

	Environmental Analysis Teaching and Research Laboratory	Date: 8/15/2016	Number: 39 v.01
	Standard Operating Procedure	Title: DNA Isolation from Soil – MoBio PowerSoil DNA Kit	
	Approved By: TBD	Revision Date: August 22, 2016	

1. DNA Extraction

1.1 This procedure is to extract DNA from soils using a MoBio extraction kit.

1.2 The applications of this SOP include ribosomal DNA, community profiling and PCR/qPCR for specific DNA sequences from soil microbes.

2. Summary of Method

Soil samples are homogenized by mechanical treatment or collected in a manner that does not require additional treatment (i.e. samples suspended in water/solvent).

The PowerSoil® DNA Isolation Kit is effective at removing PCR inhibitors from even the most difficult soil types. Environmental samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

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3. Acknowledgements

- 3.1** This SOP was originally written by Aparna... to extract DNA from surface waters. Initial test of the methods were done by Alejandro Guerrero in the summer of 2016.

4. Definitions

- 4.1** All reagents and equipment are self explanatory in lab.

5. Biases and Interferences

- 5.1** The extraction process is supposed to isolate DNA from other cellular or other organic materials. However, proteins and phenols...
- 5.2** When measuring the DNA yield, these compounds may interfere with the measurements and thus, suggest you have more DNA than was extracted in reality.
- 5.3** Measuring the absorbance ratios of several wavelengths gives a reasonable estimate of DNA purity. See the QA/QC section to measure these contaminants.

A260/A280 Ratio Nucleic acids, DNA and RNA, absorb at 260nm. For a pure sample, a well defined peak (no shoulders or wiggles) at 260nm is expected. Several factors, however, can influence the accuracy of the 260/280 and 260/230 ratios. Readings from very dilute samples will have very little difference between the absorbance at 260 and 280nm leading to inaccurate ratios. The type(s) of protein present will also have an effect. Absorbance in the UV range by proteins is primarily the result of aromatic ring structures. Phenol and other contaminants can also absorb at 280 nm and can affect the ratio calculation. Phenol absorbs with a peak at 270nm. **Nucleic acid preparations uncontaminated by phenol should have an A260/A280 ratio of around 1.8.** The pH of the solution can also affect the 260/280 ratio, with acidic solutions having a lower ratio of up to 0.2-0.3 and alkaline solutions having an increased ratio by a similar amount.

A260/A230 Ratio Pure RNA has an A260/A280 ratio of 2.0, therefore if a DNA sample has an A260/A280 ratio of greater than 1.8 this could suggest RNA contamination. The 260/280 ratio is a secondary measure of nucleic acid purity. This ratio for pure samples are often higher than the respective 260/280 ratio values. Strong absorbance around 230nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. A ratio of 260nm to 230nm can help evaluate the level of salt carryover in the purified DNA. The lower the ratio, the greater the amount of salt present. As a guideline, **the A260/A230 ratio should be greater than 1.5, ideally close to 1.8.** Urea, EDTA, carbohydrates and phenolate ions all have absorbance near 230nm. A reading at 320nm will indicate if there is turbidity in the solution, another indication of possible contamination.

6. Health and Safety

6.1 The risks involved with using this kit are mainly related to the chemicals that are in use. As listed below, safety precautions should be taken at all times, but especially when handling hazardous reagents. While following safety protocol, it is advised that the materials and equipment are kept organized to avoid contamination and thus yield good results.

6.2 Avoid all skin contact with reagents in this kit.

6.3 In case of contact, wash thoroughly with water. Do not ingest. See Safety Data Sheets for emergency procedures in case of accidental ingestion or contact.

6.4 All SDS information is available upon request (760-929-9911) or on our web site at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

6.5 WARNING: Solution C5 contains ethanol. It is flammable.

6.1. Safety and Personnel Protective Equipment

6.6 In addition, to appropriate laboratory attire, always wear gloves, laboratory coat, and goggles when following this SOP.

7. Personnel & Training Responsibilities

7.1 Training is required before this SOP can be used.

7.2 Researchers using this SOP should be trained for the following SOPs:

- SOP01 Laboratory Safety
- SOP03 Handling of Hazardous Materials
- SOP09 Using Balances, Pippettes, and Glassware
- SOP10 Quality Control and Quality Assurance
- SOP14 Microcentrifuge
- SOP16 Using Laboratory Refrigerators and Freezers

8. Required Materials

Equipment

8.1 Bead homogenizer – BM Fast-Prep 24 Homogenizer

8.2 Microcentrifuge with rotor capable of reaching 10,000 g

8.3 Piettors 2 μL - 750 μL

8.4 Vortex

8.5 Sharpies

8.6 Laboratory Notebook

Reagents

8.7 Reagents are provided by MoBio PowerSoil DNA Kit

Consumables

8.8 RNAase free gloves (cat # ???)

8.9 Disposable pipette tips (for 100 preps)

- 100 — 2 μL tips
- 50 — 200 μL tips
- 700 — 1000 μL tips
- 50 — 5000 μL tips

8.10 Weighing Paper

8.11 Scupula, spatula, tweezers

9. Estimated Time (batch of 12)

9.1 This procedure requires approximately 5-6 hours depending on the number of samples, and the time it takes to homogenize starting material and complete other preliminary steps.

