Protocol for Making an Agarose Gel.

For preparation of 0.8% agarose gel

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Amount of 0.5%TBE	Agarose	Gel Red
50 ml	0.4 g	1.5 ul
75 ml	0.6 g	2.25 ul
100ml	0.8 g	3 ul

For preparation of 1.0% agarose gel

Amount of 0.5%TBE	Agarose	Gel Red
50 ml	0.5 g	1.5 ul
75 ml	0.75 g	2.25 ul
100ml	1.0 g	3 ul

For preparation of 2.0% agarose gel

Amount of 0.5%TBE	Agarose	Gel Red
50 ml	1.0 g	1.5 ul
75 ml	1.5 g	2.25 ul
100ml	2.0 g	3 ul

The small orange gel rigs are used to create 50 ml gels.

All others are used to create 75/100 ml gels.

In top right drawer of Gel rig area you will find combs to insert and create wells in your gel. Determine the amount of wells for each gel you create.

If using small orange 50 ml rigs, you can create 6, 10 or 12 wells. If using the large orange, green or black 75/100 ml rigs, you can create 12, 16 or 20

If using the large purple 75 ml rig, you can create 30 or 40 wells.

Gel Prep Protocol

wells.

Example for 0.8% agarose 50ml gel:

- 1. Take a 250 ml flask and fill with 50 ml of 0.5% TBE.
- 2. Add 0.4 g of agarose from upper cabinet in media prep room.
- 3. Swirl the agarose evenly in the liquid.
- 4. Microwave solution for 1 min.
- 5. Swirl 250 ml flask for a few seconds (flask will be hot, use a heat protective glove).
- 6. Microwave solution for 1 more min.

- 7. Take out flask and make sure all agarose has dissolved. (no clear flakes).
- 8. Wearing gloves, open GelRed container from in upper cabinet in media preproom.
- 9. Add 1.5 ul of GelRed to your solution in the 250 ml flask.
- 10. Swirl GelRed into solution.
- 11. Make sure your gel rig has a tray in it (the tray should have rubber seals on either end. To help with correct placing, wet down rubber ends with some 0.5% TBE along with wetting the gel rig walls with 0.5% TBE. The tray should create a seal along sides of gel rigs. Check to make sure the rubber seal has not come loose to prevent leaking.).
- 12. Pour solution into gel tray.
- 13. Add comb into tray for well creation.
- 14. Allow gel solidifying for 20-25 min.
- 15. Remove tray from gel rig and place back in gel rig with wells on top.
- 16. Pull out plastic comb
- 17. Add 0.5% TBE into gel rig until liquid is over gel.
- 18. Load lanes.
- 19. Place lids on gel rigs. Should run black (negative) on top to red (positive) on bottom.
- 20. Set gel machine to 96 volts and start
- 21. Should take 45 min.