



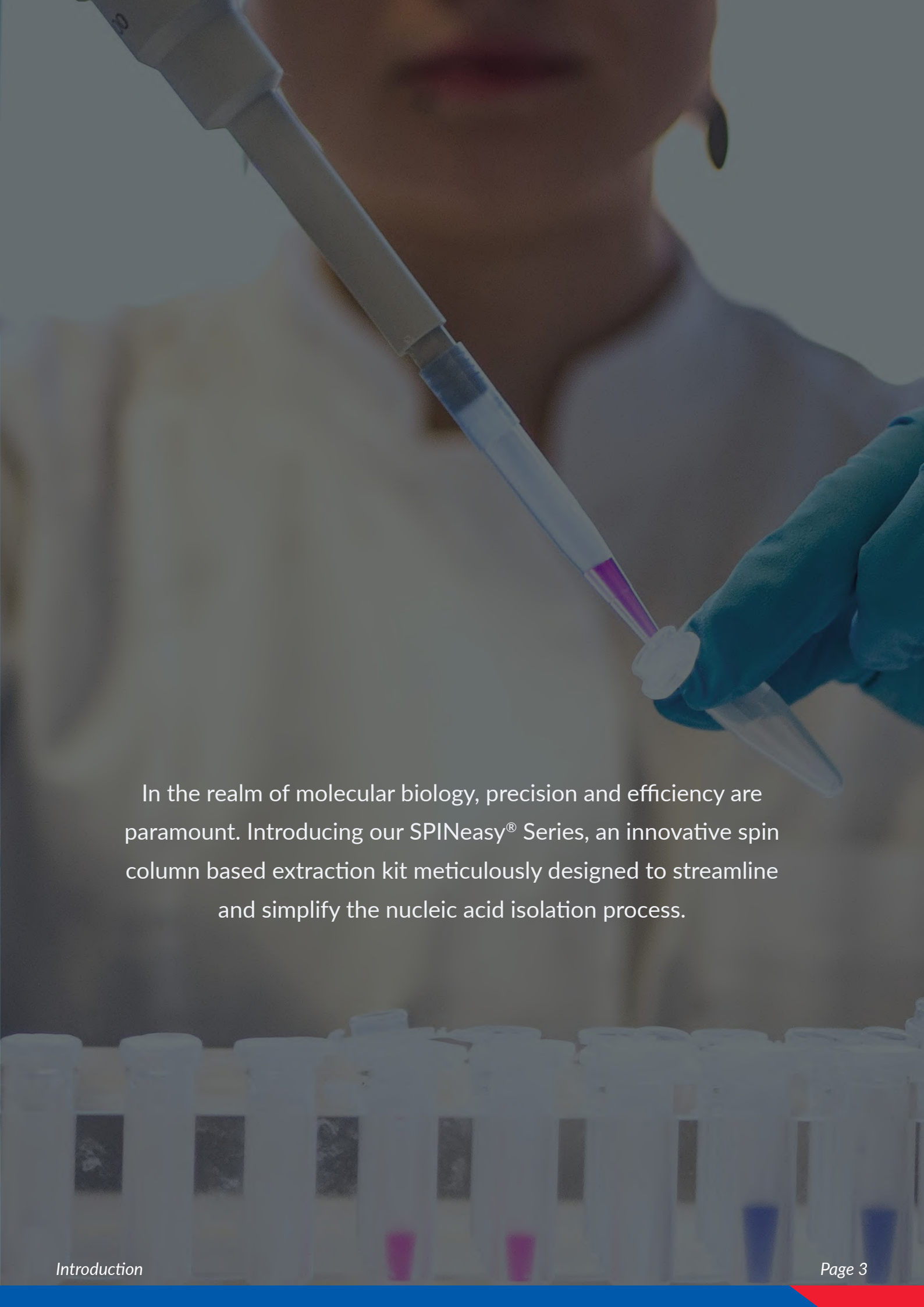
SPINeasy[®] **Case Studies**

202410-25



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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 colored 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)

