Bulk Samples Preparation for Extraction

Bulk fractions need to be prepared before DNA extractions. Sessile fractions need to be homogenized and weighed; motile fractions need to be decanted in order to remove sediments and weighed.

We have standardized the amount of material to be extracted to:

- 10-g for the sessile fraction
- Half of the total weight after decantation for the two motile fractions

1- Sessile fraction preparation:

Materials:

- Falcon tubes containing sessile fraction
- 50-mL sterile Falcon tubes
- Cleaned and bleached spatula or sterile disposable spatulas
- Electronic scale
- Marker
- Parafilm

Protocol:

1. Wear gloves at all times. Carefully clean bench station and pipettors.
2. Centrifuge 2 of the 3 falcon tubes containing the sessile fraction at 2,500 rcf during 10 min.
3. Transfer the DMSO into a new falcon tube.
4. Homogenize the material by stirring it with a sterile spatula and transfer 10-g. into a new falcon tube for extraction.
5. Proceed to extraction (See DNA Extraction document) or store the weighed fraction topping up the matter with the DMSO removed in step 2 and freeze at -20°C until ready for extraction.

2- Motile fractions preparation:

The preparation is identical for the 106µm-500µm and the 500µm-2 mm fractions at the exception of the sieves used. We use a 45µm sieve to decant the 106µm-500µm fraction and a 100µm sieve for the 500µm-2mm fraction.

Materials:

- Tubes containing the motile bulk fraction to be decanted
- 1-Liter Erlenmeyer flask
- 45-µm and 100-µm sieves (for 106µm-500µm and the 500µm-2mm fractions respectively)
- 50-mL Falcon Tubes
- Mortar and pestle
- 10% Bleach solution in a 15-L bucket
- Source of UV light
- Source of DI water
- Parafilm
- Cleaned and bleached or sterile disposable spatulas
- Electronic scale
- 95% ethanol

Protocol:

1. Wear gloves at all time and work in a clean space.
2. Clean the flask, sieves, and mortar and pestle (20 min in the bleach solution, rinse thoroughly with DI water and if available put 20 min under UV light).
3. Empty motile fraction into the flask and fill up to 2/3 with DI water.
4. Place parafilm over the top of the flask and shake vigorously while holding the parafilm in place. Immediately pour the water out onto the appropriate sieve in order to remove all floating organic particles.
5. Fill the flask again to 2/3 with DI water and repeat step 4 several times.
6. Check to make sure all organic matter is removed from the sample. You may want to fill the flask and swirl the water to see if floating materials remain. If so, repeat step 4 until you are left with only sediments. Set the flask containing the sediments aside.
7. Use DI water to rinse the organic matter in the sieve and push it all to one side. Then use a spatula to collect all matter and place it into a clean, pre-weighed tube.
8. Using the scale, weigh the tube and organic matter.

9. Using the spatula, split the matter evenly between two tubes. If the weight of the matter exceeds 20 grams, split it evenly between an even number of clean tubes (so that half of the tubes will be extracted and half of the tubes are kept as a back-up). The content of any tube should not exceed 10 grams.
10. Fill the back-up tubes with 95% ethanol, label and seal with parafilm. Store in the freezer at -20ºC.
11. For each "extraction" tube, empty the material into the mortar and use a small amount of ethanol to rinse the remnants from the tube into the mortar. Grind the sample with the pestle until the sample is well homogenized. Return the ground sample to its tube by rinsing it out of the mortar with 95% ethanol. Label the tube and seal with parafilm. Store in freezer at -20ºC until ready for the extraction.
12. Repeat step 11 for any remaining "extraction" tubes for this sample.
13. Empty the sediments from the flask onto the sieve. Use DI water to rinse the sediments and push them to one side. Use the spatula to collect all of the sediments and transfer them to a tube (or multiple tubes to keep them less than half full; may reuse original sample tubes). Label, seal with parafilm and store in freezer at -20ºC for further examination.
14. Dispose of the spatula and repeat step 1 & 2 before starting the next sample.

Illustrations:



Steps 3 to 6: Organic matter is removed from original sample through decantation.



Step 7 to 11: Organic matter is weighed and half is grinded for DNA extraction.