SPINeasy®
Case Studies

MP BIOMEDICALS

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202312-18

CONTENT PAGE

In the realm of molecular biology, precision and efficiency are paramount. Introducing our SPINeasy® Series, an innovative spin column based extraction kit meticulously designed to streamline and simplify the nucleic acid isolation process.

CASE STUDY 1

DNA Extraction from Red Soil Samples Using SPINeasy® DNA Pro Kit for Soil

Red soils contain large amounts of clay and are generally derived from the weathering of ancient crystalline and metamorphic rock. They are named after their rich red color, which can vary from reddish brown to reddish yellow as a result of their high iron content. Typically, red soils contain low biomass which poses a challenge for DNA extraction; in addition, their high humic acid content may also result in colorized eluted DNA.

Overview

Sample Type: Red clay soil and red sand soil

Aim of the study: Optimization of DNA extraction from red soils

Application: NGS

Materials: FastPrep-24 5G instrument; **SPINeasy® DNA Pro Kit for Soil**; Competitor kit (Q company)

Protocol and Parameters

1. DNA extraction was performed with **SPINeasy® DNA Pro Kit for Soil** and Competitor Q Kit by following the protocol.
2. For the **SPINeasy® DNA Pro Kit for Soil**, there were a few optimized steps being done as follows:
   - 2.1. Weigh 0.5 g of soil sample and add it into a Lysing Matrix YB tube.
   - 2.2. Add 900 µL of Lysis Buffer S1 and 10 µL of RNase A. Homogenize with FastPrep-24 5G at 5m/s for 35s or vortex @2,500-3,000 rpm for 20 min. Centrifuge for 2 min @≥15,000 xg.
   - 2.3. Transfer the supernatant into a 2 mL centrifuge tube, add 200 µL of Inhibitor Removal Buffer S2, invert and mix 10 times, then centrifuge for 2 min @≥15,000 xg.

2.4. Transfer the supernatant into a 2 mL centrifuge tube. Add 750 µL of Buffer S3, invert and mix 10 times.

2.5. Load the lysate into the Column S, centrifuge for 10s @≥15,000 xg and discard the flow-through. Repeat once.

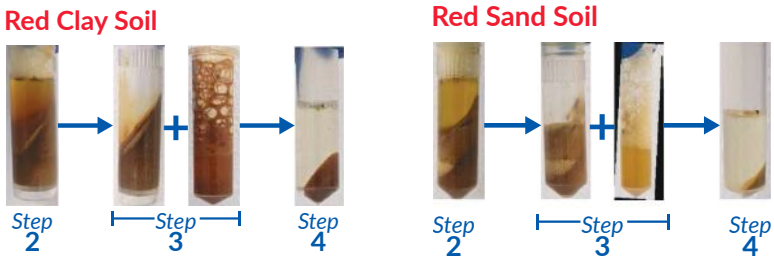
2.6. Transfer the Column S into a new 2 mL collection tube. Add 600 µL of Buffer S4 onto the center of the column membrane, then centrifuge @≥15,000 xg. Discard the flow-through and place the Column S back into the same 2 mL collection tube.

2.7. Add 750 µL of Buffer S5 into the center of the column membrane, then centrifuge for 1 min @≥15,000 xg.

2.8. For the rest of the steps, follow the existing protocol.

Results

1. Using the customized protocol of **SPINeasy® DNA Pro Kit for Soil**, the supernatant appeared to be clear at step 4 (of the protocol).



2. The performance of both DNA extraction kits (**SPINeasy® DNA Pro Kit for Soil** and Competitor Q kits) was tested with red soil samples in several independent experiments. In each experiment, 0.5 g of soil was used per extraction. In addition to spectrophotometer, a fluorometer was used to quantify the extracted DNA in samples with low DNA concentration (the reading from spectrophotometer tends to be inaccurate for these samples).

