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Appendix:

Grind the sample using one of the following methods:

1. Manually scroll and oscillate on the scroll oscillator for 10 min at the maximum speed.
2. Shake the Lysis Tube horizontally for 10 min at maximum speed on a vortex oscillator equipped with a 1.5-2 mL horizontal centrifuge Tube holder (keep the Tube horizontal). If the number of samples exceeds 12, extend 5-10 min.
   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 the Qiagen PowerLyzer 24 Homogenizer, homogenize at 2000 RPM for 30 sec, pause for 30 sec, and homogenize again at 2000 RPM for 30 sec.
5. When using FastPREP-24 of MP Biomedicals, the recommended speed is 6.0 and the time is 40 sec.

Magbead Soil And Stool DNA Kit

Cat. No. : CW2556M (96 preps)

Shipping and Storage : Buffer RIL 2-8℃
other components at normal temperature.

Components

Component	CW2556M (96 preps)
Buffer QSL	85 mL
Buffer RIL	20 mL
Buffer ML	18 mL
Buffer GW1（concentrate）	80 mL
Buffer GW2（concentrate）	50 mL
Buffer EBL	25 mL
Magbeads SN	2×1 mL
RNase A	450 μL
Lysis Tubes Ⅱ	96 Tubes

Introduction

The kit provides a simple, rapid, and efficient method for extracting DNA and is suitable for soil and fecal samples. In the presence of high salt, DNA binds to the surface of the silicon-based coated Magbeads. After rinsing, highly purified DNA is eluted in Buffer EBL or deionized water. The purified DNA has good purity (A260/280 ratio between 1.7-1.9) and high integrity (>15 KB), which can be used for next-generation sequencing, quantitative PCR, microarray detection and other downstream experiments. The kit can be matched with CWE2100 or CWE3200 32 channel nucleic acid extractor and CWE9600 or CWE960 96 channel nucleic acid extractor, simple and rapid high-throughput extraction, greatly reducing the experimenter's workload and human error in the experiment.

Reagents to Be Supplied by User

1. Constant temperature mixer
2. 2/15 mL magnetic stand
3. 32 channel nucleic acid extractor
4. 96-channel nucleic acid extractor
5. 96 DW Plate
6. 8 Channel Comb
7. Spin Tips Pack
8. Absolute ethanol, isopropyl alcohol
9. Vortex oscillator or tissue grinder

Protocol

1. Sample pre-processing

1.1 Lysis the Tube briefly so that the beads settle at the bottom.

1.2 A. Add 0.1-0.3 g soil or faecal sample to the Lysis Tube, add 740-820 μL Buffer QSL and 4 μL RNase A, screw the Tube lid tightly, and vortex briefly to mix.

B. For non-lysis stool storage solution (e.g. CWY041S and CWY041M), add 200μL -600μL solid-liquid mixture to the Lysis Tube, centrifuge at 13000 rpm for 1 min, and discard the Lysis solution (if the amount of solid after centrifugation is too small, it can be reabsorbed. But should not exceed 0.3g). 620 μL Buffer QSL and 4 μL RNase A are added, the tube cap is tightened, and briefly vortexed to mix.

1.3 Place the Lysis Tube in an oscillating grinding device with a 2 mL adapter and handle the Lysis Tube using the optimized grinding conditions for your equipment (see Appendix).

1.4 The Lysis Tube is Lysis Tube and then shaken at 1200 rpm at 70 ℃ for 10 min on a thermostatic mixer. This was followed by centrifugation at 13,000 rpm for 2 min to precipitate solid particles. Transfer 540 μL of the supernatant to a new 2 mL centrifuge tube.

1.5 180 μL Buffer RIL was added, vortexed for 5 sec, and centrifuged at 13000 rpm for 2 min. Note: The Buffer RIL should be removed before use and stored at 2-8 ℃ immediately after use.

1.6 Manual extraction according to Part 2, automatic nucleic acid extraction according to Part 3 or 4.

2. Manually extract data

2.1 Add 160 μL Buffer ML, 480 μL supernatant of Step 1.5, 320 μL isopropyl alcohol and 20 μL Magbeads SN successively into the centrifuge tube. After mixing by vortex oscillation for 5 sec, the centrifuge tube is placed on a constant temperature mixer at 25℃ and 1600 rpm for 10 min. Note: Before adding Magbeads SN, swirl for 20 seconds to mix thoroughly. Magbeads SN can be premixed with isopropyl alcohol in the above-mentioned volume according to the number of samples and then added.

2.2 Place the centrifuge tube on the magnetic rack for 1 min. After Magbeads are completely adsorbed on the side wall of the centrifuge tube, discard the solution completely (keep the centrifuge tube fixed on the magnetic rack).

