applied biosystems
by life technologies™

MicroSEQ® ID Microbial Identification Software

Version 3.0

Getting Started Guide

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

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About This Guide

Purpose

The *Applied Biosystems MicroSEQ® ID Microbial Identification Software Version 3.0 Getting Started Guide* is an installation guide and tutorial. It provides:

- Installation instructions to help the Administrator set up the MicroSEQ® ID software.
- Brief, step-by-step tasks to help users quickly learn the MicroSEQ ID software. The tasks use tutorial data that is supplied on the software CD-ROM.

To get the most out of this *Getting Started Guide*, we recommend that you:

- Review the "Software basics" chapter; see page 7
- Determine whether you are using a System or Lite version of the MicroSEQ ID software; see page 8
- Determine which user group you belong to; see page 10
- Proceed with tutorial tasks; see page 45
- Advance to data analysis; see page 75

Version 3.0 features

With MicroSEQ® ID Microbial Identification Software Version 3.0, you can:

- Start and process a MicroSEQ ID Run directly with the Applied Biosystems 3500 Series Data Collection (DC) Software v1.1., without running AutoAnalysis Manager software.
- Perform an analysis and automatically receive (Auto-ID) or manually enter a specimen identification.
- View phylogenetic relationships between specimens by:
  - Calculating the genetic distance for selected sequence pairs in an unrooted phylogenetic tree
  - Zooming in/out on the horizontal branches in the phylogenetic tree diagram as needed
- Search entries in MicroSEQ ID software validated and custom libraries.
- Manage a series of sequence edits (multiple Undo).
- Export multiple projects at one time.
- Copy an entire sample or consensus sequence using the MicroSEQ ID software analysis toolbar and paste it into the BLAST® database search window.
- In the Raw view for sample data files, zoom in and out on the traces in the raw data profile.
- View, archive, and restore audit records for all users at the application level (Administrators only).

Other basic features remain unchanged in MicroSEQ ID software and have not changed in version 3.0; with MicroSEQ ID software you can:

- Display MicroSEQ ID validated libraries.
- Search and analyze specimens across projects.
- Perform an analysis by:
  - Aligning any combination of sequence types
  - Using an improved display of concise alignment
  - Generating a phylogenetic tree from sequences in FASTA format
- Customize reports.

MicroSEQ ID Microbial Identification Software Version 3.0 supports 3500 Series (3500/3500xL) and 3130 Series (3130/3130*xl*) Genetic Analyzers.

Additionally, MicroSEQ ID software reads the .ab1 files, and consequently supports data analysis, although not autoanalysis, from the following instruments:

- 310 Genetic Analyzer
- 3100 Series Genetic Analyzers
- 3730 Series Genetic Analyzers

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

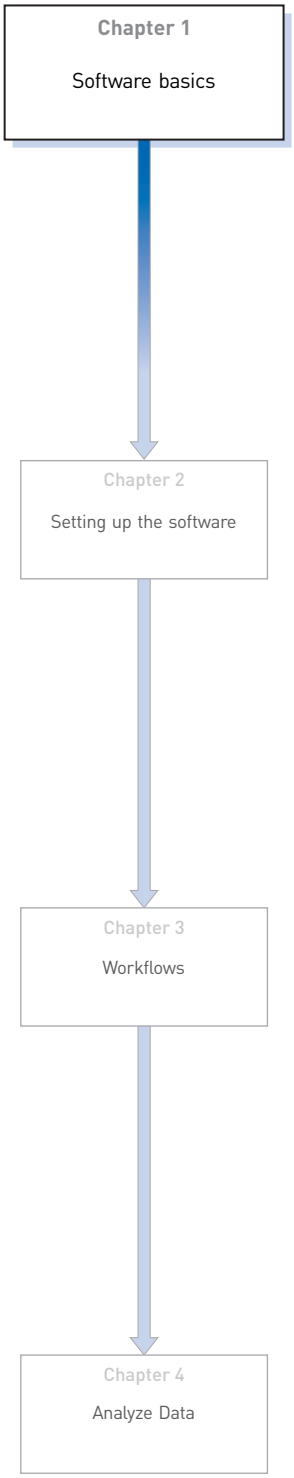
CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument.

1 Software basics



This chapter covers:

- What is the MicroSEQ® ID software? 8
- System vs. Lite. 8
- Compatibility matrix 9
- User groups and privileges 10
- User workflow. 11
- Tutorial data 12
- Software structure 13
- Software toolbars 17

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

What is the MicroSEQ® ID software?

MicroSEQ® ID Microbial Identification Software Version 3.0 is a tool for identification of bacteria and fungi. The software analyzes data generated using a MicroSEQ® ID sequencing kit and a Life Technologies capillary-based genetic analyzer.

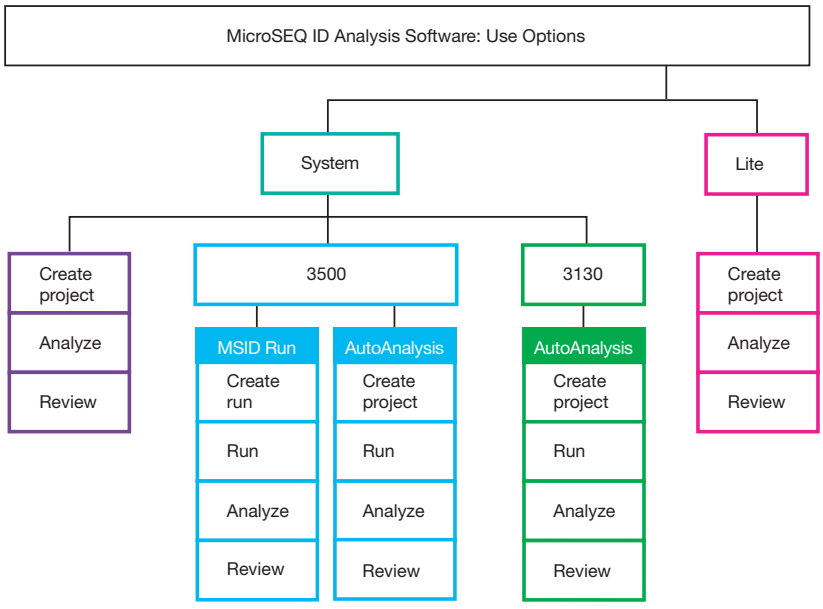
To identify unknowns, the MicroSEQ ID software:

- Compares the sequence of unknown isolate data to the sequences of known bacteria or fungi stored in a library.
- Generates a final identification list of organisms that are the closest matches to the unknown.
- Calculates the similarity between the known and unknown sequences in the list and reports that similarity in terms of percentage.
- Identifies the specimen based on interpretive guidelines (Auto-ID); final identification is based on parameters that are either automatically or manually set in the software.

System vs. Lite

In addition to the System version of MicroSEQ ID software, a Lite version is also available for use on a stand-alone PC (not connected to the sequence analyzer). Use the Lite version if you plan to perform simple analyses. Use the System version if you plan to run complete analyses on the 3500 Series (3500/3500xL) or 3130 Series (3130/3130*xl*) Genetic Analyzers. Refer to the workflow below to determine when to use the System version and when to use the Lite version.

Note: Verify minimum System or Lite requirements (see page 20) prior to running the software.



Note: The MicroSEQ ID software system workflow is optimized for 3130 and 3500 Series Genetic Analyzers using POP-6™ polymer and a 50-cm array. For instructions on using POP-7™ polymer, refer to the instrument user guide provided with your analyzer.

Compatibility matrix

Version 3.0 is compatible with the following versions of data collection and secondary analysis software:

MicroSEQ ID software installation type	MicroSEQ ID software version‡	Data Collection (DC) software version	Secondary analysis software version supported			
			Sequencing Analysis§	Variant Reporter™§	GeneMapper®#	SeqScape®#
Lite	New v3.0	N/A	N/A	N/A	N/A	N/A
System	New v3.0	3500 Series DC v1.1	v5.4	v1.1	v4.1	v2.7
	Upgrade v3.0					
	New v3.0	3130 Series DC v3.0	v5.3.1		v4.0	v2.6
	Upgrade v3.0					

‡ The same installer is used in all cases regardless of whether an upgrade or a new install is being performed.

§ Autoanalysis not supported; Autoanalysis Manager is not installed.

If MicroSEQ ID software is installed, autoanalysis is not supported; incompatibility with installed version of AutoAnalysis Manager. For MicroSEQ ID Software v3.0 to interact properly with AutoAnalysis Manager, do not install SeqScape software or GeneMapper software after MicroSEQ ID software is installed.

IMPORTANT! For MicroSEQ® ID software v3.0 to interact properly with AutoAnalysis Manager, do not install SeqScape® Software or GeneMapper® Software after MicroSEQ ID software is installed.

User groups and privileges

Three user groups are defined in the software: Administrator, Scientist, and Analyst. Each of these user groups has the privileges listed in the table below.

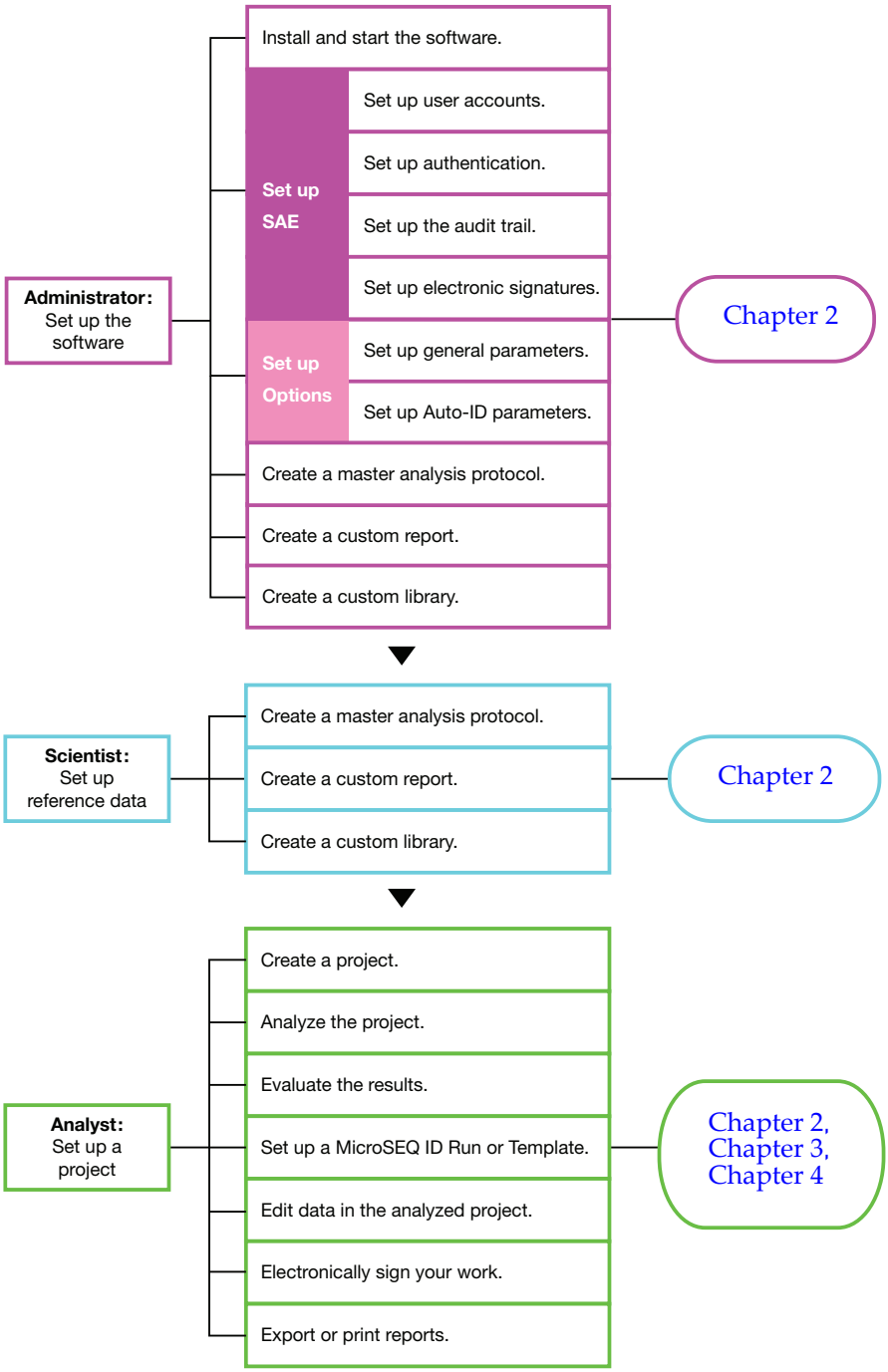
For a more detailed description of the allowed privileges for each user level, in the MicroSEQ ID software, see the User tab of the SAE Manager dialog box.

User group	Privileges
Administrator	All Scientist and Analyst privileges, *plus*: • Install and start the software • Set up Auto-ID parameters • Set up users • Set up authentication settings • Set up auditing trail • View, archive, and restore application level audit records • Set up electronic signatures
Scientist	All Analyst privileges, *plus*: • Set up general defaults • Create libraries • Create analysis protocols • Set up custom reports
Analyst	• Create MicroSEQ ID runs and run templates (Applied Biosystems 3500/3500xL users only) • Create a project • Analyze the project • Evaluate the results • Edit data in the analyzed project • Electronically sign your work • Export or print reports • View Auto-ID parameters (if Auto-ID enabled)

User workflow

The workflow below provides an overview of the tasks presented in this document.

IMPORTANT! Each task should be performed in the order given by the specified user group. The Administrator and Scientist must perform their tasks before the Analyst can set up a project.



Tutorial data

When you perform the tasks in this *Getting Started Guide*, you will need to use the tutorial data found at the installation drive locations described below.

Tutorial data referenced in this guide is provided for your use in the form of the Library files described below.

Folder name	File name and extension
Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\Bacterial500Samples\ **Bacterial500CustomLibrary**	Tutorial_Bacterial500_Lib_v2.0.fsta
Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\BacterialFullGeneSamples\ **FullGeneCustomLibrary**	Tutorial_FullGene_Lib_2.0.fsta
Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\FungalSamples\ **FungalCustomLibrary**	Tutorial_Fungal_Lib_2.0.fsta

Tutorial data referenced in this guide is provided for your use in the form of the Sample files described below.

