Ministry of Higher Education
King Abdul Aziz University
Faculty of Science
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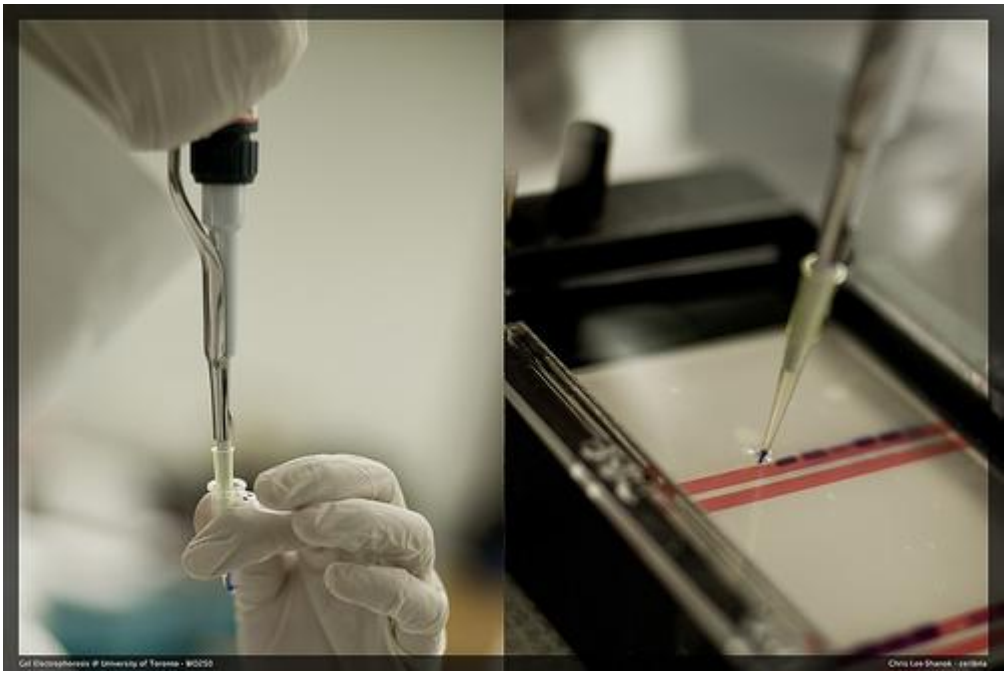
Gel Electrophoresis



Done By
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Materials needed for electrophoresis

1. DNA electrophoresis apparatus.
2. pipette loading tip.
3. 10X loading buffer (blue bromophenol stain).
4. DNA marker (ladder).
5. Beakers
6. Hot plate or microwave.
7. Distilled water.
8. Electronic balance.
9. Agar powder.
10. Spatula(lab spoon) and pasture pipette.
11. Graduated sylender.
12. DNA sample
13. Running buffer **(TBE or TAE)**

How to prepare the following:

1. **Running buffer (TBE or TAE)**

a) **0.5X TBE:**
   a. **add** 5.4 g of Tris base, 2.75 g of boric acid, and 2 ml of 0.5 M EDTA in 1 liter water solution (pH=8.0).

b) **1X TAE:** add 4.84g of Tris base, 1.14ml of glacial acetic acid, and 2ml of 0.5M EDTA in 1 liter water solution (pH=8.0).

✓ **Note, The agarose solvent should be the same with the running buffer.**

2. 6X Sample Loading Buffer

Add 1 ml of sterile H_2O, 1 ml of Glycerol, and (~ 0.05 mg) bromophenol blue powder to make the buffer deep blue

3. The agarose gel

1. Measure 1.25 g Agarose powder and add it to a 500 ml flask.
2. Add 125 ml TAE Buffer to the flask. (the total gel volume well vary depending on the size of the casting tray and the length of desired DNA).

agarose concentration versus optical range of DNA size :

Agarose concentration (%)	**0.3**	**0.6**	**0.7**	**0.9**	**1.2**	**1.5**	**2.0**
DNA (Kb)	5～60	1～20	0.8～10	0.5～7	0.9～6	0.2～3	0.1～2

3. Melt the agarose in a microwave or hot water bath until the solution becomes clear.
   Note, if using a microwave, heat the solution for several short intervals and never let the solution boil for long periods as it may boil out of the flask.
4. Let the solution cool to about 50-55°C, swirling the flask occasionally to cool evenly.
5. Add in 5-10µl of SYBR Green or SYBR gold dye or Ethidium Bromide.
6. Seal the ends of the casting tray with tape.
7. Place the combs in the gel casting tray.
8. Pour the melted agarose solution into the casting tray and let it solidify.
9. After a complete solidification (casting) put the agarose gel in the electrophoresis tank.
10. Carefully pull out the combs and remove the tape.

    Note, gels can be made several days prior to use and sealed in plastic wrap (without combs). If the gel becomes excessively dry, allow it to rehydrate in the buffer within the gel box for a few minutes prior to loading samples.

Agarose Gel Protocol

1. After putting the Prepared solid agar in the electrophoresis chamber. Pour enough running buffer into the electrophoresis tank.
   Note, The surface should be higher than the top of the gel and not overflow).So, that there is about 2-3 mm of buffer over the gel.
2. Add 6 μl of 6X Sample Loading Buffer to each 25 μl PCR reaction, and mix the sample with loading buffer sufficiently. Then, load 20 μl into the sample lane together with the marker.
   Note, Pipette 10 μl of the DNA ladder (usually marker in the first lane). Especially, if you are running multiple gels, avoid later confusion by loading the DNA ladder in different lanes on each gel.
   Note, Record the order of each sample will be loaded on the gel, including who prepared the sample (the DNA template), what organism the DNA came from, controls and ladder.
3. After sample loading, place the lid on the gel box, connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected. Then, turn on the power supply to about 100 volts
4. Set an appropriate voltage and run the electrophoresis. After approximately 35min (80V), 25min (100V), put the agarose gel in an UV detecor and record the picture.
   Note, Maximum allowed voltage will vary depending on the size of the electrophoresis chamber, it should not exceed 5 volts/ cm between electrodes!.
   Note, Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
   Note, Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye, this will take a couple of minutes (it will run in the same direction as the DNA).
5. Let the power run until the blue dye approaches the end of the gel.
6. Turn off the power.
7. Disconnect the wires from the power supply.
8. Remove the lid of the electrophoresis chamber.
9. Using gloves, carefully remove the tray and gel.

Gel Staining and documentation procedure

1. Using gloves, remove the gel from the casting tray and place into the staining dish.
2. Add warmed (50-55°) staining mix.
3. Allow gel to stain for at least 25-30 minutes (the entire gel will become dark blue).
4. Pour off the stain (the stain can be saved for future use).
5. Rinse the gel and staining tray with water to remove residual stain.
6. Fill the tray with warm tap water (50-55°). Change the water several times as it turns blue. Gradually the gel will become lighter, leaving only dark blue DNA bands. Destain completely overnight for best results.
7. View the gel against a white light box or bright surface.
8. Record the data while the gel is fresh, very light bands may be difficult to see with time.

Note, Gels stained with blue stains are stable for long periods. When de-staining is complete, remove gel from water and allow the gel to dehydrate. Dark bands can be seen for in a dried gel for weeks or months.