Bi 1x
Antibiotic Resistance

1 Overview

Almost a century since the discovery of the first commercial antibiotic, bacterial infections remain a pervasive and growing threat. This is due to the rapid, worldwide emergence of resistance to commonly used antibiotics. In this module, we will explore antibiotic resistance by studying a diversity of soil microbes. Soil is a rich source of microbial life, within which bacteria, fungi, and yeast are constantly in competition for survival. Several of these microbes produce small molecules with antibacterial activity, and many antibiotics were initially discovered in soil. In this lab, you will explore the resistance profile of local soil, search for novel antibiotic producers among the microbes you collect, and think about your findings in the context the antibiotic resistance crisis.

2 Background

2.1 Clinical Concern

Antibiotic resistance is one of the most pressing global health concerns facing humanity today. According to the CDC, every year at least 2.8 million people in the US are infected with antibiotic-resistant bacteria or fungi, and more than 35,000 people die as a result. Resistance arises from antibiotics creating selection pressure in the body—bacteria that contain or develop resistance mechanisms survive and replicate, taking up the niche left behind from other species being killed off.

A typical solution to this scenario has been to introduce secondary antibiotics to kill off microbes that were resistant to first-round antibiotics. This is why antibiotics are often prescribed in multiple rounds and need to be taken on a strict schedule. However, this can lead to the evolution of bacteria that are resistant to multiple antibiotics and are thus even more dangerous.

This phenomenon has even led to the classification of certain antibiotics as "drugs of last resort," to be used only when all other options have failed to cure a patient. However, researchers have already found species in patients that are resistant to not only one but many of these last resort drugs. This is an indication that even more such species likely exist. Resistance poses an immense clinical threat of us no longer having effective antibiotics. This is what is referred to as "the antibiotic resistance crisis." Along with climate change, it is arguably one of the greatest scientific crises facing humanity.

2.2 Antibiotic Resistance Mechanisms

As you explored in the Luria-Delbrück module, organisms can acquire mutations that allow them to become resistant to the effects of molecules that are supposed to be toxins, such as antibiotics. In that module, you saw the effects of base-pair level mutations, but this is not the only way organisms can acquire resistance.

Another possibility for gain of resistance is horizontal gene transfer (HGT). In this process, bacteria exchange genetic information by conjugation. If a bacterium happens to contain a plasmid (a small non-genomic piece of DNA coding for a few proteins) conferring resistance to a given antibiotic, it is possible for the plasmid to be copied, then transferred to a neighboring bacterium through a structure called the pillus. If this happens, the recipient bacterium will now also contain and express resistance genes. Resistance genes, generally, are genes whose products are able to either deactivate antibiotic molecules or drive a cellular mechanism that prevents uptake of the antibiotic. For example, some bacteria produce enzymes that bind to and inactivate antibiotics. Or, some species bulk up their cell wall in the presence of toxins to prevent uptake of antibiotics altogether.

Bacteria also tend to contain genes encoding for efflux pumps, which act to pump toxic molecules out of the cell. These genes might be expressed constitutively, or could be induced or overexpressed according to environmental stimuli. Efflux pumps are an especially concerning mode of resistance because they are not substrate-specific like an antibiotic-deactivating enzyme would be. Efflux pumps can function on virtually all classes of clinically relevant antibiotics.

2.3 Significance of Soil

Soil microbes are a traditional source for antibiotic discovery. The first commercially produced antibiotic, penicillin, was discovered in the soil fungus *Penicillum*. Since then, several commonly used antibiotics, including the ones you will test out in this lab, were originally derived from soil microbes. Soil is teeming with life, and it is one of the deepest reservoirs of microbial diversity. Just one gram of soil can contains tens of thousands of taxa. Soil environments put myriad organisms in close contact with each other, which lends to the ease of transmission of resistance genes by horizontal gene transfer when resistant organisms are introduced. The introduction of antibiotics to soil drives natural selection for resistant species.

In addition, selection pressure from the environment contributes to the evolution of microbes that themselves produce antimicrobial substances to kill off their competitors. Soil can contain many such antibiotic producers. Microbes classified as *Pseudomonas* or *Streptomyces* are well-known examples. The ability to find antibiotic producers in soil is of particular interest because this could provide alternatives to currently over-used antibiotics.

It's also worth noting that there is a universe of life in soil that we don't know

about yet. In fact, it is estimated only about 1% of all the microbes in soil are culturable in a traditional lab setting. So, there are still lots of microbes with interesting capabilities waiting to be discovered!

3 Protocol

This experiment will take place over a few weeks. You will collect a local soil sample and test the susceptibility of microbes in your sample to some common antibiotics. You will also test whether your samples happen to contain any antibiotic producers capable of killing non-resistant *E. coli*-K12. Finally, you will sequence your soil samples to better understand the communities you collected.

NOTE: Proper safety protocols are important in this module. Soil can contain opportunistic pathogens, and we will be growing up antibiotic resistant bacteria. You do not want to release these things into the environment or spread them around the lab, so make sure to wear gloves when performing this experiment and keep paper towels and 10% bleach handy. Wipe down surfaces with paper towels and 10% bleach solution (or Clorox wipes) whenever you are done working each day, and dispose of pipettes and inoculation loops in containers containing 10% bleach.

