



Shoreline Wave™ V1-V9 PCR Amplify Protocol

Set A (16 Samples)

Prepare amplicons for sequencing using single-step PCR amplification of the full-length 1500bp 16S rRNA gene for microbial community profiling up to 112 samples. This kit contains unique barcoded primer sets for processing 16 samples. One additional kit contains 96 unique barcoded primer sets for processing another 96 samples. Every sample will have a unique barcode at the completion of the PCR reaction for sequencing all the samples together.

Products

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

1. Shoreline Wave™ V1-V9 PCR Amplify Set A (96 Barcodes) Cat. No. **WAVEV19-A**
2. Shoreline Wave™ V1-V9 PCR Amplify Set Z (16 Barcodes) Cat. No. **WAVEV91-Z**

Additional Items Needed for 16 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. Two colored 8-tube strips with barcoded primers, labeled with sample barcode number
Barcodes: 1-8 **Yellow** tube strip, 9-16 **Green** tube strip
2. 2X PCR Premix (**blue cap**)

Shoreline Wave™ PCR Amplify Protocol

1. **Add 10 µL 2X PCR Premix (blue cap)** to each barcoded primer tube.
2. **Add 10 ng – 100 ng Purified genomic DNA** to appropriate barcoded primer tube.
3. **Adjust remaining volume with water so that the PCR reaction final volume is 20 µL**
4. 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:
 - 4.1. **Make sure tubes are capped**, then flick gently until uniform blue color is achieved in each tube.
 - 4.2. Spin tubes briefly to ensure all fluid is at the bottom of the tube
5. 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
	63°C for 0:45 (Ramp speed 4°C/sec)
	72°C, 1:30
72°C, 3:00	Final extension



Shoreline Wave™ V1-V9 PCR Amplify Protocol
Set A (16 Samples)

6. 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 ~1500bp.

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** 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

This document and its contents are proprietary to Shoreline Biome, LLC and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Shoreline Biome. Shoreline Biome does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

SHORELINE BIOME DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY SHORELINE BIOME IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

All names, logos, and trademarks are the property of their respective owners.