MicroSEQ™ Full Gene 16S rDNA Identification

USER GUIDE

using:
MicroSEQ™ Full Gene 16S rDNA PCR Kit and
MicroSEQ™ Full Gene 16S rDNA Sequencing Kit

Catalog Numbers 4349155 (PCR kit) and 4347484 (Sequencing kit)

Publication Number 4393009

Revision H

ThermoFisher SCIENTIFIC

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 4393009

Revision	Date	Description
H	13 July 2021	Update to the recommended thermal cycler. See "Instrument platforms" on page 5.
G	11 January 2021	Update for the launch of MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer (Cat. No. A49382).

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™ Full Gene 16S rDNA PCR Kit and the Applied Biosystems™ MicroSEQ™ Full Gene 16S rDNA Sequencing Kit provide all of the reagents necessary for the amplification and sequencing 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 software (3500/3500xL Genetic Analyzer) or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer compares the sequence to the validated MicroSEQ™ 16S rDNA Full Gene Library, then generates an identification report. Variations found within the full 16S region are sufficient to identify most bacteria to the species level.

Instrument platforms

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

- Applied Biosystems™ VeritiPro™ 96-well Thermal Cycler
- 3500/3500xL Genetic Analyzer or SeqStudio™ Genetic Analyzer

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

Contents and storage

Table 1 MicroSEQ™ Full Gene 16S rDNA PCR Kit (Cat. No. 4349155)

Contents	Amount	Storage
MicroSEQ™ Full Gene 16S rDNA PCR Master Mixes	Three tubes, each containing a different Master Mix. Each tube is sufficient for 20 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™ Full Gene 16S rDNA Sequencing Kit (Cat. No. 4347484)

Contents	Amount	Storage
MicroSEQ™ Full Gene Forward Sequence Mixes	Three tubes, each corresponding to one of the PCR Master Mixes from the PCR kit, sufficient for a total of 15 reactions	–25°C to –15°C
MicroSEQ™ Full Gene Reverse Sequence Mixes	Three tubes, each corresponding to one of the PCR Master Mixes from the PCR kit, sufficient for a total of 15 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.

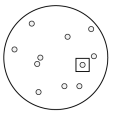
Required materials not supplied

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

VeritiPro™ 96-well Thermal Cycler

▼

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

VeritiPro™ 96-well Thermal Cycler

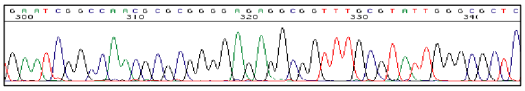
▼

Perform electrophoresis
Configure instrument, then prepare and run samples

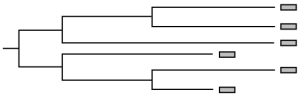
SeqStudio™ Genetic Analyzer or 3500/3500xL Genetic Analyzer

▼

Analyze data



MicroSeq® ID Analysis Software



Collect and prepare samples

Important procedural guidelines

- Review "Good laboratory practices for PCR and RT-PCR" on page 25.
- 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 17.

Amplify the full 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 25 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.

IMPORTANT! For each sample or control, prepare three PCR reactions, one for each of the three PCR Master Mixes. See Figure 1.

Reaction type	Volume for one reaction
Negative controls	• 15 µL PCR Master Mix 1, 2, or 3 • 15 µL negative control (provided with kit)
Samples	• 15 µL PCR Master Mix 1, 2, or 3 • 15 µL of 1:100 dilution of PrepMan® Ultra supernatant
Positive controls	• 15 µL PCR Master Mix 1, 2, or 3 • 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 26). 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.

