**Aliquot Protocols for Astrangia Poculata Isotope Samples**

Adapted from Putnam lab protocols by T. Lindsay

*\*This protocol replaces PBS with DI to reduce salts to improve Isotope methodology\**

Materials

* 3 x 1.5 mL microcentrifuge tubes per sample
* Centrifuge
* Homogenizer
* 1000 uL Pipet and tips
* DI type II
* 2 Disposal containers (for tips and excess supernatant)

Protocol

1. Thaw samples.
2. Label three 1.5-mL microcentrifuge tubes for each sample.
   1. Side of tube:

* Project name
* Full Sample Name
* One tube each: “CHL”, “SYM”, and “HOST”
  1. On lid:
     + Sample Number
     + One tube each: “CHL”, “SYM”, and “HOST”

1. Homogenize the tissue until there are no visible chunks.
2. Pipette 1mL to each of the CHL and SYM tubes.
3. Return leftover sample to freezer.
4. Centrifuge both 1.5 mL tubes at 13,000g for 3min.
5. From the CHL and SYM tubes, pipet off the supernatant into the HOST tube (fill tube until full then discard any remaining liquid).
6. To the symbiont pellet from the SYM tube, add 1 mL DI type II to resuspend pellet. Pipet up and down to fully dissolve pellet.
7. Place the three tubes in their respective freezer boxes and store at -80˚C until use.

A diagram of a test tube

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