Benchling

UPDATED 9.10.2020 15:57

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Introduction

GEL/EtBr room: take new gloves there and throw them away in the bin next to the door afterwards! Wear glasses!

Materials

- stored in 4°C glass window fridge P_1 38:
  - 2 homemade ladders (100kb and 1kb) neon yellow racket
  - Loading dye

Procedure

Prepare GEL

1. take appropriate sized cast and combs= #wells according to your sample size nr. + 2 for the two ladders
2. seal the ends of the cast with tape, prevents leakage (see picture)
3. from stock in the 60°C oven: take TAE bottle (which has already 1%EtBr in it) and fill cast up to around 1cm height.
4. if there are air bubbles along the combs: pop them with a pipettetip
5. let gel dry for 30 min

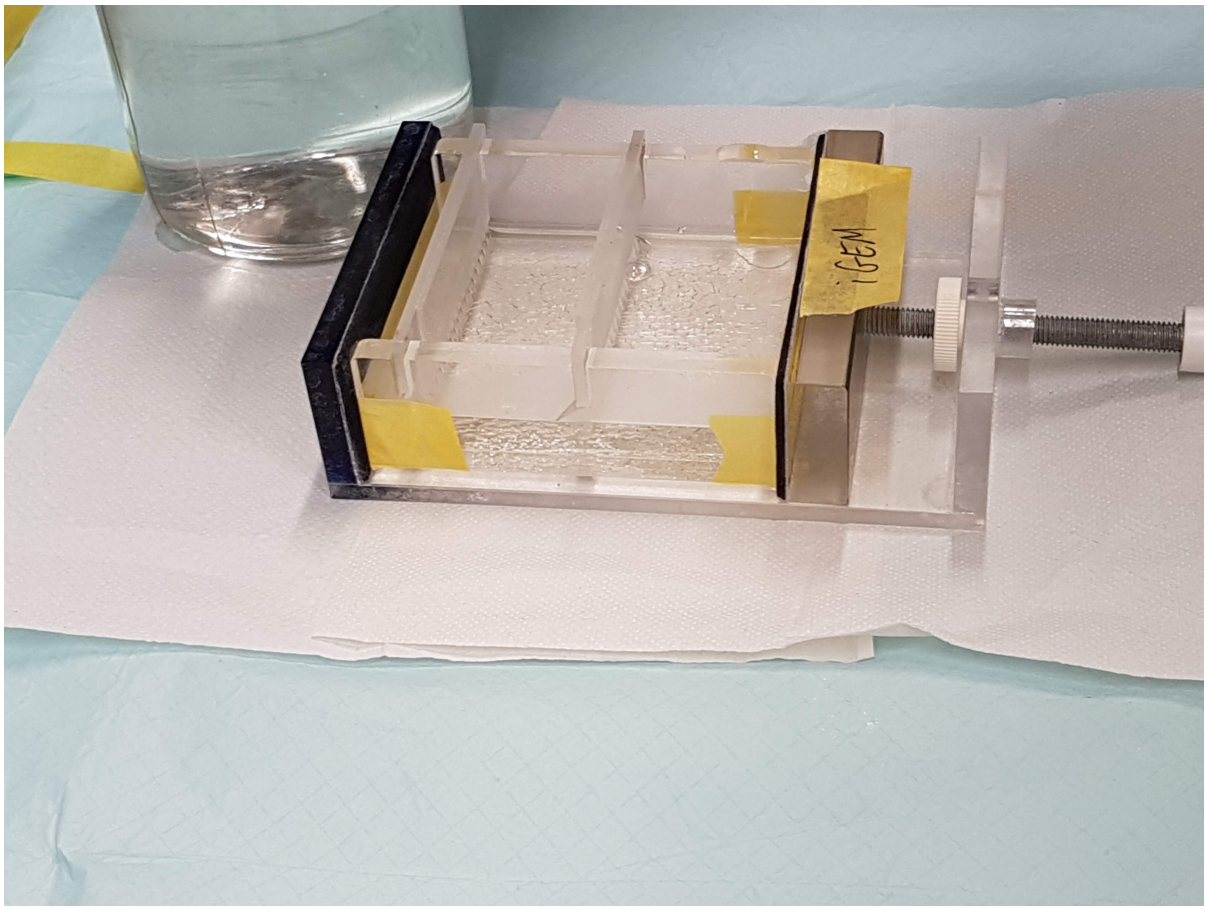
Dye your samples

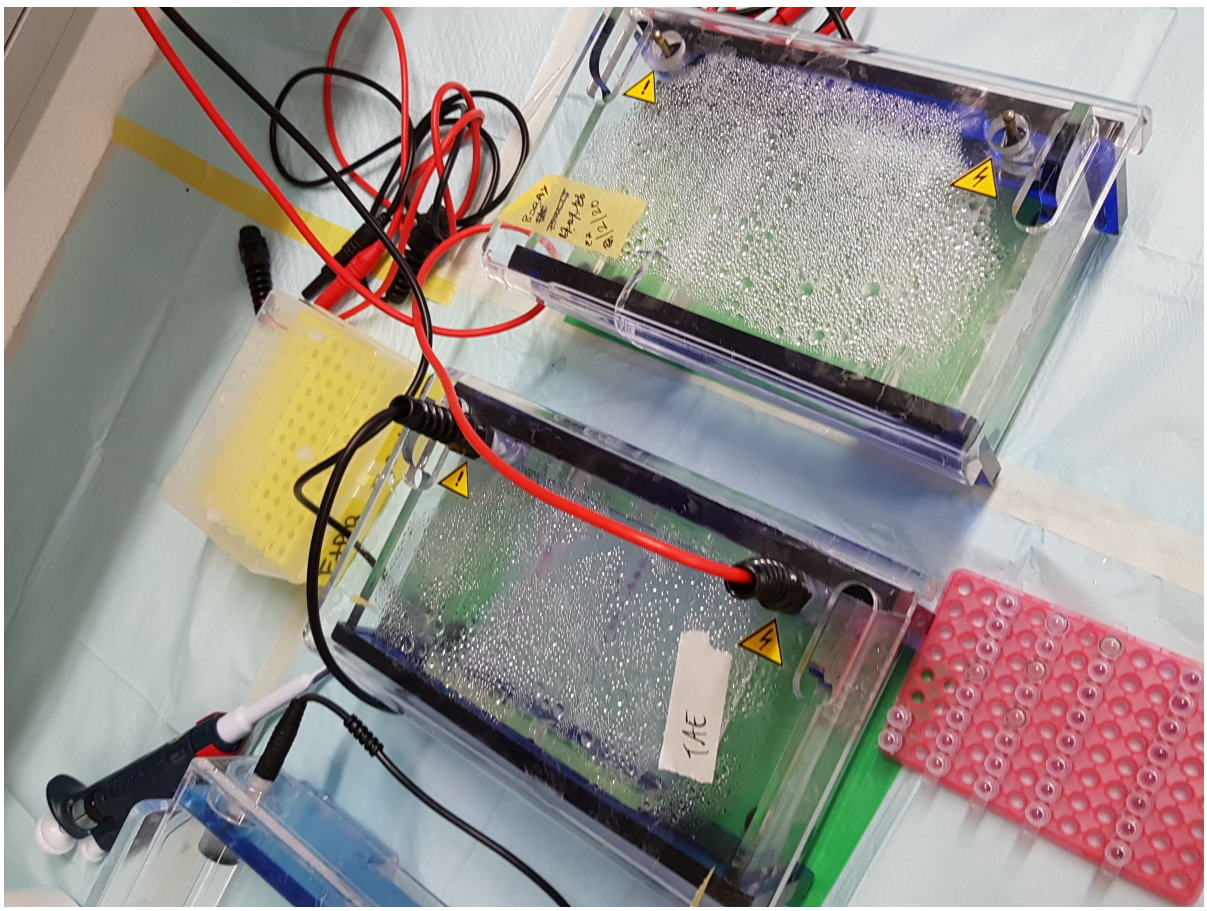
while the gel is drying:

