01_01 Agarose Gel Preparation

DONNERSTAG, 13.5.2021

Goal-Setting

- Preparation of an agarose gel

Terms / abbreviations

- TAE-Buffer = Tris-acetate-EDTA-Buffer
- TBE-Buffer = Tris-borate-EDTA-Buffer

Risk areas

Harzard symbols

Required materials and / or information

- Chemicals:
  - Agarose low EEO 0,09 - 0,13, AppliChem
  - Roti GelStain Red, Roth
  - SYBR Gold, ThermoFisher
  - TAE-Buffer (1x)
    Update 26.05.21: TBE-Buffer (1x)
- Materials:
  - Casting tray
  - Cling film (plastic wrap) to store gels
  - Falcon Tube 50 mL, Sarstedt
  - Heat gloves
  - Pipettes, Eppendorf
  - Schottflask 250 mL, Labsolute
  - Well combs (different sizes available)

Templates, devices, software

- 60 °C cupboard, Memmert
- Fridge
- Microwave

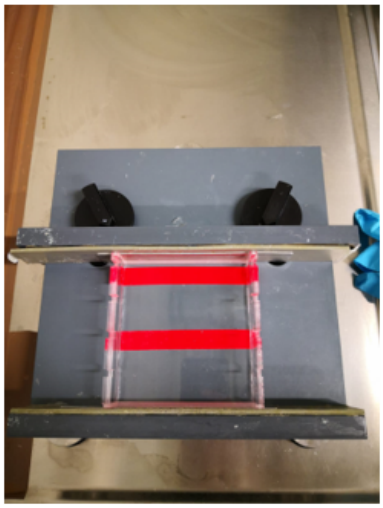
Preliminary work

- 00_03 1x TAE Buffer Recipe
- 00_02 10x TBE Buffer Recipe

Operation

1. Weigh in 1 g of agarose in a 250 mL Schott flask (2% or 3% gels: add 2 or 3 g of agarose respectively)
2. Add 100 mL TAE-Buffer (1x)
   Update 26.05.21: use TBE-Buffer (1x)
3. Mix gently
4. Put agarose mix in the microwave for 1-2 min (keep an eye on the microwave and take it out when it starts to foam!)
   - Put a paper towel under the Schottflask before starting the microwave
5. Store in 60 °C cupboard or use directly
6. Fill the required amount of agarose into a Falcon Tube
7. Add Roti GelStain Red (1.5 µL for 15 mL Agarose, 3 µL for 30 mL and 5 µL for 50 mL) and pour in casting tray
   Update 10.05.21: SYBR Gold post-staining is used --> there is no need to add any stain before casting
8. While the gel is still liquid, create wells with comb
9. Let it solidify until milky-white color
10. If it is not used immediately, please wrap a foil around the gel and store it in the frigde (please add manufacturing date + percent agarose of the gel + "iGEM")

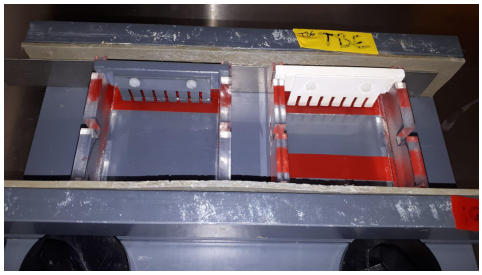
Construction



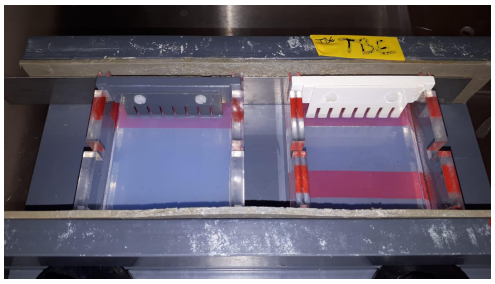
Build-up for pouring a gel

Cupboard

Freshly poured gel



Solidified gel



Disposal

- Buffer can be discarded in black canister on ground next to trash cans in gel electrophoresis room
- Solid gels can be discarded into white cans right next to the door (S1 waste)

Troubleshooting

- The boiled agarose should be completely liquid (Step 4!)
- Do not invert the Falcon Tube, because that will create bubbles. Just shake it a bit (Step 7!)

Follow-up work

- 01_03 Performing Agarose Gel Electrophoresis