

Isolation of DNA and RNA from Soil using Two Different Methods Optimized with Inhibitor Removal Technology® (IRT)



Two major difficulties facing environmental scientists are the inability to adequately stabilize the microbial profiles of samples collected during field work and the carry-over of inhibitors to PCR and other enzymes into the final nucleic acid. Many other manufacturers' methods do not have a process to specifically remove these inhibitors. Irreplaceable samples collected from remote locations, such as soils, sediments, biofilms and biomats are abundant in humic acids, fulvic acids, polysaccharides, and polyphenols. Incomplete removal of any of these species will result in inhibition in enzymatic assays and inaccuracy in metagenomic analysis. Using the patented Inhibitor Removal Technology® (IRT), removal of PCR inhibitors from the final DNA or RNA is accomplished in even the most difficult samples. To overcome the inability to preserve and stabilize the microbial community gene expression profile in soils during collection, the LifeGuard™ Soil Preservation Solution was developed. LifeGuard™ allows for high quality and high yields of total

Materials and Methods

LifeGuard™ Soil Preservation Solution, cat# 12868-100, 100 ml

RNA PowerSoil® Total RNA Isolation Kit, cat# 12866-25, 25 preps

RNA PowerSoil® DNA Elution Accessory Kit, cat# 12867-25, 25 preps

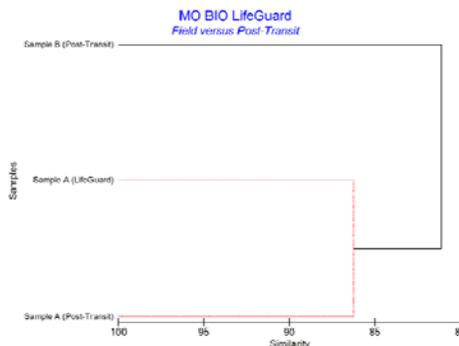
PowerSoil® DNA Isolation Kit, cat# 12888-50, 50 preps, cat# 12888-100, 100 preps

Results

Preservation of microbial communities in Antarctic Dry Valley soil using LifeGuard™ Soil Preservation Solution

Figure 1: In collaboration with Dr. Charles Lee from the University of Waikato, microbial community analysis was performed with Antarctic Dry Valley soils collected on site and the same soil shipped unstabilized. All samples were shipped at 4°C and stored for 30 days. RNA was extracted using the RNA PowerSoil® Total RNA Isolation Kit and T-RFLP analysis performed for 16s rRNA. Results demonstrated that Sample A soil preserved and unpreserved were 86% similar in the number of phylotypes. The unpreserved sample A had additional phylotypes, indicating that the fluctuations in temperature during transit resulted in changes to the original community metatranscriptome.

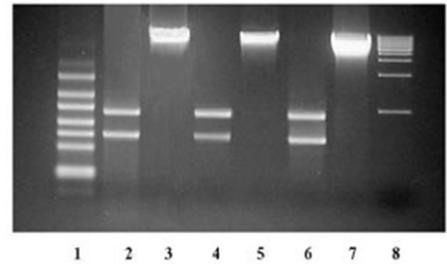
Sample B is a Dry Valley soil collected at a totally different site, and as expected, it gives a completely different transcriptional profile.



T-RFLP results from LifeGuard™ stabilized soil indicate the soil microbial community was preserved during transport from Antarctica to New Zealand.

Isolation of high yields and quality RNA and DNA from the same soil sample using the DNA Accessory Kit

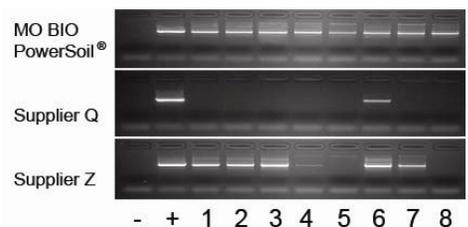
Figure 2: Using the DNA Accessory Kit with the RNA PowerSoil® Total RNA Isolation Kit, it is possible to isolate high molecular weight DNA suitable for metagenomic analysis from the same soil sample as used for RNA extraction. Because the RNA PowerSoil® Kit uses anion-exchange technology for purification, mechanical shearing forces are not applied to the DNA upon elution. DNA is collected in a separate elution buffer by gravity flow and precipitated to yield DNA at the desired final concentration. Isolation of nucleic acids is maximized when using the combination of the two kits together.



Lane 1: ladder; lane 2: mulch RNA; lane 3: mulch DNA; lane 4: strawberry field RNA; lane 5: strawberry field DNA; lane 6: compost RNA; lane 7: compost DNA; lane 8: ladder.

The PowerSoil® DNA Isolation Kit gives 100% success

Figure 3. The PowerSoil® DNA Isolation Kit remains the most highly cited purification method for soil in use today. The PowerSoil® DNA Isolation Kit is a fast and easy method that starts with only 0.25 gram of sample. Inhibitors are removed using IRT allowing for the highest success rates in PCR of any commercial method. The PowerSoil® DNA Isolation Kit is fully compatible with bead beating technology if harder beating for lysis of tough organisms is desired. For more technical help on how to modify your PowerSoil® DNA Kit for tough organisms, call or e-mail the experts at MO BIO.



PCR amplification of genomic DNA isolated from nine different soils using the PowerSoil® DNA Isolation Kit and kits from two other suppliers. Only the MO BIO kit gives 100% success.

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