applied biosystems

MicroSEQ™ 500 16S rDNA Identification

USER GUIDE

using:
MicroSEQ™ 500 16S rDNA PCR Kit and
MicroSEQ™ 500 16S rDNA Sequencing Kit

Catalog Numbers 4348228 (PCR kit) and 4346480 (Sequencing kit)
Publication Number 4346298
Revision G

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. 4346298

Revision	Date	Description
G	31 August 2018	Update trademark, legal, and manufacturer information. Update the list of purification products in "Purify extension products" on page 12.
F	24 October 2015	Corrected amplification thermal cycling conditions.
E	August 2015	Updated legal and contact information.

Contents

Product Information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product information

The Applied Biosystems™ MicroSEQ™ 500 16S rDNA PCR Kit and the Applied Biosystems™ MicroSEQ™ 500 16S rDNA Sequencing Kit provide all of the reagents necessary for the amplification and sequencing of the first 500 base pairs of the 16S ribosomal RNA gene (rDNA). The DNA sequence of the unknown is deciphered by capillary electrophoresis on an Applied Biosystems™ Genetic Analyzer. MicroSEQ™ ID Analysis Software compares the sequence to the validated MicroSEQ™ 16S rDNA 500 Library, then generates an identification report. Variations found within the first 500 base pairs of the 16S region are sufficient to identify most bacteria to the species level.

Note: The MicroSEQ™ Full Gene 16S rDNA Identification is recommended if you need a full 16S rDNA sequence to identify a bacterial species.

Instrument platforms

For optimum performance of the MicroSEQ™ 500 16S rDNA Identification, use the:

- Applied Biosystems™ Veriti™ 96-Well Thermal Cycler
- Applied Biosystems™ 3500 or 3130 Series Genetic Analyzer

For information on older instruments that can also be used, see Appendix B, "Additional supported instruments".

Contents and storage

Table 1 MicroSEQ™ 500 16S rDNA PCR Kit (Cat. No. 4348228)

Contents	Amount	Storage
MicroSEQ™ 500 16S PCR Master Mix	One tube sufficient for 50 PCR amplifications	On receipt: –25°C to –15°C After first use: 2–8°C in a PCR clean room
Positive Control, *E. coli*, 1 ng/µL	One tube sufficient for 5 positive-control assays	
Negative Control, water	One tube sufficient for 5 negative-control assays	

Table 2 MicroSEQ™ 500 16S rDNA Sequencing Kit (Cat. No. 4346480)

Contents	Amount	Storage
MicroSEQ™ 500 16S Forward Sequence Mix	Two tubes sufficient for a total of 55 reactions	–25°C to –15°C
MicroSEQ™ 500 16S Reverse Sequence Mix	Two tubes sufficient for a total of 55 reactions	

Storage guidelines

- Avoid excess freeze-thaw cycles. Aliquot reagents in smaller amounts, if necessary.
- Before each use of the kit, allow the frozen reagents to thaw at room temperature or on ice.

  IMPORTANT! Do not heat the reagents.

- Whenever possible, keep thawed reagents on ice during use.
- Mix the reagents by gently vortexing the tubes. Centrifuge the tubes briefly to collect all liquid at the bottom of the tube.

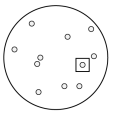
Required materials not supplied

Contact your local MicroSEQ™ ID representative for a list of additional materials and equipment required.

Methods

Workflow

Collect and prepare samples
Harvest bacterial colony, isolate DNA, then dilute DNA for PCR




▼

Amplify DNA
Prepare reactions, perform amplification, analyze PCR products (*optional*), then purify PCR products

Applied Biosystems™ Veriti™ Thermal Cycler

▼

Perform cycle sequencing
Prepare reactions, perform cycle sequencing, then purify extension products

Applied Biosystems™ Veriti™ Thermal Cycler

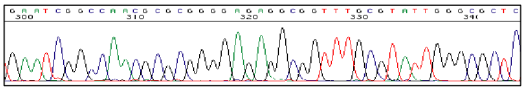
▼

Perform electrophoresis
Configure instrument, then prepare and run samples

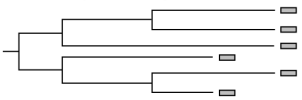
Applied Biosystems™ 3500 or 3130 Series Genetic Analyzer

▼

Analyze data



MicroSeq® ID Analysis Software



Collect and prepare samples

Important procedural guidelines

- Review "Good laboratory practices for PCR and RT-PCR" on page 22.
- When the isolated DNA (in PrepMan™ Ultra supernatant) is not in use, store it at –15 to –25°C . Before use, thaw, then vortex and centrifuge the stored supernatant. Alternatively, cover and store the supernatant at 4°C for up to 1 month.

Isolate genomic DNA from samples

Isolate bacterial genomic DNA from bacterial colonies using PrepMan™ Ultra Sample Preparation Reagent. See the *PrepMan™ Ultra Sample Preparation Reagent Protocol* for additional information.

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.

