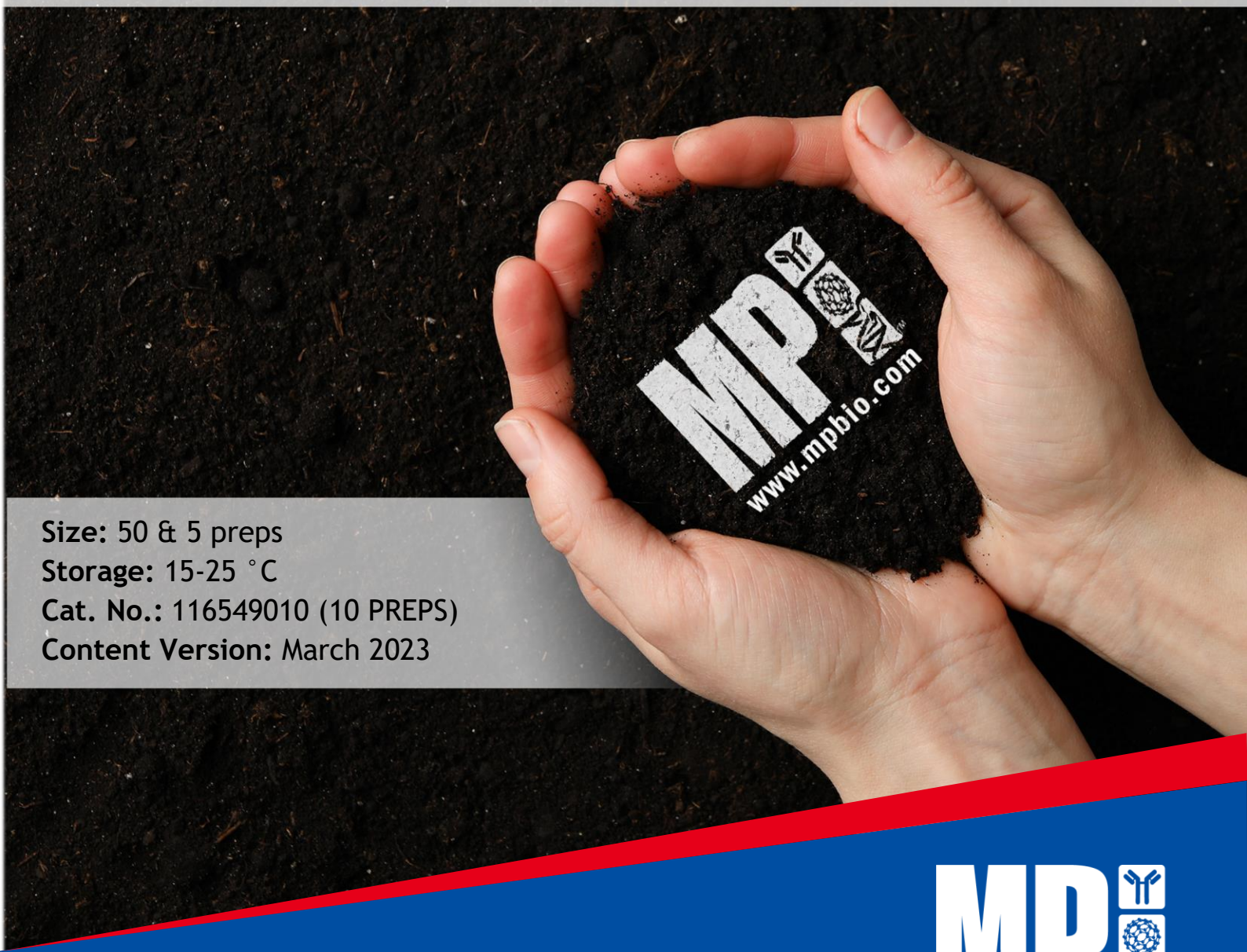
Instruction Manual
Research Use Only

SPINeasy DNA Maxi Kit for Soil

Isolation of microbial genomic DNA from large quantities of soil



Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116549010 (10 PREPS)
Content Version: March 2023

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1. Introduction to SPINeasy DNA Maxi Kit for Soil

Soil samples are a complex environment characterized by large variations of microbial load and diversity that prove to be challenging to analyze due to a large presence of inhibitory compounds such as humic acid, heavy metals, and other aromatic components.

