**Salivary Sheath Staining Protocol**

-**Chemicals:** dH2O, 95% Ethanol, 99% Acetic Acid, 85% Lactic Acid, Glycerol, Acid Fuchsin

-**Glassware** for stock solutions, Petri dishes and histological cassettes for leaves, pipettors

-**Leaf** section that a bug has fed on

-**Wear gloves, goggles, lab coat, and apron. Acids burn things.**

1) Prepare McBride’s staining solution:

*-*Calculate necessary volume of solution (8 ml x number of samples)

-Mix 50 ml Ethanol with 100 ml of Acetic Acid (1:2 ratio)

-Dissolve 0.002 g of Acid Fuchsin in 100 ml of distilled water (0.2 % aqueous solution)

-Add 5 milliliters of the Acid Fuchsin to each 100 ml of Ethanol/Acetic Acid solution

2) Staining: place leaflet(s) inside cassettes, label petri dishes (date and trial #)

-Submerge cassettes with McBride’s stain solution (~8 ml per sample)

-Cover and leave **48-72 hours**

-If leaf veins appear pink, transfer to clearing solution

-If solution has evaporated, add more McBride’s stain

3) Prepare clearing solution

-Mix 50 ml Glycerol and 50 ml Water to 100 ml Lactic Acid (1:1:2 ratio)

-Add Mix solution thoroughly before using

 4) Clearing: gently blot leaf samples and submerge in clearing solution

-Cook in oven at 90-100 ℃ until samples are transparent

**Slide Preparation**

-**Chemicals:** Demineralized H2O, Polyvinyl Alcohol, Glycerol, Tris buffer powder

**-Equipment: w**ater bath, pH Meter, drying over, gloves

-**Glassware** for stock solutions, cleaned and labelled glass slides, coverslips

-**Cleared Leaf Tissue**

1) Prepare stock 0.0995 M Tris buffer solution:

-Add 2.42 g Tris powder to 95 ml demineralized water

-Add 3.0 ml of 1 M HCl to solution

-Using a pH meter, continue adding drops of 1 M HCl until pH is 8.5

-Add demineralized water until total volume is 100 ml

2) Prepare stock Polyvinyl Alcohol-Glycerin solution:

- Mix 3g Glycerol with 1.2 g Polyvinyl Alcohol until dissolved

-Add 3 ml of distilled water

-Add 6 ml of Tris buffer solution

-Heat container in 50℃ water bath until completely dissolved, leave overnight

3) Gently take leaflets out of clearing solution and blot dry

4) Add a small drop of PVA-G to slide, place leaf on top

-Add another drop to top of leaf then gently put coverslip on leaf (droplet side down) at an angle to avoid bubbles

5) Heat slides at 50-70 ºC to dry, seal coverslip with nail polish.

-Store slides flat