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Electrophoresis

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[1]NTHU_igem

iGEM NTHU

igemnthuth

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Protocol status: Working
We use this protocol and it's working

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Abstract

Separate the PCR products to verify the presence of the target DNA fragment and determine whether its size is correct.

Materials

- PCR product samples
- Agarose powder
- 25× TAE buffer (containing Tris base, sodium acetate, EDTA)
- ddH_2O
- Ethidium bromide (EtBr, 10 mg/mL)
- DNA loading dye (containing bromophenol blue, glycerol, etc.)
- DNA ladder (e.g., 100 bp or 1 kb marker)
- Mini gel electrophoresis system
- Electrophoresis chamber (with comb)
- Gel casting tray
- Microwave oven
- UV or blue-light gel documentation system
- Micropipettes (pipetman) with sterile tips
- Tubes
- Plastic plate (for gel transfer)

Preparation of TAE Buffer (skip if already available)

1 Dissolve 121 g Tris base, 10.25 g sodium acetate, and 18.6 g EDTA in 750 mL distilled water.

2 Adjust the pH to 7.8 using glacial acetic acid.

3 Add ddH_2O to bring the final volume to 1000 mL.

Preparation of Agarose Gel (0.8% agarose)

4 Assemble the mini gel electrophoresis system according to the instrument's instructions.

5 Dilute 25× TAE buffer with distilled water to prepare 1× TAE buffer (25× TAE buffer : ddH_2O = 1:25, v/v).

6 Dissolve 0.2 g agarose in 25 mL of 1× TAE buffer to make a 0.8% agarose solution.

7 Heat the solution in a microwave until the agarose is fully dissolved. Allow it to cool to about 45 °C.

8 Pour the solution into the gel casting tray to a thickness of about 7.5 mm. Immediately insert the comb. Avoid bubble formation and ensure the surface is level and the thickness uniform.

9 Leave at room temperature for about 20 minutes until solidified. Carefully remove the comb to finish gel preparation.

Sample Preparation and Loading

10 Place the prepared agarose gel into the electrophoresis chamber.

11 Pour in enough 1× TAE buffer to completely cover the gel until it reaches the marked level inside the chamber.

12 On a piece of parafilm, mix 1 µL DNA loading dye with 5 µL PCR product in a tube, then load the mixture into the wells using a micropipette. Separately, load 2 µL of DNA ladder into another well as a size reference.

13 Connect the power supply, turn the switch to “ON,” and set the voltage to 100 V. Ensure that DNA migrates from the negative electrode (black) toward the positive electrode (red). (*Check electrode orientation carefully.*)

14 Run electrophoresis for about 30–45 minutes until the dye front migrates to about 1.5 cm from the bottom of the gel. When finished, switch the power supply to “OFF” and disconnect the electrodes. (*Do not allow the dye to run off the gel.*)

15 Using a plastic tray, transfer the gel onto the UV transilluminator plate. Visualize the DNA bands under UV light and record the results with a gel documentation system.