IMPORTANT! The ideal colony size is 2–3 mm. For smaller colonies, decrease the amount of PrepMan™ Ultra Sample Preparation Reagent to 50 µL from the 100 µL suggested in the protocol.

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.

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: For samples with low biomass, make a smaller dilution (for example, use 195 µL of nuclease-free water to make a 1:40 dilution). The minimum recommended dilution for the PrepMan™ Ultra supernatant is 1:10.

   Note: If the PrepMan™ Ultra supernatant is colored (typically a shade of black or green), PCR inhibition may occur. See "Troubleshooting" on page 14.

Amplify the 16S rDNA region

Important procedural guidelines

- Select the appropriate tubes or 96-well plates for your thermal cycler. See your instrument user guide (available at **thermofisher.com**).
- Using strip caps instead of 96-well adhesive plate covers may help reduce cross-contamination.
- Before preparing the PCR reactions, review "Good laboratory practices for PCR and RT-PCR" on page 22 and "Storage guidelines" on page 6 for sample and reagent handling instructions.
- If necessary after performing PCR or purifying PCR products, cover and store the PCR products at –15°C to –25°C until you are ready to use them.

  Note: PCR products are stable for 6 months or longer at –15°C to –25°C.

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 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)

   Note: To help avoid cross-contamination, we recommend that you pipet components in the following order: negative controls, samples, positive controls. If possible, leave empty cells between different reaction types.

3. Use strip caps and the capping tool, or adhesive film and the sealing tool, to cap the tubes or plate (see "Seal the PCR plate" on page 23). Vortex, centrifuge briefly, then place the tubes or the plate in the thermal cycler.

   IMPORTANT! Apply significant downward pressure on the sealing tool in all steps to form a complete seal.

Perform the amplification run

1. Set the appropriate ramp mode for your thermal cycler:
   - Veriti™ 96-Well Thermal Cycler—9600 emulation

     Note: To use the 9600 emulation mode, select **Tools Menu ▸ Convert a Method ▸ 9600 Emulation Mode**, then enter the thermal cycling conditions. See the *Veriti™ Thermal Cycler User Guide* for details.
   - 9800 Fast Thermal Cycler—Std
   - GeneAmp™ PCR System 9700—9600 emulation (9600)

2. 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 ∞

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.

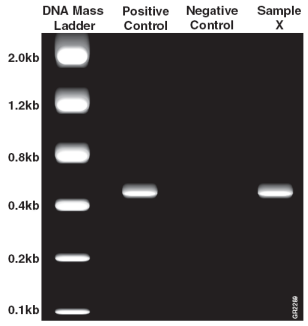
   Note: Centrifuging helps avoid cross-contamination from liquid remaining on the caps or plate covers.

(Optional) Analyze PCR products

Analyze PCR products to confirm the presence of amplified DNA, or to estimate the PCR product yield. The cycle-sequencing protocol works best with 5 to 20 ng of amplicon input.

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.



IMPORTANT! If your samples show no PCR product, PCR inhibition is the most likely cause. See "Troubleshooting" on page 14.

Purify PCR products for cycle sequencing

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

IMPORTANT! Follow the guidelines for the starting sample volume for cleanup as directed in the product literature.

Perform cycle sequencing

Cycle sequencing occurs when successive rounds of denaturation, primer annealing, and primer extension in a thermal cycler result in the incorporation of dye terminators into extension products. The products are then loaded into a genetic analyzer to determine the 16S rDNA sequence. For additional information about cycle sequencing chemistries, refer to the *DNA Sequencing by Capillary Electrophoresis Chemistry Guide*.

Important procedural guidelines

- Select the appropriate tubes or 96-well plates for your thermal cycler. See your instrument user guide (available at **thermofisher.com**).
- Using strip caps instead of 96-well adhesive plate covers may help reduce cross-contamination.
- If you are using a CentriSep™ Spin Column to purify extension products (see "Purifying Extension Products" on page 15), hydrate the column with highly purified (nuclease free) water during the cycle sequencing run.
- If necessary, cover and store the unused portions of the purified PCR products at –15°C to –25°C until you are ready to use them.

  Note: PCR products are stable for 6 months or longer at –15°C to –25°C.
- If necessary, cover and store the extension products at 4°C overnight or at –15°C to –25°C for up to 1 week before purifying them.

Prepare cycle sequencing reactions

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

   Note: Centrifuging helps avoid cross-contamination from liquid remaining on the caps or plate covers.

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.

   Note: To help avoid cross-contamination, pipet components in the following order: negative controls, samples, positive controls.

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:
   - Veriti™ 96-Well Thermal Cycler—9600 emulation
   - 9800 Fast Thermal Cycler—Std
   - GeneAmp™ PCR System 9700—9600 emulation (9600)

3. 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 ∞

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.

   Note: Centrifuging helps avoid cross-contamination from liquid remaining on the caps or plate covers.

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.

