**Silver Stain Protocol**

**Solutions:**

Solution 1: 1% glutaraldehyde in water (make fresh, need 5mL for 1 gel, prepare in fume hood)

Solution 2: 0.15g Silver Nitrate in 94.3mL water, dissolve then add 0.7 mL ammonia and mix (enough for 2 gels, prepare in fume hood)

Solution 3: Sodium Hydroxide, 1.5g in 100mL water (use stock)

Solution 4: Citric Acid, 200mg in 100mL water (use stock)

Solution 5: Formaldehyde, 2mL of 37% stock in 100mL water (make fresh, need 0.625mL per gel, prepare in fume hood)

**Glutaraldehyde & formaldehyde are toxic.**

**Procedure:**

(Volumes are for 1 gel)

Dilute your regular molecular weight marker 10X and load only 1uL of diluted marker

Use a clean gel box

1. Fix: after the gel has finished running, fix in destain solution (27mL methanol + 6mL HACO + 27mL water) for **at least 20 minutes**. Do not microwave. Longer than 20 minutes is fine.
2. Wash: many changes of MilliQ water, **15 minutes**
3. Pretreat:
   1. Pretreat solution: 20mL methanol + 5mL ethanol + 22.5mL water + 2.5 mL Solution 1
   2. Discard last change of water, add pretreat solution, gentle agitation for **20 minutes**
4. Silver Stain:
   1. Silver solution: just before use, add 2.5mL solution 3 to 50mL of Solution 2 and mix (some ammonia will release, so do this in the hood)
   2. Discard pretreat solution, add silver stain, gentle agitation for **14 minutes**
5. Wash: several changes of water, **5 minutes**
6. Develop:
   1. Developer solution: 47.5mL water + 0.625mL Solution 4 + 0.625mL Solution 5
   2. Discard last change of water, add developer solution to gels, swirl until the bands have come up, but the background has not (**2-10 minutes**), **immediately quench with water**
7. Wash several times with water, **5-10 minutes**
8. Store in destain solution until ready to dry, equilibrate gels with water before drying

**Notes:**

Use MilliQ for every step involving water

Do not use a beaker

Don’t touch the gel after fixing