Gel Electrophoresis(1% Agarose gel)

Materials for preparation of 1% gel:

1. RNase-free tubes and filter tips
2. General purpose agarose, molecular biology grade
3. Tris base
4. Boric acid
5. 0.5 M EDTA
6. Gel loading Die[1]
7. Ethidium bromide (EtBr), stock concentration of 10 mg/mL)
8. Gel electrophoresis casting tray
9. Gel electrophoresis power supply
10. Gel electrophoresis comb
11. UV light source

Methods:

N.B. Here we will show you gel preparation of 160 ml (our tray is hold up to 160 ml gel).

1. For the preparation of 5x TBE buffer: Add 54 g of Tris base, 27.5 g of boric acid and 20 mL of 0.5 M EDTA. Add H_2O until the total volume reaches 1L. [2]
2. Dilute and heat 160 ml 1X of TBE buffer while stirring with magnetic stirrer in a beaker.
3. Add 1.6 g of Agarose to the beaker.
4. Wait until the agarose is melted completely.
5. Add 8 µL of EtBr (approximately 0.2-0.5 µg/mL final concentration) and stir carefully without making bubbles.
6. Put the comb of the tray and pour the gel into the casting tray. Avoid making bubbles.
7. Wait for 25-30 min until the gel solidifies.
8. After the gel is solidified, remove the comb and put the gel it into the Electrophoresis gel box unit. Fill the gel box with the same buffer (TBE) with which you made the gel, until the gel is submerged.
9. Load 8 µL of DNA ladder in the first well (lane) of the gel.
10. Combine 2.5 µL of gel loading die or equivalent with each of your PCR product and put it into the additional wells of the gel.
11. Run the gel at 110-120V for 35-32 min or 200V for 22 mins
12. Turn off the power supply, disconnect the electrodes from the power source, and remove the gel from the gel box.
13. Analyse the DNA fragments in your gel using a UV light source.

N.B. **If some of the bands in the gel shows a desired band, this PCR product is used in the subsequent template generation 2nd PCR.**

Note:

1. Instead of Gel Loading Die you can use 30% Glycerol or the one supplied with the PCR *Taq* polymerase, 10X CoralLoad loading buffer (Quigen, Taq Polymerase core Kit).
2. For more protocols check Cold Spring Harbor Protocols: http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8458.full.