Perform electrophoresis of extension products

Important procedural guidelines

- Use only the 50-cm capillary array length regardless of the instrument that you are using. Refer to your instrument user guide for more information.
- If you are not using a 3500 or 3130 Series Genetic Analyzer, select the appropriate parameter settings from the table in "Electrophoresis settings for additional supported instruments" on page 21. Refer to the *MicroSEQ™ ID Analysis Software Online Help* for more information.
- Cover and store any unused purified extension products at 4°C overnight or at –15°C to –25°C for up to 1 week.

Configure the instrument for electrophoresis

1. Configure your data collection software:
   - **Applied Biosystems™ 3500 Series Genetic Analyzers**—Use MicroSEQ™ ID Analysis Software Version 3.0 (or greater)
   - **Applied Biosystems™ 3130 and 3130*xl* Genetic Analyzers** —Use MicroSEQ™ ID Analysis Software Version 2.0 (or greater)
   - **For all other instruments**—Contact your local MicroSEQ™ ID representative

   Note: See "Additional documentation" on page 18 for a list of MicroSEQ™ ID documentation.

2. 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.

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" on page 24.

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

2. Prepare reactions:
   a. Pipet at least 15 µL of each purified extension product or control into separate wells in a 96-well plate.

   b. Pipet 15 µL of Hi-Di™ Formamide into each blank well that is injected together with samples.

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

   Note: Centrifuging removes bubbles from the bottom of the wells.

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

   Note: If you are not using autoanalysis with a 3500 or 3130 Series Genetic Analyzer, refer to the *MicroSEQ™ ID Analysis Software Getting Started Guide* for data analysis instructions.

Troubleshooting

Observation	Possible cause	Recommended action
No PCR product	• No biomass *or* • Fungal sample *or* • PCR inhibition *or* • Cells were not disrupted by the PrepMan™ Ultra method *or* • Incorrect dilution	1. If no PCR product is seen, use more bacterial cells. 2. If the problem persists, the isolate you are trying to identify may be fungi. Amplify the sample with the Fast MicroSEQ™ D2 LSU rDNA Fungal PCR Kit (Cat. no. 4396787). 3. If the problem persists, make one or more new dilutions of the PrepMan™ Ultra supernatant, then run several PCR reactions of each dilution to increase your chance of obtaining a PCR product of the correct size. If the PrepMan™ Ultra supernatant is: • **Clear**–Make smaller dilutions (1:40 or 1:10) of the original PrepMan™ Ultra supernatant. • **Colored (typically a shade of black or green)**– Make the following dilutions: – Smaller dilutions (1:40 or 1:10) of the original PrepMan™ Ultra supernatant. – A 1:1000 dilution of the original PrepMan™ Ultra supernatant. 4. If you do not obtain a PCR product from any of the diluted samples, try one of the following solutions: • Use a DNA extraction kit to isolate pure DNA. *or* • Use the bead-beating method to isolate fungal genomic DNA or bacterial genomic DNA.

Observation	Possible cause	Recommended action
Signal is too high	Too much amplicon in the sequencing reaction	Dilute the purified extension product with Hi-Di™ Formamide, then perform a new run. If you ran purified extension product that was: • **Not diluted**—Dilute the purified extension product at a ratio of 1:1. • **Diluted at a 1:1 ratio**—Dilute the purified extension product at a 1:10 ratio. • **Diluted at a 1:10 ratio**—Dilute the purified extension product at a 1:40 ratio. See "Prevent evaporation during electrophoresis" on page 24.
Absence of signal/blank electropherogram	Sample evaporation	See "Prevent evaporation during electrophoresis" on page 24.
The sequence is short and/or the first part of the sequence is very bright and off-scale and the remainder has very low intensity	• High starting amount of DNA *or* • Too much DNA template in the sequencing reaction	1. Decrease the amount of bacterial cell material using one of the following methods: • Use a smaller colony or pellet. • Further dilute the PrepMan™ Ultra supernatant. 2. If the problem persists, estimate the PCR product yield on agarose gel and use 5–20 ng of amplicon for sequencing as described in "(Optional) Analyze PCR products" on page 10.
Both results and raw data show occasional high spikes for all four dye colors	Bubbles in the capillary	Check the instrument maintenance and troubleshooting guides.
Large regions of overlapping sequence *or* cannot call bases for large regions of sequence	• Pipetting more than one template per well *or* • DNA sample is contaminated (that is, the DNA is derived from more than one species of bacteria) *or* • The organism has multiple copies of the rDNA gene, and some copies have insertions or deletions	1. Prepare new reactions, then repeat electrophoresis. 2. If the problem persists, sub-culture the organism to pure culture, then repeat identification. 3. If the problem persists, clone the PCR product (using a kit such as the TOPO™ PCR Cloning Kit) before performing sequencing.
Small regions of overlapping sequence	In bacterial species with multiple copies of the rRNA gene, the gene can be polymorphic, resulting in overlap of up to 1% of the sequence	No action needed.

Frequently asked questions

Sensitivity and quantitation

What is the sensitivity of the MicroSEQ™ 500 16S rDNA Identification?

