Shoreline Wave™ StrainID™ PCR Amplify Protocol

Set A (96 Samples)

Prepare amplicons 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 96 unique barcoded primer sets for processing 96 samples. One additional kit contains unique barcoded primer sets for processing up to 16 additional samples. Every 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 Wave™ StrainID™ PCR Amplify Set A (96 Barcodes) Cat. No. **WAVESID-A**
2. Shoreline Wave™ StrainID™ PCR Amplify Set Z (16 Barcodes) Cat. No. **WAVESID-Z**

Additional Items Needed for 96 Barcode Kits: (Not included; see Suggested Supplier list below)

1. Additional Items **Required** for Amplify:
   a. Qiagen MinElute® PCR Purification Kit
   b. Sterile Laboratory Grade Water
   c. Sterile 100% Ethanol
2. 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 Wave™ PCR Amplify Protocol

1. 96-well PCR plate with 96 barcoded primers in wells A1-H12
2. 2X PCR Premix (**blue** cap)
3. PCR sealing film (1 sheet)

Shoreline Wave™ StrainID™ PCR Amplify Protocol

1. Remove the plate seal from the Amplicon Primer plate.
2. **Add 10 µL** 2X PCR Premix (**blue** cap) to each position in the 96-well plate. (Use a Repeater pipette.)
3. **Add 10 ng – 100 ng Purified genomic DNA** to each of the 96-wells.
4. **Adjust remaining volume with water so that the PCR reaction final volume is 20 µL**
5. Using the PCR sealing film provided with the kit, cover the plate and seal tightly with no gaps.
6. Primers will dissolve off the bottom of each well. Observe that blue color is uniformly distributed throughout 20 µL reaction, if not, tap plate gently until uniform blue color is achieved in each well.
7. Spin plate briefly if reaction mix is not completely at the bottom of the wells.
8. Transfer the plate to PCR machine; close and lock the lid. Run PCR protocol:

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

9. 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 96-well plate *(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 **80 µL** of pooled sample with 400 µL of Qiagen PB buffer.
4. Transfer **all** 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 room temp, 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)

Fisher Scientific: Biotech Grade Water BP24854