The SPINeasy® DNA Maxi Kit for Soil has been optimized to enable fast and convenient isolation of pure microbial DNA from large amount of soil. All types of soil samples are compatible with this kit, including those with high microbial load and humic acid content such as compost (up to 10 g) or those with low microbial load such as sand (up to 20 g). The SPINeasy® DNA Maxi Kit for Soil effectively lyses various microbiome population such as bacteria, fungi, viruses, protists with a higher purity and reduced processing time. The isolated DNA is fully compatible with downstream PCR and NGS applications (16S and whole genome) without the need of further inhibitor removal step.

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Kit Specifications at a Glance

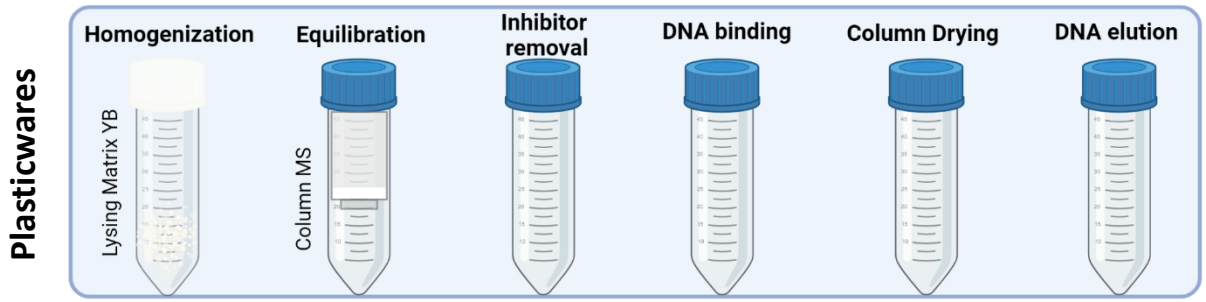
Technology	Silica membrane technology
Format	Maxi spin column
Vacuum manifold	Yes
Sample	Soil with high/low microbial load
Sample amount	up to 10 g (high biomass e.g., compost) or 20 g (low biomass e.g., sand/clay)
Typical yield	up to 800 µg (experimentally observed)
Elution volume	< 2 mL
Preparation time	90 min

2. Kit Components and User Supplied Materials

2.1 SPINeasy DNA Maxi Kit for Soil Component

Components	10 PREPS (Cat.No.: 116549010) Package	Cat. No.
Equilibration Buffer	2x28 mL	112075904
BigPrep™ Lysing Matrix YB	10x50 mL	116549011
Buffer MS1A	180 mL	116549012
Buffer MS1B	2x35 mL	116549013
Buffer MS2	2x30 mL	116549014
Buffer MS3	2x130 mL	116549015
Buffer MS4	200 mL	116549016
Buffer MS5	180 mL	116549017
Elution buffer	2x30 mL	116549021
Buffer MS6	0.4 mL	116549018
Column MS	10 ea	116549019
50 mL Tubes	4x10 ea	116549020
Quick-start protocol	1 ea	-
Instruction Manual	Available www.mpbio.com	-
MSDS & CoA	Available www.mpbio.com	-

For faster processing, it is advised to pre-position the plasticwares and columns used during the extraction as depicted below



2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No. 116005500) with BigPrep™ adapter 50 mL tube (Cat. No. 116002525) or Vortex mixer. Do not use tape or hand to hold the matrix during homogenization as it could lead to reduced performance and poor reproducibility.
- Swing bucket centrifuge that can spin 50 mL centrifuge tubes @ ≥3,000 g (~4000 rpm). Higher speed is preferred.
- Pipette controllers and Nuclease-free serological pipette.
- (Optional) a commercial vacuum manifold with luer connectors connected to a vacuum pump.

3. Storage and Kit Stability

All the components and reagents of the SPINeasy® DNA Maxi Kit for Soil can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ If Buffer MS1B has precipitated, heat at 37˚C until precipitate dissolves.
- ❑ The SPINeasy® DNA Maxi Kit for Soil requires a centrifuge capable of spinning 50 mL tubes at 3,000 x g using swing-out rotor to obtain optimal results.
- ❑ **Store Buffer MS2 at 2-8˚C upon receiving the kit**.
- ❑ For faster processing, use vacuum manifold.
- ❑ The sample can be transferred from tube to tube by pouring throughout the entire homogenization and binding procedure to reduce plastic waste.