As long as you start from a visible colony or cell pellet, MicroSEQ™ kits will work.

Can I use the MicroSEQ™ 500 16S rDNA Identification to quantify fungi or yeast?

No. The PCR is an endpoint assay.

Sample preparation and storage

Which kits should I use to identify yeast samples?

Use the Fast MicroSEQ™ D2 rDNA Fungal Identification or the MicroSEQ™ D2 rDNA Fungal Identification to sequence and identify yeast samples.

What is the best way to prepare yeast samples?

Prepare yeast samples using the PrepMan™ Ultra Sample Preparation Reagent or bead-beating method, just as you would prepare bacterial samples. Extra dilutions of the fungal DNA supernatant are sometimes necessary.

Are there alternative methods for preparing genomic DNA?

If the PrepMan™ Ultra Sample Preparation Reagent method does not successfully disrupt cells, you can use the bead-beating method to isolate genomic DNA.

Alternatively, you can use a DNA extraction kit (available from various vendors) to isolate pure DNA.

Can I use less PrepMan™ Ultra Sample Preparation Reagent if I start with a smaller colony?

Yes. The ideal colony size is 2–3 mm. For smaller colonies, you can decrease the amount of PrepMan™ Ultra Sample Preparation Reagent to 50 µL from the suggested 100 µL in the *PrepMan™ Ultra Sample Preparation Reagent Protocol*.

Can I enrich my genomic DNA by using less PrepMan™ Ultra Sample Preparation Reagent?

Yes. However, be careful not to overload the PCR mix. Enriched samples tend to have more cellular and other debris, which can interfere with PCR.

At what temperature should I store my PrepMan™ Ultra-isolated DNA?

Store isolated DNA at –15 to –25°C. (Alternatively, you can safely keep it overnight at room temperature or at 4°C.)

Contamination

How can I tell if my sequence is representative of a single species?

The DNA sequence from a single species should be distinct (easy to call base pairs), without significant regions of overlapping sequence.

If my initial DNA sample is contaminated (that is, it comes from multiple species), how can I sequence my PCR product?

Clone the PCR product using a kit such as the TOPO™ TA Cloning™ Kit (Cat. no. K4575-J10) .

Overlapping sequences

My sequence has large regions of overlap (>5% mixed bases). What does this mean?

See Troubleshooting, "Large regions of overlapping sequence or cannot call bases for large regions of sequence" on page 15.

My sequence has small regions (less than or equal to 1% of overlap). What does this mean?

See Troubleshooting, "Small regions of overlapping sequence" on page 15.

PCR product size

Can I always expect the same size PCR product for all species?

PCR products can vary from the expected product size, depending on the species.

Expected product sizes for the:

- **MicroSEQ™ Fungal Kits** – 1 band at 300–500 bp
- **MicroSEQ™ 500 Kits** – 1 band at 460–560 bp
- **MicroSEQ™ Full Gene Kit** – 1 band at 460–560 bp and 2 bands at 700–800 bp

Can I increase the number of cycles to increase the PCR yield?

Yes, but doing so can cause additional background signal from the negative control.

Species libraries

How are species in the MicroSEQ™ libraries validated?

Please contact your local MicroSEQ™ representative to obtain a copy of the MicroSEQ™ ID Library Validation Statement for additional information.

Where does Thermo Fisher Scientific obtain the strains used to determine the reference sequencing in the MicroSEQ™ libraries?

The strains are derived from major culture collections such as the American Type Culture Collection (ATCC) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (German Collection of Microorganisms and Cell Cultures).

What is the difference between the libraries for the MicroSEQ™ Full Gene kit and the MicroSEQ™ 500 kits?

The sequences in the library for the MicroSEQ™ 500 kits are ~500 bp, which is the expected size of the PCR products for this kit. The sequences in the library for the MicroSEQ™ Full Gene kit are ~1440 bp, the maximum sequence length that the kit allows you to determine.

Additional documentation

Where can I find additional information about MicroSEQ™ ID Analysis Software?

Refer to the following documentation for MicroSEQ™ ID Analysis Software Version 2.0 or greater:

- *MicroSEQ™ ID Analysis Software Quick Reference Card*
- *MicroSEQ™ ID Analysis Software Getting Started Guide*
- *MicroSEQ™ ID Analysis Software Online Help*

Note: For additional documentation, see "Customer and technical support" on page 33.

A Ordering information

The following products are available at **thermofisher.com**.