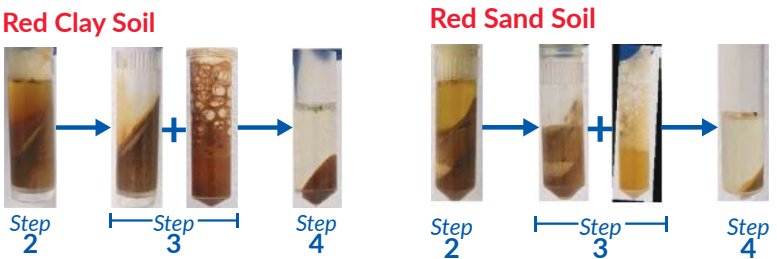
Protocol and Parameters

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 μ L of Buffer S3, invert and mix 10 times.
- 2.5. Load the lysate into the Column S, centrifuge for 10s @ \geq 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 μ L of Buffer S4 onto the center of the column membrane, then centrifuge @ \geq 15,000 xg. Discard the flow-through and place the Column S back into the same 2 mL collection tube.
- 2.7. Add 750 μ L of Buffer S5 into the center of the column membrane, then centrifuge for 1 min @ \geq 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).

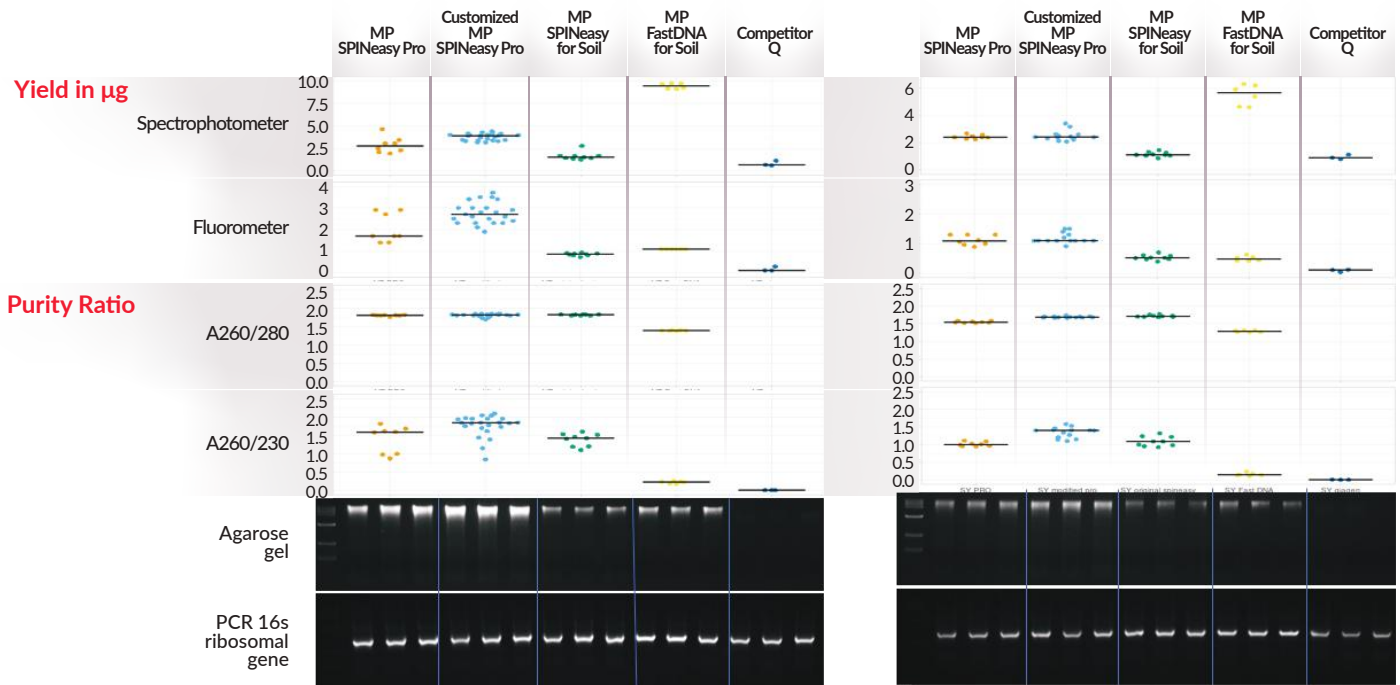


Figure 1. Summary of yield, purity, agarose gel electrophoresis, and PCR results from the DNA extracted with **SPINeasy[®] DNA Pro Kit for Soil** (following normal protocol and customized protocol) and Competitor Q kits.