Specimen	Folder name	File name and extension
MicroSEQ Bacterial 500 (*S. aureus*)	Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\ Bacterial500Samples\BacterialFullGene_Saureus	Specimen1_F.ab1
		Specimen1_R.ab1
MicroSEQ Full Gene (*B. cereus*)	Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\ BacterialFullGeneSamples\BacterialFullGene_Bcereus	Specimen1_1F_3500.ab1
		Specimen1_1R_3500.ab1
		Specimen1_2F_3500.ab1
		Specimen1_2R_3500.ab1
		Specimen1_3F_3500.ab1
		Specimen1_3R_3500.ab1
MicroSEQ Full Gene (*P. syringae*)	Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\ BacterialFullGeneSamples\FullGenePsyringae	E01_11.1F_2005-01-19.ab1
		E01_11.1R_2005-01-20.ab1
		E01_11.2F_2005-01-19.ab1
		E01_11.2R_2005-01-20.ab1
		E01_11.3F_2005-01-19.ab1
		E01_11.3R_2005-01-20.ab1
MicroSEQ Fungal (*C. globosum*)	Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\ FungalSamples\Cglobosum	MicroSeq042803_C03_FUN_7 R_05.ab1
		MicroSeq042803_G01_FUN_7 F_13.ab1
MicroSEQ Fungal (*S. cerevisae*)	Drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\ FungalSamples\Fungal_Scerevisae	Specimen1_F_3500xl.ab1
		Specimen1_R_3500xl.ab1

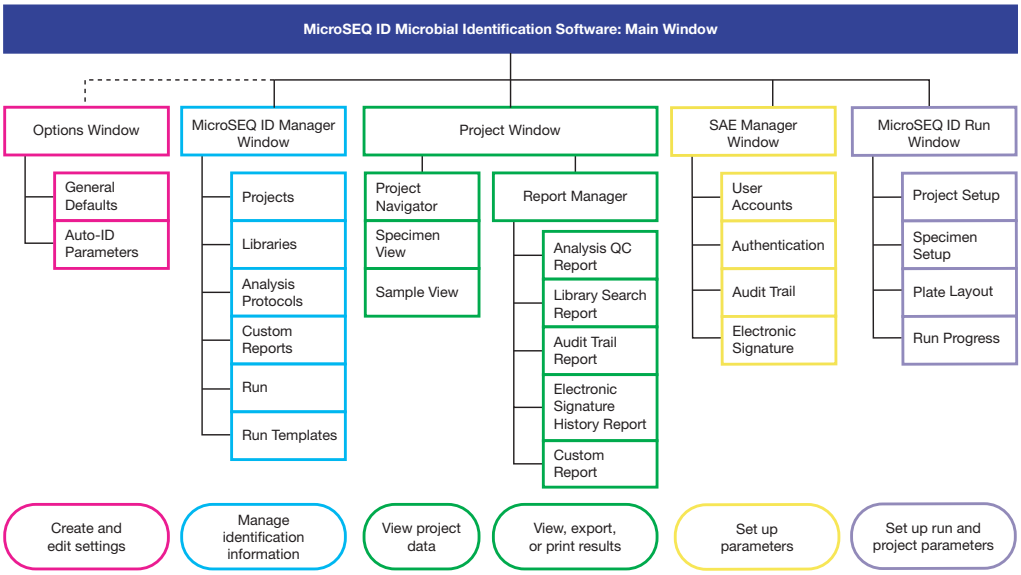
Note: The tutorial data was electrophoresed on an Applied Biosystems 3500xL Genetic Analyzer running POP-6™ polymer on a 50-cm array using the Fast MicroSEQ ID 50_POP6xl instrument protocol. The unknown isolates were sequenced using MicroSEQ ID chemistry.

Software structure

To perform the tasks in this chapter of the *Getting Started Guide*, you access the:

- Options Window; accessed from the Tools menu, see page 14
- MicroSEQ ID Manager Window; accessed from the MicroSEQ ID software main window, see page 15
- Project Window; accessed from the MicroSEQ ID software main window, see page 16

Note: Tasks surrounding the SAE Manager Window and the MicroSEQ ID Run Window will be covered in Chapters 2 and 3, respectively.

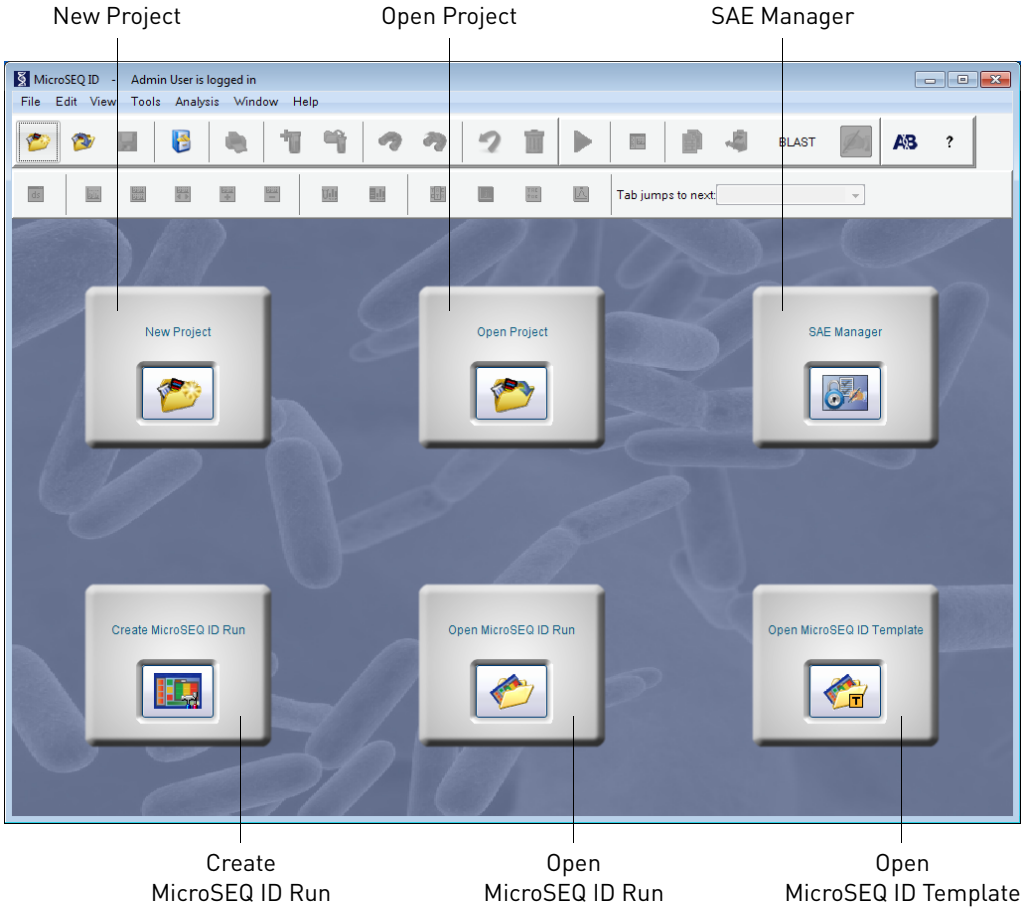


MicroSEQ ID software main window

Use the MicroSEQ ID software main window to:

- Create and open MicroSEQ ID runs and templates (Applied Biosystems 3500/3500xL users only).
- Create, open, and analyze projects.
- Display the Project View, Specimen View, or Sample View.
- Display the Report Manager to display library search results and other reports.
- Set default display settings that are saved with your user account.
- Change your password.
- Access the MicroSEQ ID Manager to manage MicroSEQ ID runs and templates, projects, libraries, analysis protocols, and custom reports.

- Access the Options dialog box via the Tools menu, to view or set Auto-ID and general system settings.
- Access the SAE Manager to manage security, audit, and electronic signature functions (Administrators only).

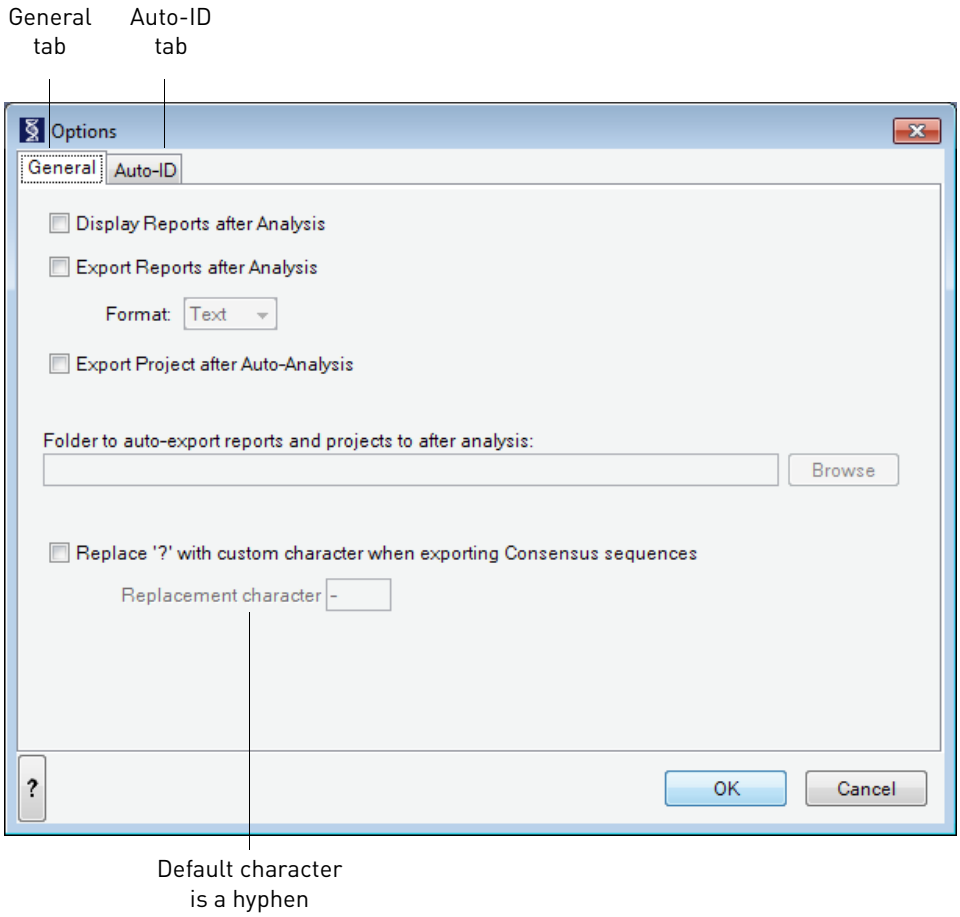


Options window

In the MicroSEQ ID software main window, select **Tools ▸ Options** to open the Options dialog box.

Use the Options dialog box to:

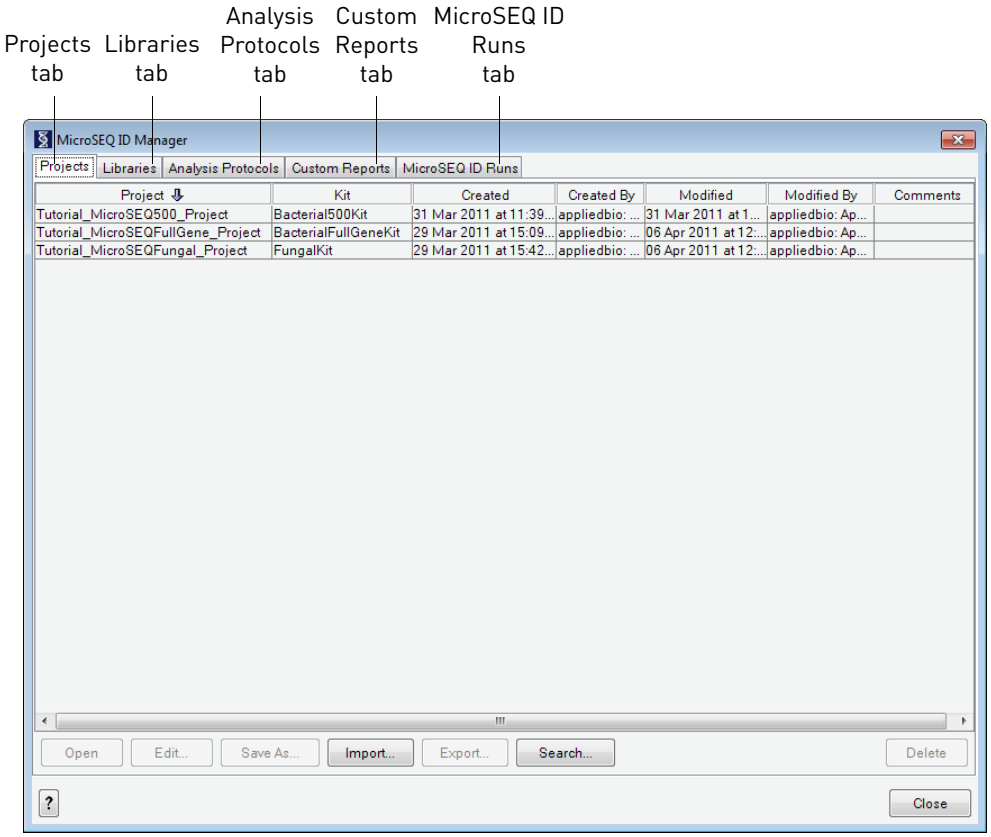
- Set the export and report defaults for the current user.
- Enable or disable Auto-ID for all projects for all projects and edit Auto-ID settings for MicroSEQ® ID kits.



MicroSEQ ID Manager window

Use the MicroSEQ ID Manager window to enter and manage the information necessary to perform analyses on your projects:

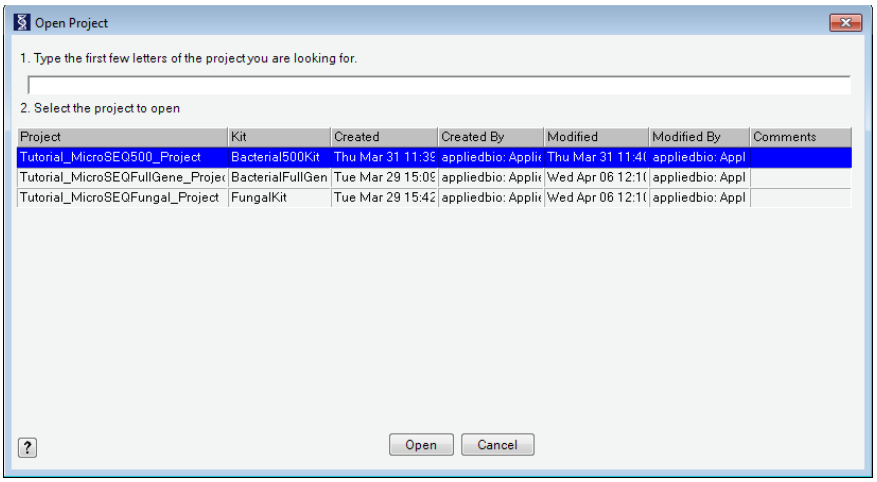
- Open, edit, copy, delete, search, import, and export projects
- Create, open, copy, and delete MicroSEQ ID Runs and MicroSEQ ID Run templates (Applied Biosystems 3500/3500xL users only)
- Create, edit, copy, delete, import, and export custom libraries, analysis protocols, custom reports, MicroSEQ ID Runs, and MicroSEQ ID Run templates



Project window

Use the Project window to:

- Import specimens/sample files
- Edit data using the Properties and Settings of the Project or Specimen
- View, export, or print project data (Project Navigator, Specimen View, or Sample View)
- Access the Analysis Protocol Viewer to view analysis information
- Access the Report Manager to view, export, or print project analysis results

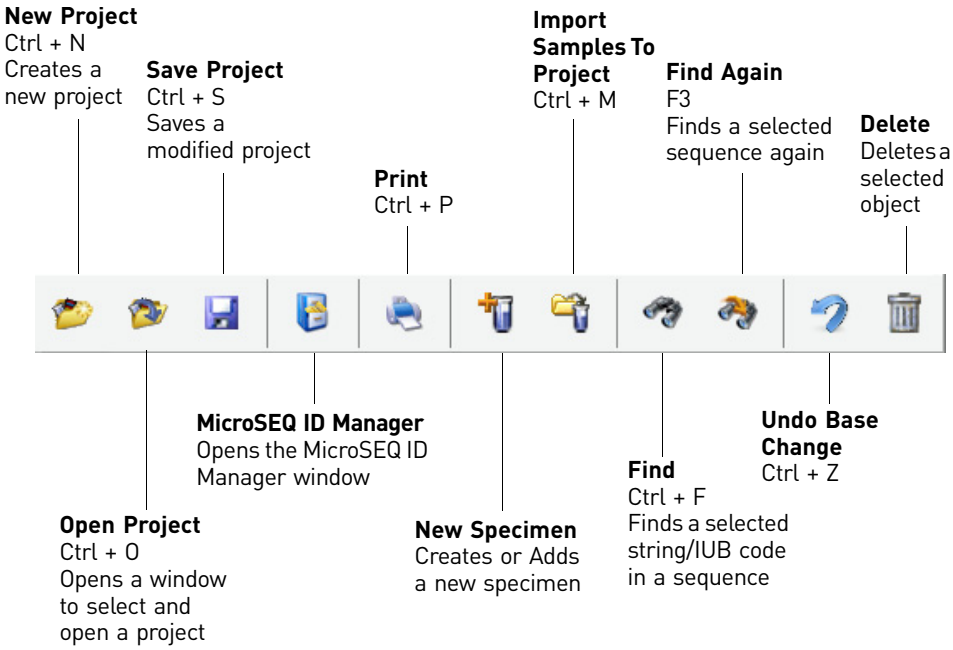


Software toolbars

The MicroSEQ ID software toolbars display buttons for software functions that you are likely to use often. Refer to the diagrams on pages 17 to 18 for the names, descriptions, and keyboard shortcuts for each button.