Product	Description	Cat. No.
Fast MicroSEQ™ D2 LSU rDNA Fungal PCR Kit	This kit is the PCR component of the FAST MicroSEQ™ Fungal Identification System containing enough reagents for 50 PCR amplifications of fungal DNA unknowns. The sequencing component of the kit is required for species identification.	4382397
MicroSEQ™ D2 rDNA Fungal PCR Kit	This kit is the PCR component of the MicroSEQ™ Fungal Identification System containing enough reagents for 50 PCR amplifications of fungal DNA unknowns. The sequencing component of the kit is required for species identification.	4349153
MicroSEQ™ D2 rDNA Fungal Sequencing Kit	This kit is the sequencing component of the MicroSEQ™ Fungal Identification System, which provides an easy-to-use DNA sequence-based method to identify most fungi. It includes the primers needed to sequence the PCR products generated using the PCR component.	4347481
Fast MicroSEQ™ 500 16S rDNA PCR Kit	This kit is the PCR component of the MicroSEQ™ Bacterial Identification System containing enough reagents for 50 PCR amplifications of bacterial DNA unknowns. The sequencing component of the kit is required for species identification.	4370489
MicroSEQ™ 500 16S rDNA PCR Kit	This kit is the PCR component of the MicroSEQ™ Bacterial Identification System containing enough reagents for 50 PCR amplifications of bacterial DNA unknowns. The sequencing component of the kit is required for species identification.	4348228
MicroSEQ™ 500 16S rDNA Sequencing Kit	This kit is the sequencing component of the MicroSEQ™ Bacterial Identification System, which provides an easy-to-use DNA sequence-based method to identify most bacteria. It includes the primers needed to sequence the PCR products generated using the PCR component. There are enough reagents for 55 sequencing reactions.	4346480
MicroSEQ™ Full Gene 16S rDNA PCR Kit	This kit is the PCR component of the MicroSEQ™ Bacterial Identification System containing enough reagents for 50 PCR amplifications of bacterial DNA unknowns. The sequencing component of the kit is required for species identification.	4349155
MicroSEQ™ Full Gene 16S rDNA Sequencing Kit	This kit is the sequencing component of the MicroSEQ™ Bacterial Identification System, which provides an easy-to-use DNA sequence-based method to identify most bacteria. It includes the primers needed to sequence the PCR products generated using the PCR component. There are enough reagents for 55 sequencing reactions.	4347484

Product	Description	Cat. No.
PrepMan™ Ultra Sample Preparation Reagent	PrepMan™ Ultra Sample Preparation Reagent was developed for the rapid preparation of DNA template from Gram-negative food-borne pathogens for use in PCR amplification reactions. These samples often have high lipid content that can inhibit PCR amplification of the template. Using a simple boil and spin protocol, PrepMan™ Ultra Sample Preparation Reagent efficiently inactivates PCR inhibitors, significantly reducing the need to repeat the template preparation step.	4318930
MicroSEQ™ ID Analysis Software Version 3.0	This easy-to-use software enables you to identify and classify unidentified bacterial or fungal sequences by comparing them to a validated microbial library.	Contact your local MicroSEQ™ ID representative

B Additional supported instruments

We recommend that you use the Applied Biosystems™ Veriti™ 96-Well Fast Thermal Cycler and the Applied Biosystems™ 3500 or 3130 Series Genetic Analyzer with the MicroSEQ™ kits.

However, the MicroSEQ™ kits can also be used with:

- Applied Biosystems™ GeneAmp™ PCR System 9700 thermal cycler

  Note: An amplification run using a GeneAmp™ PCR System 9700 can take up to 20 minutes longer than a run using the Veriti™ or 9800 Fast Thermal Cycler.
- Applied Biosystems™ 9800 Fast Thermal Cycler
- Applied Biosystems™ 3730 and 3730*xl* DNA Analyzers
- Applied Biosystems™ 3100 and 3100-Avant™ Genetic Analyzers
- Applied Biosystems™ 310 Genetic Analyzer

Electrophoresis settings for additional supported instruments

Instrument	Filter Set	Run Module	Base-caller	DyeSet/Primer (Mobility File)
Applied Biosystems™ 3730 and 3730*xl* DNA Analyzers	E	StdSeq50_POP7	KB.bcp	KB_3730_POP7_BDT v1.mob
Applied Biosystems™ 3100 and 3100-Avant™ Genetic Analyzers	E	StdSeq50_POP6_1	KB.bcp	KB_3100_POP6_BDT v1.mob
Applied Biosystems™ 310 Genetic Analyzer	E	Seq POP6 (1 mL) E.md4	KB.bcp	KB_310_POP6_BDT v1_50Std.mob

C Supplemental procedures and guidelines

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Seal the PCR plate

Seal the plate with strip caps

IMPORTANT! Apply significant downward pressure on the sealing tool in all steps to form a complete seal.

Note: Use of strip caps instead of 96-well adhesive plate covers may help reduce cross-contamination.

To use the rolling capping tool:

- Roll the capping tool across all strips of caps on the short edge, then the long edge of the tray.
- Roll the capping tool around all outer rows of strips of caps.