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

Option 1

Further purification with SPINeasys[®] 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 + SPINeasys [®] DNA Purification Kit (Buffer P1)
4	M3P1	28.5	1.6	1.11	FastDNA SPIN Kit for Soil + SPINeasys [®] DNA Purification Kit (Buffer P1)
5	M2HA	63.4	1.89	2.09	FastDNA SPIN Kit for Soil + SPINeasys [®] DNA Purification Kit (Buffer P1HA)
6	M3HA	16.8	1.83	1.33	FastDNA SPIN Kit for Soil + SPINeasys [®] 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 **SPINeasys[®] DNA Purification Kit**. M2P1 and M3P1 indicated the samples that were treated with Buffer P1 from **SPINeasys[®] 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/ μ L)	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 μ L 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.

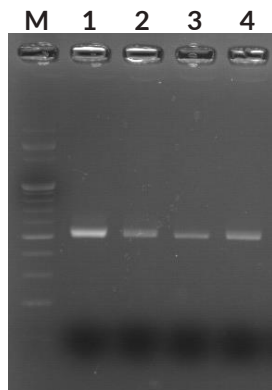
Results

- 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

Sequences producing significant alignments:

	Score (Bits)	E Value
FQ696856.1 16S rRNA amplicon fragment from a soil sample (ferrals...	758	0.0
LC546634.1 <i>Bradyrhizobium</i> sp. S0-263 gene for 16S ribosomal RNA, ...	756	0.0
LC546606.1 <i>Bradyrhizobium</i> sp. S0-153 gene for 16S ribosomal RNA, ...	756	0.0
MT522397.1 <i>Bradyrhizobium</i> sp. strain TUTSW251 16S ribosomal RNA g...	756	0.0
MT522395.1 <i>Bradyrhizobium</i> sp. strain TUTSW253 16S ribosomal RNA g...	756	0.0
MT522394.1 <i>Bradyrhizobium</i> sp. strain TUTSW254 16S ribosomal RNA g...	756	0.0
MT522392.1 <i>Bradyrhizobium</i> sp. strain TUTSW285 16S ribosomal RNA g...	756	0.0
MT522391.1 <i>Bradyrhizobium</i> sp. strain TUTSW261 16S ribosomal RNA g...	756	0.0
MT522389.1 <i>Bradyrhizobium</i> sp. strain TUTSW290 16S ribosomal RNA g...	756	0.0
MT522386.1 <i>Bradyrhizobium</i> sp. strain TUTSW250 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/ μ L)	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

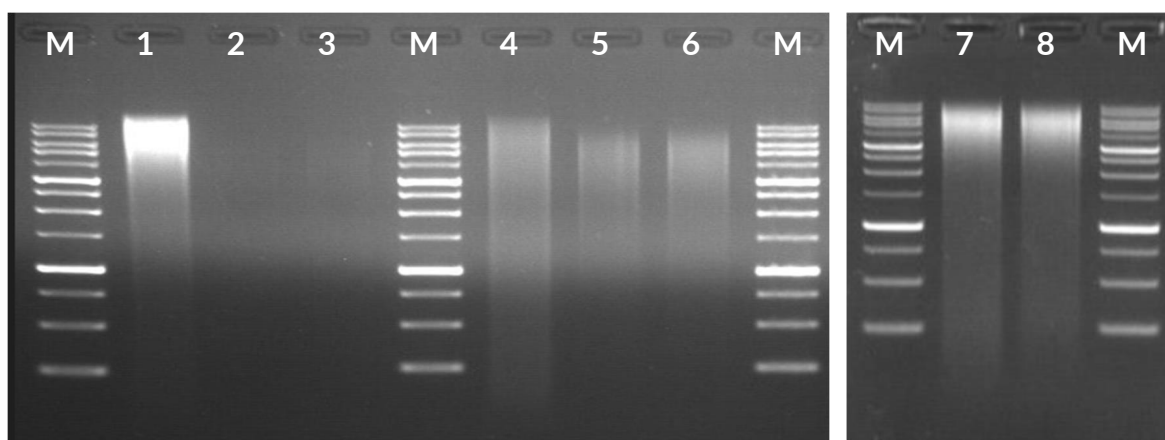
Methods

- 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/ μ L)	Yield (μ g)
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).

CASE STUDY **6**

DNA Extraction from **Yeast** Samples Using **SPINeasy® DNA Kit for Yeast**



Yeast, comprising single-celled eukaryotic microorganisms within the Dikarya subkingdom of the Fungus kingdom, exhibit remarkable diversity with over 1500 species. Traditionally, yeast has found applications in various sectors, including food, cosmetics, medicines, and biotechnology. Recent studies are expanding our understanding of yeast's impact on the gut microbiome, while investigations into its role in the oral and skin microbiome are gaining attention (Caetano, et al.). Publications regarding fungal infections has increased dramatically over the years showing the importance of such studies. The challenge of extracting them is due to the highly rigid cell wall that prevents a successful lysing of the cells, or retrieving the genomic DNA. Thus, developing an effective strategy to overcome the challenges associated with yeast sample extraction has become a prevalent issue encountered in laboratory settings.

