Expert in Soil DNA Extraction

---- D3142 HiPure Soil DNA Kit

Introduction

Soil samples contain a large number of microorganisms, the vast majority of which cannot be directly cultivated for reproduction and research. Extracting DNA from soil samples is the most effective method for studying soil microorganisms. At present, there are mainly direct and indirect methods for extracting microbial DNA from soil samples. The direct method refers to placing soil samples in the lysis solution, and using effective wall breaking methods to release all microbial DNA into the lysis solution, followed by separation and extraction, such as Zhou's method. Indirect method refers to placing soil in a buffer, such as Buffer PBS, to separate microorganisms from the soil and then extract DNA. The indirect method can greatly reduce the impact of humic acids and heavy metal salts on DNA extraction in soil, but this method will lose many microorganisms and the resulting DNA is not the entire genome (metagenome) of the soil sample. Currently, few researchers have adopted this method. Extracting DNA directly from soil samples can maximize the likelihood of obtaining the entire genome, but this method faces the following issues:

1. Humic acid pollution. The soil, especially in forests and grasslands, is rich in humic acids. Humic acid is a series of organic molecules, some of which are very similar to nucleic acid molecules and difficult to remove during purification. Trace amounts of humic acid pollution can lead to downstream applications such as PCR and enzyme digestion failure.

2. Lysis method. Soil samples contain various microorganisms, such as bacteria and fungi. Gram positive bacteria and fungi both contain very thick bacterial walls, and effectively breaking down the cell walls of these microorganisms is crucial for extracting high-yield metagenomic DNA. Due to the complexity of soil samples, it is not feasible to use enzymatic methods (such as lysozyme, wall breaking enzyme, snail enzyme) or liquid nitrogen grinding, as the soil contains various metal ions or inhibitory factors that inactive the digestive enzymes, or the presence of sand particles in the soil makes liquid nitrogen grinding difficult.

3. The DNA yield is difficult to control. Soil samples would have significant changes in the number and variety of microorganisms due to fertility, inferiority, high moisture content, dryness, or depth of sampling. In a small range of soil samples, the DNA content often varies by thousands of times. In addition, certain chemical components in soil, such as heavy metal salts and clay substances, can cause a decrease in DNA yield.

Magen's HiPure Soil DNA Kits are currently the most optimized kit for soil DNA extraction. The kit adopts glass bead grinding method and thermal shock chemical wall breaking method, which can be carried out in the point vortex instrument without special bead grinding instrument, and is suitable for a wide range of laboratories. The Absorber Solution in the reagent kit is a humic acid adsorbent exclusively developed by Magen Company, which can efficiently remove various humic acid pollutants. In addition, an alcohol-free silica gel column purification method is also used to efficiently remove various soluble metal salts and other soluble inhibitory factors from the soil. The kit has successfully extracted from the following soil (partially based on customer feedback): soil from forests in nature reserves (30 to 40 years old forest soil with a surface layer of 30-50cm deciduous layer), mangrove soil, grasslands, farmland, seabed mud, sludge, mineral area soil, organic matter contaminated soil, pond mud, garbage mud, air conditioning pipeline deposits, etc.

Experiment methods

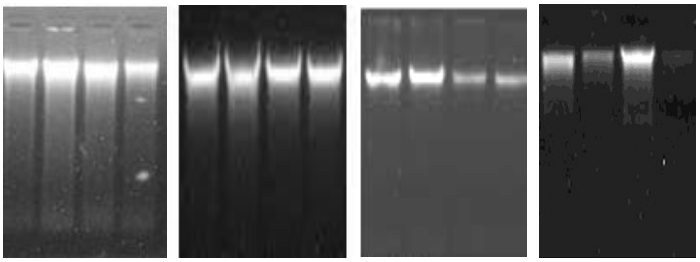
To demonstrate the superiority of this kit, we selected the following different samples for DNA extraction experiments, with each sample repeated 3 times.

- Forest samples: Forest soil in the nature reserve (Guangdong Heishiding Nature Reserve) and Hainan Island Mangrove Reserve
- Mineral area samples: underwater soil (wet) and surface soil (dry) in the mineral area
- Cultivated land samples: paddy soil, vegetable soil
- Organic pollution surface samples: sewage ditch sediment and soil in household waste storage areas

Experiment Result

1. DNA electrophoresis results

Take 10 μl purified genomic DNA, analyze by 0.8% agarose gel 80V electrophoresis for 30 minutes. The results are as follows.

