

## **Penstemon Phenotyping**

End result:

- 3 flowers with the following:
  1. 4 photographs
  2. Nectar volume & nectar concentration information
  3. ½ flower (with staminode & 1 long stamen) + carpel in EtOH in tornado tube
  4. 2 short stamens with nectary in 1.5 mL tube in fridge
- 3 flowers in envelopes with silica gel for anthocyanin extractions
- 3 flowers in EtOH with the corolla intact; carpel removed
- 1 bud in the -80 freezer; 10 mm bud

## **Nectar + photographed flowers**

1. Write out sticky note for photographs and write plant and flower information in the notebook.
2. Cut flower off after the sepals on the plant.
3. Check that flower is open and without any deformities/mutations. Do not use any with spray damage. If you find that there are abnormalities when dissecting open the flower, delete any images and nectar measurements and discard the flower.
4. Take out sepals and carpel from the back of the flower.
5. Remove any excess nectar with a microcapillary tube from the back of the corolla. Set aside.
6. Take photos of the top, side, and front of the flower.
7. Dissect open flower by ripping between the top 2 petals, and the bottom 3 petals.
8. Remove any remaining nectar from on top of the stamens or underneath. Be sure to remove nectar from the surface of the nectary.
9. Measure the amount of nectar present in the flower and record.
10. Remove anthers from stamens and remove the sepals from the carpel.
11. Lay the dissected floral organs so they are flat and photograph.
12. Measure nectar concentration with the refractometer using all of the nectar present. Be sure to check the cleanliness before measuring the first time of the day. Clean with a kimwipe and milliQ after each measurement.
  - a. Note: if you cannot get a reading due to low volume, you can add an equal volume of milliQ water then do the math to calculate the percentage.
13. Place ½ of corolla (with staminode attached) as well as 1 long stamen (preferably attached to the corolla) and the carpel with stigma into tornado tube with EtOH. When 3 flowers have been completed for the plant, will be put into fridge (-80 room).
14. Place both short stamens with nectary tissue into 1.5 mL tube with EtOH. Place in fridge (lab).
15. Mark off that the flower has been completed (checkmark + FV) and that the tissues have been placed in EtOH. Discard any remaining flower parts.