Supplementary Procedure: DNA Extraction from Soil & Stool

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Introduction

Extracting DNA from soil samples for PCR and qPCR is complicated because of the the release of humics. This method uses a modification of ZyGEM Bacteria extraction procedure and relies on a short differential sedimentation of solids in a proprietary buffer. This buffer is designed to dislodge bacteria from biofilms but it is isotonic to protect more fragile species.

METHOD

Preparation

- All manipulations should be performed in a clean-room or a PCR hood.
- Use only certified RNAse/DNA-free tubes and reagents and wash surfaces likely to come into contact with the samples in 0.05% Hypochlorite bleach. Rinse thoroughly with RNAse/DNA-free water.
- To reduce possible contamination, store *prep*GEM and *RNA*-GEM at -20°C once opened

Differential sedimentation step

1. Add 20 mg of soil or stool to a 1.5 ml tubes
2. Resuspend in 500 µl of Wash buffer

   (Provided as 5x WASH BUFFER)
3. Vortex for 1 min.
4. Centrifuge gently at **200 rcf** for 30 seconds to sediment the solids but leave a suspension of cells. **NOTE: This is a very slow centrifugation.**
5. Transfer the supernatant to a new tube.
6. Centrifuge at full speed for 2 min.
7. Carefully pipette away all of the wash buffer.

It is important to remove **all** the wash buffer. A second short spin is recommended and the last drop removed with a pipette.

8. Resuspend pellet in 100 µl of water.

Extraction

1. Make up the following extraction cocktail
   a) 10 µl of 10x Buffer (GREEN)
   b) 1 µl of Lysozyme @ 10 mg / ml.
   c) 1 – 2 µl of *prep*GEM
   d) 38 µl water
   e) 50 µl of cell suspension
2. Cycle:

37°C	15 min
75°C	15 minutes
95°C	5 minutes

3. Vortex.
4. Centrifuge at full speed for 5 min

- PCR/qPCR using 1 µl or less in a 25 µl reaction

Variations

1. The method provided is a starting point only. For different types of samples, it will need to be optimised further.
2. If inhibitions remains a problem, add PVP to your PCR mix to a final concentration of 0.5%.
3. Try different amounts of extract in your PCR. Sometimes, less is best.

NOTE: At ZyGEM, we take great effort to remove all bacterial DNA from our reagents. However, this is not always the case with other vendors. If you are using universal 16S rRNA gene primers, be aware that many laboratory reagents (including PCR reagents) contain trace amounts of bacterial DNA. Make sure that appropriate negative controls are carried out in order to evaluate the scale of the problem.

For more information email us on: support@zygem.com