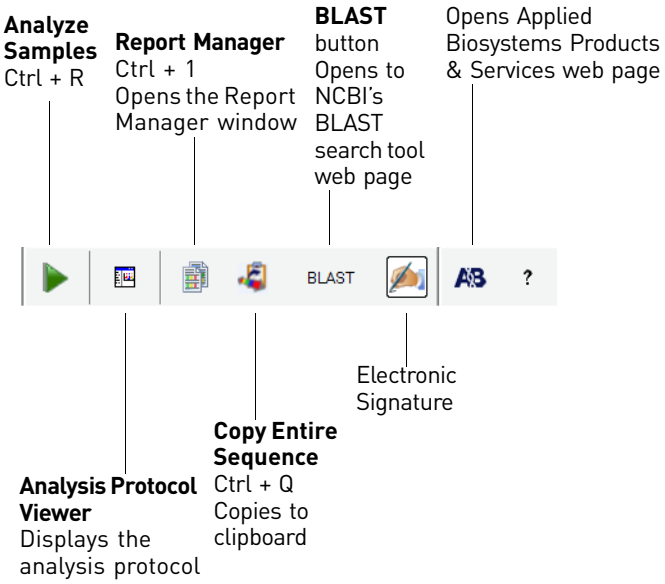
General toolbar

The General toolbar contains general processing tools for creating projects.



Analysis toolbar

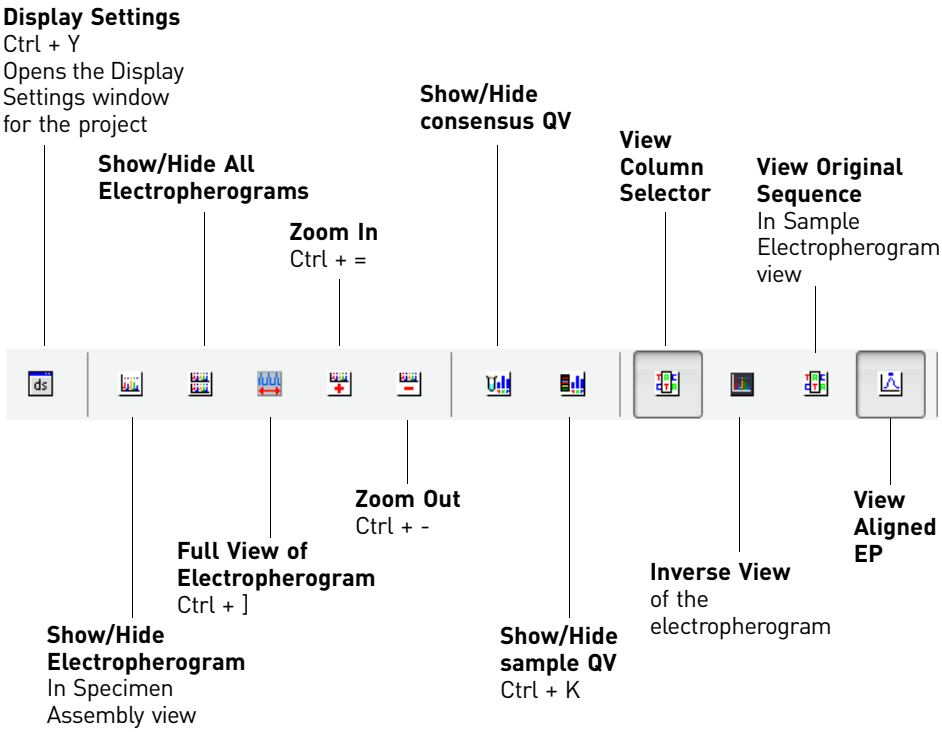
The Analysis toolbar contains analysis tools for the projects you create. A project or a run needs to be open in order to access the analysis tools.



The BLAST button links you to the National Center for Biotechnology Information (NCBI) website that maintains the Basic Local Alignment Search Tool (BLAST™; *Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997 Sep 1; 25(17):3389–402. Review*).

Display toolbar

The Display toolbar contains viewing options for the projects you create. A project or a run needs to be open in order to access the display tools.



Illegal characters

The following are not allowed in user names or file names:

- Spaces
- \ / : * ? " < > | &
- Diacritical characters

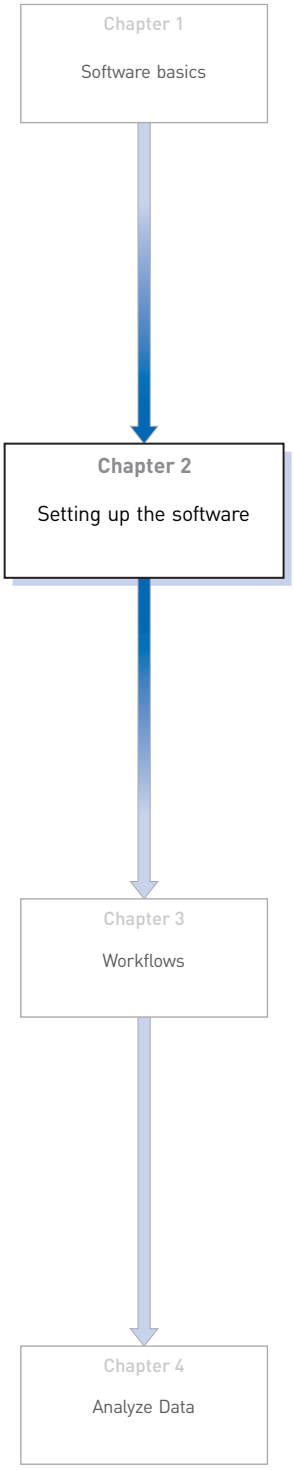
  Note: Diacritical characters are found in some non-English alphabets. An example of a French diacritical character is Ç (C cedilla).

Note: If you use a space or any of these characters in a user name or file name, an error message is displayed. You must remove the invalid character to continue.

Limit the use of the following:

- [ ] ‘ ~ ! @ # $ % ^ ( ) _ , . - + = ; { }

2 Setting up the software



IMPORTANT! You must have Administrator privileges to perform the tasks in this chapter.

An Administrator must install the MicroSEQ® ID software and use it for the first time to set up the software for the Analyst, Scientist, or other Administrator users.

This chapter covers:

- Before you begin 20
- Install MicroSEQ® ID Microbial Identification Software Version 3.0. 27
- Set up user accounts. 29
- Set up authentication 31
- Set up the audit trail. 31
- Set up electronic signatures. 33
- Set Auto-ID 37
- Set options 36
- Create a custom report. 39
- Create a custom library 43

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

Before you begin

Register the MicroSEQ® ID software

License and warranty

Before you begin, read your rights and responsibilities in your Software Warranty Information. During the installation process, you must accept the terms and conditions of the Software License Agreement before the software can be installed.

Register the MicroSEQ ID software

Registering your software enables Life Technologies to send you notification of software updates and any other information for MicroSEQ ID software owners.

IMPORTANT! Be sure to record your product registration code in the field below. If you need to reinstall the software you will need this registration code.

Registration Code:

3500 Series Data Collection software license

For information on managing the 3500 Series Data Collection software license, see "Manage the 3500 Series Data Collection software licenses" on page 106.

Hardware and software requirements

Minimum system requirements

Note: In general, the more memory, the larger the screen size, and the more processing power in your system, the better the software performance.

System component	Minimum requirements
CPU	1.86 GHz or faster with an Intel® Pentium® IV processor
CD-ROM drive	Any
Operating system	Microsoft® Windows® 7 OS *or* Microsoft® Windows Vista® OS with Service Pack 1 *or* Microsoft® Windows® XP OS with Service Pack 3
Regional and language options	English (United States) **Windows® 7** – Control Panel ▸ View by: Category ▸ Clock, Language, and Region ▸ Region and Language **Windows Vista®** – Control Panel ▸ Regional and Language Options ▸ Administrative ▸ Change system locale **Windows® XP** – Control Panel ▸ Regional and Language Options ▸ Region Options

System component	Minimum requirements
RAM	At least 2 GB
Disk space	4 GB Storage requirements depend primarily on the quantity of data to be generated and stored. It is common to store many MicroSEQ ID software project files on the analysis computer. Because MicroSEQ ID software stores data files in the area where the program is installed, you should install the software on a partition with enough space for the projects and their files.
Monitor	• 1024 × 768 resolution is recommended • 17-inch monitor or larger is recommended
Ethernet card	10/100 Network Interface Card (NIC) with RWU

Hard drive partitions

During install, files are installed in:

- *drive letter:*\AppliedBiosystems\MicroSeqID

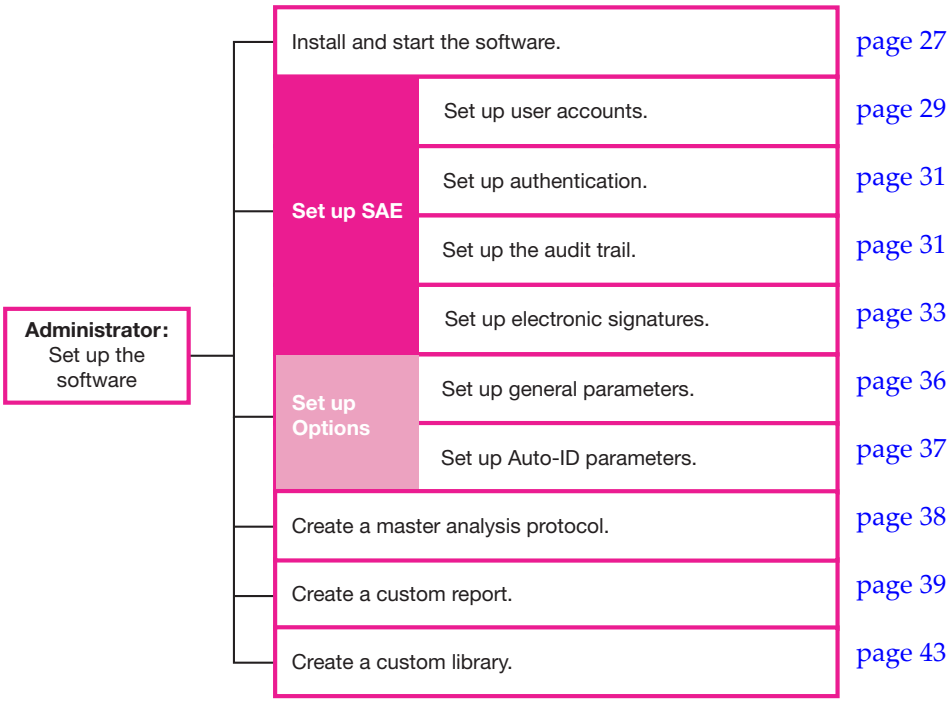
IMPORTANT! We strongly recommend that you install the MicroSEQ ID software on a drive other than the C drive, where the operating system is typically installed. Various memory-related issues have been observed when <10% of the C drive capacity is available. The MicroSEQ ID software and other applications may exhibit erratic behavior unless >200 MB (~10%) of the drive is free. This erratic behavior may also occur if either drive C or D is severely fragmented.

The drive letter is determined by the following conditions:

If the computer...	The installer selects drive...
Is not connected to a genetic analyzer	• D (default) *or* • C (if D drive is not available)
Has Data Collection software that is connected to either a 3500 Series or 3130 Series Genetic Analyzer	E

Workflow for software setup

IMPORTANT! Perform each task in the order given.



Install and start the software

Perform installations of the MicroSEQ® ID Microbial Identification Software Version 3.0 on the following platforms:

- Windows 7; see page 23
- Windows Vista; see page 24
- Windows XP; see page 25

Log on requirements

To install the MicroSEQ® ID Microbial Identification Software Version 3.0, you must:

- Log on to the local computer (not a network domain)
- Use a Windows® Administrator user account (unrestricted access)

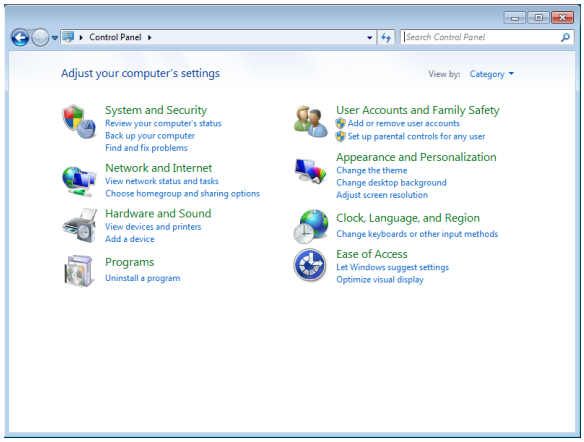
  Note: After the software is installed, you can run the MicroSEQ® ID software without using an Administrator account.

