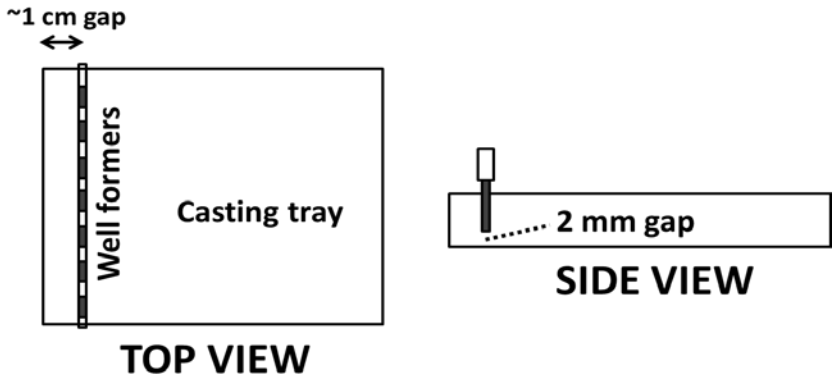
Protocol for making and running agarose gels

1. Figure out how many samples you have, and choose well-formers and gel volume accordingly: the smallest gel tank uses 60 ml agarose and takes up to 10 samples, the largest tank uses 150 ml agarose and takes up to ~40 samples (if two rows of well-formers used). Remember to leave a lane for the molecular weight marker!

2. Decide what agarose concentration and what buffer are appropriate. For many gels, 0.5 x TBE buffer, and 1% agarose will be fine. However, if you are trying to separate very small DNAs (<500 bp), you can increase up to 2% agarose, and if you are trying to separate large DNAs (>5 kb), you can decrease down to 0.7% agarose. Using 1 x TAE instead of 0.5 x TBE will also allow better resolution of large DNAs. If you need to cut out DNA from the gel for cloning or sequencing, you need to use 1 x TAE instead of 0.5 x TBE (borate interferes with later enzyme reactions).

3. Decide whether you need very accurate band-sizing or not. If you do not (e.g. just want to see band or no band when screening clones), you can put the detection dye (GelRed or GelGreen or HydraGreen) into the gel. If you do need high accuracy, instead use post-staining.

4. Set up the gel-casting tray, either by masking-taping the ends of the tray (old black gel box), or placing in the plastic end-formers (newer clear gel boxes). Place the well-former (comb) such that it is level and straight, and sitting ~2 mm above the base of the gel forming unit. It is crucial that the well formers are NOT TOUCHING the casting tray – in that case, your wells will have holes in the bottom and you will lose your samples. See pics. In some cases you can adjust the height of the well formers, in others you can't.



5. Using a small, clean, dry conical flask, weigh out the appropriate amount of agarose powder (NOT AGAR!) for the correct and % agarose gel that you intend to make, then pour in the appropriate volume of TBE or TAE buffer. Put in microwave, and heat on high for 1 minute. Put on heat resistant gloves, then take out to examine whether all the agarose is dissolved. If not, replace for another 30 sec on high power, and repeat examination and heating until all dissolved.

5. Cool the conical flask down under a stream of cold water, while swirling. Be careful not to get tap water INTO the flask. After about 20 seconds, see if you can hold the flask comfortably in your hand (no glove) – if it is too hot to hold, continue cooling, re-check every 20 seconds or so. When the flask feels warm but not uncomfortably hot, it is ready to pour (this is approx. 45-50°C). If you cool it too much, it may set solid. In this case, microwave again (as above) in 30 sec increments to re-dissolve.

6. (if appropriate): add GelRed or GelGreen dye to the gel (use ~ 0.5 µl stock per 50 ml gel).

7. (if using plastic end-formers): using a p-1000 pipette, pipette a thin line of agarose along the bottom edge of the end-formers, where they meet the base of the casting tray – the aim is to seal this crack through which agarose can potentially leak out. Allow 30 sec or so for this agarose seal to set.

8. Pour the molten agarose into the casting tray. Use a single smooth motion, don't stop and start. Stop when the agarose is 3/4 of the height of the 'teeth' on the well-forming comb. Or alternatively, stop when you judge the wells are deep enough to hold the amount of sample that you need to load. What you DON'T want to do is over-fill the agarose so that it goes over the teeth – this will result in a channel that connects all the wells, and subsequent cross-contamination of the samples.

9. Allow the gel to set. This takes about 15 minutes, but there are a few ways to speed this along. If using the black gel tanks you can fill these with buffer (to half-way up the masking tape height on the casting tray), to get faster cooling. OR with either type of gel box, you can set up the whole casting tray / gel box in the cold room, and do the gel-pouring there.

10. When gel is set, pour a little of the appropriate buffer (TBE or TAE) over the top, then carefully pull out the well formers (straight up, don't yank them side to side or forwards and back). The reason for the buffer is to stop the wells collapsing on themselves (this can happen with thin wells at lower % agarose). Then pull out the end-formers or take off the masking tape.

11. Fill the gel tank up with the appropriate buffer so that it fills the reservoirs on both sides of the gel, and so that it *just* covers the gel (by 1-2 mm).

12. Choose the appropriate molecular weight marker. For DNAs <1 kb, use the NEB 100 bp ladder. For DNAs from 1 kb – 10 kb use the NEB 1 kb ladder. For DNA's 10 kb-30 kb, use the lambda/HindIII digest ladder. You may need to load two different ladders, one on each side of the gel. For GelRed staining, load ~ 200 ng ladder (= 8 µl of a 1/20 dilution of stock at 0.5 µg / ml… check this!). For ethidium staining you need about double this much DNA ladder.

13. Prepare a row of spots of blue loading buffer on a Parafilm strip, of appropriate volume and number. This is a 5 x buffer, so e.g. you will need 2 µl spots if you are loading 10 µl samples.

14. Sketch in your labbook a map of which samples will be loaded in which locations. Avoid the first and last wells if possible, these tend not to run as straight as the more central wells.

15. Mix your first sample with the first blue spot, and load into the first well. Steady the pipette tip with the finger of your non-pipetting hand to ensure accurate dispensing. The tip needs to be <u>just inside</u> the well, don't push it all the way down in the well. Change tips, mix up the next sample with blue dye, and load again. Repeat for all samples, including the marker.

17. Put the lid on the gel box, check that the terminals are connected correctly (negative terminal should be closest to the wells, positive terminal is far from the wells, ie. <u>R</u>un towards <u>R</u>ed). Run the gel at ~150 volts (small gel box) up to ~250 V (large gel box). Check that you have current (non-zero milliamps), and gas bubbles at the electrodes (in clear tanks). If not, check all the wire connections. You can run the gels slower than this to get better resolution of bands (maybe!).

18. Stop the gel when the fast-running blue dye (bromophenol blue) (= usually the only blue dye) is near the end of the gel (this may take 30-60 min). You may need to run longer to get good separation of large products (>5 kb). Run for shorter time for small products (<500 bp), or they may run off gel.

19. If you added GelRed to gel, it is now ready to image on the transilluminator (gel can then be discarded in regular garbage bins). Otherwise, post-stain in a solution of GelRed (5 µl per 100 ml water) for 1 hour, or in ethidium bromide (1 mg/L final conc) for 30 min, before transilluminating. Can destain in water to enhance contrast (~half the staining time). Ethidium gels need to be disposed of as hazardous waste. Also note that can't use ethidium on some transilluminators due to lab rules.