MicroSEQ™ 500 16S rDNA Identification

Catalog Numbers 4348228 (PCR kit) and 4346480 (Sequencing kit)

Pub. No. 4393013 **Rev.** B

Note: For safety and biohazard guidelines, see the "Safety" appendix in the MicroSEQ™ 500 16S rDNA Identification (Pub. No. 4346298). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Procedures

1 Isolate genomic DNA from samples

a. Obtain the sample, then add PrepMan™ Ultra Sample Preparation Reagent:

If starting from a ...	Follow this procedure ...
Culture broth	1. Pipet 1 mL of culture broth (containing less than 10^7 cfu/mL of bacteria) into a new 2-mL screw-cap microcentrifuge tube or any other microcentrifuge tube that can be tightly closed. 2. Centrifuge the sample for 2 minutes in a microcentrifuge at maximum speed. Aspirate and discard the supernatant. 3. Add 100 µL of PrepMan™ Ultra Sample Preparation Reagent, then close the cap tightly.
Culture plate	1. Select a small sample amount (2–3 mm) from an isolated colony by using a 1 µL loop or the straight end of a 1 µL loop. 2. Suspend the cells in 100 µL of PrepMan™ Ultra Sample Preparation Reagent in a 2-mL screw-cap microcentrifuge tube or any other microcentrifuge tube that can be tightly closed.

b. Vortex the sample for 10 to 30 seconds.

c. Heat the sample for 10 minutes at 100°C in a heat block, then cool the sample to room temperature for 2 minutes.

d. Centrifuge the sample for 2 minutes in a microcentrifuge at maximum speed.

e. Transfer 50 µL of the supernatant into a new microcentrifuge tube.

2 Dilute genomic DNA for PCR

a. Pipet 495 µL of nuclease-free water into a 1.5-mL microcentrifuge tube.

b. Add 5 µL of the PrepMan™ Ultra supernatant to obtain a 1:100 dilution.

Note: If the PrepMan™ Ultra supernatant is colored (typically a shade of black or green), see "Troubleshooting" in the *MicroSEQ™ 500 16S rDNA Identification User Guide*.

3 Prepare the PCR reactions

a. Vortex the diluted supernatant to mix the tube contents.

b. Using the volumes that are shown in the table, prepare samples and controls in MicroAmp™ reaction tubes or 96-well plates.

Reaction type	Volume for one reaction
Negative controls	• 15 µL PCR Master Mix • 15 µL negative control (provided with kit)
Samples	• 15 µL PCR Master Mix • 15 µL of 1:100 dilution of PrepMan® Ultra supernatant
Positive controls	• 15 µL PCR Master Mix • 15 µL positive-control DNA (provided with kit)

c. Use strip caps and the capping tool, or adhesive film and the sealing tool, to cap the tubes or plate. Vortex, centrifuge briefly, then place the tubes or the plate in the thermal cycler.

4 Perform the amplification run

a. Set the appropriate ramp mode for your thermal cycler:
- Veriti™ 96-Well Thermal Cycler—9600 emulation
- 9800 Fast Thermal Cycler—Std
- GeneAmp™ PCR System 9700—9600 emulation (9600)

b. Set the thermal cycling conditions:

Initial step	Each of 30 cycles			Final extension	Final step
	Melt	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95°C 10 minutes	95°C 30 seconds	60°C 30 seconds	72°C 45 seconds	72°C 10 minutes	4°C ∞

c. Set the reaction volume to 30 µL, then start the run.

d. Before removing the caps or adhesive film, briefly centrifuge the tubes or plate.

5 *(Optional)* Analyze PCR products

a. Load 5 µL of PCR product per lane on a 2% agarose gel separation (such as E-Gel™ available from **thermofisher.com**), or prepare your own gel.

b. Use the Mass Standard Ladder to estimate the PCR product yield. In a positive control or sample, a single fragment ranging from 460 to 560 bp in size should be detected on a gel. Actual fragment size depends on the bacterial species. No product should be visible in a negative control reaction.

6 Purify PCR products for cycle sequencing

Remove unused dNTPs and primers from each PCR product with ExoSAP-IT™.

