Polyacrylamide Gel Electrophoresis & Silver Staining

This process instruction characterizes the standard processing of a polyacrylamide gel electrophoresis and the consecutive silver staining. This instruction permanently ensures a constant quality of the analysis of PCR products.

Description

In a polyacrylamide gel electrophoresis nucleic acids are separated according to their size and charge, using a gel matrix in an electric field. To interprete obtained results a DNA-standard is carried along. For visualizing the separated DNA-fragments a silver staining with silver nitrate ($AgNO_3$) has to be done.

Materials

ELECTROPHORESIS

- Electrophoresis chamber including 8% Polyacrylamide gel cassette
- Power supply
- Pipette 10 µL, 100 µL, 200 µL, 1000 µL
- Powder-free gloves

SILVER STAINING

- Staining vessel
- Shaker
- Light screen
- Digital camera

Chemicals

ELECTROPHORESIS

Running buffer 5 x TBE

Working solution 1x TBE:

400 mL 5 x TBE

1600 mL deionized H2O

DNA-standard (100 bp DNA Ladder, Fermentas, G210A), -20 °C

Working solution:

25 µL DNA standard ladder

100 µL 6 x Orange Loading Dye Solution

400 µL H_2O for preparing DNA-standard mix

Loading Dye: 6 x Orange Loading Dye Solution (Fermentas, R0631), -20 °C

Working solution:

200 µL 6x Orange Loading Dye solution

800 µL H_2O

SILVER STAINING

Silver nitrate solution (4-8 °C)

Stock solution 10%:

1 g silver nitrate in 10 mL deionized water

Make aliquots of 0.5 ml each and store at 4-8 °C in the dark

Working solution 0.1%:

1 time re-usable (store at 4-8°C in the dark)

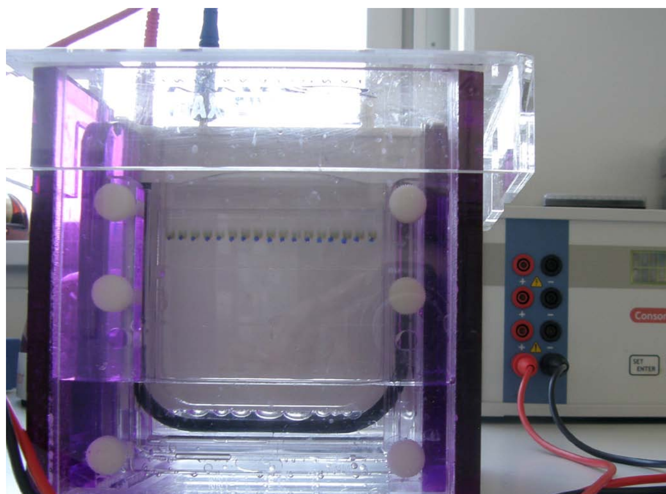
0.5 mL stock solution in 50 mL deionized water

Developer solution

Working solution, use only one time:

Dissolve 3 NaOH drops in 50 mL deionized water, add 150 µL formaldehyde

ATTENTION! Formaldehyde is toxic. Work carefully and wear gloves, laboratory coat and safety glasses. Formaldehyde contaminated reagents and materials have to be collect and disposed by hazardous waste.



Polyacrylamid gel electrophoresis

Preparation

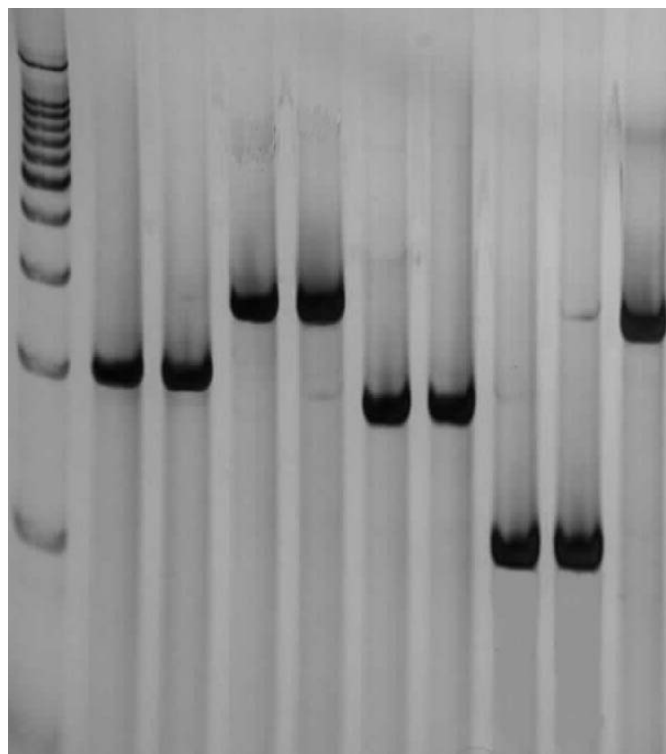
- Take gel out of storage vessel (1% TBE)
- Remove the tape strip, maybe also free the gap from remaining glue
- Insert precisely one gel and one plastic dummy or 2 gels respectively on each site of the cassette (comb to the inside) and tighten steady
- Place cassette in the chamber (contacts at the same site)
- Fill the inner part of the system totally and the outer part with about ¼ running buffer (gap of the cassette must be covered with buffer)
- Remove the comb carefully, remove bubbles using a syringe

Sample Loading

- Add 4 µL Loading Dye mix onto each PCR reaction on the AmpliGrid. Due to the physical conditions, the aqueous solutions will merge immediately.
- Load 3.5 – 4 µL of each and of DNA standard mix respectively each in one well
- Close the chamber with cover and connect it with the power supply
- Pay attention to the right poles at the power supply
- Run the gel at 250V for approximately 30 min

Silver Staining

- Add silver nitrate solution
- Let shake 5 min at 150 rpm if using fresh silver nitrate working solution and 10 min for 1-time used solution
- In the meantime prepare developer solution
- Remove silver nitrate solution for storage in the refrigerator (4-8°C) or into hazardous waste (containing heavy metal and formaldehyde)
- Wash gel one time with deionized water
- Remove wash water into hazardous waste
- Add developer solution
- Let shake 10-15 min at 150 rpm until desired intensity of bands is reached and remove developer into hazardous waste
- Wash gel two times with deionized water and discard wash water into hazardous waste
- Store gel in deionized water
- Take a photo of the gel on the light screen with the digital camera



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