02_03 Silver Staining DNA in PAA Gels

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Goal-Setting

- Post-electrophoresis staining of a PAA gel in a sensitive silver stain

Terms / abbreviations

- PAA = Polyacrylamide
- STP = Sodium thiosulphate pentahydrate
- RT = Room temperature

Risk areas

- Wear acetic gloves when dealing with acids
- Mix solutions under fume hood

Hazard symbols

Required materials and / or information

- Chemicals:
  - Acetic acid CH_3COOH, Carl Roth
  - Formaldehyde CH_2O, Merck
  - MilliQ water, Sartorius arium pro VF
  - Silver nitrate $AgNO_3$, Merck in toxic cabinet!
  - Sodium thiosulphate pentahydrate $Na_2S_2O_3$, Sigma-Aldrich
  - Sodium Carbonate Na_2CO_3, AppliChem
- Materials:
  - Six 250 mL glass bottles, Labsolute
  - Waste bottles (if needed)
  - Measuring cylinder
  - Spatula
  - Plastic staining tray

Templates, devices, software

- Analysis balance, Kern ABJ 220-4NM
- Ice bath or fridge
- Platform rocker, Heidolph DUOMAX 1030

Preliminary work

- 02_02 Performing PAA Gel Electrophoresis
- 01_03 Performing Agarose Gel Electrophoresis

Operation

Preparation of all solutions

1. Fixer solution
    a. Dilute 7.5% glacial acetic acid with deionized water (4.5 mL in 60 mL water)
    b. Storage at RT
    c. Caution: Avoid inhaling the vapor
2. Formaldehyde solution
    a. 9 mL formaldehyde is added with 51 mL deionized water to 60 mL
    b. Solution must be made fresh!
    c. Formaldehyde is inactivated when cooling, so only use at RT
    d. Caution: Components of this solution are toxic, handle and dispose of with care
3. Silver solution
    a. Dissolve 0.06 g silver nitrate in 60 mL deionized water
    b. Solution must be made fresh!
4. STP stock solution
    a. Dissolve 0,312 g STP in 50 mL water
    b. Solution must be made fresh!
5. Developer solution
    a. Dissolve 1.8 g sodium carbonate in 60 mL deionized water
    b. Solution must made fresh and used at 8 °C
6. Developer stop solution
    a. Dilute 7.5% glacial acetic acid with deionized water (4.5 mL in 60 mL water)
    b. Storage at RT
    c. Caution: Avoid inhaling the vapor

Nucleic acid fixation

1. Place the gel into the staining tray
2. Add fixer solution and rock on a platform for 5-10 min
    a. Longer times for thicker gels up to 30 min
    b. Fixation is important to immobilize the DNA molecules in the acrylamide gel matrix to avoid diffusion and subsequent image blurring.
3. Carefully decant the solution, taking care not to damage the gel or touch the gel surface
4. To wash the gel, pour MilliQ water to cover the gel, rock the staining tray for 2 min
5. Decant solution and wash for two more times (total 3 washes)

Formaldehyde pre-treatment

1. Add formaldehyde solution to the gel and rock for 5-10 min
    a. For thicker gels use up to 30 min
    b. Formaldehyde pre-treatment is important for stain sensitivity and maximum image contrast
2. Decant solution

Silver impregnation

1. Add sufficient silver solution to cover the gel in the staining tray to a depth of ~5 mm
2. Gently rock the staining tray continuously on a platform rocker for 20 min
    a. The recommended silver concentration cannot be reduced without affecting sensitivity and contrast. A careful examination of silver impregnation times showed that optimal staining was achieved after ~20 min. However, as little as 10 min is sufficient for high-quality staining without significant loss of sensitivity. Impregnation times can be increased up to ~60 min, butgreater than ~90 min can cause severe image loss.
3. Following silver impregnation, carefully decant the solution, taking care not to damage the gel or touch the gel surface

    a. Caution: The silver solution is toxic and should be disposed of with care. Avoid spilling the solution, as it will permanently stain most surfaces.
4. Rinse residual silver solution from the surface of the gel by rinsing with ~100 mL of deionized water for 5-10 seconds
    a. Do not rinse the gel longer than 15 s, as this step removes silver from the gel

Image development

1. Add STP stock solution at rate of 50 µL per 100 mL to the developer solution
    a. Cool this mix by putting it into a 4 °C refrigerator before usage
    b. Low temperature slows the kinetics of development and avoids overdevelopment
2. Add sufficient developer solution to cover the gel in the staining tray and rock for 3 min
3. Decant the developer carefully

Stopping the image development

1. Add sufficient developer stop solution to cover the gel in the staining tray to a depth of ~5 mm.
    a. The developer stop solution must be cooled to 4 °C before usage
2. Allow the gel to sit in developer stop solution for 5-10 min
3. Decant the developer stop solution and rinse the gel with deionized water
4. Photograph the gel
5. Store the gel (optional)
    a. Polyacrylamide gels can be hung from a corner using an 'alligator clip' to dry overnight
    b. Store the dried gel in a commercially available 'zip-lock' plastic bag.

Disposal

- Observe all federal, state and local environmental regulations
- Here: Formaldehyde solution must be disposed in black canister in electrophoresis room
- Here: Silver solution must be disposed in the iAMB

Troubleshooting

- Decreasing Na_2CO_3 concentration below the recommended levels causes higher background staining and poor image contrast. Poor staining can also result from the use of low quality or old (stale) reagents.
- The developer stop solution contains 7.5% CH_3COOH. Higher CH_3COOH concentrations can cause image fading and should be avoided.

Sources

- Jeffery, E. D. (2007). Method for full protein sequence mapping: Lc-ms sample preparation. Protocol Exchange. Advance online publication. https://doi.org/10.1038/nprot.2007.33

Follow-up work

- 04_07 Documentation with BioRad Photographer