# Application Note

**Determining the Best Homogenization Protocol for Any Soil**

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**Introduction**

Isolating DNA from soil begins with various methods to homogenize the sample. The choice of homogenization method is influenced by several factors including the texture or composition of the soil, the microbial community of interest, and the size requirement of the isolated genomic DNA. Both vortex and high powered bead beating methods efficiently lyse microbial cells, but for some applications, the use of a bead beating homogenizer may be preferred in order to achieve stronger lysis of tough organisms such as fungus and spores. When using a high-velocity bead beating instrument, glass beads are traditionally recommended because of their ability to withstand the acceleration forces without crushing the grinding matrix. Conversely, we have found that the optimal type of grinding matrix is heavily dependent on the soil type. To determine the best bead type and speed for homogenization, we evaluated two soil types for DNA purity and yield using the PowerLyzer™ 24 Bench Top Bead-Based Homogenizer with either a 0.1 mm glass bead tube or a 0.7 mm garnet bead tube. Our results demonstrate that extraction of high yield and integrity DNA in soils can vary significantly under the same bead beating conditions and that optimization for the method that achieves the best results should be considered prior to adopting a standardized homogenization protocol.

**Methods:**

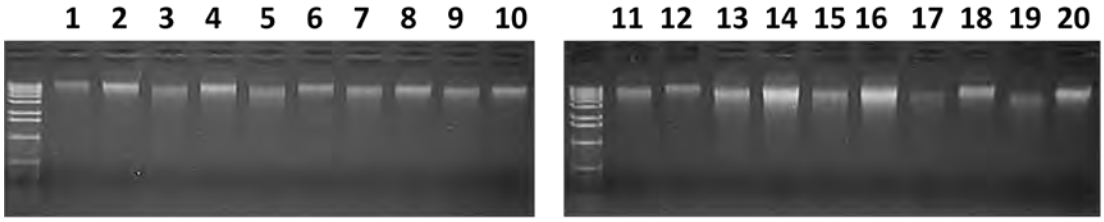
Soil samples from the California Polytechnic State University, Earth and Soil Science Department were obtained and characterized. Soil 1 consisted of 45% clay, 2.5% carbon, pH 8. Soil 2 consisted of silty soil containing 40% clay, 4.9% carbon, pH 8. DNA was isolated from 0.25 g of sieved soil according to protocol using either the PowerLyzer™ PowerSoil® DNA Isolation Kit (cat. no. 12855-50, containing glass bead tubes) or the PowerSoil® DNA Isolation Kit (cat. no. 12888-50, containing garnet bead tubes) with the PowerLyzer™ 24 Homogenizer for 45 seconds at the indicated speeds described in each figure below. DNA was eluted in 50 µl, electrophoresed in a 1% agarose gel, and analyzed using a NanoDrop® 1000 spectrophotometer.

**Results:**

To evaluate the difference in DNA yields obtained by using the garnet beads vs. the 0.1 mm glass beads with a high-velocity bead beating instrument, we compared two soils with similar characteristics. The first soil contained a high percentage of clay and a low carbon content, which typically correlates with lower microbial biomass and lower DNA yields. The second soil contained a lower percent clay and a higher carbon content, and had a higher biomass based on DNA yields.

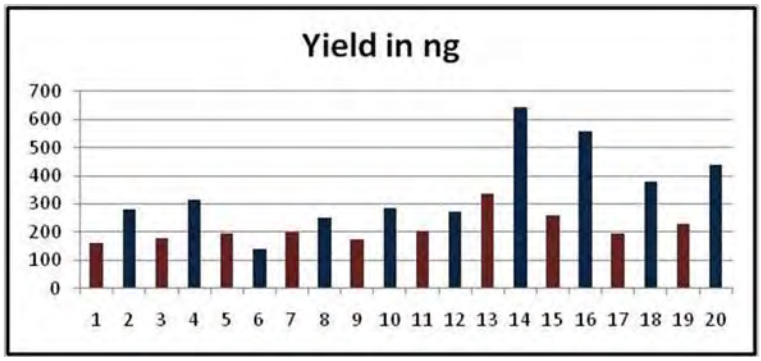
When Soil 1 was extracted using increasing speed from 2,000 RPM up to the maximum speed of 5,000 RPM for 45 seconds, distinct differences in yield were observed between the garnet and glass bead tubes (Figure 1). In general, the glass bead tubes extracted higher yields of DNA compared to garnet bead tubes, which achieved maximum DNA yields between 3,900-4,200 RPM. Beyond 4,200 RPM, DNA yields fell significantly. With garnet bead tubes, maximum DNA yield was obtained using 3,900 RPM. However, yields were only half that of the glass bead tubes at this speed.