5. Safety Precautions

Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details. Buffer MS2 contains components that may cause irritation when in contact with human tissue. Buffers MS3 and MS4 include chaotropic salts, which can form highly reactive compounds when combined with bleach. Buffers MS4 and MS5 are flammable.

6. Protocol

Add up to 10 g (high biomass, e.g., compost) or 20 g (low biomass, e.g., sand, clay) of soil to the **50 mL BigPrep Lysing Matrix YB**.

1. **Equilibration**
   Add 5 mL of Equilibration Buffer to Column MS. To be efficient, the Column MS need to be incubated with the Equilibration Buffer for at least 5 min. Spin for 2 min @ maximal speed.

2. **Homogenization**
   Add **15 mL of Buffer MS1A** and **3 mL Buffer MS1B** to the 50 mL BigPrep Lysing Matrix YB for high biomass soil. Alternatively for low biomass soil, use **12 mL of Buffer MS1A** and **6 mL Buffer MS1B**.
   Invert the tube to mix the matrix beads with the soil.
   Homogenize using Fastprep 5m/s, BigPrep, 45 sec or vortex for 20 min @ 2500-2800rpm, centrifuge for **10 min @ maximum speed**.

   Note: *The performance of the DNA output (yield, purify and DNA integrity) obtained using vortex is highly dependent on the model of vortex used. The condition stated above can be used as starting point. The homogenization time and speed may be optimized by the user. Avoid using tape or holding the matrix tubes with hand, which can result in reduced homogenization efficiency, inconsistent results, and reduced yields.*

3. **Contaminant Removal**

   Transfer the supernatant into a new 50 mL Tube (provided) without disrupting the pellet. Add **2 mL** of **Buffer MS2**, shake the tube 5X vigorously. Centrifuge for **8 min @ maximum speed**.

   Note: *the volume of Buffer MS2 is suitable for most soils. Up to 4 mL of Buffer MS2 may be used for soil with high humic acid content while 0.5 mL can be used for low biomass/contaminant soil. The volume may need to be optimized by the user as an excess of Buffer MS2 may decrease the nucleic acid yield.*

4. **Binding**

   Transfer up to **15 mL** of supernatant into a new 50 mL Tube (provided) **without disrupting the pellet**.

   Optional: *a 100 µm sterile strainer (not provided) can be used to filter floating particles if any.*

   Add 18 mL of **Buffer MS3**. If more supernatant is transferred, add 1.2 volume of Buffer MS3 instead. Shake the tube 5X vigorously. The subsequent steps can be performed using either centrifuge or vacuum manifold (faster).

Centrifuge	Vacuum manifold
Add ~15 mL of the lysate into the Column MS. Avoid transferring soil particles if any. Centrifuge for 2 min @ maximum speed. Discard the flow-through and place the column back into the collection tube. Repeat once.	Transfer the Columns MS into the vacuum manifold's luer connector. The collection tube will be used in the next. Load ~15 mL of the lysate into the Column MS while avoiding transferring soil particles if any. Apply vacuum. Repeat until all the lysate has been loaded.
5. First Wash ***Optional:*** *If soil particles are found on the membrane, add* ***2-5 mL*** *of* ***Buffer MS4*** *to the column, swirl the column and discard the buffer. Repeat if required.* Add **10 mL** of **Buffer MS4** and spin for **2 min @ maximum speed**. Discard the flow-through and place the column back into the same collection tube.	**5.First Wash** ***Optional:*** *If soil particles are found on the membrane, add* ***2-5 mL*** *of* ***Buffer MS4*** *to the column, swing the column and discard the buffer. Repeat if required.* Add **10 mL** of **Buffer MS4** and apply vacuum. Transfer back the column to its collection tube .

6. Second wash

Add **15 mL Buffer MS5**. and spin for **2 min at maximum speed**. Transfer the **Column MS** into a 50 mL Tube (provided).

7. Column drying

Spin for 8 min @ maximal speed. If soil particles are still observed on the membrane, invert the column, and tap the column against the bench to discard the debris.