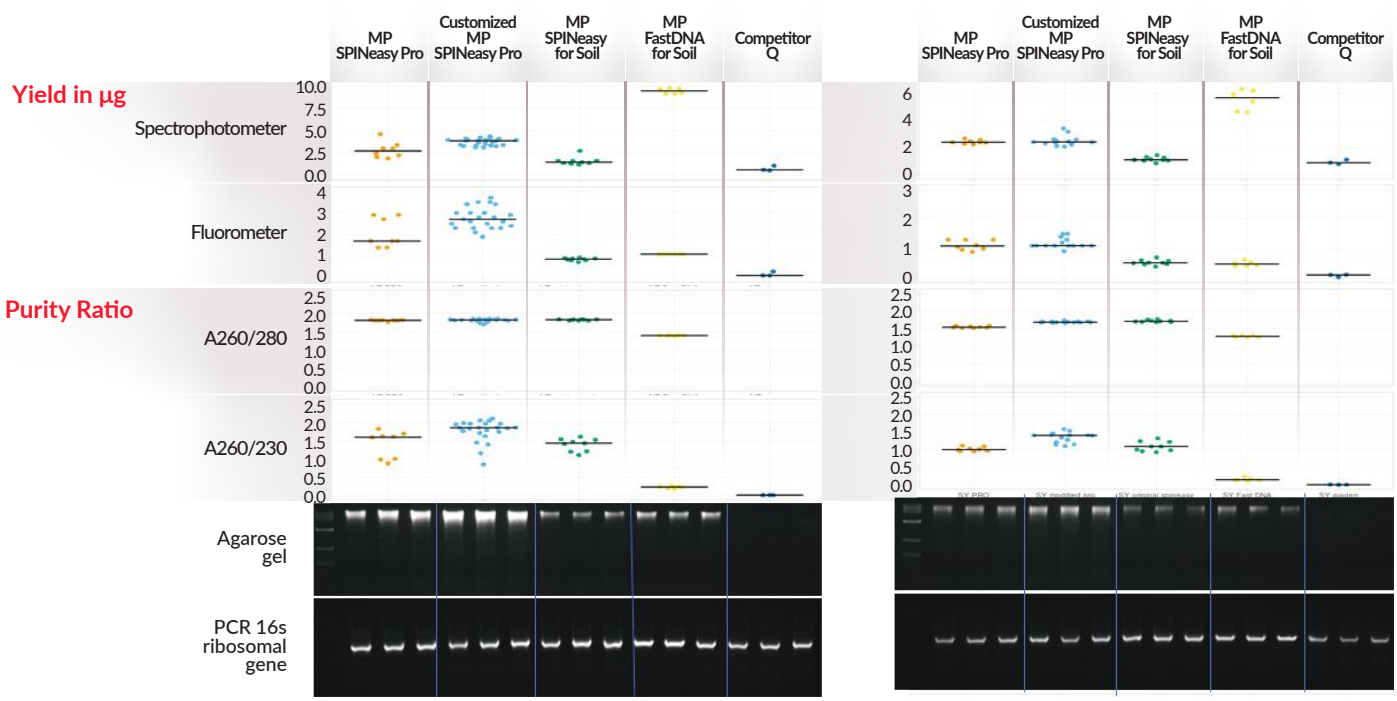


Figure 1. *Summary of yield, purity, agarose gel electrophoresis, and PCR results from the DNA extracted with **SPINeasy® DNA Pro Kit for Soil** (following normal protocol and customized protocol) and Competitor Q kits.*

Conclusion

Overall, the DNA extracted using the customized protocol of **SPINeasy® DNA Pro Kit for Soil** provided the best result in terms of yield and purity. The competitor Q kit gave poor yield with almost no detectable DNA observed on the gel electrophoresis. Nonetheless, all the samples (extracted from all DNA extraction kits) showed positive results on PCR (performed using a primer targeting a 1.5 kb region of bacteria 16s).

CASE STUDY 2

DNA Extraction from Inter-Root Soil Samples Using SPINeasy® DNA Pro Kit for Soil

FastDNA SPIN Kit for Soil was used to extract the DNA from inter-root soil and plant roots; however, the eluted DNA appeared brownish (with low A260/A280 ratio). Hence, the user was recommended the following options:

1. To use **SPINeasy® DNA Purification Kit** to further purify the eluted DNA from **FastDNA SPIN Kit for Soil**
2. To use the **SPINeasy® DNA Pro Kit for Soil** on the same sample types

