Shoreline Complete™ StrainID™ Protocol 1

Lyse, Purify, & Amplify Set Z (16 Tubes)
Fecal and Tissue Samples

Rapidly purify microbial DNA from fecal and tissue samples and prepare for sequencing using single step PCR amplification of the EXTended 2500bp region (encompasses the 16S rRNA gene, the adjacent Internally Transcribed Spacer (ITS) region and part of the 23S gene) for microbial community profiling. This kit contains 16 unique barcoded primer sets for processing 16 samples. One additional kit contains unique barcoded primer sets for processing up to 96 additional samples. Every fecal sample will have a unique barcode at the completion of the PCR reaction for sequencing all the samples together; reads will be sorted by sample after sequencing is complete.

Products

Up to 112 different DNA samples can be barcoded and combined in a single sequencing run using the two available kits.

1. Shoreline Complete™ StrainID™ Lyse, Purify, & Amplify Set Z (16 Barcodes) Cat. No. **STRAIN-Z**
2. Shoreline Complete™ StrainID™ Lyse, Purify, & Amplify Set A (96 Barcodes) Cat. No. **STRAIN-A**

Additional Items Needed for 16 Barcode Kits: (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. 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. Additional Items **Required** for Amplify Protocol:
   a. Qiagen MinElute® PCR Purification Kit
   b. Sterile Laboratory Grade Water
   c. Sterile 100% Ethanol
3. 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)
4. Suggested items for sample QC
   a. 0.8% agarose gel with ethidium bromide or other dsDNA stain
   b. Gel loading dye to mix with samples (e.g., NEB B7024)
   c. 2kb DNA ladder

Kit Contents for Shoreline Complete™ Lyse and Purify Protocol

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

Kit Contents for Shoreline Complete™ Amplify Protocol

1. Two colored 8-tube strips with barcoded primers, labeled with sample barcode number
   Barcodes: 1-8 Clear tube strip, 9-16 Red tube strip
2. 2X PCR Premix (**blue** cap)

Important Considerations for Lyse and Purify:

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 recommends including positive and negative controls in your project to monitor lysis and amplification efficiency as well as environmental contamination. Consider using mock microbiome cells (input=10^9) and mock microbiome DNA (input 10-100ng) as positive controls and water as negative control.
5. 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 Complete™ 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 5mg (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.
   - 4.5. **Tissue Samples**
     - 4.5.1. Add **50 µL** Molecular Biology Grade water to each of the dried Lysis-1 tubes. Do not mix.
     - 4.5.2. Place up to 20mg (up to 30mg) tissue sample in Lysis-1 tubes. Do not mix.

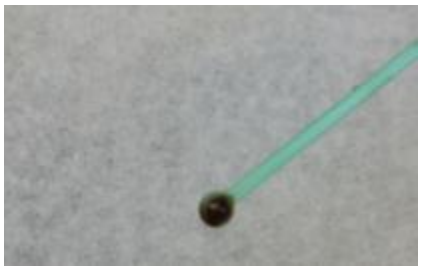

Figure 1 (left) Image shows 2 mg fecal material on a 1 µL inoculating loop.

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

5. Add **50 µL 0.4M KOH** to each tube of Lysis-1/sample mix. A precipitate will form in the tubes. Do not mix.
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 tube (**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 Lysis-1 supernatant into the clean tubes** containing Purification Buffer prepared as per the instructions in step 7.2 above; 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 sample tubes 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 of 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 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. **Incubate tubes 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 suspend beads.
23. **Incubate tubes for 2 minutes at room temperature.**
24. Return tubes to magnetic rack. Allow beads to pellet to sides for 30 - 60 seconds.
25. **Remove supernatant containing DNA** and combine with first eluate in clean, labeled tubes, avoiding pelleted beads.
26. **Dilution:**
    26.1. **For tissue Samples: Do not dilute. Store DNA at +4°C**. This is your **Shoreline Biome Lysis Mix**.