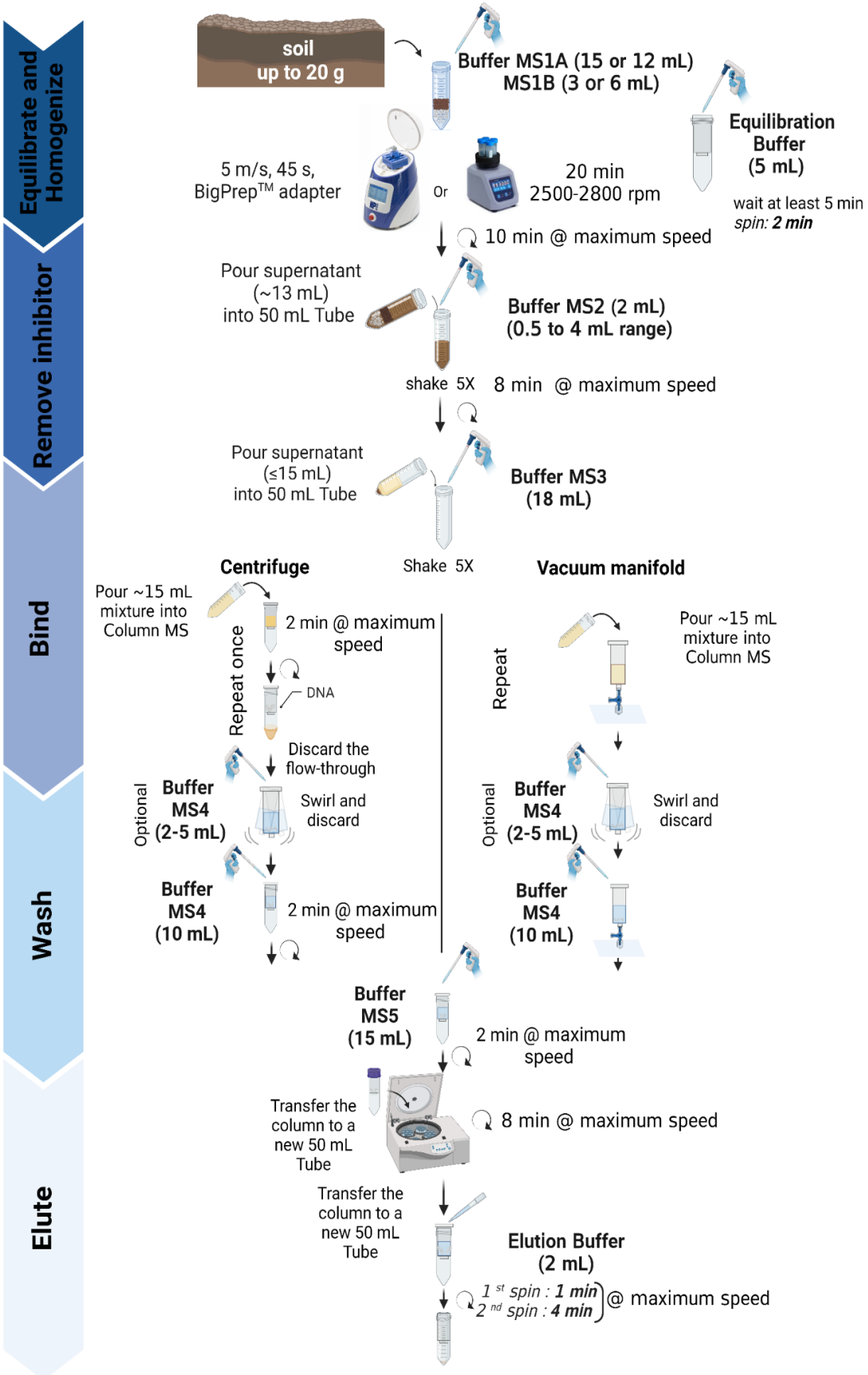
8. Elution

Transfer the column to a new **50 mL Tube** (provided). Add **2 mL** of **Elution Buffer** into the **Column MS** membrane. Incubate for ≥ 2 min, centrifuge for **1** min **@ maximum speed** and reload again the eluted DNA. Wait ≥ 5min and centrifuge for **4** min **@ maximum speed.** The expected elution volume is ~1.8 mL.

