02_01_PAA-gel-preparation (denaturating)

SONNTAG, 16.5.2021

Goal-Setting

- Preparing a PAA gel (denaturating) to separate DNA fragments

Terms / abbreviations

- APS = Ammoniumperoxodisulfate
- PAA = Polyacrylamide
- TBE = Tris-Borate-EDTA buffer
- TEMED = Tetramethylethylenediamine

Risk areas

- Health hazard/Hazardous to the ozone layer
- Acrylamide is neurotoxic, always wear gloves!

Hazard symbols

Required materials and / or information

- Chemicals:
  - Acrylamide 29:1; 40% (w/v), Roth
  - APS (10% (w/v))
  - MilliQ water, Sartorius arium pro VF
  - TBE Buffer (10x)
  - TEMED, AppliChem
  - Urea, Roth
- Materials:
  - 0.75 mm gel comb, BioRad
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  - 0.75 mm glass slides, one with edges and one without, BioRad
  - Falcon Tube 50 ml, Sarstedt
  - Grey sponge

Templates, devices, software

- BioRad MINI PROTEAN II Apparatus
- BioRad PROTEAN Tetra Cell

Preliminary work

- 00_01_10%-APS-recipe
- 00_02_10x-TBE-buffer-recipe

Operation

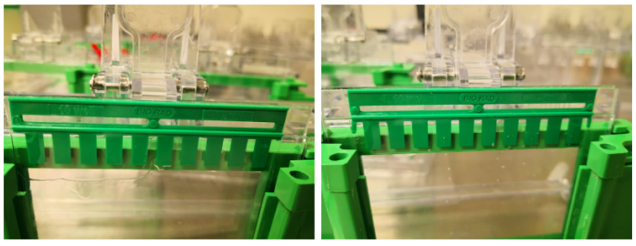
1. Set up the gel chamber by putting the two 0.75 mm glass slides together in the little plastic holder so that a gap between the slides is created
2. Turn down the hand gears so that the glass slides are tight
3. Place the two grey sponges in the big plastic holder
4. Place the little plastic holders along with the glass chambers into the big plastic holder and make sure everything is really tight
    - Test it with a bit of deionized water if the chambers are leak proof
    - Dry the chambers after that with paper!
5. Get all chemicals
6. Dependig on the percentage of the gel, mix the components according to following:

Composition of gel for different acrylamide concentrations

	A	B	C	D
1	**Component**	**5% PAA**	**15% PAA**	**20% PAA**
2	ignore this row -->	0.05	0.15	0.2
3	**40% Acrylamide [mL]**	12.5	37.5	50
4	**10x TBE buffer [mL]**	10.00	10.00	10.00
5	**Urea [g]**	42.00	42.00	42.00
6	**H2O [mL]**	25.00	25.00	25.00
7	Heat the gel in a waterbath at 60 °C until the urea is dissolved			
8	Fill the gel up to 99,5 mL using H2O and mix for 2-3 min			
9	**10% (w/v) APS [mL]**	0.5	0.5	0.5
10	**TEMED [mL]**	0.05	0.05	0.05
11	**total [mL]**	100.05	100.05	100.05

1. Mix the gel by inverting the Falcon Tube
2. APS and TEMED will start the polymerisation, so make sure that the gel chambers are ready, leakproof and dry
    - In order to get rid of air bubbles, the schottflask can be put into an UltraSound Device for a few seconds for degassing, but keep in mind that the schottflask is contaminated once it was used in the gel electrophoresis room
3. Fill up the chambers to the rim of the small glass slide
4. Stick the comb into the chamber and avoid bubble formation

Chamber with (left) and without (right) air bubbles



1. Wait until the solution has polymerised,
    - The solidity can be proofed with the rest of the solution in the Falcon Tube
    - This usually takes some time (~45 min)

2. Take the little plastic holder out of the big one and take out the glass slides
3. Gently remove the comb (not recommended if the gel should be stored)
4. If the gel is not used directly, wrap it in wet paper towels als put it in a plastic bag
5. Write down the date and the percentage of the gel and store at 4 °C in the fridge in electrophoresis room

Disposal

- TBE Buffer can be discarded in sink with a lot of water

Troubleshooting

- Use fresh APS
    - APS catalyzes the polymerization of acrylamide
    - Using old APS or APS stored above -20 °C will result in slow or incomplete polymerization
    - Keep small, fresh aliquots in the freezer

Follow-up work

- 02_02_Performing-PAA-gel-electrophoresis