Pouring an agarose gel

Introduction

Agarose gels are used to separate DNA fragments. Their most common use is

Materials

- TAE buffer, 50-100 ml
  - There is generally 1X TAE buffer at the iGEM bench in a 1L or 2L bottle.
  - If not, mix some more up from the carboy **10X stock** by the main lab gel station. (Use the 1L graduated cylinder at the gel station.)
- UltraPure agarose
- Casting tray, casting stand, combs

Procedure

Mix and melt the agarose

1. Check to see if there is a gel waiting in the fridge.
2. Determine how much molten agarose you'll need.

   A small gel cast takes 50 ml; a large gel cast is 100 ml.
3. Measure out the appropriate amount of TAE into a glass bottle or flask.
4. Add 5 μl SYBRSafe (0.5X ; stock solution is 10000X) per 100 ml of the solution and mix well.
5. Add UltraPure agarose to a final concentration of 1% (mass / volume)

   So, if you're making 50 ml gel: 0.5 g.
6. Swirl the bottle or flask to distribute the agarose.
7. Heat the solution in the microwave with frequent stirring to dissolve the agarose homogenously. ~30 seconds/100ml solution
8. Let sit until cool enough to handle.

   The agarose MUST cool some -- if it's too hot, it can warp the casting trays.
   Don't allow it to get too cold!
   PAUSE POINT - You can put the flask in the 55°C water bath almost indefinitely at this point.

Pour the gel

9. Set up the gel tray in the casting stand. Make sure the rubber gaskets are flat up against the edges of the casting tray.

10. Set up the gel co mbs to form the wells.

    Rinse the combs with water and wipe dry.
    Note for combs: 15-well combs hold about 6 ul liquid per well, 12-well combs hold about 15 ul per well, 8-well combs hold about 20 ul per well
    Taping two 8-well comb wells together results in a well that holds up to 100 ul
    Taping three 8-well comb wells together result in a well that holds up to 200 ul

11. Pour the molten agarose into the casting tray. d

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    If bubbles form around the combs, remove and re-insert.

12. Wait 30 mintues for the gel to solidify.

13. Use immediately, or place in a plastic zip-lock baggie with a little 1X TAE and store at 4°C.