Note: *For maximum yield, the second elution may be performed with 1-3 mL of fresh Elution Buffer instead. The genomic DNA can be readily used for downstream application. For extended storage, add* ***Buffer MS6*** *provided at 200X concentration to obtain DNA resuspended in 5 mM Tris and 0.1 mM EDTA (i.e., ~9 µL for 1.8 mL of DNA).*

Note: *The nucleic acid concentration of the sample is calculated from its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µL DNA/mL. Contaminations with, RNA, protein, salt, ethanol and humic acids or other non-nucleic acid contaminants contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real DNA concentration. When measured using a UV spectroscopy, a ratio of A260/A280 between 1.80–1.90 and A260/A230 >1.8 indicates pure DNA. A260/A280 and 260/230 ratio above 2.0 indicate RNA contamination. Conversely, an A260/A280 ratio below 1.8 indicates protein contamination. Additionally, a low A260 / A230 ratio indicate increasing humic acids, but also proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reaction.*

7. Flow Chart


Equilibrate and Homogenize
Remove inhibitor
Bind
Wash
Elute
soil
up to 20 g
Buffer MS1A (15 or 12 mL)
MS1B (3 or 6 mL)
Equilibration Buffer (5 mL)
wait at least 5 min
spin: 2 min
5 m/s, 45 s,
BigPrep™ adapter
Or
20 min
2500-2800 rpm
10 min @ maximum speed
Pour supernatant (~13 mL) into 50 mL Tube
Buffer MS2 (2 mL)
(0.5 to 4 mL range)
shake 5X 8 min @ maximum speed
Pour supernatant (≤15 mL) into 50 mL Tube
Buffer MS3 (18 mL)
Shake 5X
Centrifuge
Vacuum manifold
Pour ~15 mL mixture into Column MS
2 min @ maximum speed
Repeat once
DNA
Discard the flow-through
Optional
Buffer MS4 (2-5 mL)
Swirl and discard
Buffer MS4 (10 mL)
2 min @ maximum speed
Repeat
Pour ~15 mL mixture into Column MS
Optional
Buffer MS4 (2-5 mL)
Swirl and discard
Buffer MS4 (10 mL)
Buffer MS5 (15 mL)
2 min @ maximum speed
Transfer the column to a new 50 mL Tube
8 min @ maximum speed
Transfer the column to a new 50 mL Tube
Elution Buffer (2 mL)
1 st spin : 1 min
2 nd spin : 4 min
@ maximum speed

8. Data

The SPINeasy® DNA Maxi Kit for Soil has been developed to handle wide range of soil samples, regardless of their microbial load or level of contamination. This kit is highly effective in extracting DNA from low or high biomass samples, as evidenced by its optimal yield and A260/280 and A260/230 ratio (Figure 1).

