ARMS Processing – Plate scraping

 Make sure that all the containers and tools used have been bleached and rinsed between ARMS processing. Wear gloves at all times; do not touch the water or the plates with bare skin.

During this step, all sessile communities will be bulk collected from all the ARMS plates to assess their overall taxonomic diversity using next-generation sequencing. Bulk samples must be treated with care as to not introduce exogenous DNA in these fractions.

Materials:

- Laboratory gloves
- Clean photo tray
- Clean sharp paint scrapers
- 1 squeeze bottle containing filtered seawater
- 1 squeeze bottle containing 90% ethanol
- 1 food blender capable of blending ice
- 1 40-microns nytex mesh net
- 1 waste bucket
- 3 50 ml falcon tubes (6 tubes if 2 preservatives are used DMSO + ethanol)
- 2 Spatulas (bleached or disposable)
- 150 ml Salt saturated DMSO buffer (1L d'EDTA, 0.5L de DMSO, 0.5L sterile H2O, Sodium Chloride until saturation) (optional: 150 ml 90% ethanol).
- 1 2-Liters whirlpack or bigger depending on the overall volume of the sessile community
- 1 pencil
- 1 marker
- Laboratory tape
- Parafilm

Procedure:

1. Using gloves, place the first plate in the clean photo tray and scrape all sides (including the fine borders) into the photo tray
2. If needed, use the squeeze bottle to quickly rinse off the plate with filtered seawater. Only use a small quantity of water
3. Repeat step 1 and 2 for all the plates. If several persons are scraping, 2 photo trays can be used

4. Transfer the content of the tray carefully into the blender and blend the sessile fraction at full strength for 15 seconds or until the fraction is well homogenized
5. Pour the blended fraction into the mesh net on top of the waste bucket and wash several times with filtered seawater. The matter may need to be stir to let the water go through
6. Squeeze the liquid through the mesh to dry the fraction as much as possible.
7. Transfer the dried fraction back into the photo tray and mix to homogenize with the spatula
8. Place ~10 ml of matter into each of the three falcon tubes and fill with DMSO. Shake vigorously to homogenize
9. Label the tubes and close with parafilm. Place in the freezer
10. If possible, place another ~10 ml of matter into three additional falcon tubes and fill with 90% ethanol. Shake vigorously to homogenize
11. Label the tubes and close with parafilm. Place in the freezer
12. The remaining matter can be dried immediately and weighed for biomass quantification and/or put into a whirpack bag and kept frozen until further analysis

Illustrations:

All sides of the plates are scraped into a clean photo tray

The matter is blended until well homogenized

The blended fraction is poured into a mesh rinsed and dried out

The fraction is placed back into the tray

Subsamples are preserved