**Isolation of DNA from soil containing a high clay content using the PowerLyzer™ 24 Homogenizer.**




Lane 1: Garnet 2000 rpm
Lane 2: Glass 2000 rpm
Lane 3: Garnet 2200 rpm
Lane 4: Glass 2200 rpm
Lane 5: Garnet 2500 rpm
Lane 6: Glass 2500 rpm
Lane 7: Garnet 2800 rpm
Lane 8: Glass 2800 rpm
Lane 9: Garnet 3200 rpm
Lane 10: Glass 3200 rpm

Lane 11: Garnet 3500 rpm
Lane 12: Glass 3500 rpm
Lane 13: Garnet 3900 rpm
Lane 14: Glass 3900 rpm
Lane 15: Garnet 4200 rpm
Lane 16: Glass 4200 rpm
Lane 17: Garnet 4600 rpm
Lane 18: Glass 4600 rpm
Lane 19: Garnet 5000 rpm
Lane 20: Glass 5000 rpm



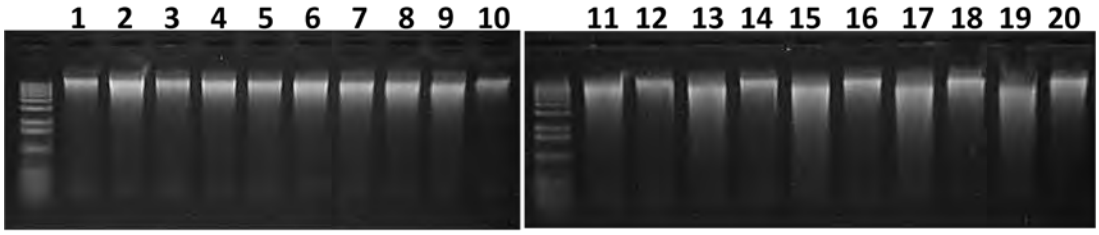

Red= garnet
Blue= glass

**Figure 1. DNA isolation from clay soil (soil 1) with increasing speed for 45 seconds**. DNA yields remain consistent with either glass or garnet bead tubes until speeds reach 3,900-4,200 RPM where maximum DNA recovery is achieved using glass bead tubes. DNA yields decline when the bead beating speed exceeds 4,200 RPM, indicating that too much force can be detrimental to DNA recovery.

To compare these results with another soil, a silty soil (soil 2) containing 40% clay and double the carbon content was evaluated. DNA yields and integrity were analyzed (Figure 2) using identical protocols as previously described. DNA yields were similar between glass and garnet bead tubes between 2,500-3,200 RPM and appeared as expected with high molecular weight products on an agarose gel. Garnet bead tubes performed best at 3,200 RPM but beyond that speed, DNA integrity was negatively affected. Maximum yields, as measured by the NanoDrop® 1000 spectrophotometer, were achieved with garnet

bead tubes at 4,200 RPM. However, the resulting sheared DNA may have resulted in a higher absorbance at $A_{260}$ and therefore, falsely indicating an increase in DNA yield. Glass bead tubes performed best at low speed (2,000 RPM). Beyond 3,200 RPM, the DNA yield dropped significantly. Although DNA recovery using glass beads at the higher speeds reached the same yield (according to the NanoDrop® 1000 spectrophotometer) as that obtained at 2,000 RPM, the yield and quality at higher speeds are not as consistent at the lower speeds (according to agarose gel electrophoresis). This data demonstrates that a different bead type may be acceptable for some soils but homogenization speed must also be optimized and adjusted accordingly to each sample type.