Verify user accounts

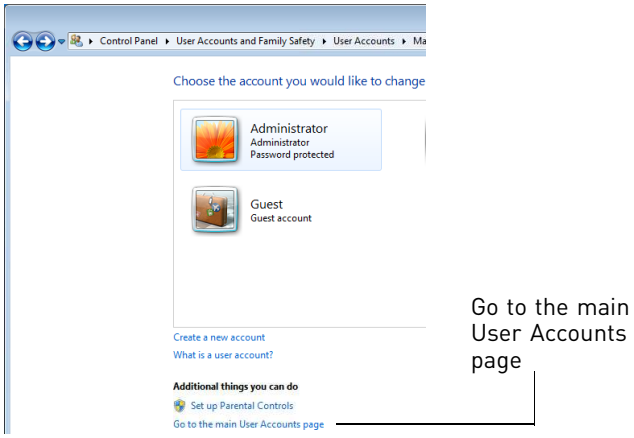
Windows 7

1. On the desktop, click  ▸ **Control Panel**.

2. Double-click **User Accounts and Family Safety ▸ Add or remove user accounts**.

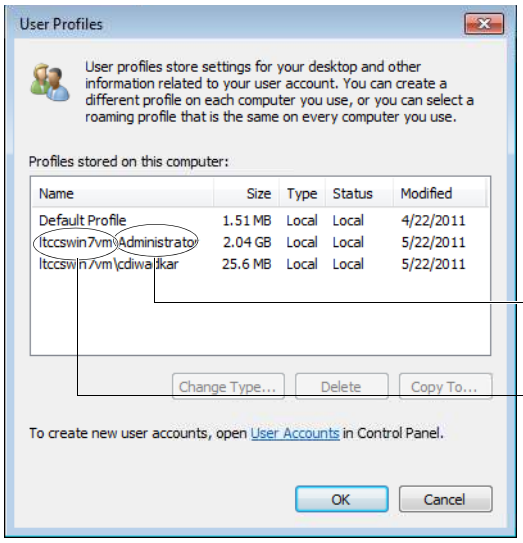


3. Double-click **Go to the main User Accounts page**.



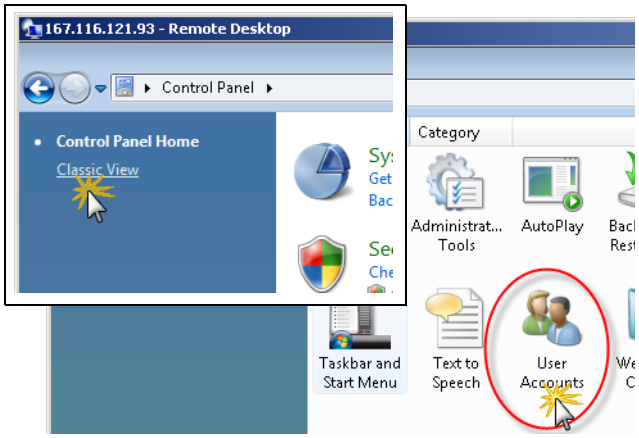
4. Double-click **Configure advanced user profile properties**.

5. In the Users Profiles dialog box, verify that the user account belongs to the Administrators group and the domain name is the same as the computer name.

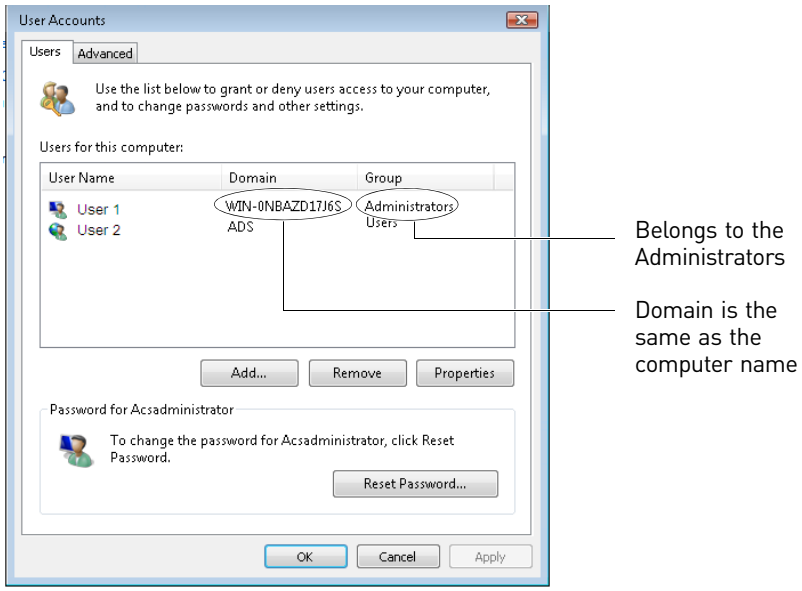


Windows Vista

1. On the desktop, click ▸ **Control Panel**.
2. Double-click **Classic View**.
3. Double-click **User Accounts**.

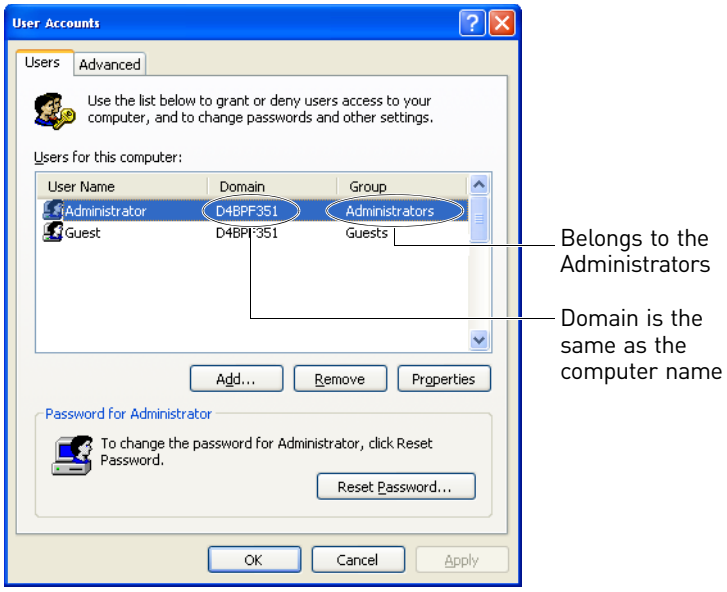


4. In the Users tab, verify that the user account belongs to the Administrators group and the domain name is the same as the computer name.



Windows XP

1. On the desktop, select **Start** ▸ **Control Panel** ▸ **User Accounts**.
2. In the Control Panel window, double-click the **Users** tab.
3. In the Users tab, verify that the user account belongs to the Administrators group and the domain name is the same as the computer name.



Prepare for installation

For System or Lite installations

1. Ensure that your system meets the minimum requirements (see page 20). Check that you have at least 4 GB of free disk space to accommodate the MicroSEQ ID software, and sufficient space for all projects and their sample files.
2. Log in to Windows® OS with local Administrator privileges.

IMPORTANT! For MicroSEQ® ID software v3.0 to interact properly with AutoAnalysis Manager, after MicroSEQ ID software is installed, install GeneMapper® Software and then SeqScape® Software.

For System installations only

If you plan to perform autoanalysis or MicroSEQ ID runs with the system, make sure to install and start the appropriate Data Collection software on a 3130 Series or 3500 Series Genetic Analyzer computer before installing the MicroSEQ ID software:

3130 Series Genetic Analyzer users:

1. Install the 3130 Series Data Collection Software Version 3.0; this is the data collection software version required for autoanalysis of data from Applied Biosystems 3130/3130*xl* (3130 Series) Genetic Analyzers.

   Note: For information on installing the 3130 Series Data Collection software, see the *Applied Biosystems 3130/3130xl Genetic Analyzer Getting Started Guide* (Part no. 4352715).

2. Start the Data Collection software.
3. In the Service Console window, deselect **Instrument Service** and **Viewer** by right-clicking over the icons and selecting **Stop**. The two icons turn red.

   Note: The 3130 Series Data Collection software must be open with the Instrument Service and Viewer stopped, before installation. Before stopping Instrument Service in the console, be sure that there are no runs in progress. If necessary, select **Pause** in the Data Collection Viewer and wait until the current run stops.

3500 Series Genetic Analyzer users:

1. Install the 3500 Series Data Collection Software Version 1.1; this is the data collection software version required for autoanalysis of data from Applied Biosystems 3500/3500xL (3500 Series) Genetic Analyzers.

   Note: During 3500 Series Data Collection software upgrade or uninstall, the system prompts you to back up the datastore (the directory that contains all user-created library components) to a directory other than the installation directory. For information on installing the 3500 Series Data Collection software, see the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

2. Start the Data Collection software.
3. Look in the Windows taskbar and make sure the 3500 Data Collection Server Monitor status icon is displayed: 

-  – All services are loaded.
-  – One or more services are stopped.
- (no icon) – The Server Monitor is not started.

Note: The 3500 Series Data Collection Server Monitor must be activated before installing MicroSEQ ID software. If the Server Monitor does not start automatically, select: **Start ▸ All Programs ▸ Applied Biosystems ▸ 3500 ▸ Server Monitor**. It takes 1 to 5 minutes for the Server Monitor to start up. If the status icon does not change to  , troubleshoot using the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

Install MicroSEQ® ID Microbial Identification Software Version 3.0

1. Insert the MicroSEQ® ID Microbial Identification Software Version 3.0 CD into the computer CD-ROM drive.

   Note: If the installer does not start automatically, navigate to the CD-ROM drive and double-click **setup.exe**.

2. Follow the instructions to install the software.

3. When installation reaches the InstallShield Wizard Complete window, click **Finish**.

After the software is installed, the Administrator must log into the software to set up for additional users (see page 29).

Upgrade to v3.0

Installation

You can upgrade from MicroSEQ® ID Microbial Identification Software v2.0 or higher.

Version 1.0 libraries

MicroSEQ® ID Microbial Identification Software does not support v1.0 AB Bacterial 500, Bacterial Full Gene, and Fungal libraries.

Start MicroSEQ ID software for the first time

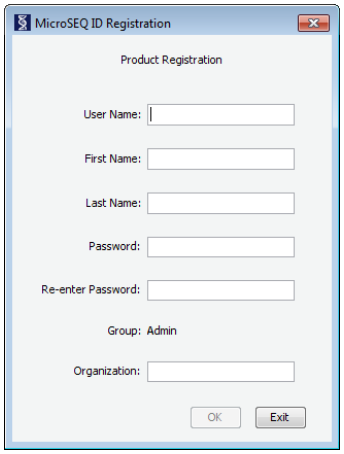
The MicroSEQ ID software requires a user login process. Logging in with a user name and password allows the software to track each user's interactions with each project.

To create a user name and password:

1. Double-click the MicroSEQ ID software desktop shortcut  .

2. In the MicroSEQ ID Registration dialog box, enter all information in the text fields. The User Name (login name) and Password must be 6 to 15 characters long. Enter a User Name that contains only alphanumeric characters. This field must contain characters that conform with the Microsoft® Windows® file system. Do not use illegal characters; for more information, see page 18.

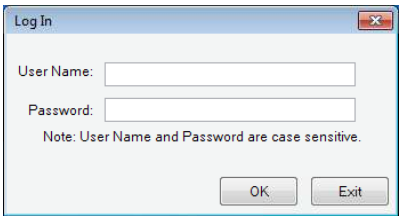
   Note: The first user created is automatically assigned Administrator privileges.



3. Click **OK**.

   While the program is loading, the splash screen appears. When the program is finished loading, the Log In dialog box opens.

4. Enter your User Name and Password again.



5. Click **OK** to open the MicroSEQ ID software main window.

Set up security, audit, and electronic signature

The SAE Manager feature of the MicroSEQ ID software allows set up of security management, audit, and electronic signature (SAE) functions by the Administrator (only).

Use the SAE Manager to create and/or edit:

- User accounts; see page 29
- Authentication; see page 31
- Audit trails; see page 31
- Electronic signatures; see page 33

Your Administrator can also export/import the SAE parameters created on your system; for information, see page 102.

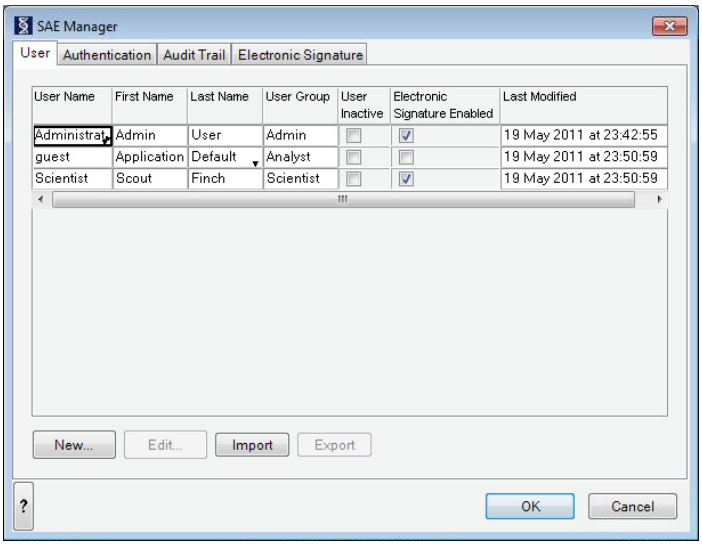
Set up user accounts

IMPORTANT! You must have Administrator privileges to perform the following tasks.

Because the MicroSEQ ID software tracks the projects and settings for each user, we recommend that you create a user account for each individual that uses the software on the computer. The User tab allows exporting of user names and access privileges for these users (see page 102).

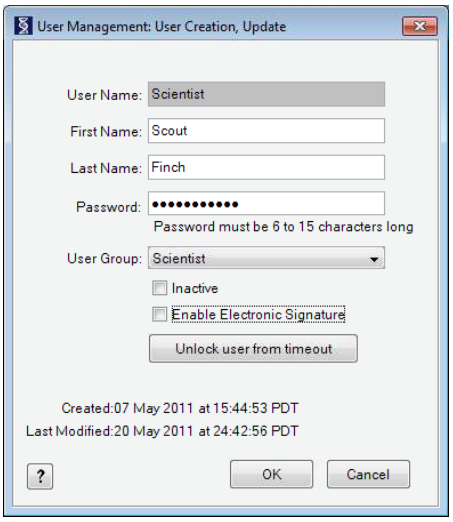
Create a user account

1. Log in as Administrator.
2. Click **SAE Manager** on the MicroSEQ ID software main window.
3. In the SAE Manager dialog box, select the **User** tab.



4. Click **New** to open the User Management dialog box.

5. In the User Management dialog box, enter the settings shown in the table below:



Parameter	Description
User Name	Enter user name (for example, Scientist, Analyst, Administrator). **Note:** The user name must be 6 to 15 characters long containing alphanumeric characters only. Do not use illegal characters; for more information, see page 18. **Note:** You can not enter the User Name of an existing user account.
First Name	Enter the user's first name.
Last Name	Enter the user's last name.
Password	Enter the user's password. **IMPORTANT!** If you plan to perform MicroSEQ ID runs with the system, you can set the MicroSEQ ID software to automatically log in to the 3500 Series Data Collection software by specifying the same user name and password in both Data Collection software and MicroSEQ ID software. You can change passwords in either application by selecting **Tools ▸ Change Password**.

6. Determine the user's privileges and then select a User Group from the User Group drop-down list.

7. (*Optional*) Select Enable Electronic Signature to give electronic signature privileges to the current user (see page 34).

8. Click **OK** to close the User Management dialog box. The new user's name appears in the list in the User tab.

9. Click **OK** to close the SAE Manager dialog box.

New users can log in after you exit the MicroSEQ ID software and restart the application.

