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Modified Three Peaks protocol - Power Soil DNA Isolation Kit

Microbiome

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We use this protocol and it's working

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Abstract

DNA extraction protocol optimized to maximize DNA yield, fragment length and microbial lysis. This protocol is a modified version of https://www.protocols.io/view/the-39-three-peaks-39-faecal-dna-extraction-method-kqdg34m9pl25/v2

The Three Peaks protocol is characterized by three separate lysis steps:
(i) a 10% SDS-based lysis,
(ii) a ProteinaseK-based lysis,
(iii) a bead-beating-based lysis (optimized power and time settings).

In between lysis steps, high-speed centrifugation collects the unlysed cell pellet at the bottom, leaving the supernatant containing DNA to be collected in a separate tube.
At the end of the lysis steps three separate tubes containing DNA in a supernatant are processed separately with a column-based DNA isolation procedure, using the Power Soil Pro Kit (QIAGEN).

The protocol yields ~40 mg (median) of total DNA/sample, ensuring enough DNA for multiple sequencing and other DNA-based procedures.

Materials

- Glass beads
- DNA/RNA Shield
- PBS
- MetaPolyzyme
- SDS
- Proteinase K
- C1 Lysis Solution
- Solution CD2
- Solution CD3
- PowerBead Tube
- Solution EA
- Solution C5
- Solution C6
- MB Spin Column
- 2 ml Collection Tube
- 1.5 ml Elution Tube

Safety warnings

This protocol uses 3x the reagents per sample usually required by the Power Soil Pro Kit

Before start

- This protocol was optimized to use Power Soil Pro Kit (QIAGEN) for DNA isolation.
  - It uses 3x the reagents per sample usually required by the Power Soil Pro Kit.
- This protocol was optimized to process 200 mg of stool.
- All mixing steps should be performed with 1000 uL pipette (1000P pipetting), or by inversion.
- All centrifugations should be performed at RT.
- Thaw MetaPolyzyme on ice. Bring C2 (Power Soil Kit) to RT.

Overview of materials

1

	Set #	End point	Volume	Content	Tube type
	set 1	discard	pellet	glass beads and solids	2ml
	set 2	first peak	600 uL	DNA shield +PBS	1.5 ml
	set 3	transfer to power bead set	pellet		2ml
	power bead set (Power Soil Kit)	bead beating		silica beads + debris	
	set 4	second peak	600 uL	PBS+ metapolyzyme +SDS+proK	1.5 ml
	set 5	third peak	600 uL	C1 lysis solution	1.5 ml

Step-wise cell lysis steps (modified Three Peaks protocol)

2 In a 1 stool : 2 solvent ratio - Combine 200 mg of fresh stool with 400 uL of **DNA/RNA Shield** in set 1 and vortex briefly.

3 Centrifuge at 5,000 rcf for 00:05:00 and transfer up to all 400 uL of **DNA/RNA Shield** added previously in step 1 - [1.1 sup] to set 2.

4 In a 1 stool **:** 1 solvent ratio - Combine the stool pellet with 200 uL of **PBS** and resuspend by 1000P pipetting.

5 Centrifuge at 5,000 x rcf for 00:05:00 and transfer up to all 200 uL of **PBS** added previously in step 4.1 - [1.2 sup] to set 2.

6 Add 700 uL **PBS** and 3-4 sterilised glass beads to the pellet. Homogenize the sample on a Tissue lyser for 2 min at 25 speed in order to suspend the sample and detach microbiota from dietary fibres [the homogenization step is repeated until faecal pellets are completely dissolved].

7 Centrifuge at low speed (35 rcf, 20 min) to separate larger fecal particles from bacteria and transfer as much supernatant as possible (~600 uL) to set 3. Throw set 1 containing glass beads and debris.

8 In a 60 solvent : 1 enzyme ratio - Add 10 uL of **MetaPolyzyme** and mix by gentle 1000P pipetting.

9 Incubate at 35 °C for 3 h with no agitation. Spheroplasts (cells without cell wall) form across incubation time.

10 In a 10 solvent:1 chemical:1 enzyme ratio - Add 60 uL 10 % (w/v) **SDS** and 60 uL of **Proteinase K** [20 mg/mL] and mix by gentle 1000P pipetting. Incubate at 55 °C for 00:30:00 with 300 rpm agitation.

11 Gently mix by flicking to ensure the solution is homogeneous before proceeding.

12 Centrifuge at 5,000 rcf for 00:05:00 and transfer up to ~ 600 uL of **PBS** added in step 5.1 [2° supernatant] to set 4.

13 Resuspend the pellet of set 3 in 00 uL of **C1 Lysis Solution** (Power Soil Kit).

14 Transfer to a PowerBead Tube (spin down still empty). Vortex briefly. Bead-beat on a Vortex adapter for 5 minutes at speed 10.

15 Centrifuge tube at 15,000 rcf for 00:02:00 and transfer up to all the C1 Lysis Solution in set 5.

16 Now perform all the following steps on set 2, set 4 and set 5 separately.

DNA isolation steps (Power Soil Pro Kit - spin column based)

17 Add 200 µl of **Solution CD2** and invert the tube for 5 times. **Note**: Solution CD2 (IRT) precipitates non-DNA organic and inorganic material including humic substances, cell debris, and proteins.

18 Centrifuge at 15,000 rcf for 00:01:00. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided). **Note**: Expect 500–600 µl.

19 Add 1:1 ratio of **Solution CD3** (600 µl) and invert the tube for 5 times. **Note**: Solution CD3 is a high-concentration salt solution. Because DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA.

19.1 If you ran 23 step twice, then add 900 uL of **Solution CD3**.

20 Load 650-670 uL of the lysate (keep the rest) onto an MB Spin Column and centrifuge at 15,000 rcf for 00:01:00. **Note**: DNA is selectively bound to the silica membrane, contaminants pass through the filter membrane.

21 Discard the flow-through and repeat step 26 with the lysate that has left. Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any flow-through onto the MB Spin Column.

22 Add 500 uL of **Solution EA** to the MB Spin Column. Centrifuge at 15,000 rcf for 00:01:00. **Note**: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the MB Spin Column filter membrane.

23 Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

24 Add 500 uL of **Solution C5** to the MB Spin Column. Centrifuge at 15,000 rcf for 00:01:00. **Note**: Solution C5 is an ethanol-based wash solution used to further clean the bound DNA from residual salt, humic acid, contaminants.

25 Discard the flow-through and place the MB Spin Column into a clean 2 ml Collection Tube.

26 Centrifuge at up to 16,000 rcf for 00:02:00. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube. **Note**: It is critical to remove all traces of Solution C5 because the ethanol in it can interfere with downstream DNA applications, such as PCR and gel electrophoresis.

27 Add 70 uL (50-100 uL) of **Solution C6** (low in salt -10 mM Tris) to the center of the white filter membrane. Centrifuge at 15,000 rcf for 00:01:00. **Note**: Placing Solution C6 in the center of the small white membrane will result in a more efficient and complete release of the DNA.

28 The DNA is now ready for downstream applications.

Acknowledgements

Josh Quick