Fast MicroSEQ™ 500 16S rDNA Identification

Catalog Numbers 4370489 (PCR kit) and 4346480 (Sequencing kit)

Pub. No. 4393012 Rev. F

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Fast MicroSEQ™ 500 16S rDNA Identification User Guide* (Pub. No. 4393007). 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

1. 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.

2. Vortex the sample for 10 to 30 seconds.
3. Heat the sample for 10 minutes at 100°C in a heat block, then cool the sample to room temperature for 2 minutes.
4. Centrifuge the sample for 2 minutes in a microcentrifuge at maximum speed.
5. Transfer 50 µL of the supernatant into a new microcentrifuge tube.

2 Dilute genomic DNA for PCR

1. Pipet 495 µL of nuclease-free water into a 1.5-mL microcentrifuge tube.
2. 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 *Fast MicroSEQ™ 500 16S rDNA Identification User Guide*.

3 Prepare the PCR reactions

1. Vortex the diluted supernatant to mix the tube contents.
2. 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 FAST PCR Master Mix • 15 µL negative control (provided with kit)
Samples	• 15 µL FAST PCR Master Mix • 15 µL of 1:100 dilution of PrepMan® Ultra supernatant
Positive controls	• 15 µL FAST PCR Master Mix • 15 µL positive-control DNA (provided with kit)

3. 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

1. Set the appropriate ramp mode for your thermal cycler:
   - VeritiPro™ 96-well Thermal Cycler—Default
   - Veriti™ 96-Well Thermal Cycler—Default
   - (3500/3500xL only) 9800 Fast Thermal Cycler—Fast (F96)
   - (3500/3500xL only) GeneAmp™ PCR System 9700—Maximum (Max)
2. Set the thermal cycling conditions:

Initial step	Each of 30 cycles		Final extension	Final step
	Melt	Anneal		
HOLD	CYCLE		HOLD	HOLD
95°C 10 seconds	95°C 0 seconds	64°C 15 seconds	72°C 1 minute	4°C ∞

3. Set the reaction volume to 30 µL, then start the run.
4. Before removing the caps or adhesive film, briefly centrifuge the tubes or plate.

5 *(Optional)* Analyze PCR products

1. 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.
2. 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™ *Express* PCR Product Cleanup Reagent (Cat. No. 75001).

7 Prepare cycle sequencing reactions

1. Before you remove the tube or plate caps, briefly centrifuge the purified PCR products.
2. 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

1. Cap the tubes or the plate, then place the tubes or the plate in the thermal cycler.
2. Set the appropriate ramp mode for your thermal cycler:
   - VeritiPro™ 96-well Thermal Cycler—Default
   - Veriti™ 96-Well Thermal Cycler—Default
   - (3500/3500xL only) 9800 Fast Thermal Cycler—Fast (F96)
   - (3500/3500xL only) GeneAmp™ PCR System 9700—Maximum (Max)
3. Set the thermal cycling conditions:

Initial step	Each of 25 cycles			Final step
	Melt	Anneal	Extend	
HOLD	CYCLE			HOLD
96°C 1 minute	96°C 10 seconds	50°C 5 seconds	60°C 1 minute 15 seconds	4°C ∞

4. Set the reaction volume to 20 µL, then start the run.
5. 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
SeqStudio™ Genetic Analyzer	1. Create a run in the MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer or the MicroSEQ™ ID Microbial Identification Software v4.0.0 by clicking **Create MicroSEQ ID Run**/**Create Run** or **Open MicroSEQ ID Template**/**Open Template** on the home screen. 2. Export the plate to the network drive. 3. Import the plate to the SeqStudio™ Genetic Analyzer.
SeqStudio™ Flex Series Genetic Analyzer	1. Create a run in the MicroSEQ™ ID Microbial Identification Software v4.0.0 by clicking **Create Run** or **Open Template** on the home screen. 2. Export the plate directly to the SeqStudio™ Flex Series Genetic Analyzer.
3500/3500xL	1. Create a run in the MicroSEQ™ ID software v3.0 or v3.1 by clicking **Create MicroSEQ ID Run** or **Open MicroSEQ ID Template** on the home screen. 2. Save the run.

11 Prepare samples and perform electrophoresis

IMPORTANT! If the electrophoresis run time is longer than 12 hours on the SeqStudio™ Genetic Analyzer or 48 hours on the 3500/3500xL Genetic Analyzer (for example, if you are injecting more than 48 wells on the SeqStudio™ Genetic Analyzer or more than 192 wells on the 3500/3500xL Genetic Analyzer), see "Prevent evaporation during electrophoresis" in the *Fast MicroSEQ™ 500 16S rDNA Identification User Guide*.

1. Before removing the tube caps or plate cover, briefly centrifuge the extension products.
2. Prepare reactions using a 1:1 ratio of purified extension product and formamide:
   a. In a 96-well plate, pipette 10 µL Hi-Di™ Formamide into each well to which you add purified extension products or controls.
   b. Pipette 10 µL Hi-Di™ Formamide into each blank well that is injected together with the samples.
   c. Add 10 µL of purified extension product or control to each well filled in step 2a, then mix by pipetting up and down.
3. Cover the plate, centrifuge, then load the plate into your instrument. Start the run.
4. When the run is complete, review the data using the MicroSEQ™ ID Microbial Identification Software v4.0.0 (SeqStudio™ Genetic Analyzer or SeqStudio™ Flex Series Genetic Analyzer), MicroSEQ™ ID software v3.0 or v3.1 (3500/3500xL), or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. 4393012 F

Revision	Date	Description
F	26 April 2023	Update for the release of MicroSEQ™ ID Microbial Identification Software v4.0.0.
E	13 July 2021	Update to the recommended thermal cycler.
D	11 January 2021	Update for the release of MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0 (Cat. No. A49382).