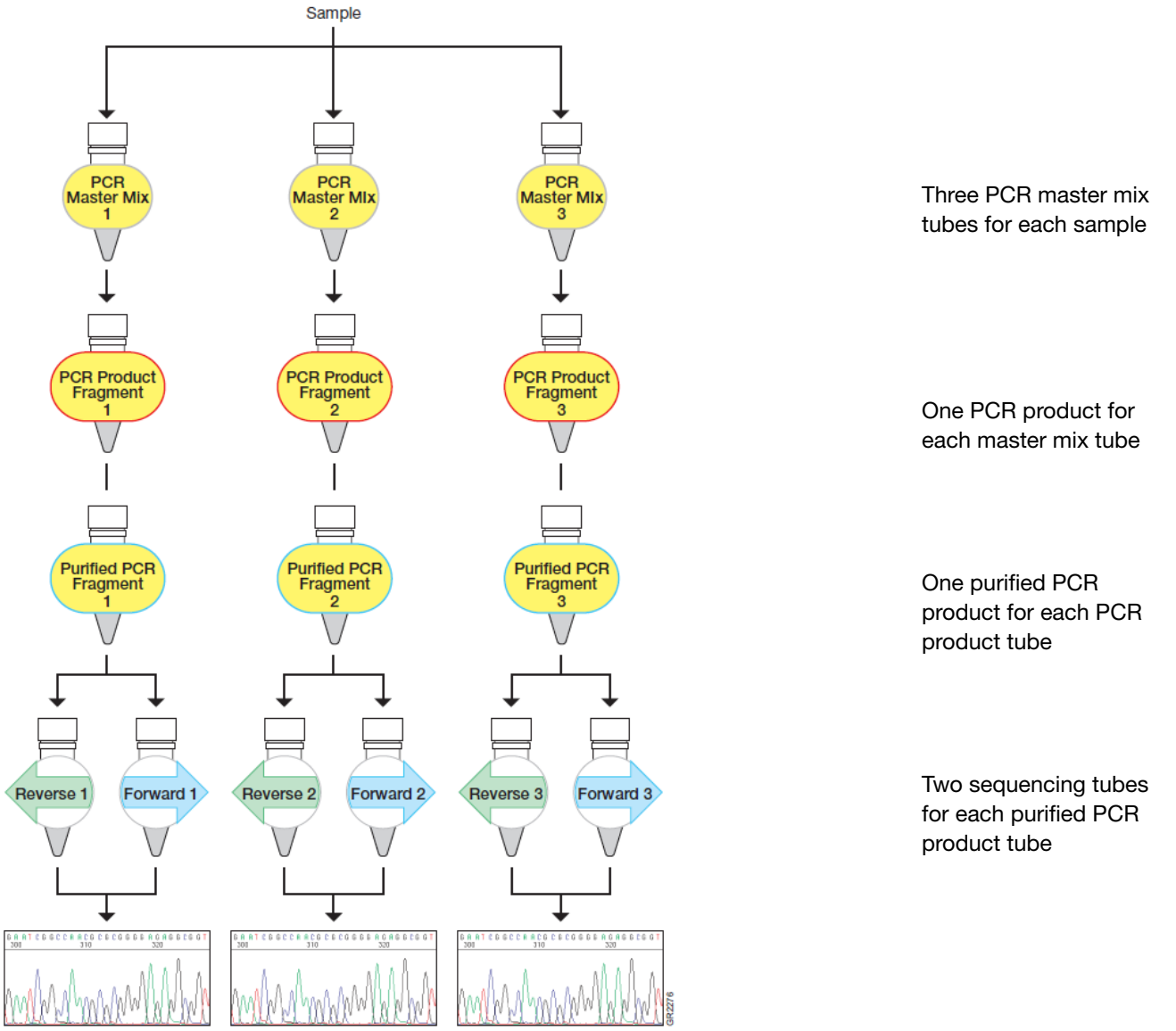


Figure 1 Use of PCR and sequencing kits

Perform the amplification run

1. Set the appropriate ramp mode for your thermal cycler:
   - VeritiPro™ 96-well Thermal Cycler—Default

     Note: To use the default mode, select **Browse/New Methods ▸ New**, then edit the thermal cycling conditions. See the *VeritiPro™ Thermal Cycler User Guide* (Pub. No. MAN0019157) for details.
   - Veriti™ 96-Well Thermal Cycler—Default
   - (3500/3500xL only) 9800 Fast Thermal Cycler—Std
   - (3500/3500xL only) 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 ng to 20 ng of amplicon entered.

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. For samples and positive controls, three fragments should be detected: one band ranging from 460 bp to 560 bp in size, and two bands ranging from 700 bp to 800 bp in size. Actual fragment size depends on the bacterial species. No product should be visible in a negative control reaction.

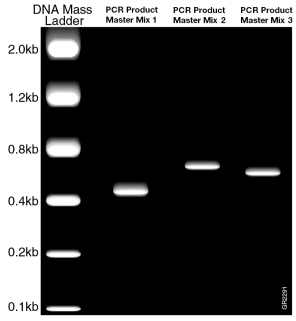


Figure 2 Mass standard ladder – samples (controls not shown)

IMPORTANT! If your samples show no PCR product, PCR inhibition is the most likely cause. See “Troubleshooting” on page 17.

