

Andreas Teske Laboratory ICoMM DNA Extraction Protocol:

Sample collection

Sediment samples were collected on-ship via APC (advanced piston coring) and frozen at -80°C (1). Samples were shipped on dry-ice to the University of North Carolina at Chapel Hill and were kept at -80°C until analysis. Sediment cores from Ocean Drilling Program (ODP) Leg 201, Site 1227, 1229 and 1231 were used in this study¹. Sites 1227 and 1229 are on the Peru Margin

DNA extraction

1. Aseptically scrape sediment surface.
2. Discard the first centimeter of sediment material.
3. Collect subsequent scrapings in a sterile container (petri dish), homogenize and weigh until there is 4 grams.
4. Prepare to use the MoBio UltraClean Soil DNA kit² (MoBio Laboratories, Inc., Carlsbad, CA, catalog number 12800-50) with the following modifications
5. Follow the manufacturer protocol:
6. To the 2ml Bead Solution tubes provided, add 0.5 gm of sample. Gently vortex to mix.
7. Add 60µl of Solution S1 and invert several times or vortex briefly.
8. Add 200µl of Solution IRS (Inhibitor Removal Solution), vortex briefly.
9. Place samples in a 65°C waterbath for 20 minutes.
10. Homogenize in a bead beater for 1 minute.
11. Centrifuge samples for 1 minute
12. Perform the remaining steps as per manufacturer protocol (step 8).
13. Pool elutions of extractions and use 1- 10 microliters for PCR template
14. **Note, for these samples PCR was done using HiFi Platinum polymerase. Pfu polymerase did not amplify these samples well.

References:

1. D'Hondt SL, Jørgensen BB, Miller DJ, *et al.* (2003) *Proceedings of the Ocean Drilling Program, Initial Reports, Leg 201 [CD-ROM]*. Available from: Ocean Drilling Program, Texas A&M University, College Station TX 77845-9547, USA.
2. Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911
email: orders@mobio.com Fax: 760-929-0109 Mail: Mo Bio Laboratories, Inc.
2746 Loker Avenue West, Carlsbad CA 92008 <http://www.mobio.com/distributors/>