26.2. **For solid fecal, liquid fecal, mouse pellet or DNA Genotek OMNIgene®•GUT Sample Collection Tube fecal samples: Dilute** samples 1:5 by **adding 160 µl 1X TE buffer** to 40 µl of combined eluted DNA for a total of 200 µl. **Store DNA at +4°C**. This is your **Shoreline Biome Lysis Mix**.

NOTES: DNA is **denatured**, due to KOH denaturation during lysis. Although Lysis Mix may retain contaminants, the Shoreline Complete™ Amplify protocol is designed to work in the presence of these contaminants. Stored Lysis Mix at +4°C for up to 10 days, or frozen at -20°C for 3 months. Avoid repeated freeze/thaws.

Shoreline Complete™ Amplify Protocol

1. **Add 10 µL** 2X PCR Premix (**blue** cap) to each barcoded primer tube.
2. **Add 10 µL** of **Shoreline Biome Lysis Mix** to appropriate barcoded primer tube.
3. Primers will dissolve off the bottom of each tube. Observe that blue color is uniformly distributed throughout 20 µL reaction; if not, perform the steps below:
   3.1. Make sure tubes are capped, then flick gently until uniform blue color is achieved in each tube.
   3.2. Spin tubes briefly to ensure all fluid is at the bottom of the tube
4. Transfer tubes to PCR machine; be sure lid is set for high profile tubes, close/lock lid. Run PCR protocol:

95°C, 3:00	Denaturation
Repeat 34X	95°C, 0:30
	59°C for 0:45 **(Ramp rate 4°C/sec)**
	72°C, 2:00
72°C, 3:00	Final extension

5. Check reactions on gel: Run 1.5 µL of sample with 5 µL diluted gel loading dye on 0.8% agarose gel in TBE, 150V for approximately 45 minutes, with DNA ladder. Band should be present at ~ 2500bp.

Qiagen MinElute® PCR Purification Protocol

1. Follow MinElute instructions for adding Ethanol (96% - 100%) to PE buffer, and label tubes.
2. Pool 5 µL of each amplified sample from the 16 tubes *(when pooling 96 sample well plate and 16 sample tubes, pool 5 µL of each amplified sample from all plates/tubes)* into a clean 1.5 mL microcentrifuge tube. **(Save 2 µL of pooled unpurified DNA for gel comparison with purified DNA in the last step below)**
3. Combine **78 µL** of pooled sample with 400 µL of Qiagen PB buffer *(if pooling more than 16 samples, combine **80 µL** of pooled sample with 400 µL of Qiagen PB buffer).*
4. Transfer **all** of the resulting solution into the MinElute column with provided 2 mL collection tube.
5. Centrifuge the MinElute column at 17,900 RCF for 1 minute or until solution has passed through the column. Discard the flow-through in the collection tube and return the column into the empty tube.
6. Add 750 µL Buffer PE to the MinElute column and centrifuge at 17,900 RCF for 1 minute; discard flow-through and return MinElute column to the collection tube. Centrifuge for 1 minute at 17,900 RCF to remove residual Ethanol.
7. Place MinElute column in a clean 1.5 mL microcentrifuge tube.
8. Add 50 µL EB Buffer (10mM Tris-Cl, pH 8.5) or water directly onto center of the MinElute membrane. Incubate column for 1 minute at RT, and then centrifuge column at 17,900 RCF for 1 minute.
9. Run 2 µL of purified DNA with 5 µL of diluted gel loading dye next to 2 µL pooled unpurified DNA with 5 µL gel loading dye on 0.8% agarose gel for 60 minutes at 100V to verify the purity of the DNA sample with no primer carry-over or contaminating gDNA.
10. This is your purified amplicon pool ready for PacBio SMRTbell™ library prep

Suggested Suppliers

Qiagen: MinElute® PCR Purification Kit (Cat. No. 28004)
BD Worldwide: (Sterile) Calibrated Disposable Inoculating Loops, Green, #220214 or #220215
Dot Scientific Inc.: 8-strip PCR tubes w/individual domed caps**,** #503-8PCR-A
Fisher: Biotech Grade Water BP24854; Potassium Hydroxide P250; TE BP24731
Permagen: 0.2 mL PCR Strip Magnetic Separation Rack 8 or 12 Strip, Cat #MSR812