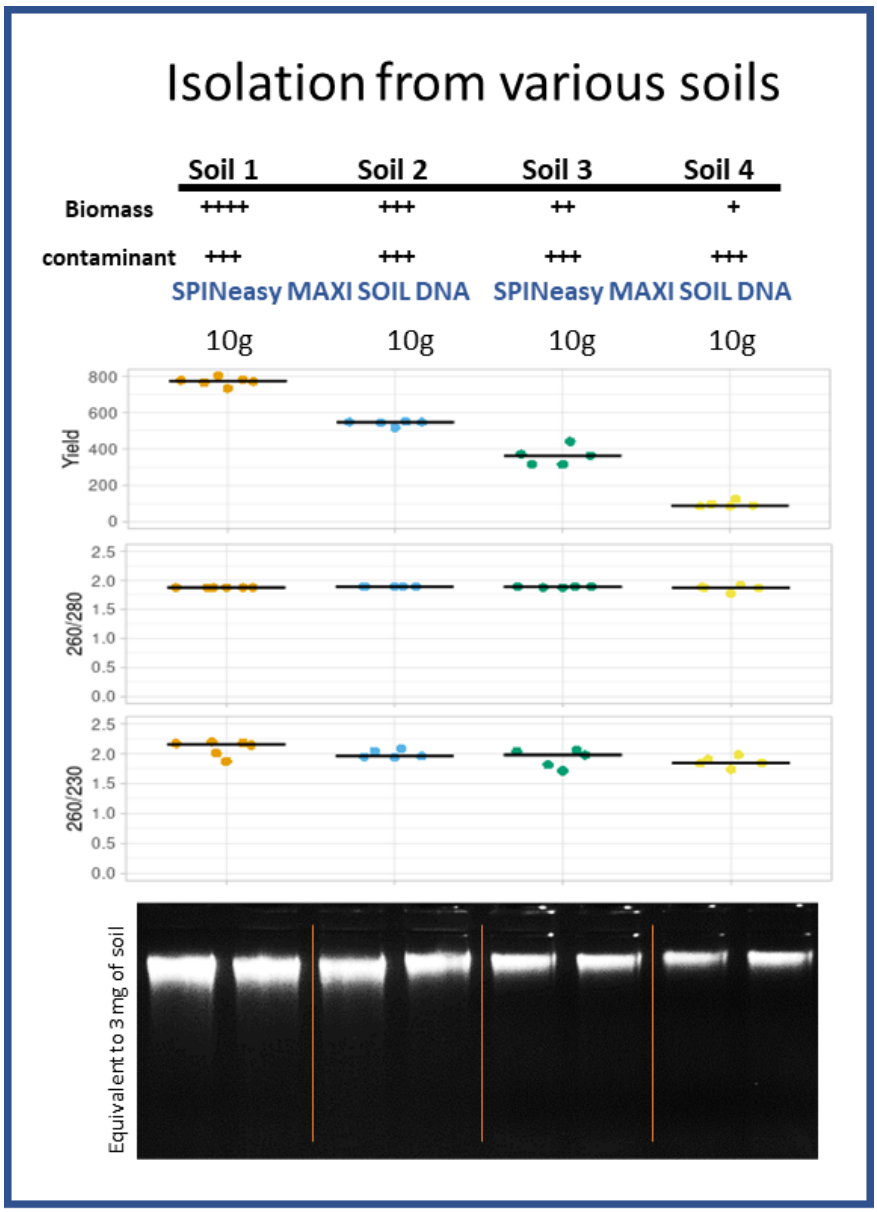


Figure 1. Soil sample with various microbial biomass/ contaminant content (10 g each) were prepared using SPINeasy® DNA Maxi Kit for Soil. The DNA yield and purity (A260/280 and A260/230 ratio) were assessed using spectrophotometer in quadruplicate. Each dot of the plot represents a single extraction. The horizontal bars indicate the median value. The DNA integrity was assessed using DNA gel.

Compared to competitor Q, the SPINeasy® DNA Maxi Kit for Soil is designed to deliver up to 800 µg of gDNA with an optimal purity in a smaller elution volume (2ml vs 5ml). With SPINeasy® DNA Maxi Kit for Soil, the DNA concentration is up to 10X higher than the competitor Q as supported by the more intense DNA bands detected in the Agilent 4150 TapeStation® profile (figure 2, bottom panel).

