Microbial probing with RedoxSensor Green for single cell genomics

Recommended protocol

Materials

1. Equipment and consumables for collecting and handling field samples under *in situ* conditions (varies by environment)
2. Sterile cryovials, at least 6 per field sample.
3. Pipettors for 1 mL, 100 µL and 1 µL.
4. glyTE cryoprotectant stock (see below).
5. RedoxSensor Green (RSG) stock, Thermo Fisher Scientific catalogue #B34954. Wrapped in foil to minimize exposure to light.
6. Permanent marker to label cryovials.
7. Liquid ultracold freezer, nitrogen dewar or dry ice.
8. Sterile 50 mL tubes (sediment and soil samples only).
9. Vortex (sediment and soil samples only).

Protocol, aquatic samples

1. All sample handling, including incubations with RSG, should be done immediately after sample collection and in a way that minimizes changes in the microbial environment, such as temperature, oxygenation, salinity, nutrient concentrations, light, etc.
2. 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. If using printed labels or tape, make sure to apply labels 24 hours in advance, to prevent dislocation of labels during freezing, and confirm label attachment prior to filling cryovials.
3. Transfer 1 mL sample aliquots to six cryovials.
4. Add 100 µL glyTE stock to three of these vials, mix gently, incubate for 1 min, then place in liquid nitrogen, ultracold freezer or on dry ice. These aliquots can be used for RSG negative controls and for analyses that do not depend on cell viability, such as staining with SYTO-9 or other DNA dye.
5. Add 1 µL RSG stock solution to the remaining three vials, mix gently. Shield RSG and samples with RSG from direct light.
6. Incubate in the dark under *in situ* conditions for 30 min.
7. Add 100 µL glyTE stock to RSG vials, mix gently, incubate for 1 min, then place in liquid nitrogen, ultracold freezer or on dry ice.
8. Store in liquid nitrogen or at -80ºC.

Protocol, sediment, soil and fecal samples

1. All sample handling, including incubations with RSG, should be done immediately after sample collection and in a way that minimizes changes in the microbial environment, such as temperature, oxygenation, salinity, nutrient concentrations, light, etc.
2. 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. If using printed labels or tape, make

sure to apply labels 24 hours in advance, to prevent dislocation of labels during freezing, and confirm label attachment prior to filling cryovials.
3. Transfer about 5 g of the sample to a 50 mL tube. Add 30 mL sterile aquatic medium that mimics the environment of the analyzed microorganisms.
4. Vortex for 30 seconds at high speed.
5. To remove large particles, let them settle and flocculate, or centrifuge briefly at 2,000 rpm.
6. Transfer 1 mL supernatant aliquots to six cryovials.
7. Add 100 µL glyTE stock to three of these vials, mix gently, incubate for 1 min, then place in liquid nitrogen, ultracold freezer or on dry ice. These aliquots can be used for RSG negative controls and for analyses that do not depend on cell viability, such as staining with SYTO-9 or other DNA dye.
8. Add 1 µL RSG stock solution to the remaining three vials, mix gently. Shield RSG and samples with RSG from direct light.
9. Incubate in the dark under *in situ* conditions for 30 min.
10. Add 100 µL glyTE stock to RSG vials, mix gently, incubate for 1 min, then place in liquid nitrogen, ultracold freezer or on dry ice.
11. 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. We have a manuscript in press that reports the use of RSG to measure **aerobic** respiration rates of individual cells of marine prokaryoplankton, in combination with genomic sequencing of the same cells. Corresponding SCGC services may be offered in late 2022/early 2023.
2. The fluorescence of RSG-labeled cells declines over time. We are currently testing the impact of long-term cryopreservation.
3. Sample freezing is not recommended to hypersaline samples, due to intracellular salt crystal formation. Please consult with SCGC personnel for best ways to accommodate hypersaline samples.
4. To order SCGC services and glyTE stock, or obtain sample shipping instructions, please see scgc.bigelow.org.

Updated by Ramunas Stepanauskas, October 5, 2022