Agarose Gel Electrophoresis (updated 04/2013)

Generally, the 50 mL (large) gel rig/tray will be used to obtain the most PCR product in order to maximize results. If using the 30 mL gel rig, perform calculations accordingly to prepare a 1.5% gel.

1. 1.5% gel: add 0.75 g agarose powder to a 100 mL Erlenmeyer flask; add 50 mL TAE 1X buffer
2. Boil in microwave at 15-20 sec. intervals for 75-90 sec. (Use orange autoclave glove to handle flask)
3. Place rig in electrophoresis tank; Seal ends of tray with rubber inserts and place comb(s)
4. Add 2.0 µL cybergreen (photo-sensitive) or 1 µL if using 30 mL rig and lightly swirl
5. When solution is warm (not hot), pour into tray; allow setting for 10-15 min.

Add DI H_2O to flask and boil in microwave to remove any remaining gel; waste may be disposed of in sink.

6. Remove combs and rubber inserts
7. Add TAE 1X buffer to tank (may reuse this running buffer for 5 trials) until gel is completely covered
8. Add 5-6 µL DNA marker with dye (dye is contained within master mix); DNA marker acts as ladder for reference
9. Load 6 µL samples into each well
10. Plug in red(+) and blue(-) at ~80 V (73 V optimal) for ~1 hr (lower voltage (60) for longer run-time can be used for better separation of bands)
11. After allowing designated run-time, turn machine off, remove lid.
12. Wipe off UV Tran illuminator
13. Remove tray; slide gel out and place in illuminator
14. Always use protective shield to view
15. Take a picture using Scion image: Special → Start capturing
16. Reuse running buffer x3-5 (mark on container accordingly)
17. Remove gel with spatula and place in hazardous waste