Agarose gel electrophoresis

Materials:
Electrophoresis buffer (TAE or TBE)
Ethidium bromide solution Agarose,
electrophoresis grade 5X loading buffer

DNA molecular weight marker IV (2kg marker) (Roche Biochemicals) Size
range (0.15 to 2.1 kb)
15 fragments:
154, 154, 220, 234,, 298, 298, 394, 453, 517, 653, 1033, 1230, 1766, 2176 bp
Horizontal gel electrophoresis apparatus
Gel casting platform
Gel combs
DC power supply

Protocol:

1. Analyze PCR products by agarose gel electrophoresis. Prepare the gel, using electrophoresis buffer (TBE or TAE, 0.5 X) and electrophoresis-grade agarose (1.0%) by melting in a microwave oven or autoclave, mixing, cooling at 50ºC, and then pouring into a sealed gel casting platform, and inserting the gel comb.

2. After the gel has hardened, remove the seal from the gel casting platform and withdraw the gel comb. Place into an electrophoresis tank containing sufficient electrophoresis buffer (TBE or TAE; 0.5 X) to cover the gel (~ 1 mm).

3. Prepare DNA samples with an appropriate amount of 5X loading buffer (4 µ l PCR product, 1 µ l loading buffer) and load samples into wells with a pipettor. Be sure to include appropriate DNA molecular weight markers.

4. Attach the leads so that the DNA migrates to the anode or positive lead and electrophorese at 100 V/cm gel for 40 min.

5. Turn off the power supply after 40 minutes or when the bromophenol blue dye from The loading buffer has migrated a distance judged sufficient for separation of the DNA fragments.

6. Stain DNA by soaking the gel in ethidium bromide solution (0.5 µ g ml^{-1}) for 30 min.

7. De-stain the gl by soaking in water for 30 min. 8. Photograph the gel on a UV translluminator.