3.1 Collecting soil and preparing plates (Week 4)

First, we will all grab shovels and go digging around campus to collect our soil samples! Soil communities can vary vastly according to environmental factors, so think carefully about where you will collect your sample from. For example, bacteria living near the roots of plants (in the rhizosphere) known for constantly engaging in interesting biological behavior. We want to cover lots of different types of environments across the class so that we can look at everyone's data as a whole. However, in any case, try to choose soil patches that are relatively moist, and make sure to collect your samples from a few inches below the topsoil.

1. Once you've dug as deep as you would like with your shovel, use a 15 mL Falcon tube to collect your initial soil sample. Fill the tube to the 4.5 mL mark with loose soil. Shake to mix. Record the location where and depth at which you collected your soil.
2. Back in lab, transfer 2.5 mL of soil into a fresh 15 mL falcon tube. You should now have one tube with soil at the 2 mL mark and one with soil at the 2.5 mL mark. Label the the 2.5 mL tube of soil and give it to a TA. This will be stored for DNA extraction later on.
3. Prepare small 1.5 mL Eppendorf tubes for serial dilution. Fill each of 4 Eppendorf tubes with 900 µL of sterile water.

4. Add sterile H20 to the tube containing your soil sample until the water level reaches 10 mL. Shake to mix, then let the tube sit for a few minutes to let the soil particles settle to the bottom. Dip a pH strip into the liquid to get a pH reading. Then, using a pipette to pick up some liquid near the top, add 100 µL to one of the small tubes you prepared for serial dilution. This will be your 1/10 dilution.
5. Now, mix up and down in your 1/10 dilution tube and place 100 µl from this tube into another prepped small tube, making a 1/100 dilution. Now take 100 µl from the 1/100 dilution, and repeat with another prepped tube. Repeat once more with your last tube so that you end up with 4 dilutions: 1/10, 1/100, 1/1000, 1/10000.
6. Label two TSA + Nys plates, one with today's date, your group name/number, and "1/100 dilution" and one with "1/10000 dilution." (Nys is short for nystatin, which is an anti-fungal added to the plates to prevent them from being taken over by fungal species present in your soil.)
7. Place 100 µl of the 1/100 dilution onto one plate and 100 µl of the 1/10000 dilution onto the other.
8. Spread the liquid evenly over your plate using the round end of an inoculation loop. Take care to spread only on the surface of the agar; do not dig into it or pierce it with the loop.
9. Leave your plates to grow at room temperature.

3.2 Monitoring (Week 4)

You will want to check on your plates throughout the week that you plated them. This can be during class time or during office hours, and should not take more than a minute or two.

Observe the soil plates and note whether you see growth yet or not. Soil contains fast growers and slow growers. Antibiotic producers are more likely to be found as slow growers. If you see things that have already grown to be pretty large at this point, circle them on your plate so that you know not to pick them as potential antibiotic producer colonies.

On day 3 or 4, you will probably start to see the emergence of new types of colonies. In our tests of this experiment, we observed the presence of bright yellow colonies after a few days. You might see colonies that look fungus-like, or otherwise unique from most of the other things on the plate. These are good candidates for antibiotic producers.

If your plates are starting to become crowded with bacteria to the point where it would be hard to pick single colonies, feel free to move on to the next steps early.

3.3 Patch plate preparation (Week 5)

You will now prepare a patch plate to grow up colonies of interest. This helps ensure we have enough of each species to sequence and to pick for resistance testing.

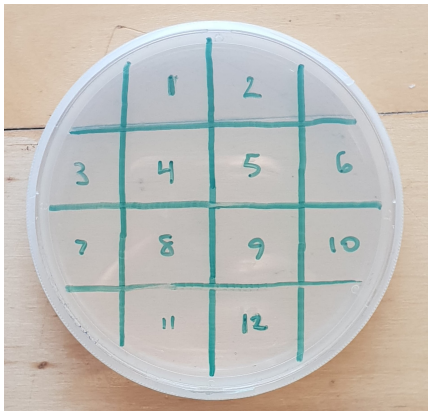


Figure 1: An example grid pattern for the patch plate you will make of soil colonies. The corner spots are not used because they are too small.

1. Draw a 4x4 grid (like in Figure 1) on the agar side of one of your 10% TSA plates and number the spots sequentially.
2. Dip an inoculation loop into the vial of *E. coli* K12 bacteria that a TA will give to you. Then spread the loop on the agar in your 10% TSA plate, only in the grid space you labeled K12. This is a test to make sure there is live, culture-able *E. coli* in your agar stab.
3. Use inoculation loops to pick colonies of interest off of your soil plate and spread them onto different squares on your patch plate. Be careful to avoid spreading to the edges and corners of your grid spaces so that the different species do not grow into each other. Leave at least one grid space empty. Also try to choose a variety of colonies (different morphologies, different colors, fast growers, slow growers, etc.). Make sure to write down which number you assigned to each colony, along with its characteristics, in your lab notebook!
4. Leave this plate out on the bench to grow at room temperature.

3.4 Resistance testing (Week 5)

You will now pick some colonies of interest from your plates to see what concentration of antibiotic they can survive. Make sure to wear gloves and be extra cautious on this day, since you have amplified all the species you isolated.

You will be given a gradient antibiotic plate that has an increasing concentration of antibiotic throughout the agar. The low and high antibiotic concentration regions

will be marked. You will streak a line of each species you want to test across the plate, and later you will be able to calculate the effective concentration of antibiotic they were able to grow to.