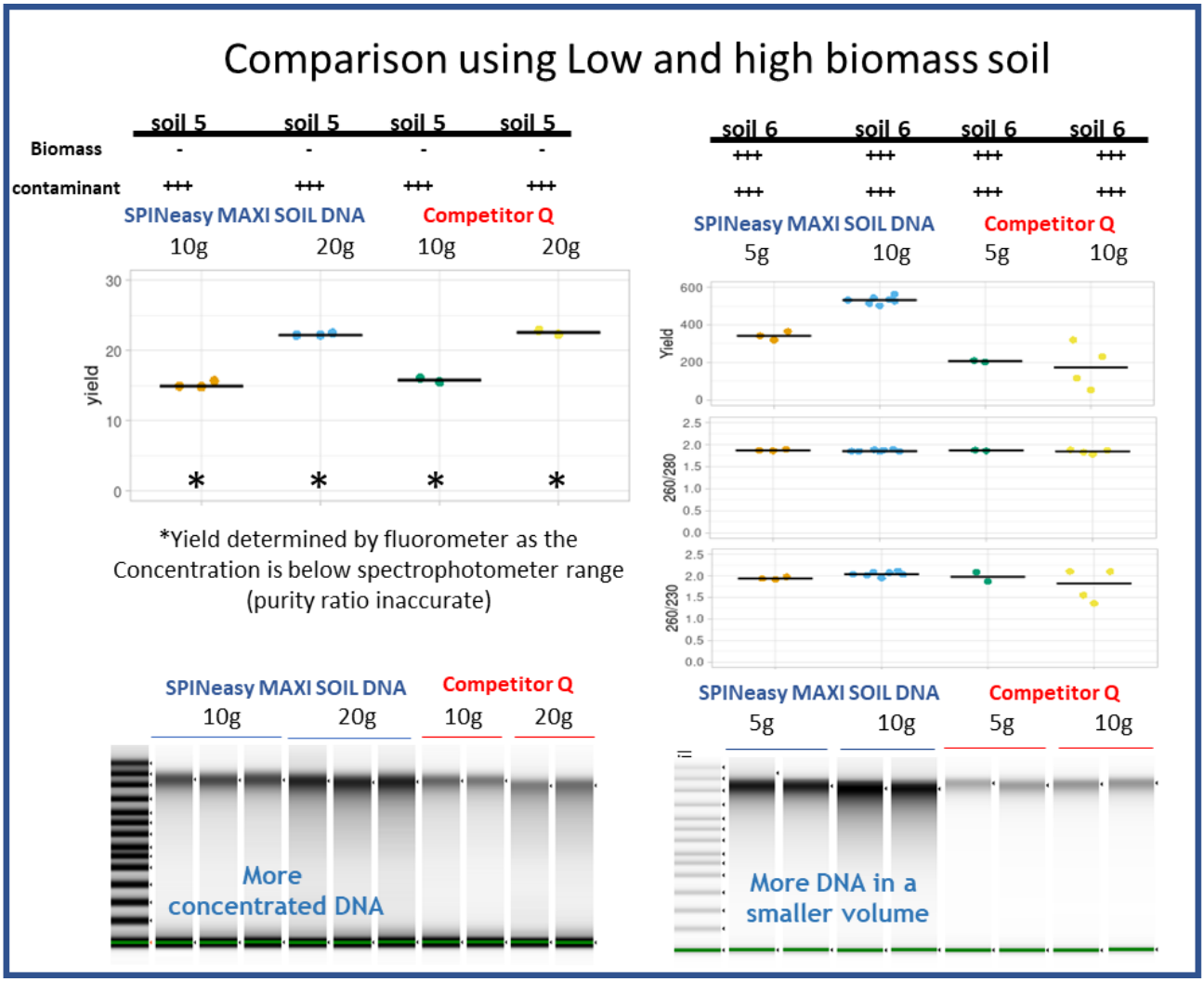


Figure 2. Low-biomass (10g and 20g, left) and high-biomass (5g and 10g, right) soil samples were extracted using SPINeasy® DNA Maxi Kit for Soil and competitor Q kit. The DNA yield and purity (A260/280 and A260/230 ratio) were assessed using spectrophotometer for the high biomass soil. For the low biomass soil, a fluorometer was used due to the low DNA concentration. Each dot of the plot represents a single extraction. The horizontal bars indicate the median value. The DNA integrity of all samples were analyzed using an Agilent 4150 TapeStation® system and Genomic DNA ScreenTape assay.

The SPINeasy DNA Maxi Kit for Soil samples derived from heavily contaminated soil can be readily used in long fragment PCR using inhibitor-sensitive DNA polymerase, supporting the absence of inhibitor (Figure 3, amplifiability). The unbiased analysis of the microbial community revealed that samples obtained using the SPINeasy DNA Maxi Kit for Soil are enriched in difficult-to-lyse gram+ firmicutes, Actinobacteriota and Deinococcota compared to a competitor Q kit (Figure 3, 16s microbial analyses, left). Moreover, the total number of bacteria identified (OTUs) and the alpha diversity were higher using SPINeasy DNA Maxi Kit for Soil samples when compared to the competitor Q kit (Figure 3, 16s microbial analyses, right).

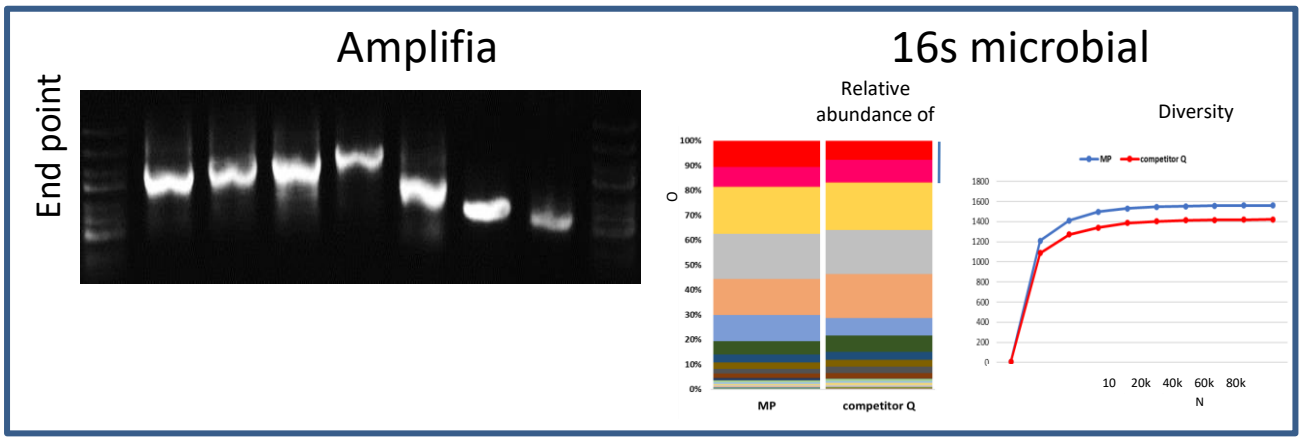


Figure 3. Amplifiability. The absence of inhibitor was assessed using inhibitor-sensitive PCR and undiluted sample as well as quantitative PCR.