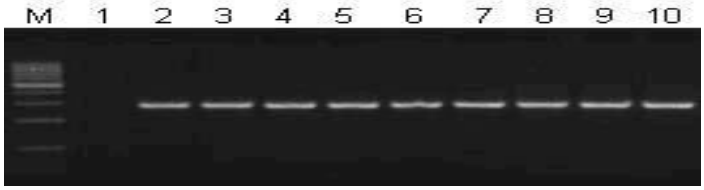


2. Analysis of DNA purity and yield

Soil	A260	A280	A230	A320	A260/ A280	Yield μg
6	0.1792	0.1041	0.1075	0.0207	1.7	9
7	0.2107	0.1195	0.1257	0.0178	1.8	11
4	0.1244	0.0730	0.0759	0.0159	1.7	6
5	0.1477	0.0855	0.0900	0.0236	1.7	7
2	0.2640	0.1739	0.2149	0.0685	1.5	11
3	0.2584	0.1521	0.1579	0.0354	1.7	12
8	0.0595	0.0390	0.0414	0.0121	1.5	3
1	0.0190	0.0121	0.0623	0.065	1.6	1

1. Mangrove soil 2. Forest soil in nature reserves 3. Corn soil 4. Rice field soil, surface soil (dry) in mineral areas 5. Bottom mud of sewage ditches 6. Domestic waste storage area 7. Underwater soil (wet) in mineral areas 8 Underwater soil (dry) in mineral areas

3. PCR results



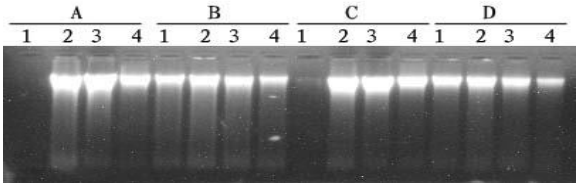
Electrophoretic maps of amplifying bacterial 16s genes, using DNA from each sample as templates.

M. 100bp DNA Ladder 1. Negative control 2. Mangrove soil 3. Forest soil in nature reserves 4. Corn soil, 5. Rice field soil, surface soil (dry) in mineral areas 6. Bottom mud of sewage ditches 7. Domestic waste storage area 8. Underwater soil (wet) in mineral areas 9. Underwater soil (dry) in mineral areas 10. Positive control, DH5a genome of Escherichia coli

F & Q

1. How does the kit break the cell wall?

This kit uses high concentration SDS lysis buffer and bead grinding method for wall breaking. The soil contains bacteria and fungi, as well as other microorganisms. Most Gram negative bacteria can lysis rapidly in SDS, while Gram positive bacteria and fungi cannot lysis in SDS. Using glass bead high-speed vortex or bead grinder can effectively lysis bacteria and fungi. In addition, the kit also adopts a thermal shock (70°C or 90-95°C) step to ensure the effective lysis of these microorganisms. To compare different methods of wall breaking, we selected four forest soils (1,2,3,4) and then used different methods (A, B, C, D) for wall breaking. The results are as follows. The results showed that using a bead grinder (A, C) to homogenize can achieve the highest yield, but sample 1 cannot obtain DNA due to lack of heating. High yields of DNA can be obtained using manual vortex and liquid nitrogen grinding assisted by heating.



A: Fastprep-24 Bead Grinder

C: 2000 Geno/Grinder Bead Grinder

(A,C After the bead grinder is completed, there is no heating step)

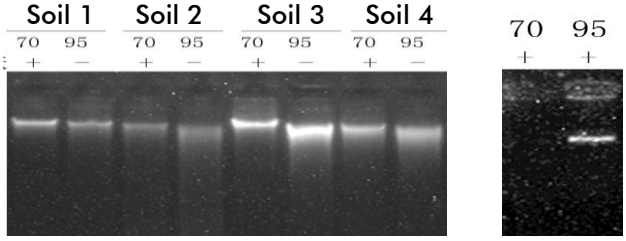
B: Use glass bead to vortex on the point vortex for 3 minutes, and then heat at 70°C;

D: Grind with liquid nitrogen and heat at 70°C.

(1,2: mangrove soil, 3,4: forest soil)

2. How much does the heating process increase production? Which temperature should be chosen, 70°C or 95°C?

The heating process is crucial for certain soils. Our experiment shows that some soils treated with an efficient bead grinder Fastprep-24 can increase DNA production 3-4 times after being treated at 70°C for 10 minutes. Some soils are not sensitive to heating. Treatment at 90-95°C for 10 minutes can increase DNA production by 10-30%. For some bacteria that are particularly difficult to lyse, such as Staphylococcus aureus, 95°C heat shock is very important. However, 95°C treatment can cause DNA degradation. We recommend heating at 95°C only when it is necessary to detect bacteria or fungi that are difficult to lyse.



The results in the left figure indicate that high-temperature treatment (95°C) can increase yield, but it can cause DNA degradation. The figure on the right shows that after inoculating Staphylococcus aureus in sterilized soil samples, only 95°C treatment is possible to obtain its DNA.

