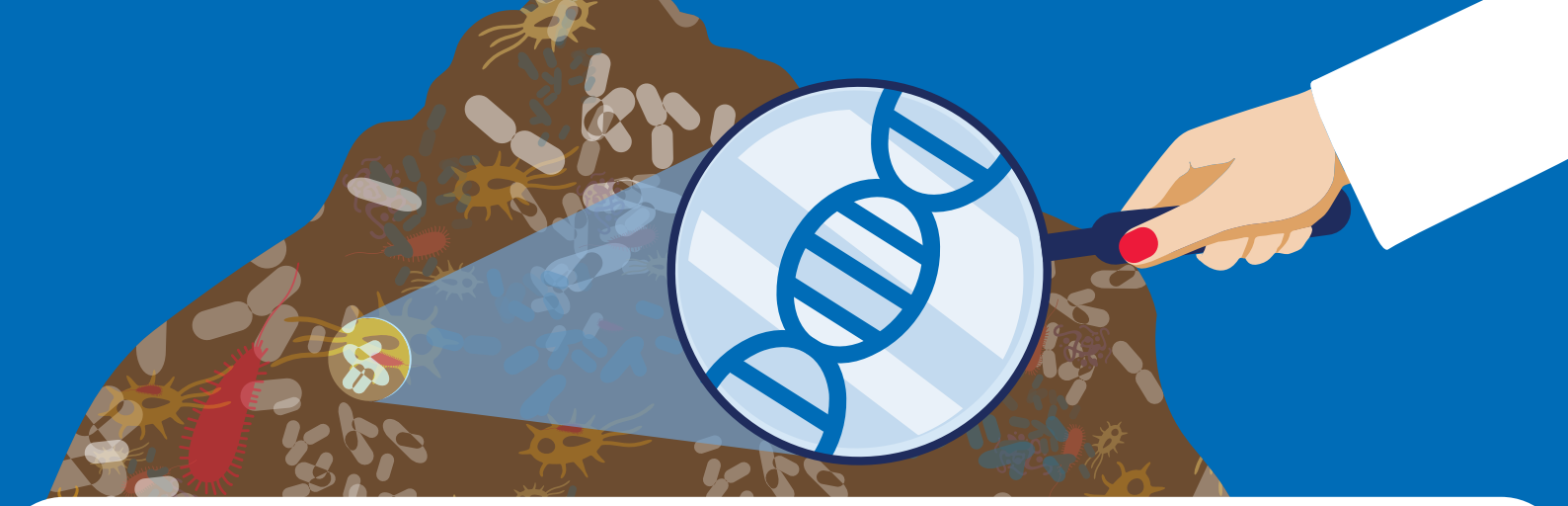


Technical Guideline

Microbial DNA isolation from soil samples

Want to improve your results?
Here is a guide to efficient and reliable microbial DNA isolations from soil samples.

Soil is complex and diverse, making it one of the most challenging sample types for microbial nucleic acid isolation. Chemical and physical properties of soil change from layer to layer, with microbiome communities and profiles varying in accordance.[1] Furthermore, the starting material of can affect downstream applications.

Considerations prior to beginning

Soils vary in consistency, texture and microbial content. Yield is not based on the amount of material processed alone and more starting material will not always increase DNA yields.

Degradation of organic matter in soil produces humic substances. Humic substances are a complex and heterogenous mix of organic molecules, some of which share chemical properties with nucleic acids, and can thus co-purify with DNA or RNA. These 'inhibitors' can interfere with downstream applications such as PCR and RT-PCR. Other examples of PCR-interfering substances found in soil are heavy metals or polysaccharides.

- Sieve soil samples to normalize texture and remove large particles. This increases DNA yield consistency.
- After collection, use your sample as soon as possible. If storage is required we recommend storing the sample at –70 to –20°C, avoiding thaw/freeze cycles.

Disruption, lysis and homogenization

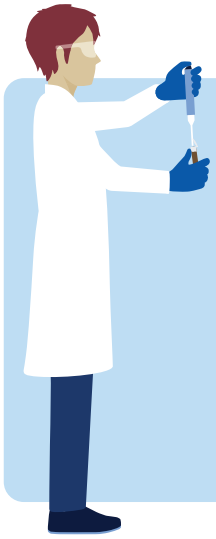
Complete disruption, lysis and homogenization are indispensable for optimal DNA yields. First, mechanical disruption using beads is the fastest and most efficient method for releasing nucleic acid from bacteria and fungi cells in soil.

We use zirconium beads with our lysis buffer for best performance in disrupting even difficult-to-lyse microbial cells such as gram-positive bacteria, archaea and fungi. As homogenization equipment, we use the TissueLyser III. Alternatively, vortex adapters clipped onto the Vortex Genie 2 will work for the disruption of a limited number of samples.

