Rapid Prep for PCR Applications Protocol 1

Lyse & Purify (96 Wells)
Fecal Samples

Rapidly lyse and purify DNA from fecal samples. This kit contains materials for processing 96 samples.

Additional Items Needed: (Not included; see Suggested Supplier list below)

1. Additional Items **Required** for Lyse and Purify
   a. One (1) sterile 96-well PCR plate
   b. Plate sealing film
   c. Sterile Laboratory Grade Water
   d. Fresh 70% ethanol
   e. TE Buffer - 10mM Tris, 1mM EDTA, pH 8.0
   f. 0.4M KOH Solution
   g. Magnetic Rack ('bar' or 'post' suggested for higher DNA yield)
2. Suggested items for rapid processing
   a. Repeater Pipette with multi-volume tips
   b. Single Channel Manual Pipettes
   c. Multichannel Pipette 0.5-10 µL working volume
   d. Centrifuge with 96-well plate adaptor
   e. Sterile inoculating loops (96)

Kit Contents for Shoreline Biome Lyse and Purify

1. Lysis-1 (96-well PCR plate, dried solution in each well)
2. Purification Buffer (two 8 mL bottles with **white** caps)

Important Considerations for Lyse:

1. Fecal samples should be thawed and stored on ice. Unmodified, frozen fecal samples will yield the best results. Storage temperatures can be -20°C to -80°C. If fecal sample has been diluted into buffer and stored at +4°C, significant degradation of the DNA in the sample can result in a few hours.
2. Shoreline Biome **lysis is pH dependent**. Samples stored in buffer with pH < 6.5 should use **at most** 10 µL input volume, otherwise the risk of suboptimal lysis affecting downstream results is high.
3. Fecal samples may contain pathogens. Handle samples using Universal Precautions. Please wear personal protective equipment and observe standard laboratory practices for handling, cleaning, and disposing of materials that come in contact with sample.
4. Shoreline Biome lysis results in ssDNA; for quantitation, please choose an appropriate kit (e.g., Qubit ssDNA assay kit #Q12012, Promega Quantifluor ssDNA system, #E3190)

Shoreline Biome Lyse and Purify Protocol

1. **Make 0.4M KOH**. Measure 0.135g KOH into 15 mL tube, then add 6 mL H_2O. 0.4M KOH expires after 7 days.
2. **Make 70% Ethanol**. Add 17.5 mL 100% ethanol to 7.5 mL sterile laboratory grade water
3. Bring Purification Buffer (**white** capped bottle) to room temperature.
4. Remove film covering the 96-well PCR plate containing dried Lysis-1.
5. **Sample Type:**
   5.1. **Frozen Solid Fecal Samples**

5.1.1. Add **50 µL** Molecular Biology Grade water to each of the dried Lysis-1 tubes. Do not mix.

5.1.2. Using sterile inoculating loop, transfer **~3 mg** of each fecal sample to the corresponding labeled well (Fig. 1 and Fig. 2). Spin each loop to disperse the sample in Lysis-1, and discard loop. Do not mix; **do not exceed 10mg of fecal sample**.

5.2. **Mouse Fecal Pellet**

5.2.1. Add **50 µL** Molecular Biology Grade water to each of the dried Lysis-1 tubes. Do not mix.

5.2.2. 4.2.2. Place up to 5 mg (1/4 mouse pellet) in each tube. **Do not add more than 10mg** (1/2 mouse pellet) Do not mix.

5.3. DNA Genotek OMNIgene®•GUT Sample Collection Tube

5.3.1. 4.3.1. Add **10 µL** of sample from the OMNIgene®•GUT Sample Collection Tube to Lysis-1. Do not mix.

5.4. **Liquid Fecal Samples stored in other buffers**

5.4.1. Add **40 µL** Molecular Biology Grade water to each of the Lysis-1 tubes. Add **10 µL (maximum)** of sample. Do not mix.

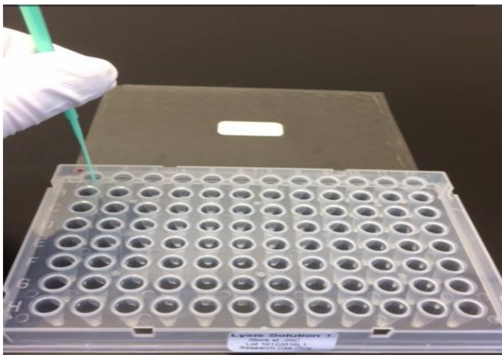

Figure 1.