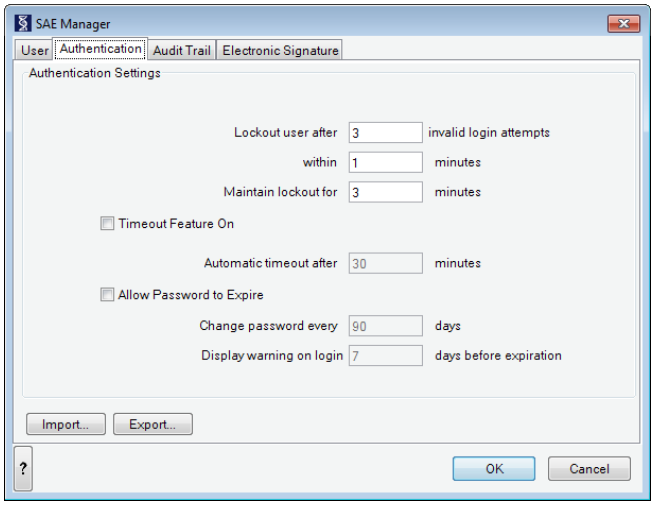
Set up authentication

IMPORTANT! You must have Administrator privileges to perform the following tasks.

The MicroSEQ ID Microbial Identification Software Version 3.0 is security, auditing, and electronic signature capable in order to ensure user integrity.

1. Click **SAE Manager** on the MicroSEQ ID software main window.
2. In the SAE Manager dialog box, select the **Authentication** tab.

   Note: The settings shown below are system defaults for authentication.
3. In the Authentication tab, enter the settings shown in the table below; leave other defaults as set:



Parameter	Description
Timeout Feature On	Select this check box to enable the timeout feature.
Allow Password to Expire	Select this check box to enable password expiration.

4. Click **OK**.

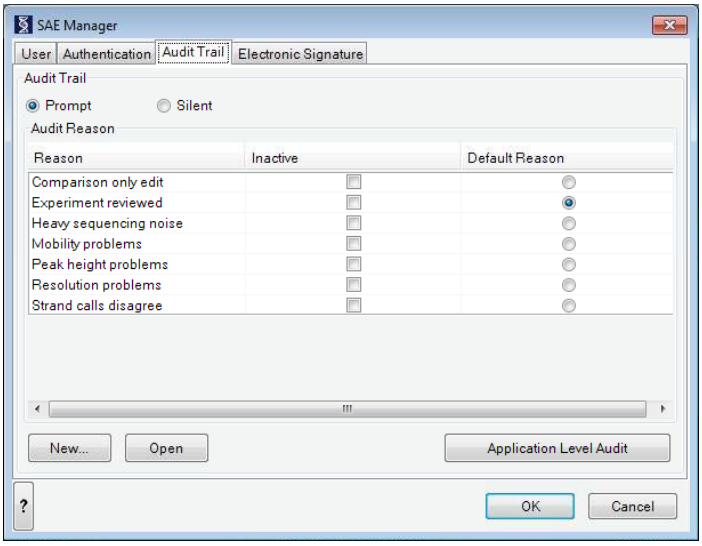
Set up the audit trail

IMPORTANT! You must have Administrator privileges to perform the following tasks.

The MicroSEQ ID software includes audit trail features to assist with tracking changes to projects and software settings. For more information, see page 91.

1. Click **SAE Manager** on the MicroSEQ ID software main window.
2. In the SAE Manager dialog box, select the **Audit Trail** tab.

3. In the Audit Trail tab, enter the settings shown in the table below; leave other defaults as set:



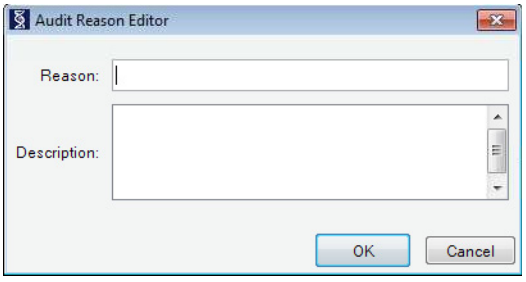
Parameter	Description
Audit Trail	Select **Prompt**. This selection enables display of the Audit Reason dialog box when a user makes an auditable change. The Audit Reason dialog box will prompt the user to select the reason for the change. **Note:** During a MicroSEQ ID Run, the MicroSEQ ID software will not prompt for an audit even though the Audit Trail state is set to Prompt.
Audit Reason	In the Default Reason column, select the first audit reason listed as the default reason.

4. Click **OK** to save the Audit Trail settings.

For information on managing the application level audit records, see page 103.

Create a new reason

1. In the Audit Trail tab of the SAE Manager dialog box, click **New** to add a new reason to the Audit Reason list. The Audit Reason Editor dialog box will appear.



2. Update the following settings:

Field	Entry
Reason	Type **Base change** as the reason for the change

Field	Entry
Description	Type **new library information** as a description of when to use the reason

Note: The description is not displayed in the Audit Reason Editor when a user is prompted to select an Audit Reason.

3. Click **OK** to save the new reason.
4. Click **OK** to save the Audit Trail settings.

Set up electronic signatures

IMPORTANT! You must have Administrator privileges to perform the following tasks.

The tutorial below walks you through the steps to set up electronic signature (eSig) features, which regulate the creation, deletion, and modification of data stored in the database.

Note: This feature is turned off by default.

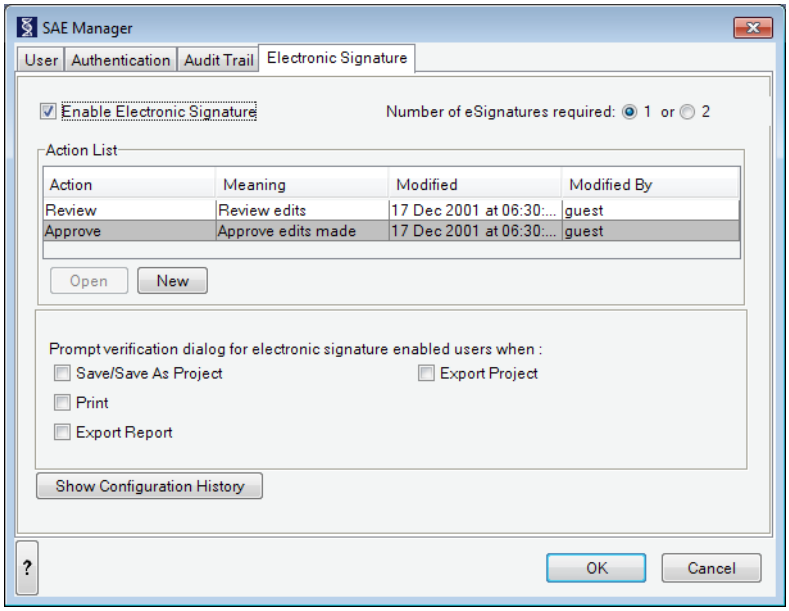
Use the electronic signature features in the SAE Manager to:

- "Enable electronic signature for the application" on page 33
- "Enable electronic signature for a user" on page 34; for each user that should have signature privileges
- "Create a new action for electronic signatures" on page 35

Enable electronic signature for the application

1. Click **SAE Manager** on the MicroSEQ ID software main window.
2. In the SAE Manager dialog box, select the **Electronic Signature** tab.

3. In the Electronic Signature tab, enter the settings shown in the table below:



Parameter	Description
Enable Electronic Signature	Select this check box to enable the Action List and the events in the Electronic Signatures Verification dialog box. **Note:** To create a new Action, see page 35.
Number of eSignatures required	Select radio button **1** for the number of eSignatures required for eSig events.

4. Select the eSig events that will prompt an Electronic Signature dialog box:
   - Save/Save As Project
   - Print
   - Export Report
   - Export Project

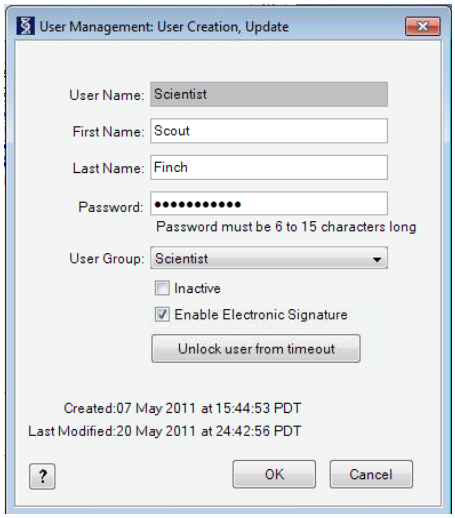
   Note: A verification dialog box will not appear for an eSig event that occurs during an autoanalysis run (3130/3130*xl* and 3500/3500xL Genetic Analyzer users) or a MicroSEQ ID Run (3500/3500xL Genetic Analyzer users only). Electronic signature is also disabled if you specify to export reports and projects after analysis is finished.

5. Click **OK** to save.

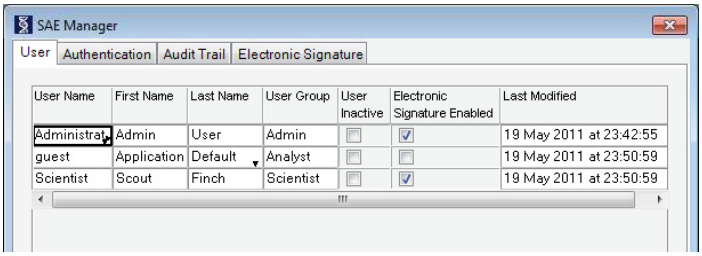
Enable electronic signature for a user

1. Click **SAE Manager** on the MicroSEQ ID software main window.
2. In the SAE Manager dialog box, select the **User** tab.
   a. Select a user, then double-click to open the User Management dialog box.

b. Select the **Enable Electronic Signature** check box, then click **OK.**



A check mark will now appear in the "Electronic Signature Enabled" column for the selected user.



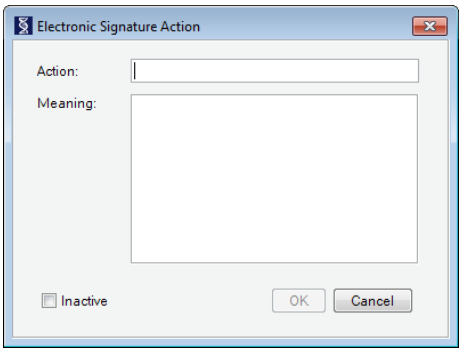
c. Repeat steps 2a and 2b for each user that requires electronic signature privileges.

3. Click **OK** to save.

Create a new action for electronic signatures

1. Click **SAE Manager** on the MicroSEQ ID software main window.
2. In the SAE Manager dialog box, select the **Electronic Signature** tab.
3. Select the **Enable Electronic Signature** check box to enable the Action List.
4. Click **New** to open the Electronic Signature Action dialog box.

5. In the Electronic Signature Action dialog box, enter the settings shown in the table below:



Parameter	Description
Action	Type **Project approved** as the name of the action to be performed.
Meaning	Type **GSG tutorial: project results approved**.
Inactive	Do not select this check box. **Note:** If you select the Inactive check box (bottom left corner), then the action becomes inactive, appearing grayed out in the Action List found in the Electronic Signature tab. In addition, the action no longer appears in the drop-down list of actions for an electronic signature.

6. Click **OK** to save.

Set options

Use the Options dialog box to:

- Set the export and report defaults; see page 37
- Enable or disable Auto-ID for all projects and edit Auto-ID settings for MicroSEQ® ID kits; see page 37

The Options parameters apply to all users.

Set up general defaults

IMPORTANT! You must have Scientist or Administrator privileges to perform this procedure.

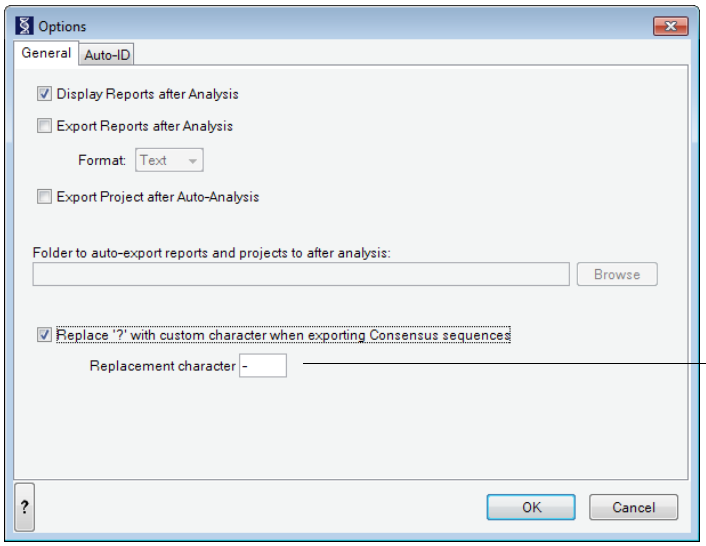
As a convenience, you can set up the general defaults to:

- Display and/or export reports after analysis
- *(System installations only)* Export projects after autoanalysis or after analysis of a MicroSEQ ID Run is finished
- Specify a directory to which reports and projects are automatically exported

  Note: If you do not specify a directory, the default directory opens to C:\.

1. In the MicroSEQ ID software main window, select **Tools ▸ Options** to open the Options dialog box.

2. In the **General** tab, select the **Display Reports after Analysis** check box.



3. Review and update the export settings in the General tab as needed. For more information on setting options parameters, refer to the *Help system*. Press **F1**, or select **Help ▸ Search** and type **options**, then select **Options parameters**.

4. Click **OK** to save your defaults.

Set Auto-ID

IMPORTANT! You must have Administrator privileges to perform this procedure. A Scientist or Analyst can view, but not edit, the Auto-ID tab.

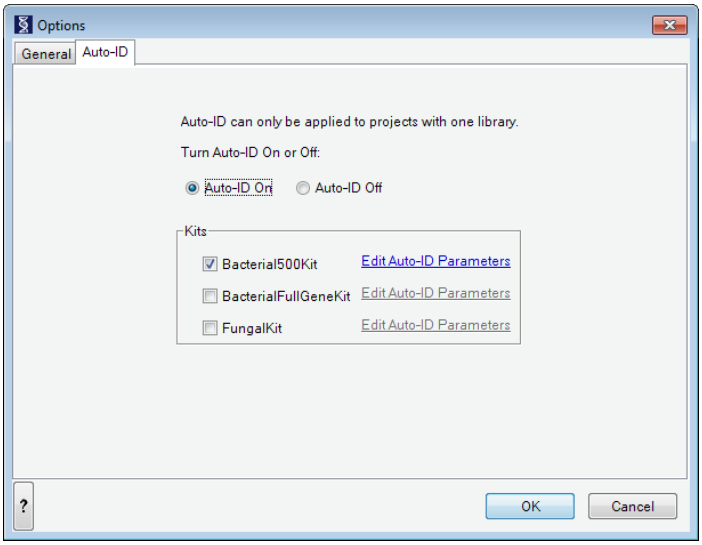
You can use Auto-ID to assign a genus or species identification to a specimen; during data analysis, the software evaluates each consensus sequence in a project against the specified Auto-ID requirements and thresholds, then assigns a specimen identification from a library. Auto-ID parameters can only be applied to projects using a single library.

1. Close any open projects.

   Note: The Auto-ID state cannot be changed while projects are open.