Overview

Sample Type: Yeast

Aim of the study: Extraction of yeast DNA

Application: qPCR

Materials: SPINeasy DNA Kit for Yeast, Competitor S Kit, sample homogenizer

Protocol

1. Homogenizing parameters was set to 6,000 rpm, 30 s, 30s pause (1 cycle)
2. DNA extraction was performed with SPINeasy DNA Kit for Yeast and Competitor S Kit by following the protocol.

Results

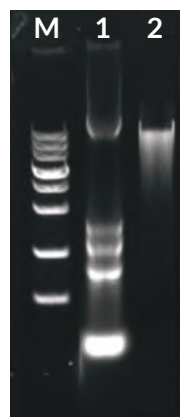
Extraction performed by SPINeasy DNA Kit for Yeast

	Yield (ng/ μ L)	Purity	
		A260/A280	A260/A230
<i>Saccharomyces cerevisiae</i>	44.76	1.78	1.53
<i>Pichia pastoris</i>	28.2	1.73	1.74

Extraction performed by Competitor S Kit:

	Yield (ng/ μ L)	Purity	
		A260/A280	A260/A230
<i>Saccharomyces cerevisiae</i>	941.6	1.91	1.14

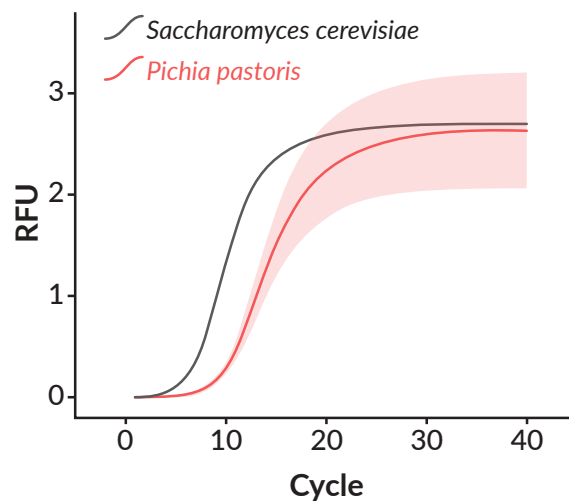
Gel electrophoresis



M marker: 15,000 bp

Lane 1: SPINeasy DNA Kit for Yeast;

Lane 2: Competitor S Kit.



Conclusion

The SPINeasy DNA Kit provides a faster and simpler yeast lysing method, facilitating efficient DNA amplification through qPCR. In contrast to Competitor S Kit, although the SPINeasy DNA Kit for Yeast may yield a lower concentration, the DNA bands from our extraction display minimal smearing in electrophoretic results, ensuring superior genome integrity. Additionally, the higher purity of A260/230 enhances the likelihood of successful outcomes in downstream applications.

CASE STUDY 7

Extraction of **Mouse Colon Tissue** RNA Using **SPINeasy® RNA Kit for Tissue (with Lysing Matrix)**



RNA extracted from mouse colon tissue is frequently employed in preclinical studies for drug discovery, offering insights into gastrointestinal physiology and providing valuable information on biomarkers and gene expression that can reflect human biology. Initially, the user utilized the TRIzol extraction method to isolate RNA from tissue samples. However, seeking an alternative approach, they experimented with the Tiangen RNA Kit, only to find that the yield did not meet their expectations. Subsequently, the user tried the SPINeasy RNA Kit for Tissue (with Lysing Matrix) and found that the extracted RNA could finally meet the requirement for their downstream application.

