Aquatic, sediment, soil and fecal sample cryopreservation for microbial single cell genomics, using glyTE

Field sample collection

Materials

1. Cooler with crushed ice, or water with floating ice packs. Please do not use dry ice or ice packs in direct contact with the sample containers, to prevent cell damage.
2. 50 mL, sterile tubes, 1 per field sample.
3. Permanent marker to label sample tubes.

Protocol

1. Label 50 mL tubes with a permanent marker
2. For aquatic samples, fill 50 mL tubes with sample, place on ice and transport to the lab, maintaining cold but not frozen. Process as soon as possible.
3. For sediment, soil or fecal samples, transfer about 5 g sample to a 50 mL tube, place on ice and transport to the lab, maintaining it cold but not frozen. Process as soon as possible.

Sample cryopreservation

Materials

1. Sterile cryovials, at least 3 per field sample.
2. Pipettors for 1 mL and 100 uL.
3. Vortex (for sediment and soil samples only).
4. Sterile-filtered buffer with similar salinity to field samples. For freshwater sediment and soil samples of regular salinity, please use 1x PBS. For marine samples, please use sterile-filtered seawater.
5. glyTE cryoprotectant stock.
6. Permanent marker to label vials.
7. Either liquid nitrogen or -80C freezer.

Protocol, aquatic samples

1. Label sample cryovials using a high-quality, permanent marker or freezer-compatible adhesive labels. Please make sure the label is legible and contains sufficient information to identify the PI and the sample by an external facility. Prepare at least 3 replicate vials for each sample.
2. Transfer 1 mL sample to each cryovial.
3. Add 100 µL glyTE stock to each cryovial.
4. Mix gently, incubate for ~1 minute at ambient temperature.
5. Store in liquid nitrogen or at -80ºC.

Protocol, sediment, soil and fecal samples

1. Label sample cryovials with a permanent marker or freezer-compatible adhesive labels. Please make sure the label is legible and contains sufficient information to identify the PI and the sample by an external facility. Prepare at least 3 replicate vials for each sample.
2. Add about 30 mL sterile sample buffer (e.g. PBS or sterile seawater, depending on sample salinity) to 50 mL tubes containing field samples.
3. Vortex for 30 seconds at high speed.
4. Centrifuge for 30 s at 2,000 rpm, to remove large particles.
5. Transfer 1 mL supernatant to each cryovial.
6. Add 100 µL glyTE stock to each cryovial
7. Mix gently, incubate for ~1 minute at ambient temperature.
8. Store in liquid nitrogen or at -80ºC.

glyTE stock recipe

1. Mix the following:
    a. 20 mL 100x TE pH 8.0
    b. 60 mL deionized water
    c. 100 mL molecular-grade glycerol (use a syringe)
2. Pass the glyTE stock through a 0.2 micrometer pore size filter
3. Store at -20ºC.

Important notes

1. Cells should not be exposed to significant changes in ionic strength (salinity) during sample processing.
2. Quick sample handling prior to cryopreservation reduces the risk of DNA degradation.
3. This method is not applicable to hypersaline samples, due to salt crystal formation during freezing. Please consult with SCGC personnel for best ways to accommodate hypersaline samples.
4. When ready to send samples to SCGC for analysis, please:
    a. Complete and return a sample request form.
    b. Notify SCGC of your intent to ship so we can coordinate receipt.
    c. Ship frozen (cryopreserved) samples on dry ice [*and for sediment samples, please also send an additional package of cold (unprocessed) sediment samples*]. For additional shipping instructions, please see www.bigelow.org/scgc.

Updated by Ramunas Stepanauskas, July 8, 2016