16S Microbial Analyses. The hypervariable *region V4* of the *bacterial 16S rRNA gene* was amplified using DNA extracted from a high biomass soil using the 4 extraction kits depicted in the legend. Sequences were obtained using a NovaSeq PE250 platform and analyzed using the Qiime 2 pipeline. The relative abundance of bacterial species compilated from 3 technical replicates is shown on the left. The percentage indicates the average proportion of gram-positive bacteria. The rarefaction curves corresponding to each method are depicted on the right. The alpha diversity was measured by the number of operational taxonomic units (OTUs) identified (vertical axis) following the sequencing depth (horizontal axis).

9. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**

Problem	Possible Cause	Recommendation
Low DNA Yield / reduced DNA integrity	Absence of second elution with the same eluate or fresh buffer	An increase of DNA yield by ~20% can be expected by eluting twice.
	The sample have been stored before extraction	Fresh sample is preferred to obtain optimal yield and integrity.
	No salt added to eluted DNA	Freeze/thaw cycles may degrade DNA stored in Elution buffer. It is recommended to add MS6 Buffer provided at 200X concentration to obtain DNA resuspended in 5 mM Tris and 0.1 mM EDTA
	Lost of DNA during the inhibitor removal step.	For low biomass soils or samples with low level of contaminant, reducing the volume of Buffer MS2 may improve the overall yield. The volume of Buffer MS2 is sample dependant. Use 1.5 mL of Buffer MS2 as starting point if the amount of contaminant is unknown.
Low A260/230 or A260/280 ratios	High level of contaminant in the sample	Centrifuge for 10min @ maximum speed at Step 3 (contaminant removal). Increase the volume of Buffer MS2 and/or perform an additional wash using Buffers MS4 and MS5. Alternatively, reduce the amount of sample.
	Clogged column	Reduce the amount of sample.
	Soil particles trapped on the membrane	Debris may remain floating in the supernatant despite the inhibitor removal step and loaded into the column. Those debris can be removed by 1) washing the column using Buffer MS4 (step 4 optional) and 2) inverting the Column MS after drying and tapping the column against the bench.
	Contamination of the column's membrane	Ensure that all traces of wash buffer are removed from the column's rim prior to elution. A kleenwipe™ may be used to clean the column wall before drying (step 6).

High A260/280 or A260/230 ratios and low molecular weight contaminants observed in DNA gel	Possible RNA contamination	The optimized binding condition employed in the SPINeasy® DNA Maxi Kit for Soil enable specific binding of gDNA and thus, the amount of RNA contaminant that is co-purified is low. These low amounts are usually not detectable using canonical detection technique and do not affect most downstream applications. However, a RNase treatment can be performed in the homogenization by adding 500 µL of RNase A (10 mg/mL) to Buffer MS1B prior to bead beating homogenization.
Poor PCR Performance	High concentration of DNA	Dilute the sample. Large amount of DNA sample is inhibitory for PCR large amount of DNA molecule in the confined space of the reaction vessel is known to lead to false priming, exhaustion of the magnesium ions, primer(s), dNTP(s) and obstruct the passage of the large Taq polymerase molecules. If PCR using undiluted sample is required, check enzyme specification and manufacturer instruction or choose alternative PCR enzyme with strong strand displacement activity. If PCR can be done using diluted sample, the amount of DNA to be used is specific to each PCR enzyme and may need to be optimized by the user. However, genes in multiple copies in the genome such as ribosomal genes require much lesser DNA input. SPINeasy® DNA Maxi Kit for Soil provided positive amplifications from various samples using as much as >200 ng or as little as <0.20 ng of DNA per PCR of 20 µL using inhibitor sensitive PCR enzymes.
	Suboptimal PCR condition.	Verify PCR reagents and protocol with positive control; adjustment on reaction/cycle conditions or primer selection may be necessary following manufacturer recommendation.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat, or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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