2. In the MicroSEQ ID software main window, select **Tools ▸ Options ▸ Auto-ID** tab.

3. In the Options dialog box, enter the settings shown in the table below:



Parameter	Description
Turn Auto-ID On or Off	Select **Auto-ID On** to enable specimen identification from a library during data analysis.
Kits	As needed, if Auto-ID On is selected: • *(Administrators only)* Select the **Bacterial500Kit** check box to apply Auto-ID settings during project analysis (optionally, click **Edit Auto-ID Parameters** and enter new Auto-ID specimen requirements and thresholds for the selected kit[s]), then click **OK** to save your changes. • *(Scientists or Analysts only)* Click **View Auto-ID Parameters** to view the current Auto-ID specimen requirements and thresholds for the selected kit(s), then click **OK**.

4. Click **OK**.

Create a master analysis protocol

IMPORTANT! You must have Scientist or Administrator privileges to perform this procedure.

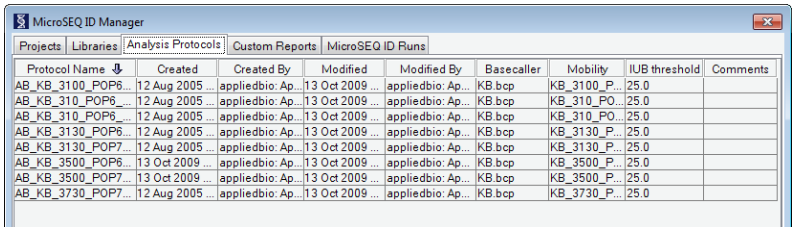
A master analysis protocol, also referred to as an analysis protocol, is a collection of settings that determine how the MicroSEQ ID software:

- Calls pure and mixed bases using Basecalling settings
- Generates calls according to the international standard IUB code for heterozygous positions using Mixed Bases settings
- Identifies poor-quality data to be excluded from the clear range using Clear Range settings
- Filters data used to generate the consensus sequence using Filter settings

This tutorial requires that you create a master analysis protocol and specify settings.

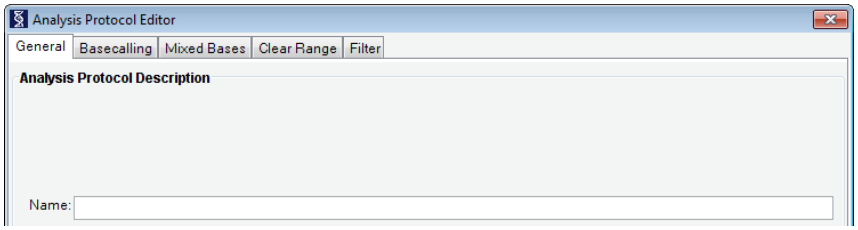
IMPORTANT! To create a master analysis protocol, you must access the Analysis Protocol Editor from the MicroSEQ ID Manager. This selection ensures that the protocol will be available for selection in other projects. The master analysis protocol *is not applied* to any projects that are reanalyzed.

1. Select **Tools** ▸ **MicroSEQ ID Manager** ▸ **Analysis Protocols** tab.



2. Click **New** to open the Analysis Protocol Editor.

3. In the Analysis Protocol Editor, enter the settings shown in the table below; leave other defaults as set.



Parameter	Description
Name	Type **GSG_AnalysisProtocol**; spaces are not allowed, use the underscore character. Do not use illegal characters; see page 18.
Comments	Type **GSG tutorial: create a master analysis protocol**.

4. Complete setup of your new master analysis protocol by setting parameters in the remaining tabs of the Analysis Protocol Editor; refer to the *Help system* as needed:
   - Basecalling
   - Mixed Bases
   - Clear Range
   - Filter

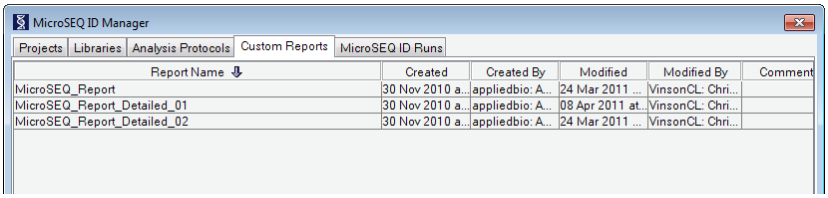
5. Click **OK** in the Analysis Protocol Editor.

Create a custom report

IMPORTANT! You must have Administrator or Scientist privileges.

In this tutorial we walk you through the steps for creating a new custom report based on an existing custom report; the new custom report will be referred to in Workflow 3 on page 69.

1. Select **Tools** ▸ **MicroSEQ ID Manager** ▸ **Custom Reports** tab.



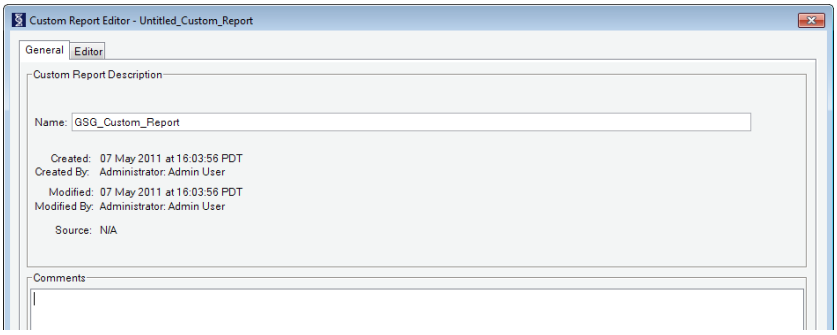
2. Select **MicroSEQ_Report** from the Report Name column, click **Save As** and enter **GSG_CustomReport** as the name for the report, then click **OK**.

   Note: You can also copy an existing custom report and save it for future use by selecting a custom report from the Report Name column, clicking **Save As**, entering a new name for the report, then clicking **OK**.

3. Click **Save As** and in the Custom Report Name field of the Save 'MicroSEQ_Report' As dialog box, replace **MicroSEQ_Report_Copy** with **GSG_CustomReport**.

4. Click **OK**.

5. Click **Edit** and in the General tab of the Custom Report Editor dialog box, enter the settings shown in the table below; leave other defaults as set.



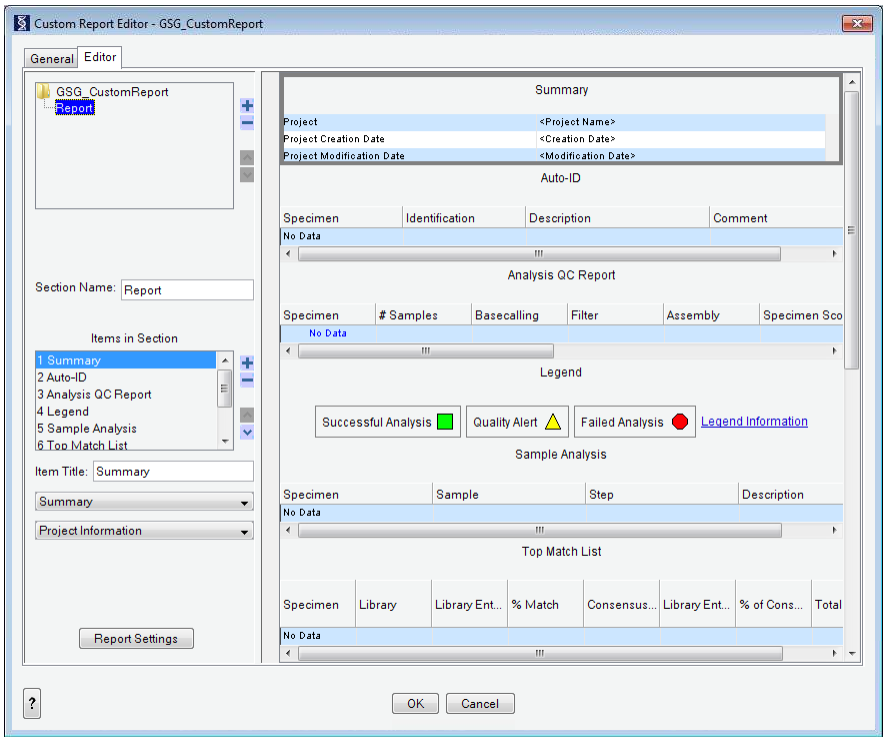
Parameter	Description
Name	Type **GSG_CustomReport**; spaces are not allowed, use the underscore character. Do not use illegal characters; see page 18.
Comments	Type **GSG tutorial: create a custom report**.

6. Click **OK**.

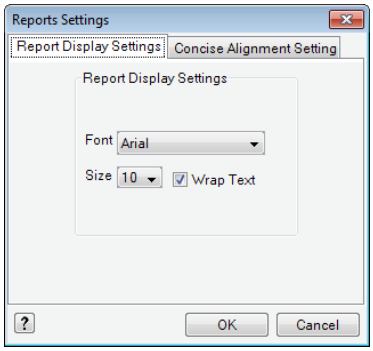
Set report display settings

1. Click the **Editor** tab of the Custom Report Editor window for GSG_CustomReport, the custom report created above.

2. (*Custom Reports only*) Click **Report Settings** in the lower portion of editor tab (at left) of the Custom Report Editor window.



3. In the **Report Display Settings** tab of the Report Settings dialog box:



   a. Change the font type to Helvetica; the default font type is Arial.
   b. Change the font size to 9; the default font size of 10.

4. In the **Concise Alignment Settings** tab of the Report Settings dialog box, select **2** as the number of top matches to display; the default is 1.

5. Click **OK** to close the Report Settings dialog box.

6. Click **OK** to close the Custom Report Editor.

Customize preinstalled reports

Use the Custom Report Editor to generate your own custom report based on one of the following three preinstalled custom reports provided with the MicroSEQ ID software:

Custom report name	Report content
MicroSEQ_Report	This is a single report with the following sections: • Auto-ID • Analysis QC Report • Sample Analysis • Top Match list • Phylogenetic Tree • Audit Trail Report • Electronic Signature Report
MicroSEQ_Report_Detailed_01	Items from the following standard reports: • **Analysis QC Report** – Project summary, specimen analysis table and legend, sample analysis table, assembly table • **Library Search Report** – Specimen summary, top match list, concise alignment table, phylogenetic tree, library table • **Audit Trail and Electronic Signature Report** – Project level audit trail report, electronic signature report • **Annotation Report** – Sample information
MicroSEQ_Report_Detailed_02	Items from the following standard reports: • **Analysis QC and Library Search Report** – Project summary, specimen analysis table and legend, sample analysis table, top match list, concise alignment table, phylogenetic tree, assembly table • **Audit Trail, Electronic Signature, and Annotation Report** – Project level audit trail report, electronic signature report, specimen information, run information

Create a custom library

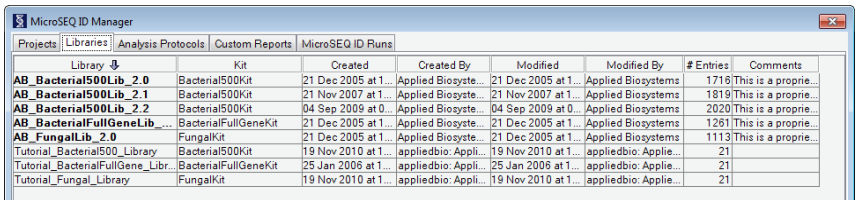
During project analysis, the MicroSEQ ID software compares the consensus sequence to library sequences in order to generate a list of the closest matches. You may use our custom or proprietary libraries or you can create your own custom library of consensus sequences. Additionally, you can create a custom library of consensus sequences based upon the specimen names of the libraries searched.

Below we walk you through the steps to create a custom library using tutorial data and a tutorial library specifically created for the tutorial procedures.

For information on the items below, refer to the *Help system*.

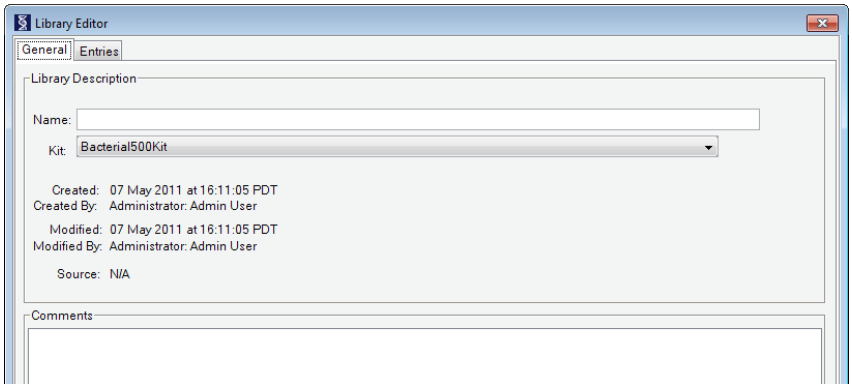
- Accessing a GenBank file
- Importing a FASTA file into a new library
- Importing FASTA file into a custom library
- Adding a consensus sequence to a custom library
- Searching library entries

1. Select **Tools** ▸ **MicroSEQ ID Manager** ▸ **Libraries** tab.



2. Click **New** to open the General tab of the Library Editor.

3. In the General tab, enter the settings shown in the table below; leave other defaults as set.



Parameter	Description
Name	Type **GSG_TutorialLibrary**; spaces are not allowed, use the underscore character. Do not use illegal characters; see page 18.
Kit	Select **Bacterial500Kit** from the drop-down list.
Comments	Type **GSG tutorial: create a custom library (Bacterial 500 Kit)**.

4. Select the **Entries** tab, then click **Import** to open the Import Library Sequence Entries browser.

5. Import the library file:

   a. In the Files of type field, make sure **FASTA format (*.fsta or *.fasta**, depending on your file extension) is selected.

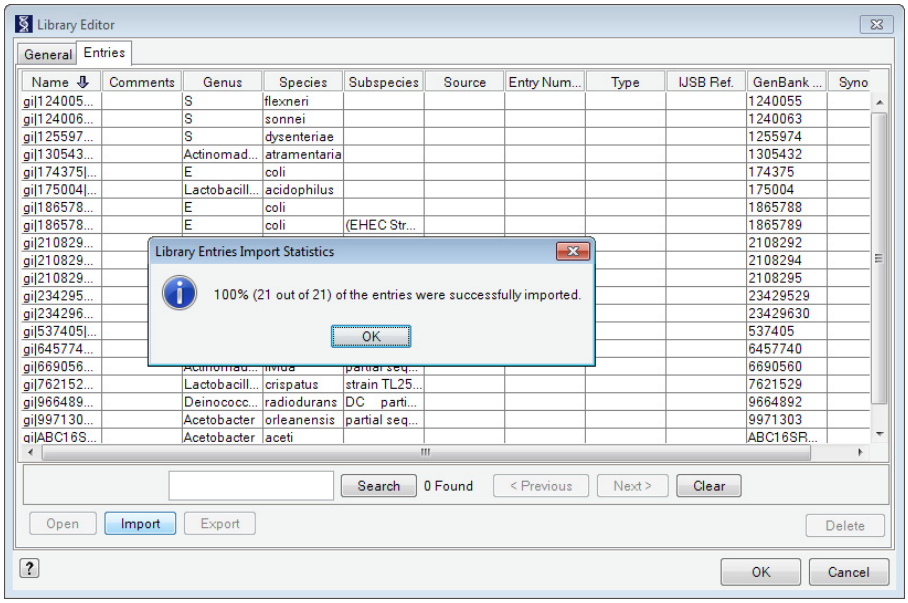
   b. Navigate to the installation drive location and select **Tutorial_Bacterial500_Lib.fsta**:

      - *drive letter:*\AppliedBiosystems\MicroSeqID\Tutorial Data\Bacterial500Samples\Bacterial500CustomLibrary

      Note: When creating your own custom libraries, you can import information from any location.

   c. Click **Import**.