7 Prepare cycle sequencing reactions

a. Before you remove the tube or plate caps, briefly centrifuge the purified PCR products.

b. In reaction tubes or a 96-well plate, prepare separate forward- and reverse-sequencing reactions for each PCR product and control:
- **Forward-sequencing reaction**—Combine 7 µL of purified PCR product or control with 13 µL forward sequence mix.
- **Reverse-sequencing reaction**—Combine 7 µL of purified PCR product or control with 13 µL reverse sequence mix.

8 Perform the cycle sequencing run

a. Cap the tubes or the plate, then place the tubes or the plate in the thermal cycler.

b. Set the appropriate ramp mode for your thermal cycler:
- Veriti™ 96-Well Thermal Cycler—9600 emulation
- 9800 Fast Thermal Cycler—Std
- GeneAmp™ PCR System 9700—9600 emulation (9600)

c. Set the thermal cycling conditions:

Each of 25 cycles			Final step
Melt	**Anneal**	**Extend**	
CYCLE			HOLD
96°C 10 seconds	50°C 5 seconds	60°C 4 minutes	4°C ∞

d. Set the reaction volume to 20 µL, then start the run.

e. Before removing the tube or plate caps, briefly centrifuge the extension products.

9 Purify extension products

After cycle sequencing, use one of the following products to remove excess dye terminators, non-incorporated nucleotides, and primers from the extension products. Select an appropriate purification product depending on whether you performed cycle sequencing in tubes or a plate. Follow the guidelines and procedures that are supplied with the kits.

For cycle sequencing in ...	Purify using ...[1]
8-strips kit	• MicroSEQ™ ID Purification Combo Kit v2.0 8-strips Kit (includes ExoSAP-IT™ *Express* PCR Product Cleanup Reagent) , Cat. No. A35854 or • MicroSEQ™ ID Ultra Sequencing 8-strips Kit, Cat. No. A33246
96-well plates	• MicroSEQ™ ID Purification Combo Kit v2.0 (includes ExoSAP-IT™ *Express* PCR Product Cleanup Reagent) , Cat. No. A35852 or • MicroSEQ™ ID Ultra Sequencing Cleanup Plates Kit, Cat. No. A33245

[1] Contact your local MicroSEQ™ ID representative for additional options.

10 Configure the instrument for electrophoresis

Configure the instrument as described in the following table:

Instrument	Procedure
3500/3500xL	Create a plate using the MSID plate template in the 3500 Series Data Collection software. This plate template contains an instrument protocol/run module (POP-6™ polymer) and a base-calling protocol optimized for MicroSEQ™ ID applications.
3130/3130*xl*	Specify: • **Filter Set**—E • **Run Module**—StdSeq50_POP6_1[1] • **Base-caller**—KB.bcp • **DyeSet/Primer (Mobility File)**—KB_3130_POP6_BDT v1.mob

[1] You can use POP-7™ polymer with the StdSeq50_POP7 run module and the KB_3130_POP7_BDT v1.mob file. However, this instrument configuration reduces data quality within the first 40 bases on the 5′ end of the sequence.

11 Prepare samples and perform electrophoresis

IMPORTANT! If the electrophoresis run time is longer than 24 hours (for example, if you are injecting more than 40 wells on a 4-capillary instrument or more than 160 wells on a 16-capillary instrument), evaporation may occur. Therefore, we recommend that you add formamide to the reactions. See "Prevent evaporation during electrophoresis" in the *MicroSEQ™ 500 16S rDNA Identification User Guide*.

a. Before removing the tube caps or plate cover, briefly centrifuge the extension products.

b. Prepare reactions:
   1. Pipet at least 15 µL of each purified extension product or control into separate wells in a 96-well plate.
   2. Pipet 15 µL of Hi-Di™ Formamide into each blank well that is injected together with samples.

c. Cover the plate, centrifuge, then load the plate into your instrument. Start the run.

d. When the run is complete, review the data using the MicroSEQ™ ID Analysis Software.

Limited product warranty

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The information in this guide is subject to change without notice.

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Revision	Date	Description
B	31 August 2018	Update template and legal information. Update the list of purification products in "Purify extension products" on page 3.
A	December 2017	Baseline for this revision history.