2.3 Remove the centrifuge tube from the magnetic rack. Add 750μL Buffer GW1 (please check whether absolute ethanol has been added before use). Votex for 1 min or votex for 5 sec. and then shake on a constant temperature mixer at 25 ℃ and 1600 rpm for 2 min (ensure that Magbeads are mixed during the oscillation). Then place the centrifuge tube on the magnetic rack for 1 min. After Magbeads are completely adsorbed on the side wall of the centrifuge tube, gently reverse the magnetic bead to wash the impurities on the cover of the centrifuge tube and discard the solution completely (keep the centrifuge tube fixed on the magnetic rack).

2.4 Repeat Step 2.3.

2.5 Remove the centrifuge tube from the magnetic rack. Add 750μL Buffer GW2 (please check whether absolute ethanol has been added before use) and then votex for 1 min or votex for 5 seconds and then shake on a constant temperature mixer at 25℃ and 1600 rpm for 2 min (ensure that Magbeads are mixed during the oscillation). Then place the centrifuge tube on the magnetic rack for 1 min. After Magbeads are completely adsorbed on the side wall of the tube, gently reverse the magbead to wash the impurities on the cover of the

centrifuge tube and discard the solution completely (keep the centrifuge tube fixed on the magnetic rack).

2.6 Repeat Step 2.5.

2.7 Keep the centrifuge tube fixed on the magnetic rack, further remove the solution on the bottom and cover of the centrifuge tube with a pipette, and then place it at room temperature for 5-10 minutes to make the ethanol volatilize clean (the surface of the magnetic beads becomes matte and the magnetic beads do not dry).

2.8 Remove the centrifuge tube from the magnetic rack and add 50-200 μL Buffer EBL. The magnetic beads were completely suspended in the eluent by vortex oscillation and then placed on a constant temperature mixer at 56℃ and 1600 rpm for 10 min by shaking and elution, or the centrifuge tube was placed in a water bath at 56℃ and incubated for 10 min, during which the beads were oscillated for 10 sec every 3 min.

2.9 Place the centrifuge tube on the magnetic stand for 2 min. After Magbeads are absorbed on the side wall of the centrifuge tube, transfer the eluent to a new centrifuge tube with a pipette and store at -20℃ for later use.

3. Match the CWE2100 or CWE3200

3.1 Add corresponding reagents to 96DW deep well plate according to the table below.

Position	Reagent
1&7 Colume	Buffer ML: 160 μL Lysate: 480 μL Isopropyl alcohol: 320 μL
2&8 Colume	Buffer GW1: 750 μL
3&9 Colume	Buffer GW1: 750 μL
4&10 Colume	Buffer GW2: 750 μL Magbeads SN：20 μL
5&11 Colume	Buffer GW2: 750 μL
6&12 Colume	Buffer EBL: 100 μL

Note: 1. 1&7 Colume reagents are added in sequence.

2. Before using GW1 and GW2, please check whether 100% ethanol has been added.

3. Buffer GW2 and Magbeads SN can be premixed according to the volume and number of samples. Swirl for 10 SEC before use.

3.2 Place the deep-well plate and magnetic sleeve with reagents in the corresponding position of CWE2100 or CWE3200, and run the extraction program. About 40 minutes later, the program finished running.

3.3 Transfer the elution products in column 6&12 of the deep-well plate to a 1.5 mL centrifuge tube and store at -20℃.

4. Match the CWE9600 or CWE960

4.1 Add the corresponding reagents to the 96 DW deep-well plate according to the table below.

Position	Reagent
Plate 1	Buffer ML: 160 μL Lysate: 480 μL Isopropyl alcohol: 320 μL
Plate 2	Buffer GW1: 750 μL
Plate 3	Buffer GW1: 750 μL
Plate 4	Buffer GW2: 750 μL Magbeads SN：20 μL
Plate 5	Buffer GW2: 750 μL
Plate 6	Buffer EBL: 100 μL

Note: 1. Reagents in Plate1 are added in sequence.

2. Before using GW1 and GW2, please check whether 100% ethanol has been added.

3. Buffer GW2 and Magbeads SN can be premixed according to the volume and number of samples. Swirl for 10 sec before use.

4.2 Put the deep-well plate and magnetic sleeve with reagent in the corresponding position of CWE960 (magnetic sleeve in disk position 4) or CWE9600 (magnetic sleeve in disk position 8), and run the extraction program, which will be finished about 50 minutes later.

4.3 Transfer the elution product in deep well Plate 6 to a 1.5 mL centrifuge tube and store at -20℃.