9.2 Batches of 12 are easiest to follow for new researchers and takes about 5 hours, while batches of 24 are more efficient, but can take more than 8 hours.

10. Sample Collection, Preservation, and Storage

10.1 Before the extraction process, soils should be either freshly collected or frozen...
MORE here...

10.2 After extraction, the DNA shall be stored in the -80° , but be sure to follow the freezer SOP to appropriately label and store samples.

11. Procedure

Preliminary Steps

11.1 Put soils in order to facilitate an efficient weighing process.

11.2 Label **PowerBead Tubes** (provided) in the same order as soils.

11.3 Spend a few minutes homogenizing each soil before sampling.

11.4 To the PowerBead Tubes, add 250 mg (0.25 grams) of soil sample.

What's happening: After your sample has been loaded into the PowerBead Tube, the next step is a homogenization and lysis procedure. The PowerBead Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids and (c) protect nucleic acids from degradation.

11.5 Gently vortex to mix.

What's happening: Gentle vortexing mixes the components in the PowerBead Tube and begins to disperse the sample in the PowerBead Solution.

11.6 Check Solution C1. If Solution C1 is precipitated, heat solution to 60 °C until the precipitate has dissolved before use.

11.7 What's happening: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60 °C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

11.8 Add 60 μ l of Solution C1 and invert several times or vortex briefly.

Homogenization Process

11.9 If you have a MO BIO Vortex Adapter: Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog # 13000-V1-24) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.

Note: The vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-4 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

11.10 If a MB Fast-Prep 24 is available, set the time speed to 6 m/s and the time to 45 seconds. Place the tubes into the holes and lift and rotate the XXX to hold the

tubes in place. Be sure to tighten the top until the fastener cliques to singal the machine is read to be operated. Close the top and press start.

- 11.11** Place the PowerBead Tubes in the microcentrifuge. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature.

CAUTION: Do not to exceed 10,000 g or tubes may break.

- 11.12** Transfer the supernatant to a clean 2 ml Collection Tube (provided). Expect between 400 to 500 μ l of supernatant at this step. The exact recovered volume depends on the absorbency of your starting material and is not critical for the procedure to be effective. The supernatant may be dark in appearance and still contain some soil particles. The presence of carry over soil or a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry over soil and coloration of the mixture.

Note

- 11.13** Add 250 μ l of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes. What's happening: Solution C2 is patented Inhibitor Removal Technology® (IRT). It contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

- 11.14** Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

- 11.15** Avoiding the pellet, transfer up to 600 μ l of supernatant to a clean 2 ml Collection Tube (provided).

What's happening: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

- 11.16** Add 200 μ l of Solution C3 and vortex briefly. Incubate at 4 °C for 5 minutes. What's happening: Solution C3 is patented Inhibitor Removal Technology® (IRT) and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

- 11.17** Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

- 11.18** Transfer up to 750 μ l of supernatant to a clean 2 ml Collection Tube (provided). What's happening: The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

- 11.19** Shake to mix Solution C4 before use. Add 1.2 ml of Solution C4 to the supernatant.

Note: Be careful solution doesn't exceed rim of tube. Vortex for 5 seconds.

What's happening: Solution C4 is a high concentration salt solution. Since DNA binds

tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

- 11.20** Load approximately 675 μ l onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 μ l of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. **A total of three loads for each sample processed are required.**

What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

- 11.21** Add 500 μ l of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g.

What's happening: Solution C5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

- 11.22** Discard the flow through from the 2 ml Collection Tube. NOTE: It is important to avoid any traces of the ethanol based wash solution.

What's happening: This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

- 11.23** Add 100 μ l of Solution C6 to the center of the white filter membrane. Placing the Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris) which lacks salt. Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step (MO BIO Catalog # 17000-10). Solution C6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

- 11.24** Centrifuge at room temperature for 30 seconds at 10,000 x g.

- 11.25** Discard the **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required. We recommend storing DNA frozen (-20° to -80 °C). Solution C6 does not contain any EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

12. QA/QC

Analysis of DNA Yield and Purity

12.1 The Nanodrop...

12.2 Open instrument software and click on Nucleic Acid. Login the the software, username: NanoDrop and Password: Nano.

12.3 Clean machine by adding distilled water onto the platform and wipe with a Kimwipe before loading anything onto sensor.

12.4 Pipette the X μ L for a blank, using the same solution that was used for the final elution (i.e. Elution Buffer), using a 2 μ L pipettor onto the hydrophobic surface, creating a tiny droplet.

12.5 Press Blank.

12.6 Clean off surface with a Kimwipe, and repeat the previous step, but replace the elution buffer with each sample to be annalyzed. Be sure the samples are thawed out and tapped to ensure that the sample is homogenized.

12.7 Load sample and press measure.

12.8 Record the 260/280 and 260/230 ratios and the yield (nanograms/L), in your laboratory notebook. They should be around 1.8-2.0 ideally.

13. References

13.1 asd fasdf asdf asdf