SOP: SP018

Agarose gel electrophoresis

Materials and Reagents:

1. Agarose (161-3102, BioRad) (see note 1)
2. 300 ml 1X TAE buffer (40 mM Tris, 1 mM EDTA, pH 8.0)
3. DNA sample(s)
4. DNA loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 40% sucrose in water)
5. 10 µl DNA ladder (70537-3, Novagen)
6. Ethidium bromide staining solution (0.01g of ethidium bromide in 1 L of MilliQ H_2O) (see note 2)
7. Destaining solution (1 X TAE or MilliQ H_2O)
8. Gel tray and gel caster
9. Gel comb
10. Horizontal electrophoresis unit
11. Horizontal electrophoresis unit lid
12. Power supply
13. Water bath 60°C
14. Glass flask (250 ml)
15. Graduated cylinder (100 ml)
16. UV transilluminator

Protocol:

1.___ Place 0.5 g of agarose in a 250 ml glass flask (see note 3)

2.___ Add 50 ml TAE buffer.

3.___ Dissolve agarose by boiling (see note 4).

4.___ Place flask with the agarose solution in a 60°C in a water bath to cool.

5.___ Set up gel caster by inserting tray into caster bottom.

6.___ Place the comb at one end of the caster such that the comb teeth are pointing down and are ~ 2 mm above the gel tray. down into the caster.

7.___ Pour approximately 20 to 25 ml of the agarose solution into the gel caster.

8.___ Allow agarose gel to cool to room temperature and solidify. The agarose will change from being transparent to being opaque.

9.___ Pour 2 ml of TAE buffer on top of agarose gel.

10.___ Remove the gel comb by carefully lifting it vertically out of the agarose gel.

11.___ Pull the gel tray out of the caster

12.___ Set the agarose gel and gel tray in the horizontal electrophoresis unit.

13.___ Fill the reservoir with TAE buffer until the buffer just covers the agarose gel.

14.___ Mix 5 volumes of DNA sample with 1 volume of DNA loading buffer.

15.___ Load 5-20 µl of DNA sample in each well.

16.__ _Load 10 µl of a DNA ladder in one well.

17.___ Place the lid on the horizontal electrophoresis unit (see note 5)

18.___ Attach electrical wires from the horizontal electrophoresis unit to appropriate slots in power supply (red to red, black to black)

19.___ Set the power supply to 85 V, maximum Amp, and maximum Watts.

20.___ Run gel for ~1 hour, 20 minutes.

22.___ Place gel in ethidium bromide staining solution for 20-40 minutes (see note 2)

23.___ Remove gel from staining solution and place in destaining solution for 30 min to 1 hour.

24.___ Visualize the resolved DNA in the gel with a UV transilluminator (see note 6).

Notes:

1. Other manufactures of agarose may be used, but make sure the agarose is molecular biology grade.
2. Ethidum bromide is a carcinogen. Thus were gloves when working with the ethidum bromide or ethidium bromide solution. The ethidium bromide solution and stained gels must be treated as hazardous waste
3. This protocol is for 1% agarose min gels, but different sizes of DNA require different agarose concentrations and running times/voltages. For further information see the reference.
4. The agarose must be completely dissolved. To ensure that the agarose is dissolved swirl the flask and look for translucent agarose particles. If none are seen then the agarose is completely dissolved. The agarose solution is generally boiled using the microwave with 1 minute heating increments. Do not leave the microwave unattended when boiling the agarose solution. If it is heated too much it will bump and boil over.
5. The horizontal electrophoresis unit lid will have a black (-) and a red (+) electrical wire. Since DNA migrates toward the cathode make sure the lid is place on the horizontal electrophoresis unit so that the red wire is located at the bottom of the gel and the black wire is located at the top of the gel. The top of the gel is the end containing the DNA wells.
6. Always wear UV blocking eyewear when looking at gels with the UV transilluminator.

References:

1) Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual (2nd Edition). pp 6.1-6.23.