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).

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 full 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 removing 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.

   IMPORTANT! For each PCR product, use the Forward and Reverse Sequencing mixes that correspond to the PCR Master Mix used to amplify the PCR product. See Figure 1.

   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:
   - VeritiPro™ 96-well Thermal Cycler—Default
   - Veriti™ 96-Well Thermal Cycler—Default
   - (3500/3500xL only) 9800 Fast Thermal Cycler—Std
   - (3500/3500xL only) 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

- (3500/3500xL) Use the 50-cm capillary array length. See the *3500/3500xL Genetic Analyzer User Guide* (Pub. No. 100079380) for more information.
- If you are not using a SeqStudio™ Genetic Analyzer or 3500/3500xL Genetic Analyzer, select the appropriate parameter settings from the table in "Electrophoresis settings for additional supported instruments" on page 24.
- 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 according to the platform being used:
   - SeqStudio™ Genetic Analyzer—Use MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0
   - Applied Biosystems™ 3500 Series Genetic Analyzers—Use MicroSEQ™ ID Analysis Software v3.0 (or later)

   Note: See "Additional documentation" on page 21 for a list of MicroSEQ™ ID documentation.

2. Configure the instrument as described in the following table:

Instrument	Procedure
SeqStudio	**1.** Create a run in the MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer by clicking **Create MicroSEQ ID Run** or **Open MicroSEQ ID Template** on the home screen. **2.** Export the plate to the network drive. **3.** Import the plate to the SeqStudio™ Genetic Analyzer.
3500/3500xL	**1.** Create a run in the MicroSEQ™ ID software v3.0 (or later) by clicking **Create MicroSEQ ID Run** or **Open MicroSEQ ID Template** on the home screen. **2.** Save the run.

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” on page 27.

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.

   Note: Dilution in Hi-Di™ Formamide normalizes the signal strength of the sequencing reaction and stabilizes extension products. If after a 1:1 dilution you do not detect a sequencing ladder due to a low signal, run 15 µL of each sample without diluting.
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 software (3500/3500xL) or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer.

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 27.
Absence of signal/blank electropherogram	Sample evaporation	See "Prevent evaporation during electrophoresis" on page 27.
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 12.
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.

Observation	Possible cause	Recommended action
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™ Full Gene 16S rDNA Identification?

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

Can I use the MicroSEQ™ Full Gene 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 18.

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 19.

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 software or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer?

Refer to the following documentation for MicroSEQ™ ID software (3500/3500xL):

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

Refer to the following documentation for MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer:

- *MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0 Quick Reference*
- *MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0 User Guide*

Note: For additional documentation, see “Customer and technical support” on page 36.

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

(continued)

Product	Description	Cat. No.
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
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 (3500/3500xL Genetic Analyzer)	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
MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer		

B Additional supported instruments

We recommend that you use the Applied Biosystems™ VeritiPro™ 96-well Thermal Cycler and the Applied Biosystems™ 3500/3500xL Genetic Analyzer or SeqStudio™ Genetic Analyzer with the MicroSEQ™ kits.

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

- Applied Biosystems™ Veriti™ 96-Well Thermal Cycler
- 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 VeritiPro™ 96-well Thermal Cycler or 9800 Fast Thermal Cycler.

- Applied Biosystems™ 9800 Fast Thermal Cycler
- Applied Biosystems™ 3730 and 3730*xl* DNA Analyzers

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

C

Supplemental procedures and guidelines

Good laboratory practices for PCR and RT-PCR

- 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:

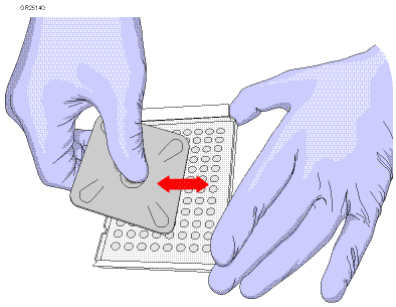
- Slip your fingers through the handle with the holes in the tool facing down.
- Place the holes in the tool over the first eight caps in a row.
- Rock the tool back and forth a few times to seal the caps.
- Repeat for the remaining caps in the row, then for all remaining rows.