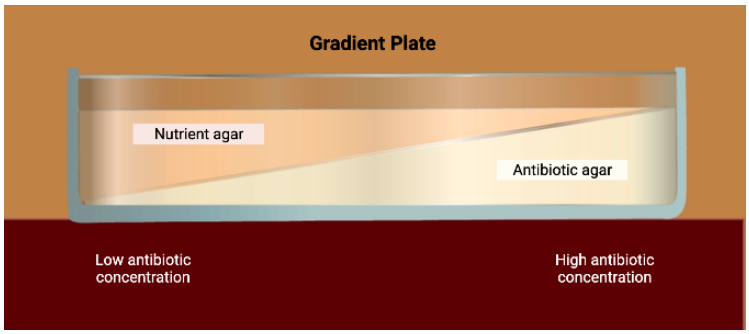


Figure 2: Schematic of gradient plate adapted from here.

1. Prepare some 1.5 mL Eppendorf tubes with 20 µL sterile water in each.
2. Pick each species of interest and pipette up and down with it in an Eppendorf tube. Try to choose colonies of roughly equal size across species. Use an inoculation loop to dip into the tube and draw a line with it on your plate. Repeat this for each colony, giving each a different number, using a different tube and inoculation loop for each, and being careful to keep the lines separate on your plate.
3. Repeat step 3 with a colony of *E. coli* K12, which will be on a plate a TA provides for you.
4. Now, let the plates sit with the lids slightly open until the lines are dry. Once they are dry, place them lid-side down on the bench. In the next day or so, you will see growth of resistant species. You should see no growth of the *E. coli* line on any of your plates. This is your control.

3.5 Antibiotic producer testing (Week 5)

You will now streak some of your soil colonies against *E. coli* to see if you found any antibiotic producers.

1. Draw a grid on a fresh 10% TSA plate and number it, repeating the pattern of the patch plate you previously made.
2. Pick up some K12 from a plate a TA will give you, and spread it over the entirety of your fresh plate using an inoculation loop. Turn the plate and spread repeatedly (without picking up more bacteria) to ensure you cover the whole plate.

3. Use the end of an inoculation loop to pick up some bacteria from one of the grid spots on your patch plate, and make a small streak in the corresponding square on your new plate.
4. Repeat for each colony.

3.6 Look at your results! (Week 5)

1. Check out your plates! On your antibiotic plates, you will see that some colonies were able to grow while others did not. Using a ruler, measure the distance from the low concentration end of the plate to the position of the most resistant colony for each species. DO NOT OPEN THE PLATE, since these bacteria are now potentially resistant to a very high concentration of antibiotic.
2. On your producer plate, you might see that some of your colony streaks have a cleared "halo" around them (Figure 2). This is a sign that they have secreted an antibiotic capable of killing the surrounding *E. coli*! Make note of any species that did this.
3. Take pictures of each of your plates.
4. Finally, report your results in the class spreadsheet/communal document for this module. There, you will specify your soil source, the characteristics of the colonies you picked, whether you had colonies that survived on each antibiotic and antibiotic concentration, and whether you got antibiotic producers. You will also include pictures of your results.

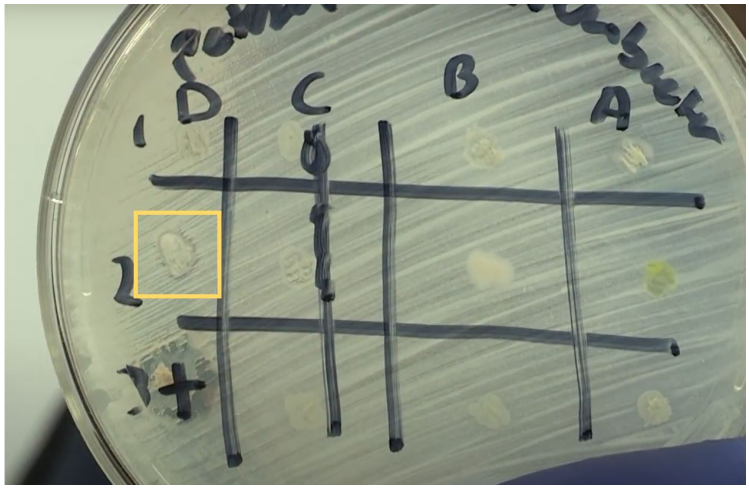


Figure 3: An example of an antibiotic producer test plate taken from here. The boxed colony is an antibiotic producer, surrounded by a thin zone of inhibition.

Don't be discouraged if you didn't find any antibiotic producers! Every soil environment is very different, and there is no way to know exactly what you will get beforehand.

3.7 Cleaning up (Week 5)

Properly disposing of all your materials is very important. While it is unlikely that you've grown anything particularly dangerous, it is important to be careful. We don't want to release resistant microbes or pathogens into the environment. So, you will use bleach to kill any bacteria on your plates before disposing of them. Since this will require you to open the plates, you need to make sure to wear proper PPE, **including an N95 mask.**

Pour 10% bleach solution over each of your plates and cover them with their lids. Let the plates sit for at least 30 minutes. After this much time has passed, dump the bleach in the sink and place the plates in the biotrash.

3.8 DNA Extraction (Week 6)

We will now use the leftover soil from your initial collection and extract microbial DNA from it using Qiagen's DNEasy PowerLyzer Powersoil kit. Soil bacteria can have really tough cell membranes that are hard to lyse, so this extraction kit is specifically designed to get them to lyse and release DNA.

