Agarose gels for visualisation of nucleic acids

FA lab

Depending on sample number, choose appropriate gel size.

Set up gel caster (mould) (run tape around edge if it might leak) and find appropriate comb.

Can test for leaking, and for appropriate gel volume, using tap water – fill, check for leaks; discard and dry.

Preparing 1% agarose gel:

1. Place correct volume of 1 x TAE buffer and agarose powder in flask.

Cast size	1 x TAE buffer (ml)	Agarose powder * (mol bio grade) (g)	Sybr Safe gel stain (only add AFTER melting)
Smallest cast (for small tank)	50	0.5	5 ul
Medium cast (for large tank)	100	1.0	10 ul
Large cast (for large tank)	150	1.5	15 ul

***** adjust weight of agarose if a 2% gel is required -> this is used for clearer separation of bands

2. Melt agarose in
   a) Microwave; on full for ~ 2 min til boiling
   b) On hot plate, with stirring flea at max temp for ~ 5 mins -> pay attention as can boil dry or spill over top (microwave easier!)

Ensure all agarose is melted (swill around flask and there should be no sugary looking streaks in the gel.

3. Leave to cool *slightly* then add appropriate volume of Sybr Safe gel stain see table.

4. Pour into gel cast; add comb and leave to cool (cover loosely with foil as Sybr safe is light sensitive)

Loading samples:

1. Typically load 5 or 10 ul (depending how much sample you have to spare)
2. Prepare loading dye by pipetting dots of correct vol. onto parafilm or a petridish.
3. Loading dye is x 5 concentrated so if loading
   a. 5ul -> 1ul dye
   b. 10ul -> 2ul dye

4. Once gel set, gently remove comb; rubber casting ends (for big tank gels) or tape but leave gel sitting in plastic mould.
5. Place in correct tank and submerge in 1 x TAE buffer (this can be re-used a couple of times but maybe top up with fresh as can lose buffering ability). Discard after > 5 runs.
6. Pipette up 4ul (or 8ul) sample and add to one dot of loading dye; pipette up and down to mix
7. Increase pipette vol to 5ul (or 10ul) and pipette up all sample+dye.
8. Load into appropriate well (note down sample order)
9. Load 5ul of ladder into first or last well (hyper ladder I for large products; hyper ladder IV for small products)
10. Place lid on ensuring red to red and black to black (gel runs from black -> red so ensure correct orientation)
11. Run small gel tank at 90V and large one at 120V
12. Takes 20 – 30 mins for gel to run; keep an eye on dye front.
13. Turn off power, remove gel from tank (still in plastic mould) and place in tray/pipette rack lid to prevent slipping off into floor -> image in imaging room downstairs
14. Discard gel in autoclave waste & clean cast & comb.