

01_03 Performing Agarose Gel Electrophoresis

DONNERSTAG, 13.5.2021

Goal-Setting

- Separation of DNA fragments of different length by gel electrophoresis

Terms / abbreviations

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

Risk areas

 Hazard symbols



Required materials and / or information

- Chemicals:
 - Agarose gel
 - Prepared samples
 - SYBR Gold DNA Gel Stain, ThermoFisher
 - TAE-Buffer (1x)
 - **Update 26.05.21:** TBE-Buffer (1x)
 - Thermo Scientific GeneRuler 50 bp DNA Ladder, ThermoFisher
- Materials:
 - Electrophoresis chamber,
 - Pipettes, Eppendorf

Templates, devices, software

- Gel electrophoresis Power Supply, Consort

Preliminary work

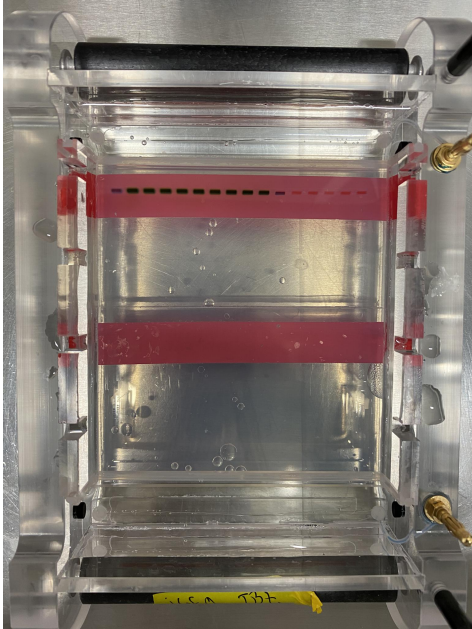
- [00_03 1x TAE Buffer Recipe](#)
- [00_02 10x TBE Buffer Recipe](#)
- [01_01 Agarose Gel Preparation](#)
- [01_02 Sample Preparation for Gel Electrophoresis](#)

Operation

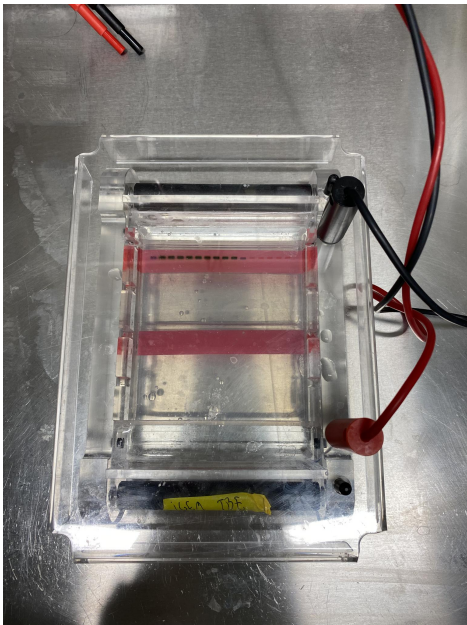
1. Fill electrophoresis chamber with 1x TAE buffer
 - **Update 26.05.21:** Fill electrophoresis chamber with 1x TBE-Buffer
 - a. It is important to use the same batch of electrophoresis buffer in the reservoirs and in the gel
 - b. Small differences in ionic strength or pH will result in buffer fronts that can greatly affect DNA migration

2. Place the tray with solid gel into the chamber
3. Remove the well comb (use both hands and take it out evenly)
4. Add additional buffer, so it covers the gel slightly
5. Fill 5 μL of desired DNA Ladder into the first gel pocket
Update 07.05.21: Use only 2 μL Ladder as SYBR Gold is very sensitive
6. Add all prepared samples into the gel pockets, in an appropriate volume (usually 10 μL)
7. Electrophoresis conditions: 90 V, until the dye runs to 3/4 of the gel (for 30 mL Gel, 2.5% agarose, fresh buffer; if there was a run before a shorter runtime can be used)

 Loaded gel in chamber



 Chamber with cover



Chamber connected to device



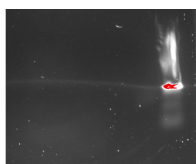
Disposal

- TAE / TBE 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

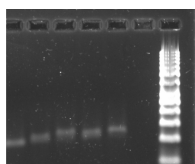
- Chambers with TAE that are already standing on the table can also be used (Step 2!)
- **Update 26.05.21:** The TBE buffer should not be used more than two times. If fresh buffer is used, leave it in the chamber; if the buffer in the chamber is used, discard it afterwards
- If the ampere does not go up or the samples run badly, using new buffer should be considered (Step 7!)

Gel electrophoresis - Troubleshooting



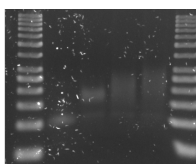
Wrong buffer

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



Smeared bands

Overloading



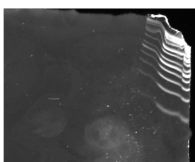
Dust particles

Wipe the surface with ethanol first

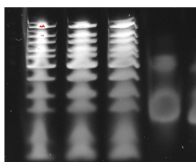


Distorted ladder

Components of TdT reaction distort the running of the ladder



Application mistake



Voltage too high

Follow-up work

- [04_07 Documentation with BioRad Photographer](#)
- [02_04 SYBR Gold Staining](#)