We need to introduce as little bias/contamination as possible. Also, we are sharing reagents. Make sure not to contaminate the reagents! Do NOT reuse tips!

1. Read the manufacturer's handbook before starting.
2. Follow the manufacturer's protocol **to the letter**. You can reference a summarized guide here. Both the quick start and handbook are reproduced at the end of this document.
3. Make sure to always label tubes properly (using Sample IDs) and record the identities of tube labels in your lab notebook.

After you finish your extraction, the TAs will send the samples out for sequencing. We will also need to sequence the individual colonies from your patch plates, so you know what you isolated. We will send the plates to the sequencing company, Laragen, who will do DNA extraction on the individual isolates for us.

3.9 Analysis

We will use QIIME2 for analysis of the populations in the soil. You will work through tutorials to familiarize yourself with the software and enable analyses.

4 Assignment

The assignment for this module is much more open-ended. We are looking at microbial communities that are largely unstudied. There is much more room for

discussion in the problems. There are fewer questions than usual, but each requires more analysis and discussion that usual. You may use the class's data in your responses.

Problem 0 (Summary)**.**
Write a summary of this experiment, its objectives, and conclusions between one paragraph and one page in length. Specifically, write about the motivation of the study and what you are looking for with respect to microbial communities in different soil conditions.

Problem 1 (Plating results)**.**
Consolidate the results of your species isolation. Show pictures of your species with labels. Make note of those you found to be antibiotic producers, showing a picture of the zone of inhibition they may have created in *E. coli* culture. Show a picture of your resistance testing plate, and make note of the approximate inhibitory concentration each species was able to grow to. The gradient in the antibiotic plates should be linear, so the ratio of the distance of max growth to the plate length should equal the ratio of inhibitory concentration to antibiotic concentration of the bottom agar layer.

Problem 2 (Population genomics results)**.**
Provide results of your analysis about the populations of various species in the soil, for the various conditions tied to the soil (depth of collection, pH, plant type, etc.). This can include principle coordinate analysis, relative abundances, etc. This is intentionally open ended, and we encourage you to explore your data and report your findings. Be sure to comment on the significance of the results, e.g., what fraction of the total population is made up by the species you found to be resistant?

This section is where most of the code cells in your notebook will be. Do not just show the code cells; you must have ample text in the markdown cells explaining what you are doing.

Problem 3 (Discussion)**.**
Expand on the results with any speculations you may have on why you saw the results you did. You may also posit other hypotheses to test and propose experiments to test them. You may choose to look into the resistance mechanisms by which a cell can gain resistance to the antibiotics we used and speculate about how likely it would be to develop resistance.

Problem 4 (Data and code)**.**
Attach all pertinent code, data, and sample images not specifically asked for in the other problems.

Appendix: Extraction protocol from Qiagen

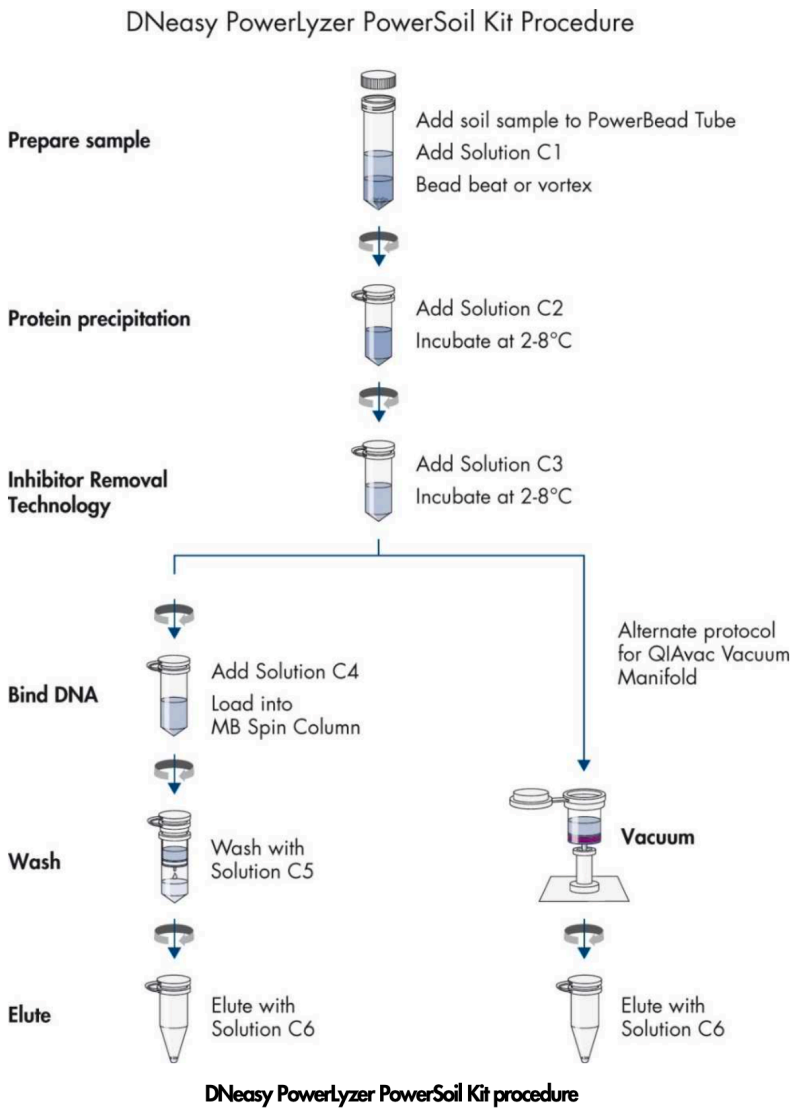


Figure 4: A visualization of the DNA extraction kit protocol, taken from the Qiagen DNEasy PowerLyzer Powersoil kit handbook.