6. Click **OK** to acknowledge the import.

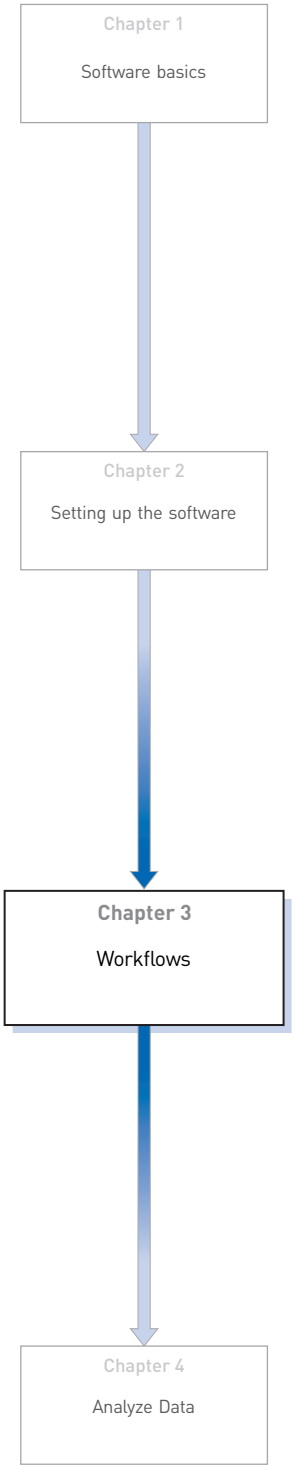


The sequences in the GSG_TutorialLibrary appear in the Entries tab of the Library Editor. Search entries using Search feature in lower portion of panel.

Note: After the library file is imported, you can double-click any sequence entry to open the Library Sequence Entry Viewer and view the entire sequence. The sequence is read-only, but you can edit all the other fields.

7. Click **OK** to save the new library and close the Library Editor. The new library appears in the Libraries tab of the MicroSEQ ID Manager window.

3 Workflows



This chapter covers:

- Before you begin 46
- Workflow 1: Using the MicroSEQ® ID Run Wizard with 3500 Series Genetic Analyzers 47
- Workflow 2: Using Autoanalysis with 3130 Series Genetic Analyzers. 57
- Workflow 3: Using the New Project Wizard to Create a Project 69

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

Before you begin

Overview

User privileges

IMPORTANT! You must have Scientist or Administrator privileges to perform the tasks in this chapter.

While both Administrators and Scientists can set up the analysis information (that is, set up libraries and analysis protocols) for the MicroSEQ® ID software, to get the most out of the workflows in this *Getting Started Guide*, a Scientist should perform the tasks in this chapter. Your selection of workflow(s) will depend on install type and your laboratory's standard operating procedures.

User accounts

Before you can log in to the software for the first time, an Administrator must create a user account for you (see page 29).

Note: Any user can create, run, and analyze projects after a user account has been set up.

Workflow overview

The workflows in this *Getting Started Guide* detail the steps necessary to create a project. In determining which of the three workflows presented here to pursue, verify which version of the MicroSEQ ID software you have installed, whether the System or the Lite version. Use this information to decide which workflow is most appropriate for your work environment.

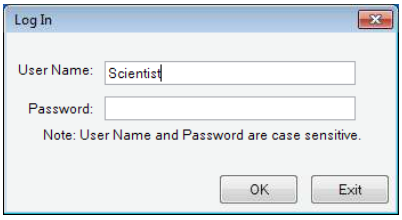
For additional information on terms used in this chapter, see "Glossary" on page 111.

System	Using the MicroSEQ ID Run Wizard with 3500 Series Genetic Analyzers	Workflow 1	page 47
	Using Autoanalysis with 3100 Series Genetic Analyzers	Workflow 2	page 57
Lite and System	Using the New Project Wizard to Create A Project	Workflow 3	page 69

Log in to MicroSEQ ID software

After your user account has been created, log into the software.

1. Start the MicroSEQ ID software by double-clicking the desktop shortcut  .
   The Log In dialog box opens, showing the last user's name.



2. Enter your user name and password, then click **OK**.

Workflow 1: Using the MicroSEQ® ID Run Wizard with 3500 Series Genetic Analyzers

This workflow covers:

- Integrating MicroSEQ ID and 3500 Series Data Collection Software 47
- MicroSEQ ID Run settings in the Data Collection software. 48
- Verify E-Signature settings in the Data Collection software (before each run) . . 48
- Verify electronic signature settings in the MicroSEQ ID software (before each run) 49
- Create a MicroSEQ ID Run 49
- Start a MicroSEQ ID Run. 54
- Monitor the MicroSEQ ID Run progress. 54
- Stop a MicroSEQ ID Run 56
- Using templates with the MicroSEQ ID Run Wizard 56

Integrating MicroSEQ ID and 3500 Series Data Collection Software

Overview

A MicroSEQ ID Run simplifies the set up, data collection, and analysis of sequencing data generated on Applied Biosystems 3500/3500xL (3500 Series) Genetic Analyzers using the MicroSEQ® ID software.

In this workflow, MicroSEQ ID software:

- Creates the run
- Uses the 3500 Series Data Collection software to start and process the MicroSEQ ID Run.
- Analyzes the data in the run

Requirements

Note: This workflow applies to installation of MicroSEQ® ID Microbial Identification Software Version 3.0 on 3500/3500xL Genetic Analyzers only (System installation); for more information, see "System vs. Lite" on page 8.

- The 3500 Series Data Collection Software v1.1 and MicroSEQ ID Microbial Identification Software Version 3.0 are installed on the same computer that is connected to a 3500 Series Genetic Analyzer.
- The 3500 Server Monitor is launched and the 3500 Services are loaded.
- "MicroSEQ" (case-insensitive) is included in names of the Data Collection instrument protocol(s) and assay(s) that will be used to analyze the data in the run.
- MicroSEQ ID software is launched, registered, and the necessary user names have been created.

Note: Alternatively, you can perform an autoanalysis run workflow using 3500 Series Data Collection software, MicroSEQ ID software, and AutoAnalysis Manager. For information on using autoanalysis with the 3500/3500xL Genetic Analyzer, refer to the *Help system*. Press **F1**, or select **Help ▸ Search** and type **autoanalysis overview**.

MicroSEQ ID Run settings in the Data Collection software

IMPORTANT! To ensure proper run processing, do not delete any MicroSEQ ID Run settings from the Data Collection software Library workflow that are currently assigned to pending MicroSEQ ID Runs (status = Not Run) or saved MicroSEQ ID Run templates.

In this tutorial you will review the factory-provided MicroSEQ ID Run settings found in the Library workflow of the Data Collection software.

Select **Library** in the 3500 Data Collection Software menu bar to access the Library workflow, then review the following factory-provided MicroSEQ ID Run settings:

Setting	Description
Instrument protocol	An instrument protocol defines the application type and instrument settings to use in the MicroSEQ ID Run. The factory-defined instrument protocols included in the 3500 Data Collection software are optimized for POP-6™ or POP-7™ polymer.
Basecalling (primary analysis) protocol	This protocol defines the analysis settings used for sequencing applications. The factory-defined primary analysis protocols included in the 3500 Data Collection software are optimized for MicroSEQ ID applications.
Results group	A results group (RG) is used to name, sort, and customize the folders in which sample data files are stored.
File name convention	An FNC specifies the naming convention for sample data files.
MicroSeqID (secondary analysis) protocol	This protocol identifies the location of the MicroSEQ ID software used to automatically analyze the MicroSEQ ID Run.
Assay	An assay specifies the instrument protocol, primary analysis protocol, and secondary analysis protocol to use in the MicroSEQ ID Run. **Note:** You can assign only one assay per plate. You can run up to two plates in a single MicroSEQ ID Run.

Note: If the factory-provided settings do not suit your needs, you can create new settings and save to the Library. For more information on this function, see the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

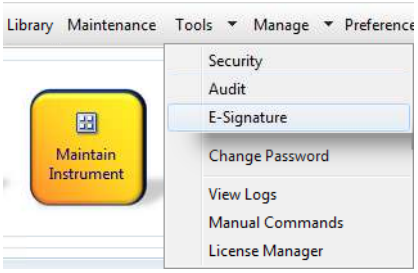
Verify E-Signature settings in the Data Collection software (before each run)

If the electronic signature function is enabled in the Data Collection software, you will need to verify that the following E-Signature settings are **not** selected (disabled):

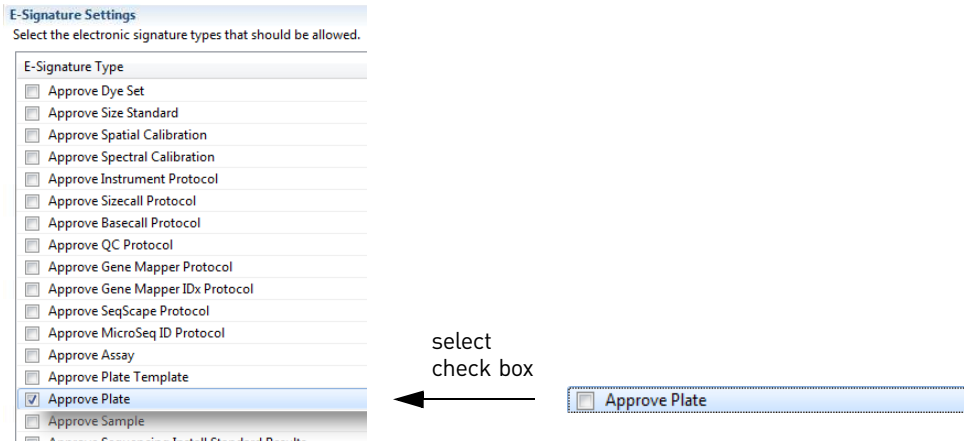
- **E-Signature Type** – Approve Plate
- **Associated E-Signature functions** – Save Plate, Start Run

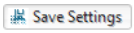
Note: If "Approve Plate" is disabled, then "Save Plate" and "Start Run" are also disabled. The E-Signature function is disabled by default. For more information on this function, see the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661).

1. In the Data Collection software menu bar, click **Tools ▸ E-Signature.**



2. If E-Signature is enabled, and the Approve Plate check box is selected (enabled), click on the check box to disable:



3. In the E-signature menu bar, click **Save Settings** 
.

Verify E-Signature settings in the MicroSEQ ID software

If the E-Signature function is enabled in the MicroSEQ ID software, verify that the following E-Signature events are not selected (disabled) by your Administrator:

- Save Project
- Export Report and Export Project (Optional, only if you specify to export reports and projects after analysis of a MicroSEQ ID Run is finished; via **Tools ▸ Options**, see page 90.)

Note: The MicroSEQ ID software does not prompt for audit during a MicroSEQ ID Run, even if the Audit Trail is set to prompt.

Create a MicroSEQ ID Run

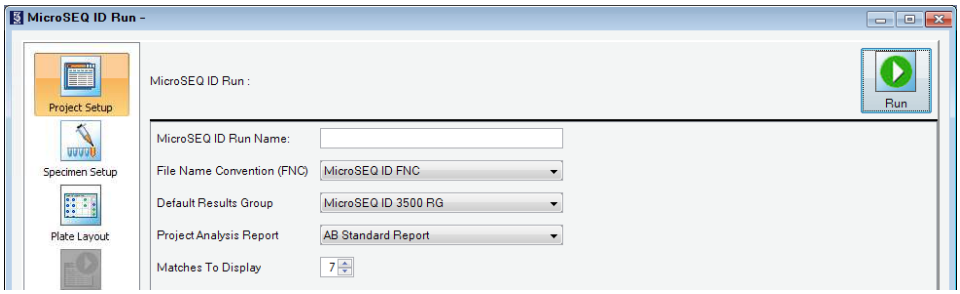
1. On the MicroSEQ ID software main window, click **Create MicroSEQ ID Run** to open the MicroSEQ ID Run Wizard.

2. If prompted, log in to the 3500 Series Data Collection software.

IMPORTANT! To automatically log in to the 3500 Series Data Collection software, set up the same User Name and Password in both 3500 Series Data Collection software and MicroSEQ ID software, then use this information to log in to MicroSEQ ID software. Users can change passwords in either application by selecting **Tools ▸ Change Password**.

Enter settings in the Project Setup tab

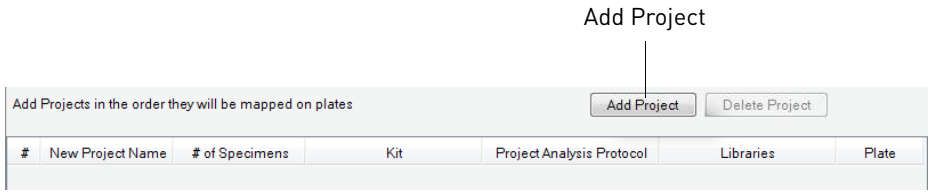
1. In the upper pane of the Project Setup tab (top left), enter the settings shown in the table below:



Setting	Description
MicroSEQ ID Run Name	Type **GSG_Run** as the name for the MicroSEQ ID Run. Do not use illegal characters; see page 18.
File Name Convention	Select **MicroSEQ ID FNC** as the naming convention to use for sample data files. **Note:** Only those FNCs with "microseq" (case-insensitive) in the FNC name are available for selection. The FNC needs to have been previously set up in the Data Collection Software in order to be available for selection; see page 48.
Default Results Group	Select **MicroSEQ ID 3500 RG** as the results group (RG) to use in order to name, sort, and customize the folders in which sample data files are stored. **Note:** Only those result groups with "microseq" (case-insensitive) in the FNC name are available for selection. The results group needs to have been previously set up in the Data Collection Software in order to be available for selection; see page 48.
Project Analysis Report	Select **AB Standard Report** as the report type to use to display your data.
Matches to Display	Select **7** as the number of library matches to display in the Project View library search results. **Note:** If Auto-ID is on (enabled) for the kit in the project, the minimum number of matches must be set to 7 for Auto-ID to occur. Administrators can change the Auto-ID state for all projects by selecting **Tools ▸ Options**.
Comments	Type **GSG tutorial: creating a MicroSEQ ID Run**.

