

**SOP: SP018.1**  
**Modified 9/1/2023 RAS**

### **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. DNA ladder (70537-3, Novagen)
6. SyberSafe DNA Gel Stain (Invitrogen, S33102)
7. Gel tray and gel caster
8. Gel comb
9. Horizontal electrophoresis unit
10. Horizontal electrophoresis unit lid
11. Power supply
12. Water bath 60°C
13. Glass flask (250 ml)
14. Graduated cylinder (100 ml)
15. UV transilluminator

#### **Protocol:**

1. \_\_\_\_ Place 0.5g of agarose in a 250 ml glass flask (see note 2)
2. \_\_\_\_ Add 50 ml TAE buffer.
3. \_\_\_\_ Dissolve agarose by boiling (see note 3).
4. \_\_\_\_ Place flask with the agarose solution in a 60°C in a water bath to cool. Add 5ul of SybrSafe DNA gel stain.
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 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  $\mu$ l of DNA sample in each well.
16. \_\_\_\_ Load 4-10  $\mu$ l of a DNA ladder in one well.
17. \_\_\_\_ Place the lid on the horizontal electrophoresis unit (see note 4)
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 to 2 hours.
24. \_\_\_\_ Visualize the resolved DNA in the gel with a UV transilluminator (see note 5).

**Notes:**

1. Other manufactures of agarose may be used, but make sure the agarose is molecular biology grade.
2. 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.
3. 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.
4. 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 placed 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.
5. 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 (2<sup>nd</sup> Edition). pp 6.1-6.23.