Quick-Start Protocol August 2016

DNeasy® PowerLyzer® PowerSoil® Kit

The DNeasy PowerLyzer PowerSoil Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C)
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use

1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
4. Bead beating options:
   A. **PowerLyzer 24 homogenizer:** Place the PowerLyzer Glass Bead Tubes into the tube holder for the PowerLyzer 24. The PowerBead Tubes must be balanced on the tube holder. Run the samples for a time and RPM suitable for your soil type.
   Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result
   B. **Vortex:** Secure the PowerBead Tubes horizontally using a Vortex Adapter tube holder (cat. no. 13000–V1–24). Vortex at maximum speed for 10 min.
   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Centrifuge Bead Tubes at 10,000 x *g* for 30 s. **Do not** exceed 10,000 x *g*.
   Note: Centrifuge for 3 min at 10,000 x *g* for clay soils or if your soil is not completely pelleted after 30 s.
6. Transfer the supernatant to a clean 2 ml collection tube (provided).

Note: Expect 400–500 µl. Supernatant may still contain some soil particles.

7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.

   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.

8. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml collection tube (provided).

9. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.

10. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml collection tube (provided).

11. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.

12. Load 675 µl of the supernatant onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow through and add an additional 675 µl of supernatant.

13. Centrifuge at 10,000 x *g* for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x *g* for 1 min.
    Note: A total of three loads for each sample processed are required.

14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x *g*.

15. Discard the flow through. Centrifuge again for 1 min at 10,000 x *g*.

16. Carefully place spin filter in a clean 2 ml collection tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.

17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-Free PCR Grade Water or TE buffer (cat. no. 17000-10).

18. Centrifuge for 30 s at 10,000 x *g*. Discard the MB Spin Column

19. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20° to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

January 2020

DNeasy® PowerLyzer® PowerSoil® Kit Handbook

For the isolation of DNA from tough soil microbes; optimized for use with bead-based homogenizers

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerLyzer PowerSoil Kit	**(50)**	**(100)**
Catalog no.	**12855-50**	**12855-100**
Number of preps	**50**	**100**
MB Spin Columns	50	2 x 50
PowerBead Tubes, Glass 0.1 mm	50	2 x 50
Solution C1	6.6 ml	6.6 ml
Solution C2	15 ml	2 x 15 ml
Solution C3	15 ml	2 x 15 ml
Solution C4	72 ml	2 x 72 ml
Solution C5	30 ml	2 x 30 ml
Solution C6	9 ml	2 x 9 ml
Collection Tubes (2 ml)	4 x 50	8 x 50
Quick-Start Protocol	1	1

Storage

The DNeasy PowerLyzer PowerSoil Kit reagents and components can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All DNeasy products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

WARNING: Solution C5 contains ethanol. It is flammable.

WARNING: Do not use bleach to clean the inside of the QIAvac® 24 Plus Manifold.

CAUTION 	**DO NOT add bleach or acidic solutions to directly to the sample preparation waste**

PowerBead Solution and Solution C4 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy PowerLyzer PowerSoil Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerLyzer PowerSoil Kit differs from the DNeasy PowerSoil Kit because it includes PowerBead Tubes with glass beads that are optimized for robust bead-based homogenizers like the PowerLyzer 24 Homogenizer (110/220V) (cat. no. 13155) as well as Fast Prep® and Precellys® instruments.

Principle and procedure

The DNeasy PowerLyzer PowerSoil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples in a fraction of the time required by traditional homogenization methods. The kit utilizes Inhibitor Removal Technology® (IRT), and is intended for use with environmental samples containing high humic acid content, including difficult soil types, such as compost, sediment and manure. Other more common soil types have also been used successfully with this kit. The isolated DNA has a high level of purity, which allows for more successful PCR amplification of organisms from the sample. PCR analysis has been used to detect a variety of organisms, including bacteria (e.g., *Bacillus subtilis*, *Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and actinomycetes (e.g. *Streptomyces*). Homogenization with the PowerLyzer 24 Homogenizer is faster than using traditional vortex methods and minimizes cross-contamination.

The DNeasy PowerLyzer PowerSoil Kit uses a humic substance/brown color removal procedure. This procedure is effective at removing PCR inhibitors from even the most difficult soil types. Environmental samples are added to a bead beating tube and homogenized rapidly and thoroughly using the PowerLyzer 24 Homogenizer. Cell lysis occurs by mechanical and chemical interaction. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. The isolated DNA is ready for PCR analysis and other downstream applications.

Optimized for homogenization with the PowerLyzer 24 Homogenizer

The DNeasy PowerLyzer PowerSoil Kit contains PowerBead Tubes with 0.1 mm glass beads, which allows for more options in choosing homogenization methods, including the use of the PowerLyzer 24 Homogenizer. The PowerLyzer's velocity and proprietary motion combine to provide the fastest homogenization possible, minimizing time spent processing samples. The 0.1 mm PowerBead Tubes are suitable for both high-velocity bead beating and vortex beating, depending on the level of homogenization desired. Alternative pre-filled bead tube options are available using harder matrices for grinding.

