13. Transfer the Spin Columns to a new centrifugal tube (provided by yourself), and drops 50-200 μL Buffer EBL or sterilized water to the middle of the Spin Columns , place at room temperature for 2-5 minutes, centrifuge at 12000 rpm for 1 minute, collect DNA solution, and store DNA at - 20 °C.

Note: 1) Incubation at room temperature for 5 minutes before centrifugation can increase production.

2) Re-extraction with another 50-100 μL Buffer EBL or sterilized water can increase production.

3) To increase the concentration of DNA, re-add the obtained solution to the Spin Columns and repeat step 13.but the total yield may be reduced.

4) The Buffer EBL does not contain chelating agents. Please store DNA at - 20 ℃.

5) Microscale PCR inhibitors in genomic DNA templates may have adverse effects on PCR reactions, which can usually be resolved by diluting DNA by 2-10 times.

Appendix: Grind the sample using one of the following methods

1. Scroll manually on the scroll oscillator for 10 minutes at the maximum speed.
2. Oscillate for 10 minutes at maximum speed on a scroll oscillator fitted with a 1.5-2 mL horizontal centrifugal Tube support (to keep the Lysis Tube placed horizontally). If the number of samples exceeds 12, extend for 5-10 minutes. Examples include vortex-Genie2 Vortex oscillators from Scientific Industries or Mobio.
3. When using Qiagen's TissueLyser II, grind at 25Hz for 10 min.
4. Using Qiagen's PowerLyzer 24 Homogenizer, homogenized for 30 seconds at 2000 rpm, paused for 30 seconds, and then homogenized again for 30 seconds at 2000 rpm.
5. When using MP Biomedicals' FastPrep-24, the recommended speed is 6.0 and the time is 40 seconds.

Version: 12/2023

Soil And Stool DNA Kit

Cat. No. : CW2091S（50 preps）

Storage Conditions: Buffer RIL 2-8°C,and other components should be stored at room temperature (10-30°C).

Components

Component	CW2091S 50 preps
Buffer QSL	45 mL
Buffer RIL	11 mL
Buffer ML	10 mL
Buffer GW1（concentrate）	13 mL
Buffer GW2（concentrate）	26 mL
Buffer EBL	13 mL
RNase A	240 μL
Lysis Tubes Ⅱ	50
Spin Columns DM With Collection Tubes	50

Introduction

This kit provides a method for extracting total DNA from soil or fecal samples, including the total DNA of cells, bacteria, parasites, and viruses in the sample. It is also suitable for extracting DNA from samples containing high concentrations of PCR reaction inhibitors. This kit uses a unique buffer system to efficiently bind DNA from the lysate to the adsorption column. Inhibitors of PCR and enzyme reactions, as well as residual impurities, can be effectively removed through washing steps. Finally, using a low salt buffer or water to elute, high purity DNA can be obtained. The purified DNA can be directly used in downstream experiments such as second generation sequencing (16S amplification and macrogenomics), library construction, PCR, qPCR, Southern Blot, and enzyme digestion molecular markers.

Reagents to Be Supplied by User

1. Constant temperature mixer - Product No.: CW2593
2. Anhydrous ethanol, isopropyl alcohol
3. Scroll oscillator or tissue grinder

Important Points Before Starting

1. Repeated freezing and thawing of samples should be avoided, otherwise the extracted DNA fragments will be smaller and the extraction amount will decrease.
2. Before the first use, absolute ethanol should be added to Buffer GW1 (concentrated) and Buffer GW2 (concentrated) in advance according to the instructions on the label of the reagent bottle.
3. Take out the buffer RIL before use, and store it at 2-8 °C immediately after use.

Procedure

1. Briefly centrifuge the Lysis Tube to allow the beads to settle at the bottom.
2. A. Add 0.1-0.3 g of soil or fecal samples to Lysis Tube, and add 740-820 µL Buffer QSL and 4 µL RNase A, tighten the cap and briefly vortex to mix.

   B. For fecal samples stored in non cracked functional fecal preservation solutions (such as CWY041S and CWY041M), 200 µL-600 µL solid-liquid mixture to Lysis Tube , centrifuged at 13000 rpm for 1 min, discard the storage solution (if the amount of solids after centrifugation is too small, it can be enriched again, but should not exceed 0.3g). Join 620 µL Buffer QSL and 4 µL RNase A, tighten the cap and briefly vortex to mix.

Procedure

3. Fix LysisTube in an oscillating grinding device equipped with a 2 mL adapter and process it according to the optimized grinding conditions used by your device (see appendix).
4. Shake the Lysis Tube on a constant temperature mixer of 70 °C and 1200 rpm for 10 minutes. Then centrifuged at 13000 rpm for 2 minutes to precipitate the solid particles. Transfer 540 µL supernatant into a new 2 mL centrifuge tube.
5. Add 180 µL Buffer RIL, vortex for 5 seconds, centrifuged at 13000 rpm for 2 minutes.

   Note: Take out the Buffer RIL before use, and store it at 2-8 °C immediately after use.
6. Add 160 µL Buffer ML、480 µL The supernatant of step 5、320 µL isopropyl alcohol to the new centrifuge tube in turn , vortex for 5 seconds.
7. Transfer the solution in the previous step 650 µL into the Spin Columns DM that have been loaded into the collection tube at 12000 rpm (~13400 × g) Centrifuge for 1 minute.
8. Discard the waste liquid in the collection tube and place the Spin Columns back into the collection tube. Repeat step 7 until the solution has been completely transferred.
9. Add 500 µL Buffer GW1 (check whether absolute ethanol has been added before use) to the Spin Columns , centrifuge at 12000 rpm for 1 minute, pour out the waste liquid in the collection tube, and place the Spin Columns back into the collection tube.
10. Add 500 µL Buffer GW2 (check whether absolute ethanol has been added before use) to the Spin Columns , centrifuge at 12000 rpm for 1 minute, pour out the waste liquid in the collection tube, and place the Spin Columns back into the collection tube.
11. Repeat step 10.
12. Centrifuge at 12000 rpm for 2 minutes and discard the waste liquid in the collection tube. Place the Spin Columns at room temperature for a few minutes to thoroughly dry.

    Note: The purpose of this step is to remove residual ethanol from the Spin Columns. The residual ethanol will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.)