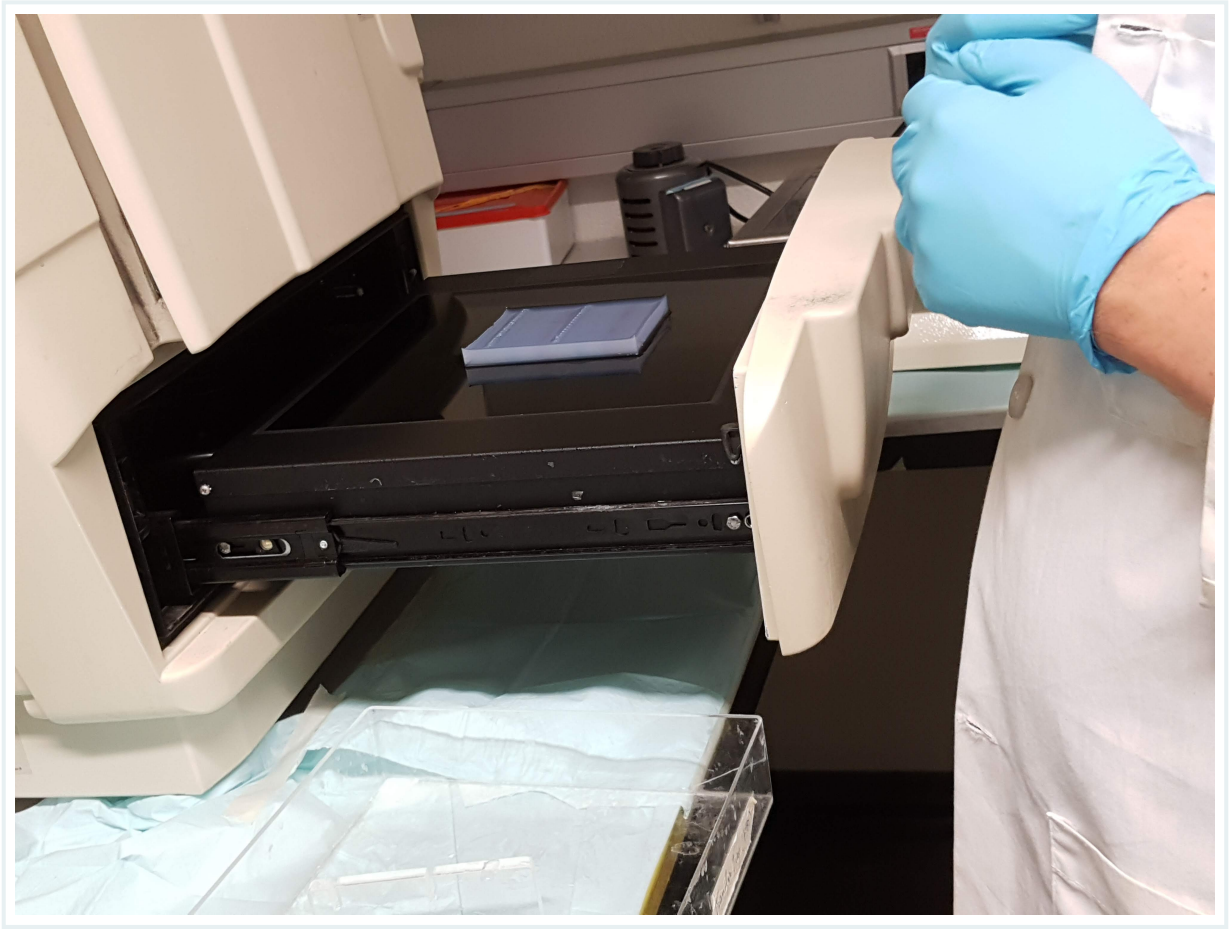
6. **purpe dye is 6 X** concentrated so need to calculate the actual conc. for your sample ml per strip (eg: 34.2 µL per strip / 6 =5.7 µL of purple dye to add to each strip. mix the dye in the tube gently by pipetting up and down.
7. **green dye is 5X** conc so: sampelvolume/ 5 = uL of dye to add

Gel loading

8. take out combs: remove brocken off pieces of gel in pockets with pipette tip if necessary, take away tape, go to electrophoresis chamber must be labeled with the same buffer you used.
9. insert **5µl** ladder 1 in first and 5µl ladder 2 in second pocket (use the gel pipette there)
10. **normal PCR:** insert **7.5 µl** sample into wells (use the gel pipette there). **For colony PCR:** put the whole **10µl** into the gel, as there will be a lot of other bacterial "stuff" in there
11. Plug in the black cable at the top of your gel and the red one at the bottom (the DNA flows towards the red (positive) Electrode
12. set Power source to 90V and let it run for around 30 min (check after 20!) until the dyefront is near the bottom of the gel
13. shut of powersource, unplug, take the gel together with the cast out and take it to the visualizing chamber. Remove the gel and insert it, close the chamber and activate first the white light so that you can place the GEIL correctly, then close it and activate UV light source.
14. afterwards: rinse combs and cast with water and let it dry near the sink, clean benches as usual.







Visualize Gel Results

15. Press Epi White
16. Click on *Live Focus*
17. Click on *wide* and ... for zooming in and out, click on *near* and far for *focus*
18. Press trans UV
19. Click *freeze*
20. Click *auto expose*
21. Transform -> invert -> display
22. go to file and export it as jpg
23. Safe file in igem folder