For the PowerLyzer 24 Homogenizer, the starting point for low-biomass and clay soils is 45 seconds at a setting of 4000 RPM. For loamy soils, such as forest soils, settings between 2500–2800 RPM provide the highest yields without compromising integrity. To use the PowerLyzer 24 Homogenizer in place of a vortex, a setting of 2000 RPM may be used for up to 5 minutes. Settings and duration of homogenization may need to be optimized for specific soil types and research projects to ensure highest yields and integrity.

Using the DNeasy PowerLyzer PowerSoil Kit with other homogenizers

Published references for using the DNeasy PowerLyzer PowerSoil Kit with a FastPrep instrument are available from technical support. For more information, please contact Technical Support at **support.qiagen.com**.

To isolate DNA using the DNeasy PowerLyzer PowerSoil Kit with FastPrep or Precellys homogenizers, use the conversion chart (see Table 1 below) to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer 24 Homogenizer, fewer cycles are required to generate the same effect using it compared to other homogenizers.

You may want to perform extractions using the PowerLyzer 24 Homogenizer at the equivalent speed and number of cycles as your current instrument and then compare the results to those obtained using less time or lower speeds to determine which settings give the best results.

To start homogenizing, use a setting of 5 on the FastPrep or 5000 RPM on the Precellys for one pulse of 45 seconds using the PowerBead Tubes provided in this kit. For fungi or other species that are difficult to lyse, a 10-minute heating step at 65°C may be performed prior to bead beating. More than one pulse of bead beating or harder beads may be used. However, keep in mind that can cause DNA integrity to decrease.

Table 1. Conversion chart for using other homogenizers with the DNeasy PowerLyzer PowerSoil Kit

PowerLyzer 24	FastPrep 24 (m/s)	Precellys 24
2500	4	5000
2600	–	5200
2700	–	5400
2800	4.5	5600
2900	–	5800
3000	–	6000
3100	5	6200
3200	–	6400
3300	–	6600
3400	5.5	6800
3500	–	–
3600	–	–
3700	6	–
3800	–	–
3900	–	–
4000	6.5	–

Note: Settings equivalent to slower than 2500 RPM or faster than 4000 RPM on the PowerLyzer 24 are not obtainable with FastPrep or Precellys homogenizers.

High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNA-binding and column-washing steps using MB Spin Columns. The QIAvac 24 Plus Manifold allows for processing of up to 24 MB Spin Column preps at a time. For additional high-

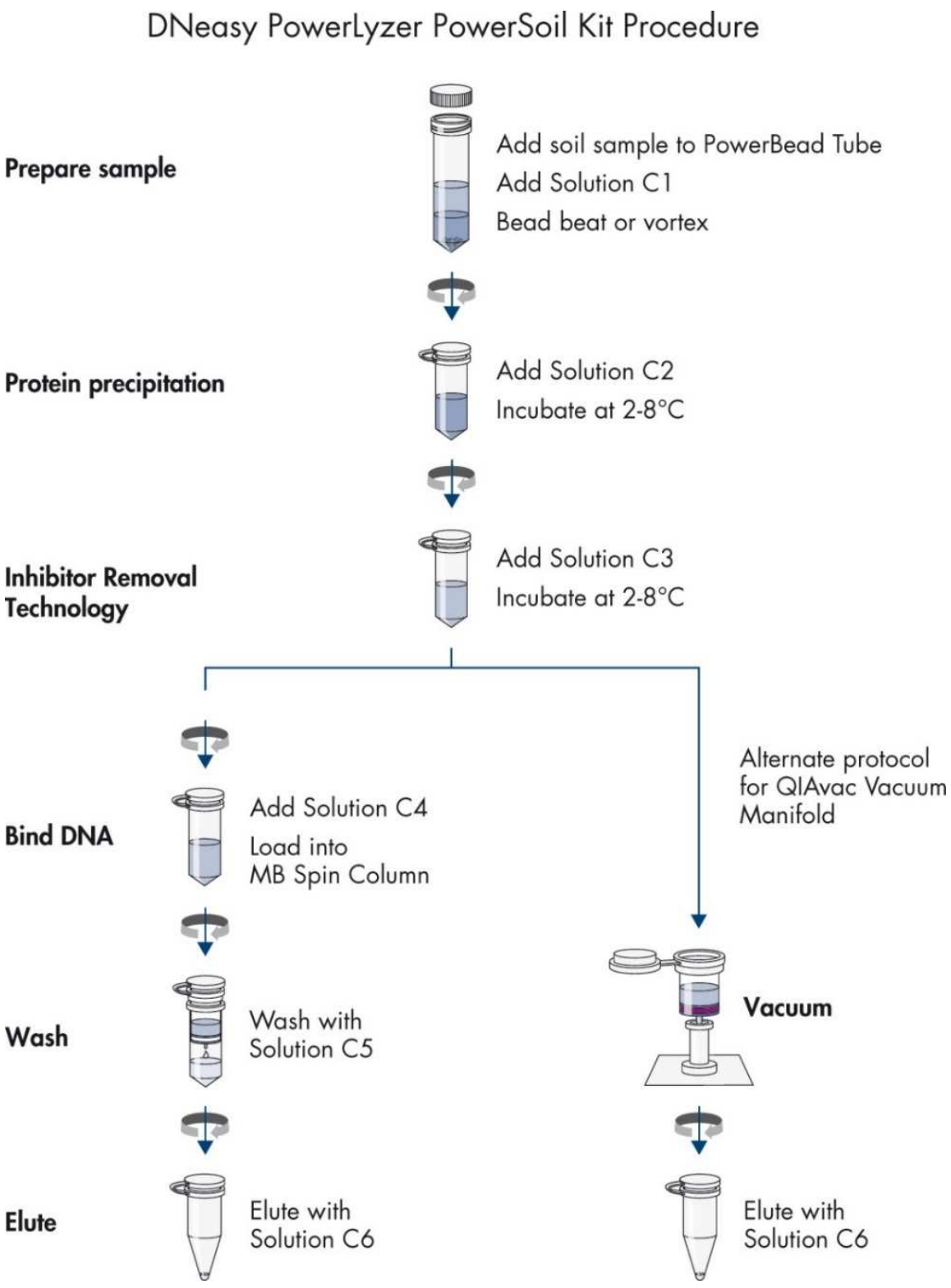
throughput options, we offer the DNeasy 96 PowerSoil Pro (cat. no. 47017) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8.5 cm x 8 cm) at 4500 x *g*. For 96-well homogenization of soil, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively.)

