02_02_Performing-PAA-gel-electrophoresis

FREITAG, 14.5.2021

Goal-Setting

- Performing PAA gel electrophoresis

Terms / abbreviations

- EDTA = Ethylenediaminetetraacetatic acid
- PAA = Polyacrylamide
- TBE = Tris-borate-EDTA

Risk areas

Hazard symbols

Required materials and / or information

- 1x TBE-Buffer (approx. 1.5 L)
- GeneRuler Ultra Low Range DNA Ladder, ThermoFisher
- PAA Gel
- Samples

Templates, devices, software

- BioRad MINI PROTEAN II Apparatus
- BioRad PROTEAN Tetra Cell
- Gel electrophoresis Power Supply, Consort

Preliminary work

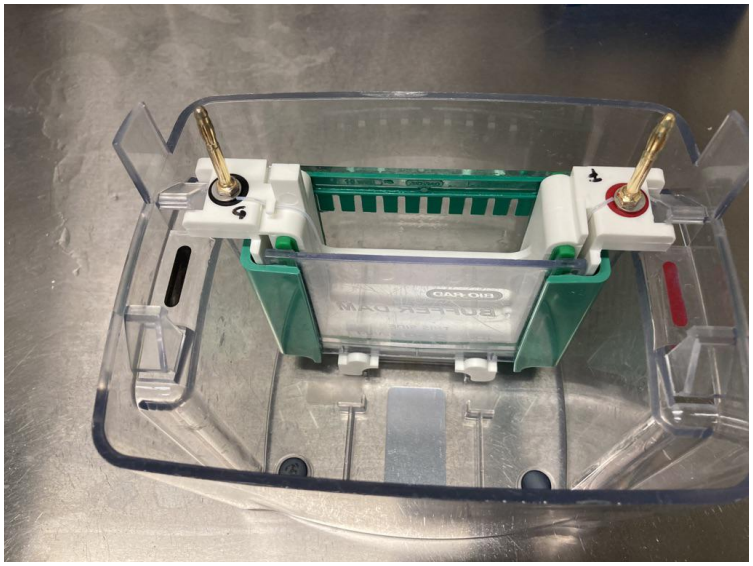
- 00_02_10x-TBE-buffer-recipe
- 02_01_PAA-gel-preparation (native)
- 02_01_PAA-gel-preparation (denaturating)
- 01_02_Sample-preparation-for-gel-electrophoresis

Operation

1. Take the gel out of the fridge and set it up in the BioRad MINI PROTEAN II Apparatus (two gel brackets à two gels fit into one apparatus)
    a. Place the small glass slides inwards
    b. Use a bracket with connective plugs!
2. Fill first the bracket, then the whole chamber up to the line with 1x TBE buffer
    a. It is important to use the same batch of electrophoresis buffer in both of the reservoirs and in the gel
    b. Small differences in ionic strength or pH produce buffer fronts that can greatly distort the migration of DNA
    c. If the bracket is filled first, leaks can be seen
    d. If leaks should be avoided completely, fill up the whole chamber up to the level of the inner bracket

    e. Make sure to create two different "rooms". The water level should not be equal!
3. Pipette 5 µL of the ladder and desired amount of prepared samples into a distinct well
    a. Be careful not to disrupt the gel; make sure the sample sinks into the well
4. Place the lid properly onto the gel electrophoresis chamber, the electrodes have to correctly connect with the BioRad MINI PROTEAN II Apparatus (see image)

BioRad MINI PROTEAN II Apparatus without lid



1. Attach the electrode cables to the Consort gel electrophoresis Power Supply, turn it on and set the right conditions
    a. Set device to desired voltage
    b. Set Watt and Ampere to max
    c. Set time
    d. Start
2. Perform gel electrophoresis at 100 V for 1 h
3. When finished, take out the BioRad MINI PROTEAN II and pour the used TBE buffer back into a schottflask (mark the number of uses of the buffer on the schottflask)
4. When finished, clean all used parts under water and place in the rack to dry

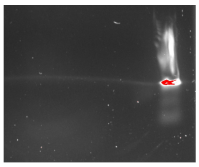
Disposal

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

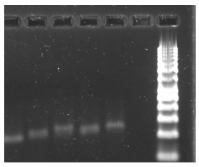
Troubleshooting

- It is important to use the same batch of electrophoresis buffer in both of the reservoirs and in the gel (Step 2!)
- For any problems use google to search for 'gel electrophoresis troubleshooting'
- Blurry bands? Too much DNA or excess salt will create smeared bands and/or streaking in the gel. Loading the correct amount of DNA (usually a maximum of 100–250 ng/mm well width) and desalting samples with a spin column prior to loading will prevent this
- Loading buffer floats away? Rinsing wells with running buffer just before loading is essential; failure to do so may prevent the loading mixture from sinking to the bottom of the well, resulting in an uneven band and delayed migration

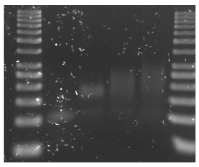
Gel electrophoresis - Troubleshooting



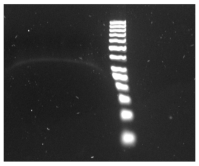
Wrong buffer
Buffer in gel and running buffer have to be from the same batch



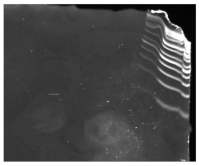
Smeared bands
Overloading



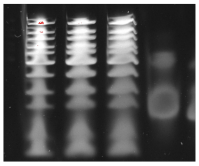
Dust particles
Wipe the surface with ethanole first



Distorted ladder
Components of TdT reaction distort the running of the ladder



Application mistake



Voltage too high

Follow-up work

- 02_04_SYBR-Gold-staining
- 02_03_Silver-staining-DNA-in-PAA-gels