Option 1

Further purification with **SPINeasy® DNA Purification Kit**

No.	Sample	Yield (ng/µL)	A260/A280	A260/230	Remark
1	M2	179.1	1.83	0.26	FastDNA SPIN Kit for Soil
2	M3	105.1	1.56	0.14	FastDNA SPIN Kit for Soil
3	M2P1	70.7	1.88	1.99	FastDNA SPIN Kit for Soil + SPINeasy® DNA Purification Kit (Buffer P1)
4	M3P1	28.5	1.6	1.11	FastDNA SPIN Kit for Soil + SPINeasy® DNA Purification Kit (Buffer P1)
5	M2HA	63.4	1.89	2.09	FastDNA SPIN Kit for Soil + SPINeasy® DNA Purification Kit (Buffer P1HA)
6	M3HA	16.8	1.83	1.33	FastDNA SPIN Kit for Soil + SPINeasy® DNA Purification Kit (Buffer P1HA)

Table 1. Summary of the yield and purity of extracted DNA (from **FastDNA SPIN Kit for Soil**) before and after further purification with **SPINeasy® DNA Purification Kit**. M2P1 and M3P1 indicated the samples that were treated with Buffer P1 from **SPINeasy® DNA Purification Kit**; M2HA and M3HA indicated the samples that were treated with Buffer P1HA.

The results (Table 1) showed that the eluted DNA from **FastDNA SPIN Kit for Soil** appeared brownish and the A260/A230 ratio was less than 1.8. There are two inhibitor removal reagents in the **SPINeasy® DNA Purification Kit**. Buffer P1 is suitable for purifying samples with slight humic acid or other PCR inhibitor contamination. Buffer P1HA is suitable for samples with large amounts of humic acid contamination. After further purification with the **SPINeasy® DNA Purification Kit**, the DNA appeared to be clear and the A260/A230 ratio in sample M2P1 and M3HA reached the optimal range. Buffer P1HA appeared to be suitable for sample M3 as it could further increase the A260/A280 ratio to 1.83.

Option 2

DNA extraction with **SPINeasy® DNA Pro Kit for Soil**

300 mg of each sample were lysed with vortex at 7,800 rpm; 30 s; 2 cycles.

The purity of DNA extracted with **SPINeasy® DNA Pro Kit for soil** appeared to be at the optimal range (as shown by A260/A280 and A260/A230 ratios) across all the samples (Table 2). The extracted DNA also appeared to be clear (Figure 1).

Sample	Yield (ng/µL)	A260/A280	A260/A230
SM2	135.8	1.9	2.16
SM3	27.2	1.81	2.06
HW10	37	1.85	2.24
XE4	13.1	1.82	2

Table 2. Summary of the yield and purity of the extracted DNA using **SPINeasy® DNA Pro Kit for Soil**

Figure 1. Comparison of DNA eluted from **FastDNA SPIN Kit for Soil** (Left) and **SPINeasy® DNA Pro kit for Soil** DNA (Right).

CASE STUDY 3

DNA Extraction from Patient with Scabies Using SPINeasy® Host Depletion Microbial DNA Kit

Human scabies is caused by an infestation of the skin by the human itch mite (*Sarcoptes scabiei var. hominis*). The most common symptoms of scabies are intense itching and a pimple-like skin rash. Scabies occurs worldwide and affects people of all races and social classes (*https://www.cdc.gov/parasites/scabies/index.html*). In this study, the laboratory was trying to extract DNA from skin scraping sample obtained from a patient with scabies.

Despite many attempts to extract the DNA using multiple extraction kits from various brands (including Supplier Q), it was unsuccessful. It was speculated that the failure might be due to the inability to break the chitin wall. Successful DNA extraction was eventually achieved with the **SPINeasy® Host Depletion Microbial DNA Kit**.

Overview

Sample Type: Skin scraping sample obtained from a patient with scabies

Aim of the study: To investigate the microbial DNA on the skin with scabies

Application: PCR, sequencing

Methods

- During the first experiment, the sample was processed according to the kit protocol.
- The method in the second experiment was slightly modified as follows:
  - During the host lysis step, the incubation time was extended.
  - During the host DNA depletion step, 2 µL of HDE solution was added, followed by vortexing.
  - For the microbial lysis step, vortexing time was extended to 10 minutes (after being transferred to a Lysing Matrix E tube).

- The quantity of samples in the first and second experiments are the same, hence the difference in the DNA yield will be mainly due to the difference in experimental methods.