Overview

- Sample Type:** Tissue
- Key words:** RNA extraction, tissue
- Aim of the study:** RNA extraction from mouse colon tissue
- Application:** qPCR
- Sample names:** Mouse colon tissues
- Materials:** Mouse colon tissues; SPINeasy RNA Kit for Tissue (with Lysing Matrix); FastPrep-24 5G

Methods

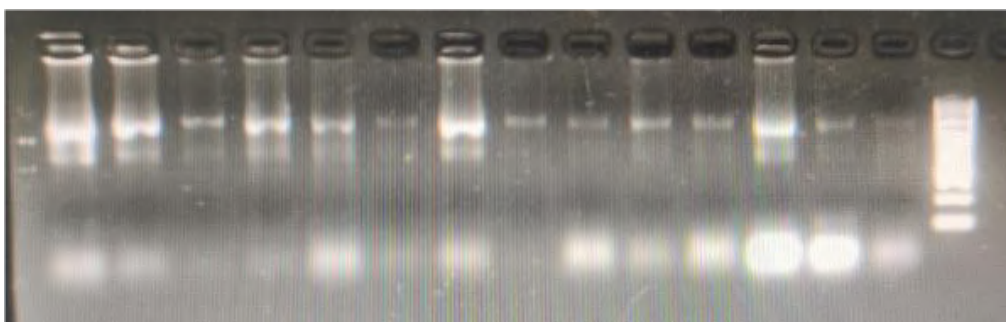
1. A sample amount of 30 mg was used.
2. Homogenization conditions were set at – 6 m/s, 15s.
3. The sample was processed according to the kit protocol.

Results

Yield/Purity

Sample ID	Concentration (ng/ μ L)	A260/A280	A260/A230
A	435	2.024	2.196
B	273	2.137	2.137
C	96	1.863	1.863
D	178	2.244	2.244
E	241	2.193	2.193
F	66	1.883	1.887
G	187	1.970	2.074
H	58	1.896	1.919
I	151	1.882	2.161
J	99	1.893	2.204
K	181	1.911	2.268
L	440	1.935	2.285
M	286	1.895	2.059
N	300	1.902	2.244

Gel electrophoresis



14 samples of various mouse colon tissues were extracted using SPINeasy RNA Kit for Tissue (with Lysing Matrix).

Conclusion

The quality and quantity of RNA extracted from the tissue plays a pivotal role in ensuring the reliability and accuracy of subsequent assays. The mouse colon tissue yielded a high quantity of RNA with consistent purity, making it well-suited for downstream experiments.

CASE STUDY **8**

Extraction of **Mouse Liver Tissue** DNA Using **SPINeasy® DNA Kit for Tissue (without Lysing Matrix)**



Mouse liver tissue samples present several challenges due to their rich composition of proteins, lipids, and inhibitory enzymes, which can interfere with DNA purification. Additionally, if the liver tissue is not thoroughly homogenized or dissolved during lysate preparation, DNA yield is often compromised. In this case, the researcher aims to conduct a functional analysis of ubiquitinating enzymes involved in the oxidative stress response by assessing mouse liver tissue. With an adequate sample volume, they successfully yielded large quantities of highly purified DNA.

Overview

- Sample Type:** Tissue
- Key words:** RNA extraction, liver tissue
- Aim of the study:** RNA extraction of liver tissue
- Application:** Gene expression, methylation
- Sample names:** Mouse liver tissue
- Materials:** Mouse liver tissue, SPINeasy DNA Kit for Tissue (without Lysing Matrix)

Methods

1. 28 mg mouse liver tissue was prepared.
2. The tissue was incubated at 65°C for 1 hour, with intermittent vortexing to ensure thorough dissolution.
3. DNA was extracted using the SPINeasy DNA Kit for Tissue (without Lysing Matrix). Results

Results

Yield/Purity

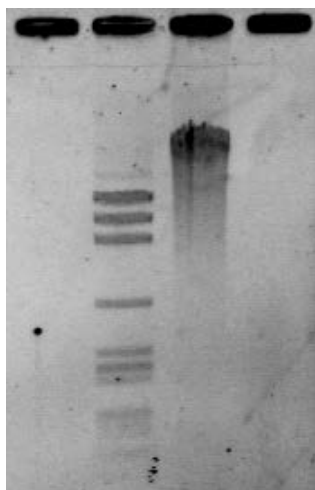


Figure 1. 28mg of mouse tissue was extracted using the SPINeasy DNA Kit for Tissue (without Lysing Matrix)

Concentration	343.6 ng/ μ L
A260/280	1.91
A260/230	2.02

Conclusion

The primary challenge in the extraction process stemmed from the nature of the sample, which required a higher incubation temperature to completely dissolve the tissue. Nevertheless, the

SPINeasy DNA Kit for Tissue demonstrated exceptional performance in achieving high concentrations and purity of DNA. Additionally, users may consider utilizing the SPINeasy DNA Kit for Tissue (with Lysing Matrix) (Cat. 116558050) for a faster and more seamless homogenization experience.



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