To use the rocking capping tool:

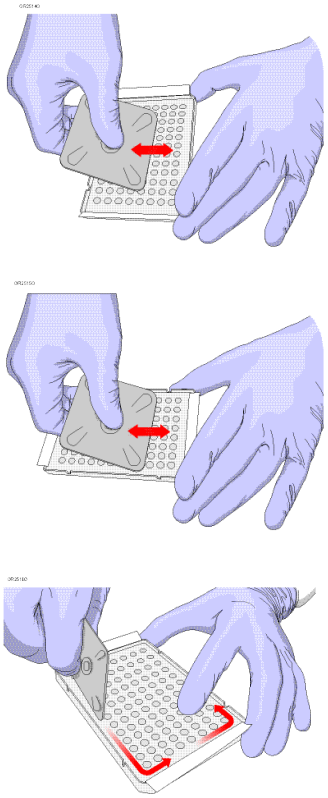
- Slip your fingers through the handle with the holes in the tool facing down.
- Place the holes in the tool over the first eight caps in a row.
- Rock the tool back and forth a few times to seal the caps.
- Repeat for the remaining caps in the row, then for all remaining rows.

Seal the plate with adhesive film

IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

1. Place an optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the *long* edge of the plate.
2. Rub the flat edge of the applicator back and forth along the *short* edge of the plate.
3. Rub the edge of the applicator horizontally and vertically between all wells.
4. Rub the edge of the applicator around all outside edges of the plate using small back and forth motions to completely seal around the outside wells.
5. Vortex the plate on the low setting for 5 seconds. If you see liquid on the well sidewalls, spin down the plate at 2000 × *g* for 20 seconds using a centrifuge with a plate adapter.

   IMPORTANT! Make sure reagents are in the bottom of the wells.



Prevent evaporation during electrophoresis

We recommend that you use Hi-Di™ Formamide to prevent sample evaporation during long electrophoresis runs. If your run time is:

- 24 hours or less, addition of formamide is not necessary
- Between 24 and 48 hours, see "Prepare a diluted sample" on page 24
- Longer than 48 hours, see "Dry-down and resuspend the sample" on page 25

Prepare a diluted sample

1. Prepare reactions using a 1:1 ratio of purified extension product and formamide:
   a. In a 96-well plate, pipette 10 µL of Hi-Di™ Formamide into each well to which you will add purified extension products or controls.

   b. Pipette 10 µL of Hi-Di™ Formamide into each blank well that will be injected together with the samples.

   c. Add 10 µL of purified extension product or control to each well filled in step 1a, then mix by pipetting up and down.

Note: If after a 1:1 dilution you do not detect a sequencing ladder due to a low signal, rerun the sample without diluting.

2. Centrifuge the plate, load the plate into your instrument, then start the run.

   Note: Centrifuging removes bubbles from the bottom of the wells.

   Note: See "Configure the instrument for electrophoresis" on page 13 for details.

3. Cover and store the unused portion of the purified extension products overnight at 4°C or for up to 1 week at –15°C to –25°C.

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

Note: If you are not using a 3500 or 3130 Series Genetic Analyzer, refer to the *MicroSEQ™ ID Analysis Software Getting Started Guide* for data analysis instructions.

Dry-down and resuspend the sample

1. Centrifuge the tubes or plate containing the purified extension products in a speed vac.

   Note: Centrifuge time and speed depend on the number of samples and the type of speed vac used. Typical times range from 30–60 minutes.

   IMPORTANT! Do not over-dry the DNA pellet, and do not use heat to dry the pellet.

2. Resuspend the DNA in 15 µL of Hi-Di™ Formamide.

   Note: Formamide disrupts hydrogen bonds in double-stranded DNA, inhibiting secondary structure and DNA conglomeration, and resulting in cleaner and more consistent electrophoresis runs.

3. Centrifuge the plate, load the plate into your instrument, then start the run.

   Note: Centrifuging removes bubbles from the bottom of the wells.

   Note: See "Configure the instrument for electrophoresis" on page 13 for details.

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

Note: If you are not using a 3500 or 3130 Series Genetic Analyzer, refer to the *MicroSEQ™ ID Analysis Software Getting Started Guide* for data analysis instructions.

D Supplemental product information

MicroSEQ™ system overview

The MicroSEQ™ Microbial Identification System combines all of the instruments, reagents, sequence libraries, and software required for automated microbial identification using DNA sequencing.

The MicroSEQ™ system is easy to use and suitable for the routine identification of all bacterial and fungal isolates, including organisms that are difficult to grow, non-viable, or unidentifiable using phenotypic methods. The MicroSEQ™ system identifies bacterial and fungal isolates from a small sample of pure culture without preliminary testing or growth on selective media.

About MicroSEQ™ ID Analysis Software

The MicroSEQ™ ID Analysis Software analyzes sequences obtained with any of the MicroSEQ™ Microbial Identification Kits.

The software assembles the 16S region rDNA sequence for the unknown, then compares the sequence with known reference 16S region rDNA sequences. For the MicroSEQ™ 500 16S rDNA Identification, data is compared to the MicroSEQ™ ID 16S rDNA 500 Library. Based on the comparison, the software provides a potential ID for the unknown bacterial species.

With the software, you can perform:

- Basecalling with assignment of quality values
- Clear-range determination, which lets you exclude data near sequence ends (typically poor-quality data) from analysis
- Assembly and alignment of sequences to generate a high-quality consensus sequence
- Comparison of the consensus sequence to the MicroSEQ™ ID proprietary libraries to generate a list of the closest matches, including percentage match scores
- Exports of projects and consensus sequences to facilitate data-sharing between collaborators

The software also has features that assist with 21 CFR Part 11 compliance requirements.