Figure 2.

6. **Add 50 µL 0.4M KOH** to each well position in the 96-well plate. Do not mix. Use of a repeater or multichannel pipette is recommended to save time. A precipitate will form in the wells.
7. **Cover the sample plate with PCR film**, load the sample plate into the PCR machine, close and lock the lid, and **heat samples to 95°C for 5 minutes**. Precipitate will dissolve.
8. **Place sample plate on ice for 2 minutes.** Precipitate will re-form.
9. While samples are on ice:

   9.1. Vortex Purification Buffer bottles (**white** cap) to re-suspend any brown magnetic beads that may have settled.

   9.2. **Add 50 µL** of **Purification Buffer** to each well of a ***clean*** 96-well plate for sample purification.

10. **After incubating sample plate on ice, spin sample plate** in plate centrifuge at 2000 RCF for 3 minutes (or 400 RCF for 7 minutes) to pellet precipitate to bottom of wells (see Figure 3 below).

Figure 3. *Pelleted precipitate in bottom of wells after spin*

11. Remove PCR film and carefully **transfer 50 µL** of supernatant **into the appropriate well** of the clean 96-well plate with Purification Buffer prepared as per the instructions in Step 9 above; and **pipette to mix.**
12. **Cover the sample plate with a _new_ PCR film**, load sample plate onto the PCR machine, close and lock the lid, and **incubate solution for 3 minutes at 50˚C** to allow DNA to bind to beads
13. After incubation, place plate on magnetic rack to pellet beads with bound DNA (~60 seconds), carefully remove PCR film.
14. Once all beads are pelleted, **remove all supernatant SLOWLY and discard**. Avoid aspirating beads.
15. **Remove plate from magnet, add 100 µL 70% ethanol** (at room temperature) to each well, and re-suspend beads by pipetting gently up and down.
16. **Return plate to magnet and allow beads to pellet** (~30 seconds)
17. **Pipette off residual ethanol and let air dry for 2 - 3 minutes**. Over-drying will make eluting DNA more difficult, so watch to ensure ethanol is mostly gone but bead pellet does not begin to shrink and crack.
18. **Remove plate from magnet.**
19. Add **20 µL of 1X TE buffer** to each well and briefly pipette up and down to re-suspend beads. Solution should turn brown in color to reflect beads mixed homogeneously throughout.
20. **Incubate plate at room temperature for 2 minutes.**
21. Place plate on magnetic rack for 30 - 60 seconds to **pellet magnetic beads. DNA is now in solution.**
22. **Transfer supernatant containing eluted DNA** into a **clean** PCR plate on ice, avoiding pelleted beads.
23. **Remove plate with pelleted beads from rack**.
24. **Add another 20 µL 1X TE buffer** onto bead pellets, gently pipette up and down to suspend beads.
25. **Incubate plate for 2 minutes at room temperature**.
26. Return to magnetic rack. Allow beads to pellet to sides for 30 - 60 seconds.
27. **Remove supernatant containing DNA** and combine with first eluate in clean plate, avoiding pelleted beads.
28. Dilute as needed for your PCR reaction.
29. Suggested dilution for solid fecal, liquid fecal, mouse pellet or DNA Genotek OMNIgene®•GUT Sample Collection Tube is 1:5. Dilute samples by adding 160 µl 1X TE buffer to 40 µl of combined eluted DNA for a total of 200 µl.
30. **Store eluted DNA at +4°C**.

NOTES: DNA is **denatured**, due to KOH treatment during lysis, which is optimal for PCR. Store Lysis Mix at +4°C for up to 10 days, or frozen at -20°C for 3 months. Avoid repeated freeze/thaws.

Suggested Suppliers

BD Worldwide: (Sterile) Calibrated Disposable Inoculating Loops, Green, #220214

Dot Scientific Inc.: 96-well plate, #353-PCR or #354-PCR

Fisher Scientific: Biotech Grade Water BP24854; Potassium Hydroxide P250; TE BP24731

Thermo Fisher: Invitrogen Dyna Mag 96 side magnetic rack, #12331D or Magnetic stand-96 #AM10027

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