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This SOP supersedes:	Approved:

Extraction of Plant Samples: Chlamydomonas

1. References:

- Lee, DoYup, Fiehn O (2008) High quality metabolomic data for *Chlamydomonas reinhardtii*. In: Plant Methods, 2008, 4:7.

2. Starting material:

- *Chlamydomonas reinhardtii*, 1mL lyophilized cell suspensions

3. Equipment:

- Eppendorf pipettes: 1-200µL and 100-1000µL
- Eppendorf tubes 2.0mL, uncoloured (Cat. No. 022363204)
- MiniVortexer, VWR.
- Speed vacuum concentration system (Labconco Centrivap cold trap)
- Ball mill MM 301 (Retsch corp.)
- Metal balls (Retsch 3 mm I.D. Cat. No 22.455.0002 or 5 mm I.D. Cat. No 22.455.0003)
- Large tweezers
- Dewar cold gloves
- Liquid nitrogen dewars
- pH paper 5-10 (EMD Chem. Inc.)
- Crushed ice
- Nitrogen line with pipette tip

4. Chemicals

- Methanol, LCMS grade
- Chloroform, HPLC grade
- Liquid nitrogen
- Water, Millipore (pure)

5. Preparation of extraction mix and material

1. Check the pH of methanol (pH 7)
2. Make the extraction solution by mixing methanol, chloroform, and water in proportions of 10 : 3 : 1
3. Rinse the extraction solution for 5 min with nitrogen, making sure that the nitrogen line was flushed out of air before using it for degassing the extraction solvent solution.

6. Homogenisation and extraction

1. Add one metal ball to each Eppendorf tube, close, and place in liquid nitrogen.
2. Take the Eppendorf tubes from the liquid nitrogen and place into the tube-holder of the grinder being careful to compensate for weight, maintaining equilibrium.
3. Shake for 30s at a frequency of 25 s-1 and check that the leaves have been ground into a fine powder. Repeat if necessary, submerging in liquid nitrogen first.
4. After grinding add 500µL of pre-chilled extraction solution to each tube one by one to prevent even partial thawing of the sample. Store all samples on ice while finishing this step.
5. Vortex the sample for 20s.
6. Centrifuge for 3min at 14,000 rcf using the centrifuge Eppendorf 5415 D.
7. Remove the whole supernatant into a clean Eppendorf tube.
8. Add 800µL of extraction solvent back into the tube with the pellet and metal ball.
9. Vortex the sample again for 20s.
10. Centrifuge for 3min at 14,000 rcf using the centrifuge Eppendorf 5415 D.
11. Remove the whole supernatant and combine with the previous supernatant.
12. Dry in the Labconco Centrivap cold trap concentrator to complete dryness and submit for derivitization.

7. Problems

To prevent contamination disposable material is used. Control pH from extraction mix.

8. Quality assurance

For each sequence perform one blank negative control by applying the total procedure (i.e. all materials and plastic ware) without biological sample. Also perform all tasks once samples/standards have come to room temperature. Finally, include a pure MSTFA vial for liner conditioning steps.

9. Disposal of waste

Collect all chemicals in appropriate bottles and follow the disposal rules.