Silver Staining SDS Gels

- Remove the stacking gel before the first fixative step.
- Do not touch the gel near samples: handle the gel by the corners.
- Use 100 mls of volume for a mini-gel. Scale up for larger gels.
- Transfer the gel to a fresh tray where indicated. Recycle trays after rinsing them with water. Otherwise add fresh solution after pouring off the used solution.
- Optional, for frequent silver staining: make up solutions as 10X stocks ahead of time.

1. Fix in 50% methanol, 10% acetic acid for 1 hr (to overnight) with one change fixative solution.

2. Wash the gel 3 X 20 minutes with 10% ethanol, 5% acetic acid.

3. Place gel in 100 mls ddH_2O containing 0.1 g $K_2Cr_2O_7$ and 20 µl of HNO_3 for 6 min. Fresh tray.

4. Rinse the gel 4 X 1 minute with ddH_2O. Make sure each rinse flows under the gel.

5. Place the gel in a solution of 0.1 g $AgNO_3$ in 50 ml ddH_2O. Fresh tray. Gently agitate for 15 minutes. Replace silver nitrate solution in same tray and gently agitate for 15 minutes again.

6. Rinse the gel 2 X 30 seconds with ddH_2O.

7. Transfer the gel to a fresh tray containing developer solution. Make up 300 mls of developer with 9 g Na_2CO_3 and 150 µl 37% formaldehyde solution (formalin). [Instead of Na_2CO_3, 24 g of $Na_2CO_3(H_2O)_{10}$ may be used.] Soak the gel in 100 mls of the solution for 30 seconds with vigorous agitation. Discard the developer, and add 100 mls of fresh developer and agitate again for 30 seconds. Repeat the change of developer solution once again. Agitate gently until the desired staining intensity is obtained. Avoid very high staining intensities.

8. Place the gel in a fresh tray containing 1% acetic acid and agitate for 2 minutes (no longer).

9. Place the gel in a fresh tray and rinse many times with ddH_2O. Gel can be stored briefly in ddH_2O.

10. Lighten the background staining as follows: Prepare the photographic reducing solutions A and B below. Add 5 mls of B to 100 mls of water and mix. Add 5 mls of A and mix. Pour this immediately into a fresh tray and transfer the gel to this tray. Gently agitate the gel in the lightening solution for only a brief period of time: 1-2 min. The lightening will continue after the gel is removed from the solution. Remove the gel from the lightening solution before the desired amount of lightening has taken place. Transfer the gel to a fresh tray containing water and rinse the gel MANY times to remove the lightening reagents. The lightening step may be repeated. It is better to repeat the lightening procedure than to lighten the gel too much with one lightening step. The second or third lightening step will lighten the gel much more rapidly than the first.

    Photographic Reducer A:
    1 g cupric sulfate ($5H_2O$)
    1 g NaCl
    Stir into 20 mls water.
    Titrate DROPWISE with NH_4OH while stirring. The solution will become cloudy white, then cloudy blue, and then clear deep blue. The endpoint is the clear deep blue color. About 1-2 mls of NH_4OH will be required. Adjust to 25 mls with ddH_2O.

    Photographic Reducer B:
    12 g $Na_2S_2O_3$
    Stir solid into water.
    Adjust to 25 mls.

11. Soak the gel in 2% glycerol for 15 minutes and dry.