The buffer should disrupt cell membranes (in combination with the mechanical disruption), be gentle enough to not denature the DNA and work regardless of soil pH. For some kits, acidic soils may need to be neutralized for optimal DNA yields, since acidic conditions are harmful to DNA. If you want to save time, the DNeasy® PowerSoil® Pro or PowerMax® Soil lysis buffers are designed to work efficiently at a wide range of pHs without prior neutralization.

Finally, disrupting cell walls releases high-molecular-weight cellular proteins and carbohydrates. Homogenization reduces the viscosity of the cell lysates by shearing these proteins and carbohydrates. Thus, complete homogenization is essential for high DNA yield.

Fungal DNA can be extracted by the standard protocol of the DNeasy PowerSoil Pro Kit. The combination of mechanical and chemical lysis reliably breaks open not only bacterial but also fungal cells.



We use an optimized lysis buffer in combination with the PowerBead Pro tubes (as provided in the DNeasy PowerSoil Pro and DNeasy PowerMax Soil kits, respectively) that are co-developed for optimal microbial lysis from any soil type. **For more information, see our soil DNA isolation video**

What about heating?

In our experience with the QIAGEN Power and Power Pro kits, heating is usually not required. The combination of lysis buffer and mechanical disruption on a high-powered bead-beating instrument leads to efficient lysis.

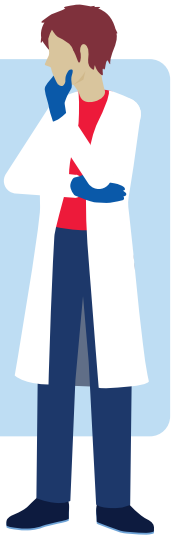
Inhibitor removal

Soil homogenate contains inhibitors, such as humic acids, the substances responsible for the sample's brown color. These impede downstream applications such as PCR and sequencing, ultimately wasting samples, time and funding. For reliable results, inhibitors should be removed prior to downstream applications. Our patented Inhibitor Removal Technology® (IRT) and patent-pending second-generation IRT precipitate humic acids and other inhibitors from the sample lysates. After inhibitor removal, samples typically look clear and perform well.

Classic phenol-chloroform treatments generally do not remove humic substances efficiently. Therefore, select a protocol with inhibitor removal.

Our Solution CD2 was stored at room temperature. Can it still be used? No. When stored at room temperature, Solution CD2 forms irreversible precipitates. You can order a new bottle (cat. no. 47016-2).

What's the difference between Power kits and Power Pro kits? All Power and Power Pro kits contain the patented Inhibitor Removal Technology (IRT), specifically designed to remove PCR-inhibitors from soil, stool, water, air and biofilm samples. "Pro" kits have a patent-pending, upgraded version of IRT performed in one step.



DNA yield after extraction

What yield should you expect? There is no easy answer. Simply put: it varies. The moisture content, organic content, collection location and sample storage will play a role in DNA yield. From our experience with "normal" soils or temperate soils (such as garden soil), yields range from 2–5 µg DNA per 250 mg soil. Sandy and clay soils tend to have very low organic content and in turn, lower DNA yields. One current hypothesis with sandy soils and clay soils is that the released nucleic acids tightly bind the soil itself.

Soil after wildfire: For investigation of the microbial life in soil after a wildfire with the DNeasy PowerSoil Pro Kit, we recommend adding Buffer ATL (100 µL) to Solution CD1 (700 µL) (alternatively, 200 µL Buffer ATL to 600 µL Solution CD1). This will help to reduce the influence of ash.

Need a break? The best place to pause in the DNeasy PowerSoil Pro protocol is after the IRT step and before adding the binding solution. The lysate can be frozen at –20°C and continued the next day.

Automation

Automation increases reliability, robustness and method standardization of research results and biomonitoring. Our kits are available in throughputs from manual to 96-well for a variety of automation platforms.

High saline samples: We've seen higher DNA yields when we washed the soil with PBS before extracting DNA. To increase yields from saline soil, wash the soil with sterile PBS as follows:

- Mix 250 mg of the soil sample with 1 mL sterile PBS in a collection tube.
- Invert a few times to mix.
- Centrifuge at 10,000 x g for 2 minutes to pellet the cells and solid material.
- Discard the supernatant.
- If needed, washes can be repeated.

More is not always better. In other words, processing more soil does not always yield more DNA. This is because the lysis buffer will be absorbed by the soil, decreasing the disruption and homogenization efficiency. The DNeasy PowerSoil Pro Kit is meant for DNA preparation from up to 250 mg of soil. For all soil types and low biomass soils (such as sandy soil) we recommend the DNeasy PowerMax Soil Kit, which can process up to 10 g.

