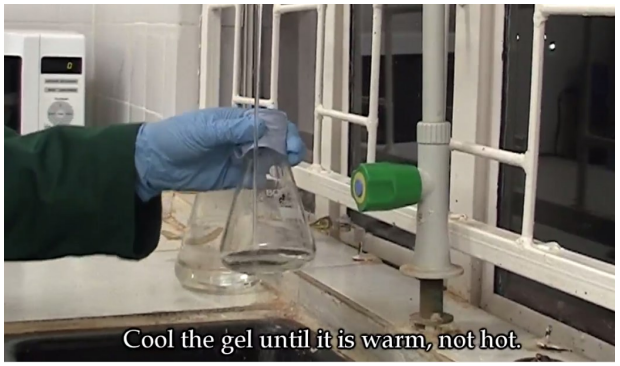
1. Preparation of the agarose gel solution:

WHAT YOU NEED
Agarose
TAE buffer, 50 or 100 bp DNA ladders, DNA loading dye / buffer
Ethidium bromide solution, 10mg/ML solution
Microwave
Gel tank, power supply
Glass flask

Instruction video on KT website in the Resources section: https://www.youtube.com/watch?v=eXkTudcrrr4

1. Choose a glass bottle or conical flask that is 2-4 times the volume of the solution because agarose boils over easily.
2. Sprinkle pre-measured agarose powder onto room temperature 1X buffer and swirl gently to mix. Weigh the bottle or flask to note the initial weight.
3. Heat the container on HIGH power for about 45 seconds or until bubbles appear. ***Caution*** The solution can become superheated and boil over. As you remove the container, give it a gentle swirl, wear gloves and hold at arms length.
4. Reheat on HIGH power until you can see that all the particles are dissolved and the solution is mixed thoroughly.
5. Check the final weight is the same as the initial volume – add distilled water to make up losses.
6. To add ethidium bromide before running your gel:
   Add ethidium bromide to melted agarose to a final concentration of 0.5 µg/mL. Do not melt agarose that already contains ethidium bromide; add it after heating when it is warm, not hot.

2. Pouring the gel:

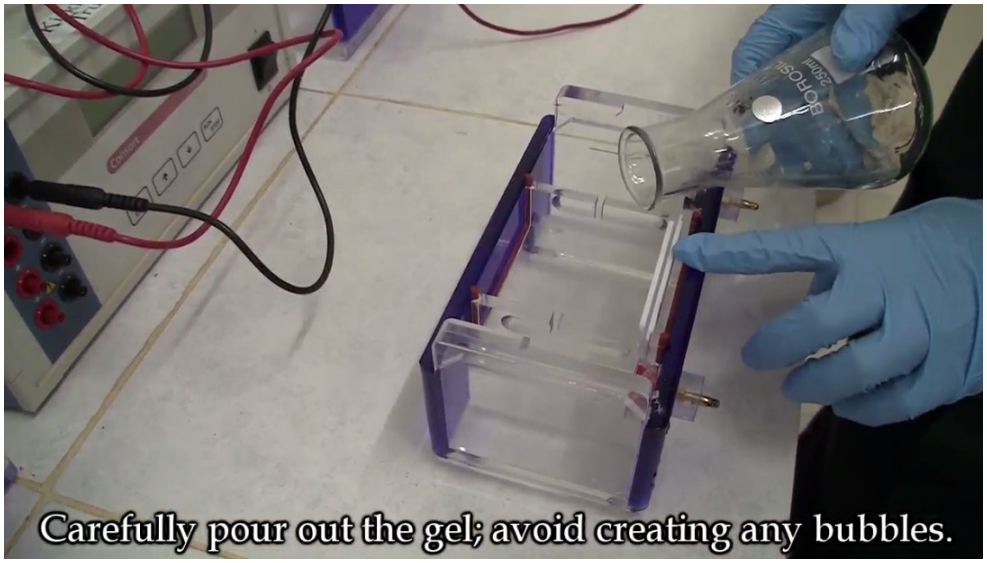


1. **IMPORTANT: Allow the agarose solution to cool to 50-60°C** [1]. If an 'in-gel' staining method is to be used, add the nucleic acid stain and swirl gently to mix thoroughly.
2. Various sizes of the horizontal gel electrophoresis units have been supplied. To cast a gel, the trays do **not** need to be taped. An electronic copy of the instruction manual can be found at www.kirkhousetrust.org, go to 'Resources → Research Resources → Equipment Manuals'.
3. Gel thickness has a profound effect on the resolution of smaller fragments – for optimal resolution cast horizontal gels **3-4 mm thick**.
4. After pouring gel leave to set for 1 hour (with the gel unit lid on if possible).

Note:

[1] **If the agarose solution is hotter than 50-60°C it will cause the Perspex tray to warp when you pour it into the gel tray.**

You can run the base of the flask under cold water to cool the gel mixture until it is hand warm.



3. Loading and running the gel:

1. Add 1 µL of DNA dye loading buffer[1] per 5 µL of sample and mix thoroughly.
2. DNA ladders 50 bp, 100 bp and 1 kb are provided ready-to-use including loading dye

Notes:
[1] Thermo Scientific 6X DNA Loading Dye is supplied in a 1.0 mL vial . Use with samples on agarose or polyacrylamide gels. It contains two dyes, bromophenol blue and xylene cyanol FF, for easy visual tracking of DNA migration during electrophoresis at 6X concentration. Composition 10 mM Tris-HCl (pH 7.6) 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol 60 mM EDTA. Add 1/6 volume of 6X DNA Loading Dye to your DNA sample.

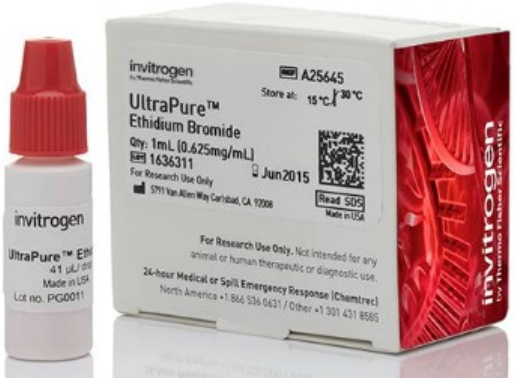
3. Buffer Depth should be 3-5mm.

   For <1 Kb voltage should be 5 V/cm[2].

   Gel should be run until the band of interest has migrated 40-60% down the length of the gel.

   Watch the process on the video—see the link on the Kirkhouse Trust website in the section:

   Resources-Training Videos-Molecular Biology



The Trust now supplies ethidium bromide solution in a dropper bottle. The concentration is 10mg/mL.

One drop is sufficient for up to 50 mL agarose and two drops for up to 100 mL

4. Photographing the gel:

See Gel Photography Protocol — page 9.

Notes:
[2] Power Supply: Voltage which is too high will cause band streaking and smearing, if the voltage is too low, it will cause band broadening due to diffusion.