthesis of specific DNA sequence, developed by Kary Mullis in 1983. It is a very simple technique for characterizing, analyzing & synthesizing any specific DNA or RNA from any source.

PCR consists of the following three basic steps:

Denaturation: During this step, two strands melt open to form single stranded DNA. this is generally carried out at 92°C-96°C.

Annealing: Annealing of primers to each original strand for new strand synthesis is carried out at 45°C to 60°C.

Extension: At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand.

Materials provided: (for 5 reactions)

The list bellow provides the information about the materials supplied in the kit.

Materials	Quantity	Storage Condition
Taq DNA polymerase	5µl	-20°C
10X Taq Buffer	50 µl	-20°C
Template DNA	5µl	-20°C
Forward Primer	5µl	-20°C
Reverse Primer	5µl	-20°C
Nuclease free water	200 µl	-20°C
1kb DNA ladder	25 µl	-20°C
dNTP mix	10µl	-20°C
6X DNA Loading Dye	25µl	-20°C
Agarose	1gm	RT
50X TAE	7.5ml	4°C
PCR Tubes	5 Nos.	RT
Ethidium bromide (EtBr)	25ul	RT

Procedure:

Add the following reagents to the PCR tubes in the following order

Nuclease free water	40µl
Template DNA (100ng/ul)	1 µl
Forward Primer (10pmol/ul)	1ul
Reverse Primer (10pmol/ul)	1 µl
10X Taq Buffer	5 µl
dNTP Mix	1 µl
Tag DNA polymerase (1U/ul)	1 µl
Total Reaction Mix	**50** µl

Mix the reagents properly, and place the tube at PCR Machine.

PCR Amplification:

Carryout the amplification in a thermo cycler for **30 cycles** using the following condition.

Initial denaturation	Denaturation	Annealing	Extension	Final extension
94°C 1min	**94°C 30 Sec**	**58°C 30Sec**	**72°C 1min**	**72°C 2min**

For 30 cycles

Preparation of 1% Agarose gel:

1. Prepare 1 X TAE by diluting appropriate amount of 50 X TAE Buffer. (for one experiment approx 200 ml buffer needed) .
2. Weight 0.5gm of Agarose and add 50ml of 1 X TAE, this gives 1% Agarose gel.
3. Boil the Agarose till dissolves completely, cooled it, when temperature is around 50°C, add 5µl EtBr in to the agarose solution and mixed well.
4. Meanwhile place the comb to the electrophoresis set and pour the Agarose solution slowly to avoid generation of air bubbles .Keep the gel undisturbed at room temperature till the agarose solidify.

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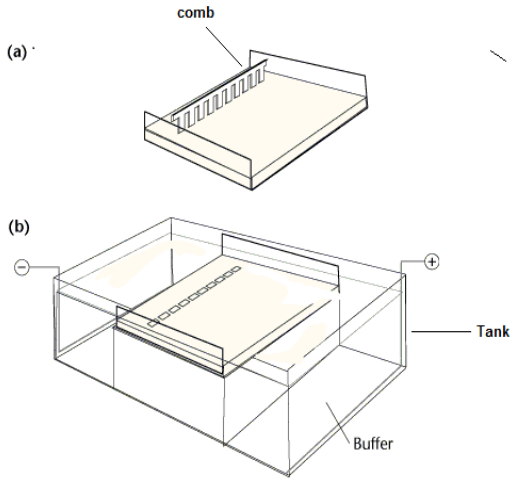
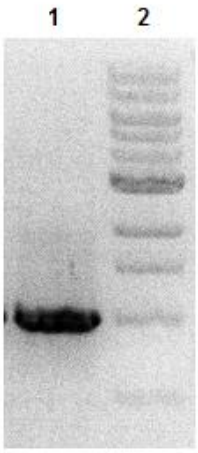
5. Pour 1 X TAE buffer into the gel tank till the buffer level stands at 0.5 to 0.8 cm above the gel surface.

6. Gently lift the comb, ensuring the wells remain intact.ace.





Lane 1: PCR Amplified 1kb DNA fragment.

Lane 2: 1kb DNA Ladder

Analysis on Agarose gel and Observation:

1. Add 5ul of 6X DNA loading Dye in to the PCR tube, Mix carefully.
2. Carefully pipette out 5µl of reaction mixture and load onto the well of 1% Agarose gel.
3. Load 5µl of 1Kbp DNA ladder (ready to use) provided. Run the sample at 100 volts till the blue dye reaches 3/4th length of the gel.
4. Visualize the gel under UV-transilluminator.