Soil microbial DNA kits

Kit	Starting amount	Recommendations for usage	Automated on	Cat. no.
DNA				
DNeasy PowerSoil Pro	250 mg	For all types of soil	QIAcube Connect (optional)	47014 47016
DNeasy PowerSoil Pro QIAcube	250 mg	For all types of soil	QIAcube Connect	47126
DNeasy 96 PowerSoil Pro QIAcube HT	250 mg	Up to 96 samples	QIAcube HT	47021
DNeasy 96 PowerSoil Pro	250 mg	Up to 96 samples	Manual processing on plates	47017
DNeasy PowerMax Soil	Up to 10 g	Low biomass soil	Manual processing in 50 mL tubes	12988-10
MagAttract® PowerSoil Pro DNA*	100 mg	Up to 96 samples	KingFisher™ epMotion®	47109 47119

*Bead Plates/tubes must be purchased separately for QIAcube HT and MagAttract Kits: Cat No. 19301 PowerBead Pro Tubes (2 mL) (50) or Cat No. 19311 PowerBead Pro Plates (4).
To learn how to use the bead plates, please watch our explainer video

DNA quality control

After you obtain a visually clear DNA eluate, measure the absorbance ratios at 260/280 nm and 260/230 nm. Ratios between 1.8 and 2.0 indicate high DNA purity. Inhibitor depletion efficiency can be controlled using a qPCR assay with an internal control. We reccomend the Quantifast Pathogen + IC kit (100) Cat. No. / ID: 211352.

Low 260/230 ratio? Carefully perform the wash steps, filling the spin column to the rim at least once with wash buffer. This removes chemicals stuck to the tube walls.

Is further cleanup after DNA preparation recommended? This is typically unnecessary when working with QIAGEN Power and Power Pro Kits. If you are using a different technology, for DNA preparation and the eluant contains inhibitors in QC test (e.g. an inhibitor sensitive qPCR assay with an internal control), the DNeasy PowerClean Pro Cleanup Kit can be used for further cleanup.

Downstream NGS

A comprehensive assessment showed that various experimental parameters can impact the 16S rRNA gene library. The representation of the microbial community can be affected by gDNA concentration and 16S rRNA gene yield.[2] For whole genome sequencing (WGS), the highest possible library complexity (i.e. the number of unique DNA fragments) reduces the amount of bias. Ideally, the library should be an accurate representation of the starting sample. Therefore, during library preparation, removing PCR inhibitors and obtaining sufficient amounts of starting input are important for minimizing the number of PCR cycles and amplification bias. This should help to capture the best possible diversity of a microbial community.[3] For automated extraction, we recommend our dedicated kits for use with the QIAcube Connect, QIAcube HT and QIAsymphony.

The Microbiome WGS SeqSets truly exhibit the Sample to Insight spirit. These all-in-one sets contain all that is needed to extract microbial DNA samples, perform sequencing and comprehensive metagenomic analysis using the Microbial Analysis Portal. Currently available for stool samples (QIAamp® PowerFecal® Pro WGS SeqSet) and soil samples (DNeasy PowerSoil Pro WGS SeqSet) these sets are available to order under a single catalog number.

- The majority of DNA fragments isolated using the DNeasy PowerSoil Pro kit tend to be 6–15 kb, with the strongest concentration seen around 8–12 kb. These fragments are well-suited for long-read sequencing. Fragment length, however, can vary with the sample type. Extended bead-beating durations can shear nucleic acids, generating fragments too short and suboptimal for NGS library preparation. Please be sure to use the durations noted in the handbook.
- During library construction, minimizing the number of PCR cycles may improve accuracy.
- To ensure NGS workflow is robust against GC bias, use mock microbial communities with a wide range of GC contents and different taxonomic classification.
- For NGS, we recommend a minimum concentration above 4×10^{-2} ng/µL gDNA input (ideally $>2 \times 10^{-1}$ ng/µL) to achieve an unbiased representation of the microbial community.[2] gDNA input levels of $\leq 1.6 \times 10^{-3}$ ng/µL introduce taxonomic biases, resulting in a definitive misrepresentation of the microbiome.
- When performing WGS on multiple samples, consider a method such as the QIAseq Normalizer to quickly normalize samples without library quantification.

References

1. Naylor, D. et al. (2022). Trends in Microbial Community Composition and Function by Soil Depth, Microorganisms
2. Multinue et al. (2018) Systematic Bias Introduced by Genomic DNA Template Dilution in 16S rRNA Gene-Targeted Microbiota Profiling in Human Stool Homogenates, mSpherReferences
3. Tourlousse et al. (2021) Validation and standardization of DNA extraction and library construction methods for metagenomics-based human fecal microbiome measurements, Microbiome

Looking for more information about our microbiome products?
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