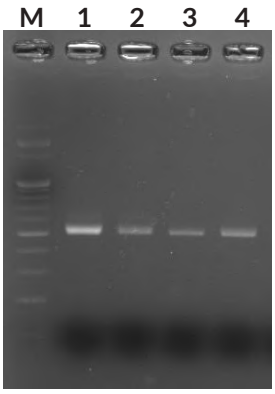
Results

- The DNA was obtained at the concentration of 0.01 ng/µL and 0.03 ng/µL (from the first and second experiments respectively). Both concentrations are sufficient for the downstream applications.
- The following primers were used for the downstream PCR amplification:

  16s 1st 341F: Tcg tcg gca gcg tca gat gtg tat aag aga cag cct acg ggn ggc wgc ag

  16s 1st 805R: Gtc tcg tgg gct cgg aga tgt gta taa gag aca gga cta chv ggg tat cta atc c

  The PCR amplification was successful for the DNA obtained from both experiments.



M indicates the 100 bp marker;

lane 1 represents sample from experiment 1;

lane 2 represents sample from experiment 2;

lane 3 represents 1/10 of sample from experiment 1;

lane 4 represents 1/10 of sample from experiment 2.

- **Sequencing result**

  The laboratory was able to confirm that the detection of Bradyrhizobium among the Scabies species was high.

```
BLASTN 2.11.0+

Reference:
Zheng Zhang, Scott Schwartz, Lukas Wagner, and Webb Miller (2000),
"A greedy algorithm for aligning DNA sequences", J Comput Biol 2000;
7(1-2):203-14.


Database: Nucleotide collection (nt)
           79,996,401 sequences; 677,779,506,543 total letters



Length=464
                                                                    Score     E
Sequences producing significant alignments:                        (Bits)  Value

FQ696856.1 16S rRNA amplicon fragment from a soil sample (ferrals...  758     0.0
LC546634.1 Bradyrhizobium sp. SO-263 gene for 16S ribosomal RNA, ...  756     0.0
LC546606.1 Bradyrhizobium sp. SO-153 gene for 16S ribosomal RNA, ...  756     0.0
MT522397.1 Bradyrhizobium sp. strain TUTSWZ51 16S ribosomal RNA g...  756     0.0
MT522395.1 Bradyrhizobium sp. strain TUTSWZ53 16S ribosomal RNA g...  756     0.0
MT522394.1 Bradyrhizobium sp. strain TUTSWZ54 16S ribosomal RNA g...  756     0.0
MT522392.1 Bradyrhizobium sp. strain TUTSWZ85 16S ribosomal RNA g...  756     0.0
MT522391.1 Bradyrhizobium sp. strain TUTSWZ61 16S ribosomal RNA g...  756     0.0
MT522389.1 Bradyrhizobium sp. strain TUTSWZ90 16S ribosomal RNA g...  756     0.0
MT522386.1 Bradyrhizobium sp. strain TUTSWZ50 16S ribosomal RNA g...  756     0.0
```

Conclusion

SPINeasy® Host Depletion Microbial DNA Kit showed superior performance in extracting the microbial DNA from the patient's skin with scabies as compared to other extraction kits on the market.

CASE STUDY 4

DNA Extraction from Rat Stool Samples Using SPINeasy® DNA Pro Kit for Feces

Overview

Sample Type: Stool samples from Rats

Aim of the study: To extract DNA from microbiome samples (rat stool)

Institute: Sunway University

Department: Sunway Microbiome Centre

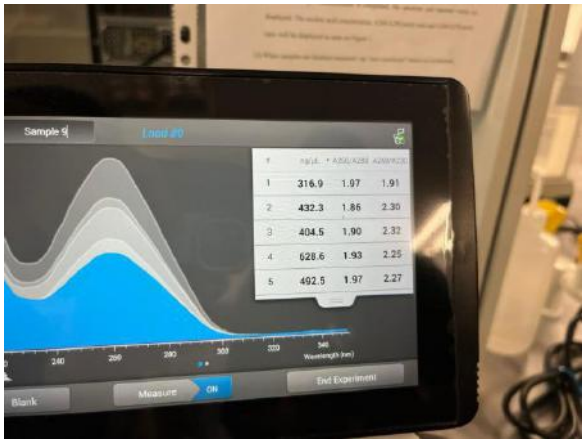
Methods

- 250 mg of stool samples were used for each extraction. There are a total of 8 preps in this study.
- The extraction procedure was performed according to the **SPINeasy® DNA Pro Kit for Feces** manual.

