# Rapid Prep for PCR Applications Protocol 1

Lyse & Purify (16 Tubes)
Fecal Samples

Rapidly lyse and purify DNA from fecal samples. This kit contains materials for processing 16 samples.

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

1. Additional Items **Required** for Lyse and Purify
   a. 8-strip 0.2 mL PCR tubes w/individual domed caps for sample lysis and purification
   b. Sterile Laboratory Grade Water
   c. Fresh 70% ethanol
   d. TE Buffer - 10mM Tris, 1mM EDTA, pH 8.0
   e. 0.4M KOH Solution
   f. 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. Mini Centrifuge for 200 µL 8-strip tubes
   e. Sterile inoculating loops (16)

**Kit Contents for Shoreline Biome Lyse and Purify**

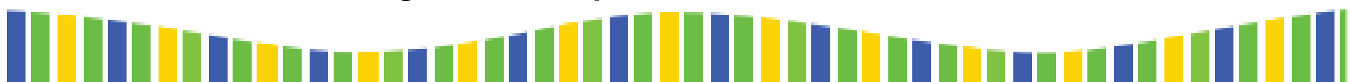
1. Two 8-tube strips of dried Lysis-1
2. Purification Buffer (two 1.5 mL tubes with **green** cap)

**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 (**green** capped tube) to room temperature.
4. **Sample Type:**
   4.1. **Frozen Solid Fecal Samples**
      4.1.1. Add **50 µL** Molecular Biology Grade water to each of the dried Lysis-1 tubes. Do not mix.
      4.1.2. Using sterile inoculating loop, transfer **~3 mg** (Fig. 1) of each fecal sample to the corresponding labeled tube. Spin each loop to disperse the sample in Lysis-1, and discard loop. Do not mix; **do not exceed 10mg of fecal sample**.



4.2. **Mouse Fecal Pellet**

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

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.

4.3. **DNA Genotek OMNIgene®•GUT Sample Collection Tube**

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

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

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

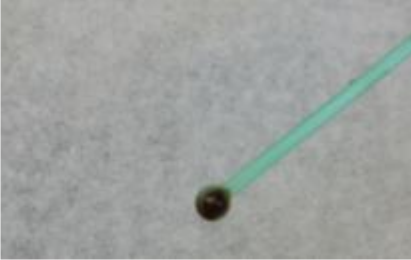
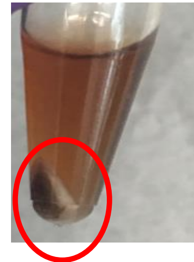


*Figure 1 (left) Image shows 2 mg fecal material on a 1 µL inoculating loop.*

*Figure 2 (right) Precipitated material after being spun down from lysis step*



5. **Add 50 µL 0.4M KOH** to each tube. Do not mix. (A precipitate will form)
6. Cap samples tightly; set PCR machine for proper tube height, close and lock the lid and **heat samples to 95°C for 5 minutes**. Precipitate will dissolve. After incubation, **allow sample to cool to RT before unlocking PCR lid** – failure to do so may result in lids popping off tubes. **While samples are cooling**:
   6.1. Label **clean 200 µL PCR tubes** for each sample
   6.2. Vortex Purification Buffer tubes (Green Cap) to re-suspend any brown magnetic beads that may have settled
   6.3. Add **50 µL of Purification Buffer** (Green Cap) to each ***clean*** tube for sample purification
7. **Transfer Lysis-1 samples from PCR machine to ice for 2 minutes**. Precipitate will re-form.
8. **Remove Lysis-1 samples from ice, and spin for 60 seconds** in mini-centrifuge to pellet precipitate (Fig. 2)
9. Carefully **transfer 50 µL** of supernatant **into the clean tubes** containing 50 µL of Purification Buffer prepared as per the instructions in Step 6 above; and **pipette up and down to mix**.
10. Cap Lysis-1/Purification Buffer samples tightly; set PCR machine for proper tube height, close and lock the lid, and **heat samples to 50°C for 3 minutes** to allow DNA to bind to beads.
11. **After 50°C incubation, place samples on magnetic rack** to pellet beads with bound DNA to side of the tube (~60 seconds)
12. Once all beads are pelleted, **remove all supernatant SLOWLY and discard**. Avoid aspirating beads.
13. **Remove tubes from magnet, add 100 µL 70% ethanol** (at room temperature) to each tube, and re-suspend beads by pipetting gently up and down.
14. **Return tubes to magnet and allow beads to pellet** (~30 seconds)
15. **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.
16. **Remove tubes from magnet.**
17. Add **20 µL of 1X TE buffer** to tubes and briefly pipette up and down to re-suspend beads. Solution should turn brown in color to reflect beads mixed homogeneously throughout.
18. **Let solution incubate at room temperature for 2 minutes.**
19. **Place tubes on magnetic rack** for 30 - 60 seconds to pellet magnetic beads. **DNA is now in solution.**

20. **Transfer supernatant containing eluted DNA** into **clean,** labeled microcentrifuge tubes on ice, avoiding pelleted beads.
21. **Remove tubes with pelleted beads from rack**.
22. **Add another 20 µL 1X TE buffer** onto bead pellets, gently pipette up and down to re-suspend beads.
23. **Incubate tubes for 2 minutes at room temperature**.
24. Return tubes to magnetic rack. Allow beads to pellet for 30 - 60 seconds.
25. **Remove supernatant containing DNA** and combine with first eluate in clean, labeled tubes, avoiding pelleted beads.
26. Dilute as needed for your PCR reaction.
27. 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.
28. **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 or #220215
Dot Scientific Inc.: 8-strip PCR tubes w/individual domed caps, #503-8PCR
Fisher Scientific: Biotech Grade Water BP24854; Potassium Hydroxide P250; TE BP24731
Permagen: 0.2 mL PCR Strip Magnetic Separation Rack 8 or 12 Strip, Cat #MSR812

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