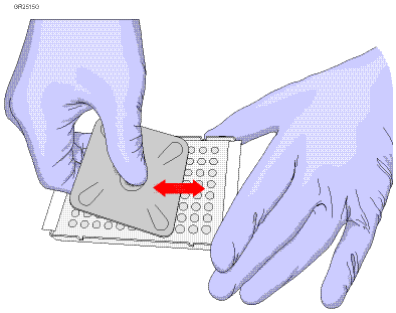
Seal the plate with adhesive film

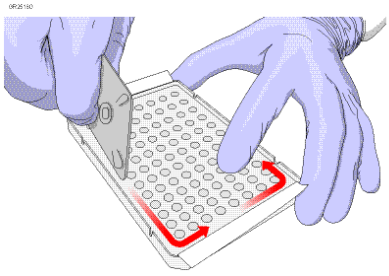
IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

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 28
- Longer than 48 hours, see “Dry-down, then resuspend the sample” on page 28

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 15 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 software or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer.

Dry-down, then 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 15 for details.

When the run is complete, review the data using the MicroSEQ™ ID software or MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer.

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 software and MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer

The 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™ Full Gene 16S rDNA Identification, data is compared to the MicroSEQ™ ID 16S rDNA Full Gene 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, see “Related documentation” on page 36.

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 3.
- **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 4.
- **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, see “Related documentation” on page 36.