Results

The DNA yield and purity (as indicated by the A260/A280 and A260/A230 ratios) were as follows:

Sample No.	Conc. (ng/µL)	A260/A280	A260/230
1	316.9	1.97	1.91
2	423.3	1.86	2.30
3	404.5	1.90	2.32
4	628.6	1.93	2.25
5	492.5	1.97	2.27
6	460.4	1.92	2.30
7	353.4	1.95	2.10
8	304.3	1.87	1.90



Conclusion

The **SPINeasy® DNA Pro Kit for Feces** is able to extract the DNA from rat stool samples with good yield and high purity. The whole extraction process can be completed within 30 to 60 minutes.

CASE STUDY 5

SPINeasy® DNA Pro Kit for Soil
VS
SPINeasy® DNA Pro Kit for Feces

Overview

Sample Type: Paddy soil, high mass soil, human soil

Aim of the study: To explore the performance of **SPINeasy® DNA Pro Kit for Soil** and **SPINeasy® DNA Pro Kit for Feces on soil** and stool samples

Institute: Apical Scientific Sdn Bhd

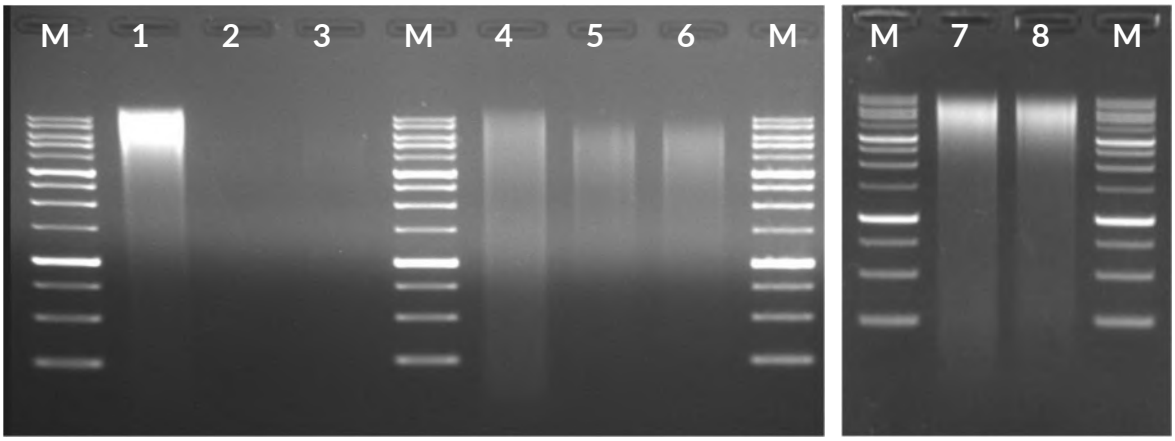
Methods

- 200 mg of samples were used for each extraction.
- The extraction procedure was performed according to the **SPINeasy® DNA Pro Kit for Soil** and **SPINeasy® DNA Pro Kit for Feces** manuals.

Results

The DNA concentration, yield, and purity (as indicated by the A260/A280 and A260/A230 ratios) were as follows:

Lane No.	Sample Type	Kit	A260/280	A260/230	Conc. (ng/µL)	Yield (µg)
1	Paddy Soil	Pro Kit for Soil	1.71	1.60	17.95	1.80
2		Pro Kit for Feces	1.69	3.22	8.85	0.89
3			1.80	-6.89	12.40	1.24
4	High Mass Soil	Pro Kit for Soil	1.90	2.08	74.85	7.49
5		Pro Kit for Feces	2.13	-0.22	1.70	0.17
6			1.84	-0.67	1.75	0.18
7	Human Stool	Pro Kit for Feces	1.87	2.26	34.80	3.48
8			1.91	2.96	47.35	4.74



Conclusion

- **SPINeasy® DNA Pro Kit for Soil** can extract the DNA from any types of soil (both low biomass and high biomass) with high yield and purity.
- **SPINeasy® DNA Pro Kit for Feces** is less effective in extracting the DNA from soil samples; nonetheless, it can extract the DNA from stool samples with high yield and purity.
- The extracted DNA from all samples could directly be amplified on PCR (data not shown).

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