For more information, refer to the *MicroSEQ™ ID Analysis Software Online Help*, *MicroSEQ™ ID Analysis Software Quick Reference Card*, and the *MicroSEQ™ ID Analysis Software Getting Started Guide* for software version 3.0 or later.

MicroSEQ™ ID proprietary libraries

MicroSEQ™ ID library sequences are carefully validated. Polymorphic positions are taken into account and included in library species.

Custom libraries

MicroSEQ™ ID Analysis Software allows you to create custom libraries using data generated by the MicroSEQ™ ID software, or using sequences from public databases. Custom libraries are easy to import and export, making information sharing convenient.

During the analysis process, you can search proprietary and custom libraries simultaneously to determine 3–20 closest matches to the sequence of your unknown bacterial species.

MicroSEQ™ ID reports

MicroSEQ™ ID Analysis Software generates four detailed reports:

- **Analysis QC Report** – Allows you to quickly scan the unknowns in a project to gather information about the samples, including the top percent identity match and specimen score to measure data quality. See Figure 1.
- **Library Search Report** – Provides more detailed information about the libraries that were searched, including a list of all the top matches and the total number of bases searched. See Figure 2.
- **Audit Trail Report** – Tracks changes made to projects after analysis.
- **Electronic Signature History Report** – Provides a summary of the electronic signatures used in a project.

All reports can be generated on project and specimen levels. In addition, the software allows you to create custom reports. For information, refer to the *MicroSEQ™ ID Analysis Software Online Help* for software version 2.0 or later.