**Isolation of DNA from a silty clay soil using the PowerLyzer™ 24 Homogenizer.**

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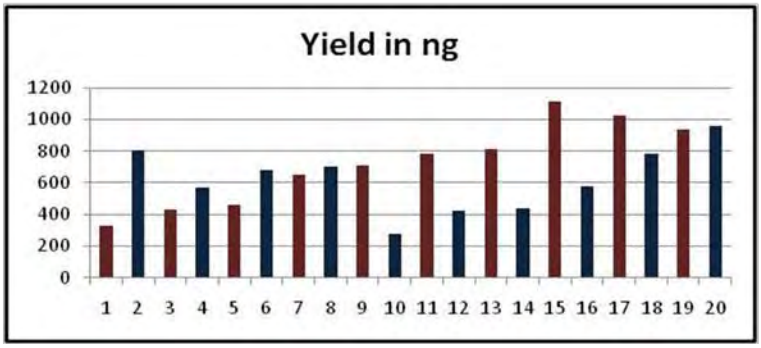


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**Figure 2. DNA from silty clay soil was extracted using the PowerLyzer™ 24 Homogenizer with increasing speed for 45 seconds.** DNA yields demonstrate that soil 2 performed better with less vigorous bead beating and that the garnet bead tubes performed equally as well as the glass bead tubes. Optimal DNA yields were achieved at lower speeds using the glass bead tubes while the garnet bead tubes required higher speeds for maximum DNA yields.

**Conclusions**

The first step required for extracting DNA from soil involves mechanical lysis and homogenization. Here, we have demonstrated that the homogenization method, the type of bead tube, and the type of soil sample each influence the outcome of DNA extraction, and that optimization is critical for complete and successful extraction results.

High powered bead beating instruments have become mainstream as the mechanical homogenization method of choice for labs requiring high throughput lysis of multiple samples in under a minute. However, velocities vary greatly within the bead tubes between various homogenizing instruments and therefore, comparing protocols from one instrument to another can be difficult. In general, high powered beat beating methods achieve higher DNA yields due to 1) the presence of sheared DNA which results in a higher $A_{260}$ UV absorbance, and 2) enhanced extraction results of non-microbial life such as plants or insects inherent in soil samples. Thus, higher yields based simply on agarose gel electrophoresis results or $A_{260}$ UV absorbance readings alone cannot be the sole factor in determining the best course of action for homogenizing a particular soil.

In this study, two soils similar in clay content but different in carbon content were evaluated for the effects of increased bead beating forces under constant time. Results indicated distinct differences between soils and bead tube types. The soil containing the higher clay content was extracted more effectively using the 0.1 mm glass bead tubes while the silty clay soil was extracted equally as well using either glass or garnet bead tubes. The higher clay soil tolerated a much harder force of homogenization and resulted in high quality, intact DNA while the silty clay soil resulted in a high level of sheared DNA with increased RPM speed. Together, these results demonstrate that every soil needs to be examined individually in order to determine the best speed of homogenization that generates the highest yields of DNA with the least sample damage. Since bead type also influences integrity, we recommend comparing garnet bead tubes to glass bead tubes to assess which bead type provides the highest yields of DNA from your soil.

More information on the differences between bead types and homogenization methods can be found in a poster presented at ASM 2010 in collaboration with the laboratory of Chris Kitts entitled, **Comparison of Microbial Populations Isolated from a Variety of Soils using Different Homogenization Methods During DNA Extraction** at http://www.mobio.com/images/custom/file/MicroPopPoster2010.pdf.

**Related Products:**

| Catalog No. | Description | Quantity |
|---|---|---|
| 13155 | PowerLyzer™ 24 Bench Top Bead-Based Homogenizer, (110/220V) | 1 unit |
| 13156 | PowerLyzer™ Tube Holder | 1 unit |
| 13157 | PowerLyzer™ Tube Holder Stand | 1 unit |
| 12855-50 | PowerLyzer™ PowerSoil® DNA Isolation Kit | 50 preps |
| 12855-50-BS | PowerLyzer™ PowerSoil® Bead Solution | 42 ml |

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|---|---|---|
| 12255-50-GBT | PowerLyzer™ Glass Micro Bead Tubes, 0.1 mm | 50 Tubes |
| 13118-50 | Glass Bead Tubes, 0.1 mm | 50 Tubes |
| 13118-400 | Glass Beads, 0.1 mm, Bulk (500 preps, 400g) | 400 g |
| 13123-05 | Garnet Beads, 0.70 mm, Bulk (500g) | 500 g |
| 13123-50 | Garnet Bead Tubes, 0.70 mm | 50 Tubes |