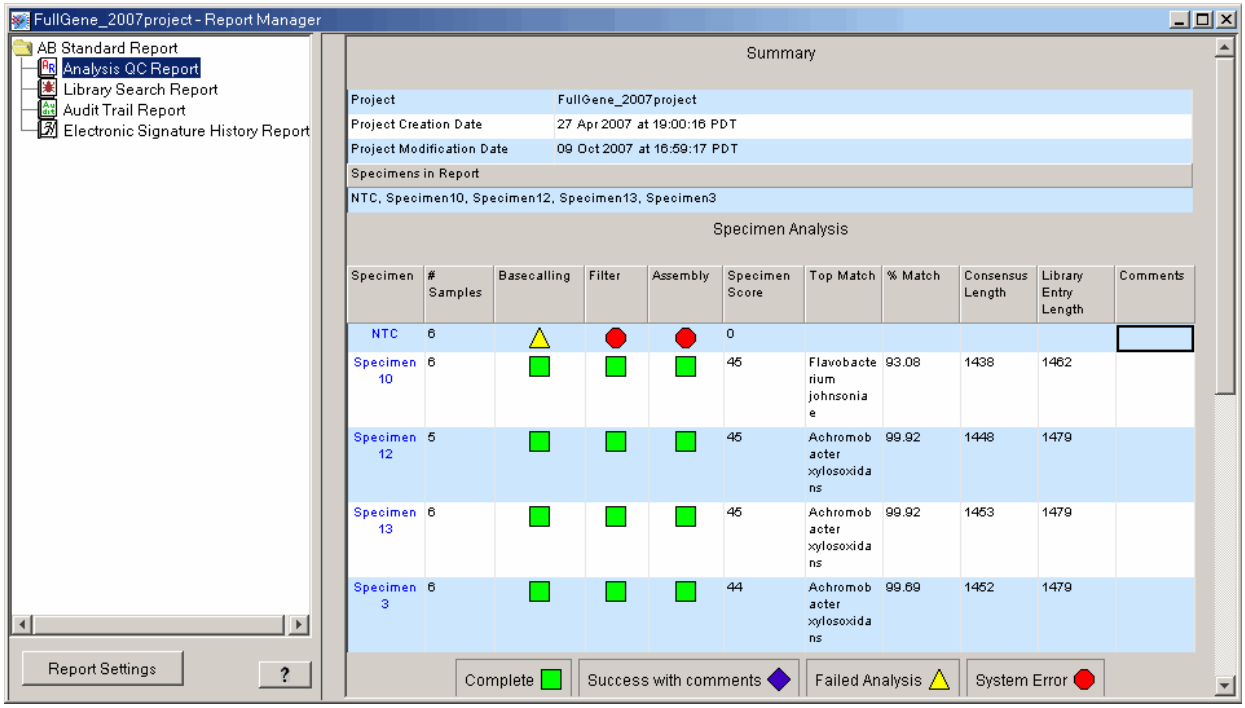


Figure 3 Example Analysis QC Report

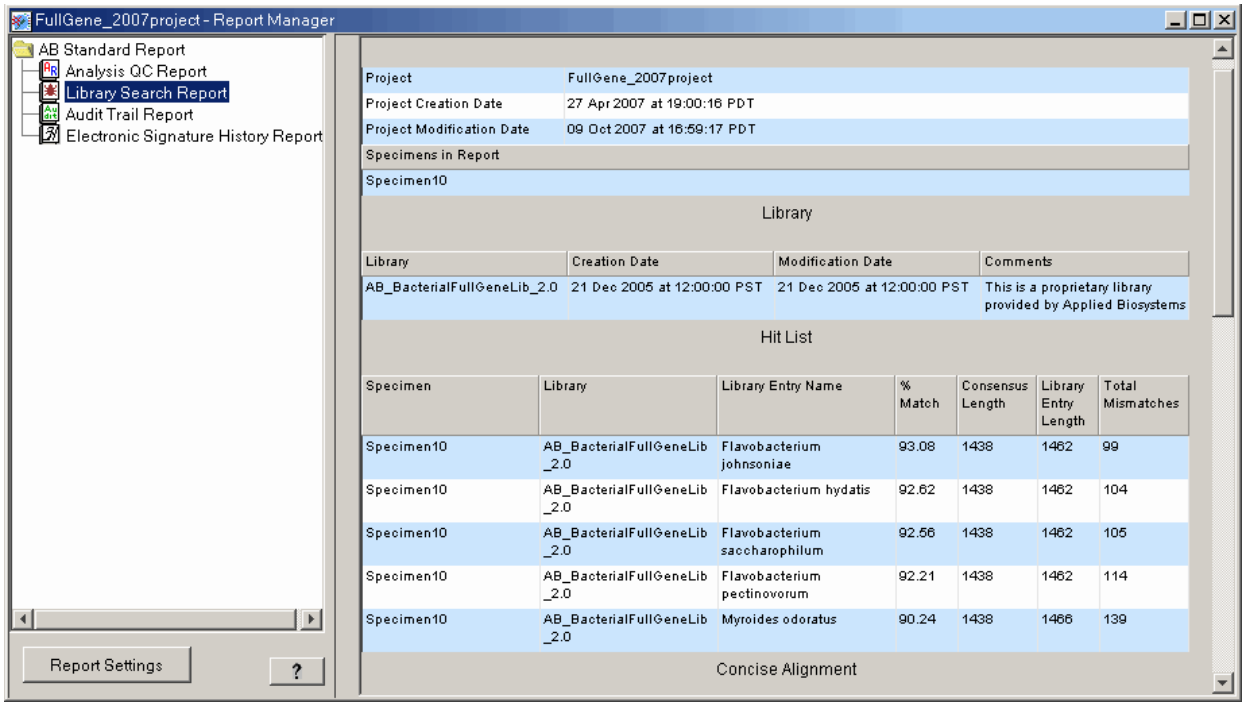


Figure 4 Example Library Search Report

About dye-labeled terminator chemistry

The MicroSEQ™ Full Gene 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.

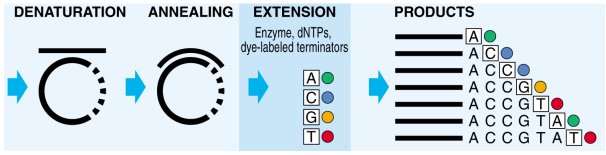


Figure 5 BigDye™ Terminator v1.1 chemistry

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 36.

E

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

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

Document	Publication number
MicroSEQ™ Full Gene 16S rDNA Identification Quick Reference	4393014
PrepMan™ Ultra Sample Preparation Reagent Protocol	4367554
VeritiPro™ Thermal Cycler User Guide	MAN0019157
DNA Sequencing by Capillary Electrophoresis Chemistry Guide	4305080
3500/3500xL Genetic Analyzer	
MicroSEQ™ ID Microbial Identification Software Version 3.0 Getting Started Guide	4465137
MicroSEQ™ ID Microbial Identification Software Version 3.0 Quick Reference Card	4465103
MicroSEQ™ ID Analysis Software v2.2 Getting Started Guide	4445126
MicroSEQ™ ID Analysis Software v2.2 Quick Reference Card	4445420
SeqStudio™ Genetic Analyzer	
MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0 User Guide	MAN0019554
MicroSEQ™ ID Software For SeqStudio™ Genetic Analyzer v1.0 Quick Reference	MAN0019555

Note: For additional documentation, see “Customer and technical support” on page 36.

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 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**.