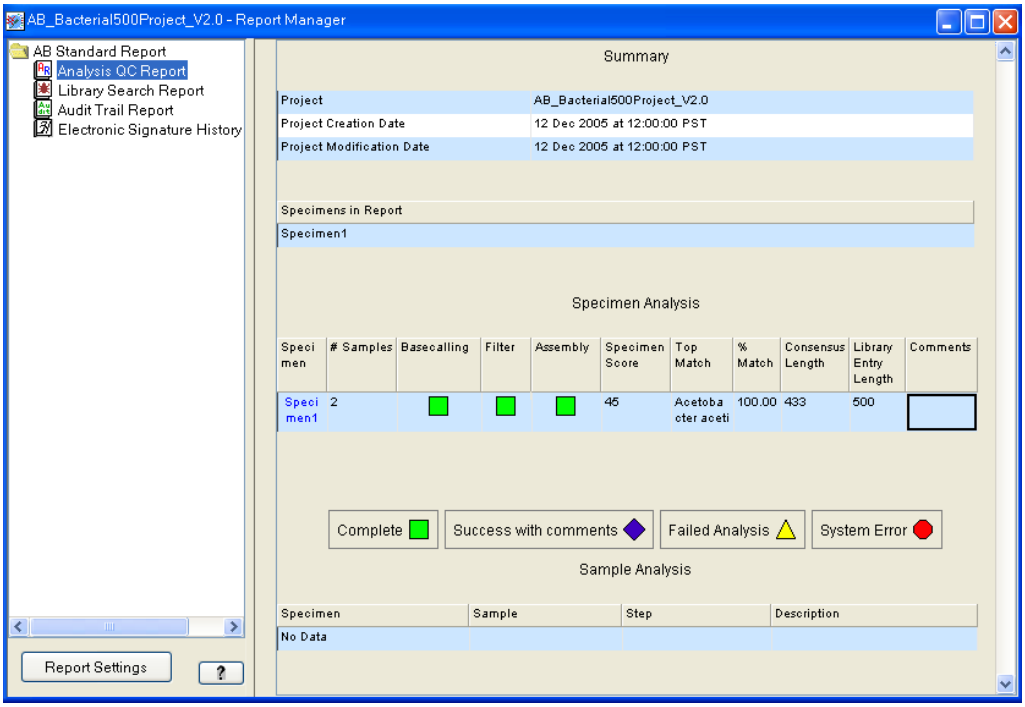


Figure 1 Example Analysis QC Report

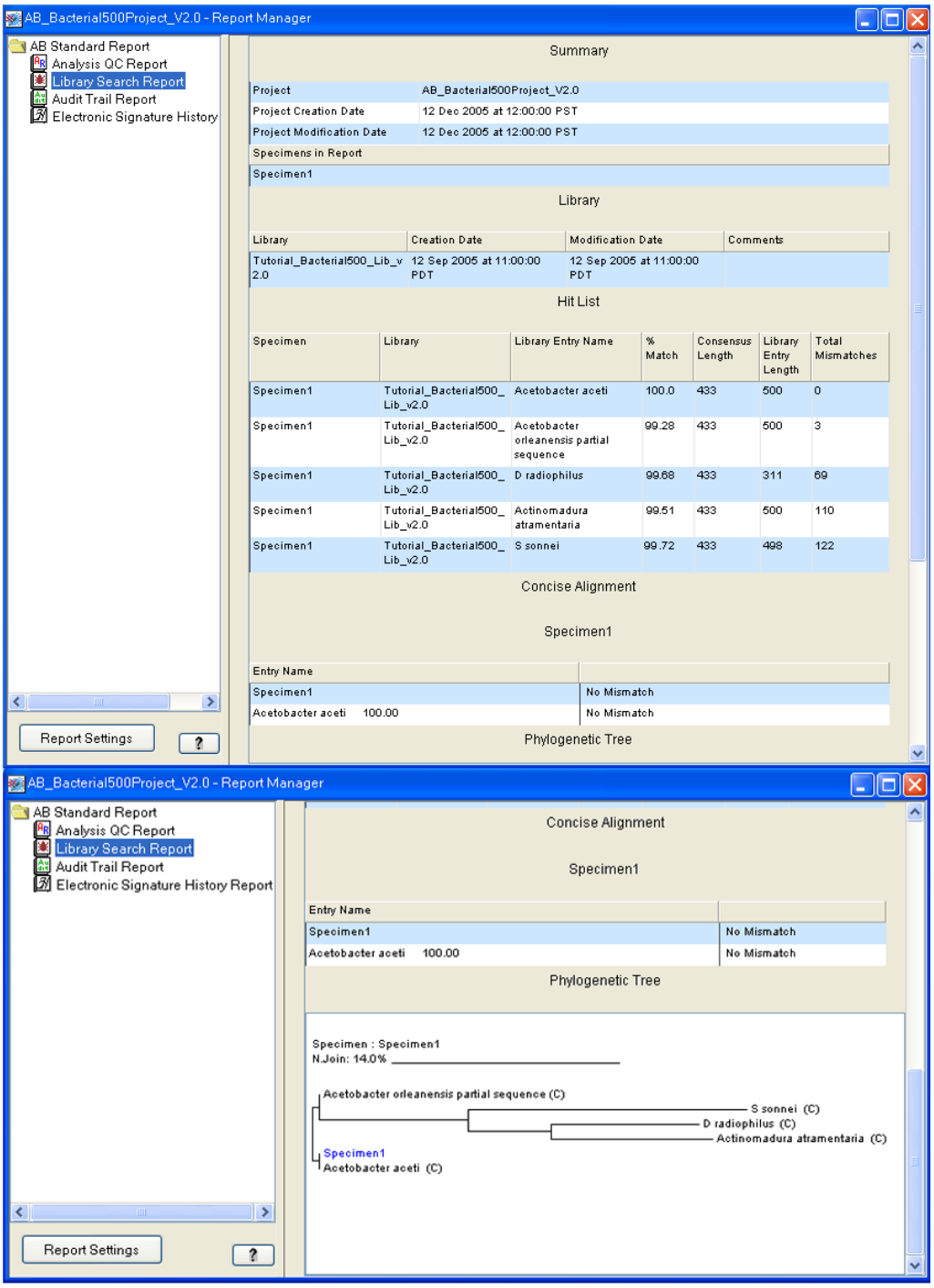


Figure 2 Example Library Search Report

About dye-labeled terminator chemistry

The MicroSEQ™ 500 16S rDNA Sequencing Kit uses BigDye™ Terminator v1.1 chemistry. Forward and Reverse Sequence Mixes contain sequence-terminating 3′-dideoxynucleotide triphosphates (ddNTPs). Each of the four ddNTPs is tagged with a different fluorescent dye. When the ddNTPs are incorporated into extension products during cycle sequencing, the extension products are simultaneously terminated and labeled with the dye that corresponds to the incorporated base, as shown in the following figure.

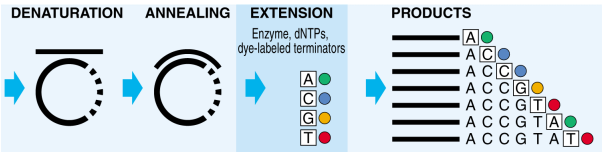


Figure 3 BigDye™ Terminator v1.1 chemistry

For more information about dye-labeled terminators and other sequencing chemistries, refer to the *DNA Sequencing by Capillary Electrophoresis Chemistry Guide*. See “Related documentation“ on page 33.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

The following related documents are available at **thermofisher.com/support**:

Document	Publication number
MicroSEQ™ 500 16S rDNA Identification Quick Reference	4393013
PrepMan™ Ultra Sample Preparation Reagent Protocol	4367554
Veriti™ Thermal Cycler User Guide	4375799
DNA Sequencing by Capillary Electrophoresis Chemistry Guide	4305080
MicroSEQ™ ID Analysis Software Getting Started Guide	v3.0: 4448336 v2.2: 4445126 v2.0: 4364623
MicroSEQ™ ID Analysis Software Quick Reference Card	v2.2: 4445420 v2.0: 4364624

Note: For additional documentation, see "Customer and technical support" on page 33.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.