

## **New Protocol for Non-Coriaceous Leaf Tissue Preparation**

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1. Take out your sample packets and line them up in order
2. Label 1.5 mL tubes
3. Place open tube in foam holder
4. Place tube and holder on scale
5. Tare scale
6. Use tweezers to pick out a leaf strip from open packet
7. Place strip in tube
8. Repeat steps 6 and 7 until scale reads 0.05 g
9. Seal packet and place in extraction samples bag
10. Use large needle's head to shatter leaf strips into small flecks (~1 to 3 mm)
11. Open bead tube
12. Pour 3 beads into a spoontula
13. Use spoontula to guide the 3 beads into the tube
14. Shut tube cap
15. Invert tube and bang cap end on counter to dislodge flecks from tube apex and to evenly distribute beads
16. Repeat steps 2 to 15 for each sample
17. Place samples into TissueLyser
18. Run TissueLyser for 60 seconds at 50 osc/sec
19. Check tubes to see if their contents are pulverized, if any tubes are ready take them out to reduce change of tube breakage
20. Rebalance remaining tubes
21. Repeat steps 18 to 20 until all tube contents are pulverized, if a tube still has large flecks and very little dust after 5 minutes of bead beating, reclassify that one as 'coriaceous' and use mortar and pestle to grind tissue

## How To Clean Metal Beads

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1. Vigorously tap used bead tubes on cap ends until all beads are dislodged from apices
2. Empty contents into large beaker
3. Repeat for all used tubes
4. Place beaker under tap spigot
5. Slowly turn on the hot water until it is at  $\frac{3}{4}$  full flow
6. Let water run until all leaf bits/goop float out of beaker (~2 minutes)
7. Turn off water
8. Slowly pour out water without losing beads (you can use a sieve if you want)
9. Add bleach solution until beads are covered
10. Swirl beads
11. Let beads sit for 30 seconds in the bleach solution
12. Place beaker under tap spigot
13. Slowly turn on the hot water until it is at  $\frac{3}{4}$  full flow
14. Let water run for 30 seconds
15. Slowly pour out water without losing beads
16. Add ethanol to beads until they are covered
17. Swirl beads
18. Slowly pour out water without losing beads (you can use a sieve if you want)
19. Place clean beads in beaker on a shelf to dry out (the sun makes them dry out faster, you could also use the hot plate if you're in a hurry, but be careful to not start a fire)
20. Once beads are dry, tap beaker to dislodge beads from bottom if they are stuck and then pour them into the bead tube