Automated purification of DNA on QIAcube Instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the DNeasy PowerLyzer PowerSoil Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.



DNeasy PowerLyzer PowerSoil Kit procedure

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- PowerLyzer 24 Homogenizer or another bead homogenizer
- Microcentrifuge (10,000 x *g*)
- Pipettors (60–750 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- 100% ethanol (for the QIAvac 24 Plus Manifold protocol only)

Important Notes

- Make sure the tubes rotate freely in your centrifuge without rubbing.
- The PowerLyzer 24 may cause marring of labels on the tops of the PowerBead Tubes. To ensure proper sample identification, label sides and tops of the tubes.

Protocol: Experienced User

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.

Procedure

1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
4. Bead beating options:

   A. PowerLyzer 24 Homogenizer: Place the PowerBead Tubes into the tube holder for the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced in the tube holder. Run the samples for a time and RPM suitable for your soil type.
   Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result.

   B. Vortex: Secure the PowerBead Tubes horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: If you are using a 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge at 10,000 x *g* for 30 s. Do not exceed 10,000 x *g*.
   Note: Centrifuge for 3 min at 10,000 x *g* for clay soils or if your soil is not completely pelleted after 30 s.
6. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 400–500 µl. Supernatant may still contain some soil particles.

7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.
8. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
9. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.
10. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
11. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.
12. Load 675 µl of the supernatant onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow-through and add an additional 675 µl of supernatant.
13. Centrifuge at 10,000 x *g* for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x *g* for 1 min.
    Note: A total of three loads for each sample processed is required.
14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x *g*.
15. Discard the flow-through. Centrifuge again for 1 min at 10,000 x *g*.
16. Carefully place the MB Spin Column in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.
17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer.
18. Centrifuge for 30 s at 10,000 x *g*. Discard the MB Spin Column.
19. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–90°C to –15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: Detailed

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.

Procedure

1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
   Note: Once the sample is loaded into a PowerBead Tube, the next step is homogenization and lysis. The PowerBead Tube will help disperse the soil particles.
2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
   Note: Vortexing mixes the components in the PowerBead Tube and begins to disperse the sample.
4. Bead beating options:
   A. PowerLyzer 24 homogenizer: Place the PowerBead Tubes into the tube holder for the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced in the tube holder. Run the samples for a time and RPM suitable for your soil type.
   Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result.

B. Vortex: Secure the PowerBead Tubes horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min. The bead beating or vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1–3 and mechanical shaking introduced at this step. By shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

Note: The PowerLyzer 24 can homogenize soils at high acceleration in only 45 s, using the glass beads to achieve lysis in less time. The time and speed for each soil may vary. PowerBead Tubes may also be used with a Vortex-Genie 2 and a Vortex Adapter (cat. no. 13000-V1-24). The Vortex Adapter is designed to be a simple platform to keep the tubes tightly attached to the vortex. Using tape to attach tubes is not recommended.

5. Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge at 10,000 x *g* for 30 s. Do not exceed 10,000 x *g*.
   Note: Centrifuge for 3 min at 10,000 x *g* for clay soils or if your soil is not completely pelleted after 30 s.
6. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 400–500 µl. Supernatant may still contain some soil particles.
7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step. Solution C2 has Inhibitor Removal Technology (IRT). It contains a reagent that can 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.

8. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
   Note: The pellet at this point contains non-DNA organic and inorganic material, including humic acid, cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.
9. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step. Solution C3 has 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.
10. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
    Note: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.
11. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.
    Note: 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 MB Spin Columns.
12. Load 675 µl of the supernatant onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow-through and add an additional 675 µl of supernatant.
13. Centrifuge at 10,000 x *g* for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x *g* for 1 min.
    Note: A total of three loads for each sample processed is required. DNA is selectively bound to the silica membrane in the MB Spin Column device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x *g*.
    Note: Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.
15. Discard the flow-through. Centrifuge again for 1 min at 10,000 x *g*.
    Note: This flow-through fraction is non-DNA organic and inorganic waste removed from the silica MB Spin Column membrane by the ethanol wash solution. The second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications, such as PCR, restriction digests and gel electrophoresis.
16. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.
17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer.
    Note: Placing Solution C6 in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column membrane. As Solution C6 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.
18. Centrifuge for 30 s at 10,000 x g. Discard the MB Spin Column.
19. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–90°C to –15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: QIAvac 24 Plus Vacuum Manifold

Important points before starting

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.
- For each sample lysate, use one MB Spin Column. Keep the MB Spin Column in the attached 2 ml Collection Tube and continue using the Collection Tube as an MB Spin Column holder until needed for the vacuum manifold protocol.
- Label each Collection Tube top and MB Spin Column to maintain sample identity. If the MB Spin Column becomes clogged during the vacuum procedure, switch to the centrifugation protocol.
- 100% ethanol will be needed for step 8 of this protocol.

