Bio Bharati Life Science Pvt. Ltd.

PCR Teaching kit

Cat# BB-MTK010S (5Reactions)
Cat# BB-MTK010 (10Reactions)

Objectives:

- To perform PCR amplification of specific target sequence from template DNA.
- To analyze 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 step is generally carried out at 92^{o}C-96^{o}C.

Annealing: In this step, primers anneal to each original strand of the template DNA for new strand synthesis. This step is carried out at 45^{o}C to 60^{o}C.

Extension: At this step the DNA polymerase synthesizes a new DNA strand complementary to the template DNA strand by adding dNTPs that are complementary to the template DNA 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 10 reactions)

Materials	Quantity	Store at
Taq DNA Polymerase	10µl	-20^{o}C
10X Taq Buffer	60 µl	-20^{o}C
Template DNA	10µl	-20^{o}C
Forward Primer	10µl	-20^{o}C
Reverse Primer	10µl	-20^{o}C
Nuclease free water	1 ml	-20^{o}C
1Kbp DNA ladder	50 µl	-20^{o}C
dNTP mix	10µl	-20^{o}C
6X DNA Loading Dye	100µl	-20^{o}C
Agarose	1gm	RT
50X TAE	15 ml	4^{o}C
PCR Tubes	10 Nos.	RT
Ethidium bromide(EtBr)	50 µl	RT

Procedure:

Add the 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 Reaction 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 in the PCR Machine

PCR Amplification: Carry out the amplification in a thermo cycler for **30 cycles** using the following conditions:

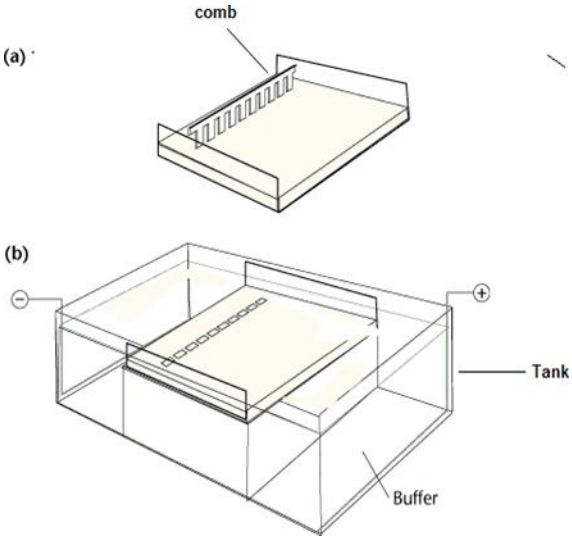
Initial denaturation	Denaturation	Annealing	Extension	Final extension
94°C	94°C	58°C	72°C	72°C
1min	30secs	30secs	1min	2min

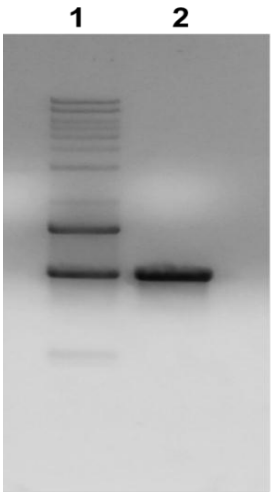
For 30 cycles

Preparation of 1% Agarose gel:

1. Prepare the gel casting tray by sealing the two ends with adhesive tape.
2. Put the comb in the appropriate place of the gel casting tray.
3. Prepare 1 X TAE by diluting appropriate amount of 50 X TAE Buffer (for carrying out one experiment approximately 200 ml buffer is needed).
4. Weigh 0.5gm of Agarose and add 50ml of 1X TAE for preparing 1% Agarose gel.
5. Boil until the agarose dissolves completely, cool it to around 50^0 C, add 5µl EtBr to the agarose solution and mix well.
6. Pour the Agarose solution slowly to avoid generation of any air bubble .Keep the gel undisturbed at room temperature till the agarose solidifies.
7. Pour 1X TAE into the gel tank till the buffer level stands at 0.5 to 0.8 cm above the gel surface.
8. Gently lift the comb, ensuring the wells remain intact.

Adapted from internet





Lane 1: 1kb DNA LADDER
Lane 2: PCR Amplified 1kb DNA fragment

Fig: Visualize the gel under UV-transilluminator.

Analysis on Agarose gel and Observation:

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