

### Notes

1. Wear gloves!
2. Thoroughly clean staining container with detergent and ddH<sub>2</sub>O before use (alternatively, you could use a large, i.e. 145mm, Petri dish)
3. All solutions should generously cover gel; mix on rotating platform
4. To have a most-clear background, it's best to fix (i.e. rinse) the gel O/N

### Staining

1. Wash staining container with detergent and ddH<sub>2</sub>O for 1 2 hours. Rinse thoroughly with ddH<sub>2</sub>O and wear gloves.
2. Rinse gel in Silver Fix once and then fix gel 2 x 15' using Silver Fix (or ON and fresh Silver Fix for 10' in morning).
3. Reducer (+100mg NaThiosulfate) for >30'.
4. Wash ddH<sub>2</sub>O 3 x 5'.
5. Silver Solution (+25µl formaldehyde stock) for 20'.
6. Develop gel: add 125µl formaldehyde to the solution; rinse gel briefly with ddH<sub>2</sub>O, pour in ~100ml Developer, swirl around, decant the Developer and replace with remaining ~150 ml Developer. Swirl in hands until desired bands appear.
7. Stop development by pipeting in 1.5 ml Glacial Acetic Acid and mixing (should bubble vigorously). Mix for 5'.
8. Wash 3 x 5' ddH<sub>2</sub>O (important or gel will stain yellow).

### Solutions

#### *Silver Fix*

- 600 ml ddH<sub>2</sub>O
- 300 ml ethanol
- 100 ml acetic acid (glacial)

#### *Reducer*

- 67 ml H<sub>2</sub>O
- 30 ml ethanol
- 3.3 ml 3M Na-Acetate pH 5.2 (molecular biology stock)
- just before use add 100 mg Na-thiosulfate

#### *Silver Solution*

- 100 ml ddH<sub>2</sub>O
- 100 mg AgNO<sub>3</sub>
- just before use add 25 µl 37% formaldehyde stock

## *Silver Staining*

### *Developer*

- 250 ml ddH<sub>2</sub>O
- 6.25g Na<sub>2</sub>CO<sub>3</sub>
- just before use add 125 µl 37% formaldehyde stock

### *Glacial Acetic Acid*

### *1 Liter ddH<sub>2</sub>O*