3. How to improve the yield of soil DNA?

The types and quantity of microorganisms contained in soil samples, as well as the organic and inorganic salts in soil samples, all directly affect DNA production. The following methods can significantly improve the yield of DNA extraction in soil:

- **Low microbial content in soil:** increase the soil sample amount to 1-2g. When increasing soil sample, it is necessary to

correspondingly increase the volume of Buffer SOL, Buffer SDS, and Buffer PS.

- **DNA adsorbents in the soil:** add skim milk powder. Soil samples contain organic substances such as clay and silicone salts, which can adsorb nucleic acids and cause low DNA production. Skim milk powder can be added to Buffer SOL until the concentration is 8-40mg/ml to reduce the adsorption of nucleic acids.
- **The soil contains heavy metal salts:** increase the soil sample amount to 2-5g. Some soluble divalent or trivalent metal salts in soil can coagulate DNA and cause DNA loss. For example, when Al3+or Zn2+ plasma is added to a DNA solution, DNA will immediately combine with these metal ions to form insoluble substances. When processing this type of sample, special metal complexing agents such as EDTA can be added to the Buffer SOL. (Buffer SOL already contains high concentrations of EDTA). Or increase the amount of soil used.

4. Why is DNA electrophoresis tailing severe?

This is due to DNA degradation. The reasons for DNA degradation are as follows:

1) The soil is rich in small organisms and easily cleavable bacteria. These small and easily lysable organisms and bacteria will cause DNA degradation in the glass bead vortex for a long time. The solution is to reduce vortex time or replace manual vortex with a high-energy bead grinder. (High energy bead grinder can break the cell wall in a short time, thus reducing DNA degradation)

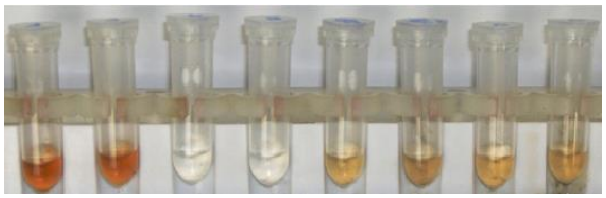
2) There are certain chemical substances present in the sample, or the sample degrades during storage.

5. Why does a large amount of precipitate form when adding isopropanol?

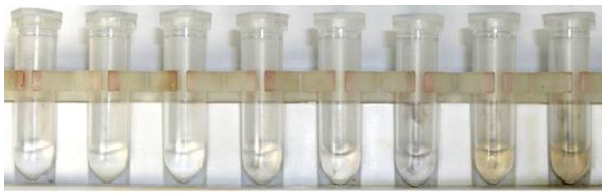
Soil samples contain various inorganic or organic molecules, while most other inorganic molecules, such as metal salts, are water-soluble. When isopropanol precipitation is added, the solubility of water-soluble metal salts decreases and precipitates are formed. This phenomenon is often encountered when processing samples from mineral areas. When using this kit, contamination of these water-soluble metal salts can be removed.

4. What is the principle and effectiveness of Absorber Solution in removing humic acid?

Absorber Solution is a pH dependent humic acid adsorbent. This adsorbent can efficiently and specifically adsorb humic acid in a pH 8.0 buffer solution. The Absorber Solution can also adsorb nucleic acid DNA below pH 7.0. Therefore, DNA must be dissolved using either Elution Buffer or Buffer TE (Ph8.0)。



(Before treating with Absorber Solution)



(After treating with Absorber Solution)

As shown in the figure, before Absorber Solution treatment, the crude soil DNA contains a large amount of humic acid pollution, with a deep color; After HTR Reagent treatment, the color of the DNA disappeared, indicating that the humic acid have been removed.

7. What other methods can be used to optimize this kit?

Due to the wide variety of soil samples, different soil samples have different effects on the pre-treatment methods. The kit uses SDS lysis buffer and glass bead grinding to lyse and pretreat soil samples, which may have no effect on some samples. At this point, users can perform rough extraction of soil DNA according to their own or other methods, and then follow the step of the kit to add Absorber Solution for adsorption and removal of humic acid, and further purify it through a column.

8. After Absorber Solution treatment, why is the DNA sample still very dark in color? What if the DNA obtained using this kit still has color?

This type of sample is often forest soil, especially soil with a thick deciduous layer. This soil accumulates rich humic acid due to the decomposition of fallen leaves. The adsorption of humic acid by Absorber Solution has a certain degree of saturation, and excessive use of soil samples or humic acid can result in residues. The adsorption step of Absorber Solution can be repeated once.

9. Can this method obtain the DNA of all microorganisms?

Not necessarily. Soil samples contain a large number of microorganisms, including bacteria and fungi, as well as other plant roots and small organisms. Some fungi, especially spore fungi, have a very thick cell wall that cannot be broken, resulting in the loss of their DNA. If it is necessary to extract the DNA of these microorganisms, we suggest strengthening the intensity and time of wall breaking.