Procedure

1. Connect the QIAvac 24 Plus to the vacuum source using the QIAvac Connecting System (for more details, refer to the *QIAvac 24 Plus Handbook*, Appendix A, page 16).
2. Insert a VacValve into each Luer slot of the QIAvac 24 Plus that is to be used. Close unused Luer slots with Luer plugs or close the inserted VacValve.
3. Insert a VacConnector into each VacValve. Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place an MB Spin Column into each VacConnector on the manifold.
5. Transfer 650 µl of prepared sample lysate (after step 11 of the centrifugation protocol) to an MB Spin Column.
6. Turn on the vacuum source and open the VacValve of the port. Hold the tube in place when opening the VacValve to keep the MB Spin Column steady. Allow the lysate to pass through the MB Spin Column completely.

7. After the lysate has passed through the column completely, load again with 650 µl of lysate. Continue until all the lysate has been loaded onto the MB Spin Column. Close the VacValve of that port.
   Note: If the MB Spin Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.
8. Add 800 µl of 100% ethanol to completely fill the MB Spin Column. Open the VacValve while holding the column steady. Allow the ethanol to pass through the column completely. Close the VacValve.
9. Add 500 µl of Solution C5 to each MB Spin Column. Open the VacValve and apply a vacuum until Solution C5 has passed through the MB Spin Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
10. Turn off the vacuum source and open an unused port to vent the manifold. If all the ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
11. Remove the MB Spin Column and place in the original labeled 2 ml Collection Tube. Place into the centrifuge and spin at 13,000 x *g* for 2 min to completely dry the membrane.
12. Transfer the MB Spin Column into a new 2 ml Collection Tube and add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-free PCR-grade water (cat. no. 17000-10) may be used.
13. Centrifuge at 13,000 x *g* for 1 min at room temperature (15–25°C).
14. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–90°C to –15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Soil processing		
a)	Amount of soil to process	The QIAGEN DNeasy PowerLyzer PowerSoil Kit is designed to process 0.25 grams of soil. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions.
b)	Soil sample is high in water content	Add soil sample to PowerBead Tube and centrifuge at 10,000 x *g* for 30 seconds at room temperature (15–25°C). Remove as much liquid as possible with a pipet tip. Resume protocol from Step 2.
DNA		
a)	DNA does not amplify	Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction. Diluting the template DNA should not be necessary with DNA isolated using the PowerLyzer PowerSoil DNA Kit; however, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.

		Comments and suggestions
b)	Eluted DNA is brown	If you observe coloration in your samples, please contact Technical Support for suggestions.
c)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 10 µl of 5 M NaCl and inverting 3–5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature (15–25°C). Decant all liquid. Remove residual ethanol in a speed vac, a desiccator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.
d)	DNA floats out of a well when loading a gel	This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 19 and not transferring liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual Solution C5.
e)	Storing DNA	DNA is eluted in Solution C6 (10 mM Tris) and must be stored at –90°C to –15°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions, such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-free PCR-grade water (cat. no. 17000-10).
Alternative lysis methods		
a)	Cells are difficult to lyse	After adding Solution C1, incubate at 70°C for10 minutes. Resume protocol from step 3.
b)	Reduction of shearing of DNA	After adding Solution C1, vortex 3–4 seconds, then heat to 70°C for 5 minutes. Vortex 3–4 seconds. Heat another 5 minutes. Vortex 3–4 seconds. This alternative procedure will reduce shearing but may also reduce yield.

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerLyzer PowerSoil Kit (50)	For the bead-based isolation of DNA from tough soil microbes	12855-50
DNeasy PowerLyzer PowerSoil Kit (100)	For the isolation of DNA from tough soil microbes, optimized for use with bead-based homogenizers	12855-100
DNeasy PowerSoil Pro Kit (50)	For the isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For the isolation of microbial genomic DNA from all soil types	47016
DNeasy 96 PowerSoil Pro Kit (384)	For the isolation of DNA from up to 384 soil samples in less than one day	47017
DNeasy PowerMax® Soil Kit (10)	For the isolation of microbial DNA from large quantities of soil with low microbial load	12988-10
DNeasy PowerClean® Cleanup Kit (50)	For secondary DNA clean-up and removal of inhibitors from heparin	12877-50
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

Product	Contents	Cat. no.
Related products		
RNeasy® PowerSoil Total RNA Kit (25)	For the isolation of high quality total RNA from all soil types	12866-25
MagAttract® PowerSoil DNA KF Kit (384)	For the automated, hands-free isolation of DNA from soil	270004-KF
Accessories		
PowerBead Tubes, Ceramic 1.4 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13113-50
PowerBead Tubes, Glass 0.5 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13116-50
PowerBead Tubes, Glass 0.1 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13118-50
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
QIAvac 24 Plus	Vacuum Manifold for processing 1–24 spin columns; includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs and Quick Couplings	19413

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.