2. Click **Save**.

3. In the lower pane of the Project Setup tab, click **Add Project** and enter the settings shown in the table below:

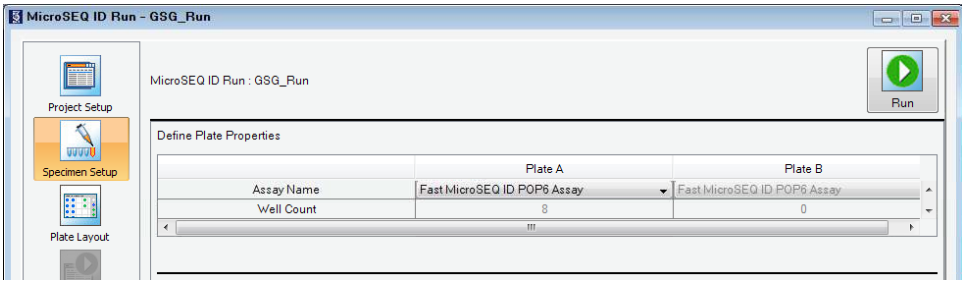


Setting	Description
New Project Name	Type **GSG_Project** as the name of the new MicroSEQ ID project in which to store the run. Do not use illegal characters; see page 18.
# of Specimens	Enter **4** as the number of specimens to add to the project.
Kit	Select **Bacterial500Kit** as the MicroSEQ ID kit to use in order to generate the sample data in the project.
Project Analysis Protocol	Select **GSG_AnalysisProtocol**, set up earlier (see page 38) as the master analysis protocol for use in project analysis.
Libraries	Click … to open the Library Selection dialog box, select the **Tutorial_Bacterial500_Library** as the library to use for project analysis, then click **OK**. **Note:** You can select one or more libraries to use for the analysis of a MicroSEQ ID project, but If the Auto-ID state is On for the kit in the project, you must select only one library for Auto-ID to occur. Only libraries that are associated with the selected Kit type are available. Library association is set when a library is created (see page 43).
Plate	Select **A** as the autosampler plate position to assign to the project.

4. Click **Next** to continue setting up the MicroSEQ ID Run.

Enter settings in the Specimen Setup tab

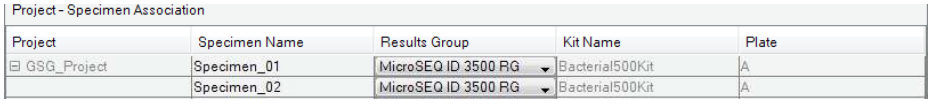
1. In the upper pane of the Specimen Setup tab, enter the **Define Plate Properties** settings shown in the table below:



Setting	Description
Plate Name	Type **GSG_Plate** as the name for the plate assigned to the project (Plate A).

Setting	Description
Assay Name	Select **MicroSEQ ID POP6 Assay** as the assay (3500xL users: select **MicroSEQ ID xl POP6 Assay**) to use for data collection and analysis. **Note:** The assays must exist in the Data Collection Software in order to be available for selection.
Well Count	This value cannot be edited. The software automatically counts the number of wells used in each plate based on the number of specimens and the kit selections you specified in the Project Setup tab. You can review the well assignments in the Plate Layout tab; see page 52.

2. In the lower pane of the Specimen Setup tab, enter the **Project - Specimen Association** settings shown in the table below:



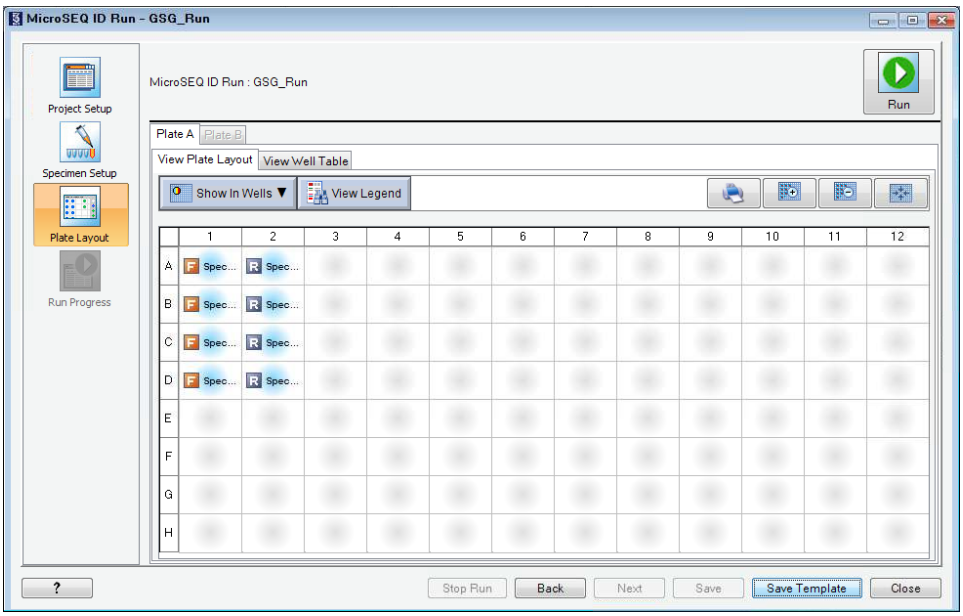
Setting	Description
Project	Review the default. Displays the project you selected on the Project Setup tab: GSG_Project (see page 50). This value cannot be edited. You can click ⊟ to collapse or ⊞ to expand the project-specimen association lists.
Specimen Name	Review the default specimen names; these can be edited as needed.
Results Group	Review the default. Displays the results group you selected on the Project Setup tab: MicroSEQ ID 3500 RG. If you do not want to use the default results group for a specimen, you can select a new MicroSEQ ID results group from the list. For the purposes of this tutorial, use the default selection.
Kit Name	Review the default. Displays the kit you selected on the Project Setup tab. This value cannot be edited.
Plate	Review the default. Displays the plate you selected on the Project Setup tab. This value cannot be edited.

3. Click **Next** to continue setting up the MicroSEQ ID Run.

View and edit the display in the Plate Layout tab

In the View Plate Layout view of the Plate Layout tab, you will view and edit the plate layout for the autosampler plate position you assigned to each project in the Project Setup tab (see page 50). By default, the Plate Layout tab shows the View Plate Layout view for Plate A.

The plate layout displays information about each well in the reaction plate in an illustration. Based on the kit(s) selected on the Project Setup tab, the software automatically assigns the samples (forward and reverse) for each specimen to adjacent wells in the plate layout, with multiple samples going down the columns.



To change the arrangement of wells in the plate:

- **Swap** – Ctrl + Click to select two wells A1 and D1, then right-click and select **Swap** to exchange positions of the two samples. The sample positions are exchanged.
- (*Optional*) **Click-Drag** – Click-drag a sample to an empty well location to move a sample. If you click-drag to a well that contains a sample, the sample is not moved. You can also **Ctrl + Click** to select multiple wells, then click-drag multiple samples to corresponding empty locations. You must click-drag at the left of or at the top of the block of the selected wells.

Note: If you add specimens to a project, the software replaces any custom well assignments in the plate layout with default well assignments. If you remove specimens, the software clears the corresponding well information, but does not move the remaining well assignments.

Finish the MicroSEQ ID Run Wizard

After completing the information in the MicroSEQ ID Run Wizard, click **Save** to save any changes to the MicroSEQ ID Run without running it (you can start the run at a later time, see page 54).

Note: You can also save the MicroSEQ ID Run as a run template, then create a new run using the template settings; see page 56.

Print the plate layout

1. Click  (**Print**). The plate layout will print exactly as it is displayed in the on-screen view.
2. Click **Close** to exit the MicroSEQ ID Run Wizard.

Note: You can only print the plate layout from a saved run.

Start a MicroSEQ ID Run

From the MicroSEQ ID Run Wizard, you can start a MicroSEQ ID Run on the 3500 Series Data Collection Software version 1.1.

1. Make sure your Administrator has properly configured the electronic signature settings in Data Collection (see page 48) and MicroSEQ ID software (see page 49).

   Note: The MicroSEQ ID software does not prompt for audit during a MicroSEQ ID Run, even if the Audit Trail state is set to Prompt. Additionally, a verification dialog box will not appear for an eSig event that occurs during a MicroSEQ ID Run, even if Electronic Signature is enabled.

2. Click **Open MicroSEQ ID Run** on the MicroSEQ ID software main window and select **GSG_Run**.

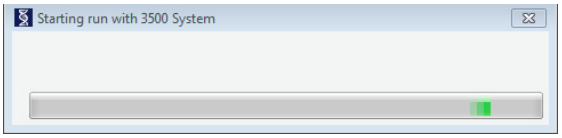
3. Prepare the plate(s) and load in the instrument.

4. In the Load Plates for Run screen of the Data Collection software, review the consumables information and the calibration information and ensure the status is acceptable for a run.

   IMPORTANT! It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.

5. In any tab of the MicroSEQ ID Run Wizard, click 

(**Run**). After all pre-run validation checks are successfully completed, the run begins and the run progress bar is automatically displayed.

   Note: It may take several seconds for the software to process the pre-run validation checks (as indicated by the progress bar). Do not close the MicroSEQ ID Run Wizard or stop the run during this time; the MicroSEQ ID Run Wizard and the MicroSEQ ID application will not close if there is a run going on.

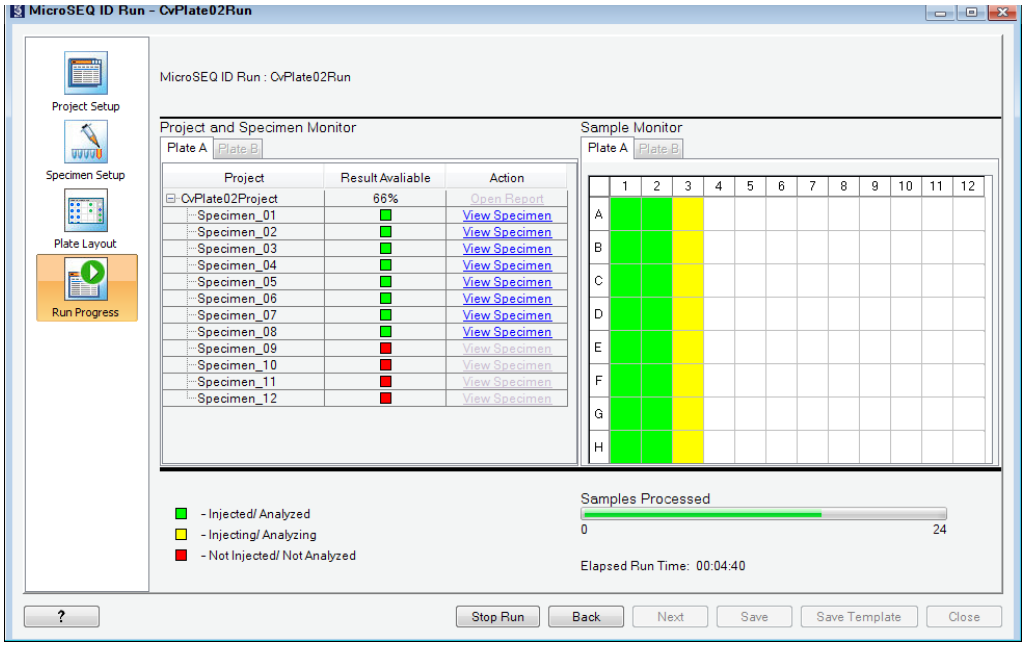
6. View the run progress as needed; see "Monitor the MicroSEQ ID Run progress" below for additional details.

   IMPORTANT! Do not run other applications when the MicroSEQ ID Run Wizard and the Data Collection software are running.

Monitor the MicroSEQ ID Run progress

From the MicroSEQ ID Run Wizard, you can view the injection, and analysis status for samples, specimens, and projects in a MicroSEQ ID Run that is currently running.

In the Run Progress tab of the MicroSEQ ID Run Wizard, monitor the status of each plate in the run:



Monitor the sample injection status

The Sample Monitor status indicators (shown in right pane of the above image) display the injection status for the associated wells (samples) in the plate view:

- (**Injected**) – The injection is complete.
- (**Injecting**) – The injection is in progress.
- (**Not Injected**) – The injection has not started.

Monitor the project and specimen analysis status

The Project and Specimen Monitor status indicators (shown in left pane of the above image) display the analysis status for each specimen in the run:

- (**Analyzed**) – The specimen analysis is complete and analysis results are available to view.
- (**Analyzing**) – The specimen analysis is in progress.
- (**Not Analyzed**) – The specimen analysis has not started.
- **% Result Available** – Displays the percentage of completed specimens in a project, based on the analysis status for each specimen.
- **Action** – Clicking an active link displays the analysis results for a completed specimen or project in the run.
  - **View Specimen** – Click to open the specimen analysis report; use this report for any of the following:

    Determine if a match was made against a validated (proprietary) library or a custom library.

    (If enabled) View the Auto-ID results for the selected specimen.

    View all library matches for the selected specimen.

    Check the top match results of the library search and determine the quality of the top match search results.
  - **Open Report** – Click to open the project analysis report selected in the Project Setup tab. This report is also available after the run is complete.

Note: During the run, you can view the specimen and project analysis reports using the active links. These views are not editable. After the run is complete, the analysis results are automatically saved to each project and the links become inactive. To view the results for a project in the run, open the project and view the reports and project information together; see page 80.

Monitor the run status

The Run Monitor status indicators (shown in lower right area of the image on the previous page) display the overall run status:

- **Samples Processed** – Displays a progress bar with the total number of samples (wells) injected and analyzed.
- **Elapsed Run Time** – If the run is in progress, displays the amount of time (hh:mm:ss) elapsed since the run started; the monitored time is logged per run (not per injection).

Stop a MicroSEQ ID Run

Note: The information shown below is not representative of the tutorial and should be considered an example only.

Use only the MicroSEQ ID Run Wizard to stop a MicroSEQ ID Run that is currently being processed on the 3500 Series Data Collection Software version 1.1.

IMPORTANT! You cannot resume a run after you stop it.

1. (*Optional, if run is minimized*) To display the MicroSEQ ID Run you want to stop running, click (**Maximize**) in the title bar of the MicroSEQ ID Run Wizard.
2. In any tab of the MicroSEQ ID Run Wizard, click **Stop Run**. Wait for the instrument to stop the run before you proceed.
3. After the run has stopped, unload the plate(s) from the instrument.

Note: We recommend that you use the MicroSEQ ID Run Wizard, and not the Data Collection software, to stop a MicroSEQ ID run. When you stop a run from the MicroSEQ ID Run Wizard, the action is recorded in the MicroSEQ ID software as a stopped run, whereas when you stop a run from the Data Collection software, the action is recorded as a completed run.

Using templates with the MicroSEQ ID Run Wizard

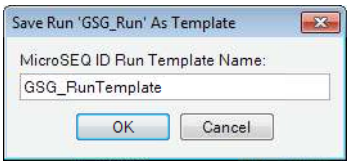
You can save time by saving the setup data in an existing MicroSEQ ID Run as a run template and then using the template to create future runs.

Save a MicroSEQ ID Run as a template

Use the MicroSEQ ID Run Wizard to save a MicroSEQ ID Run as a template.

1. Click **Open MicroSEQ ID Run** on the MicroSEQ ID software main window and open **GSG_Run**.
2. Click **Save Template** (at bottom of MicroSEQ ID Run Wizard dialog box) to save as a run template.

3. Type **GSG_RunTemplate**. By default the MicroSEQ ID Run template name shown is the same name as the MicroSEQ ID Run.



4. Click **OK**. The MicroSEQ ID Run is saved in the MicroSEQ ID Manager as a MicroSEQ ID Run template.

5. Click **Close** to exit the wizard.

Create a MicroSEQ ID Run from a template

You can select a run template from the MicroSEQ ID Manager, then create a new run using the template settings.

1. Click **Open MicroSEQ ID Template** on the MicroSEQ ID software main window and open **GSG_RunTemplate**.

2. In the Project Setup tab, enter **GSG_Run_2**.

3. Select the **Specimen Setup** tab and make sure each Plate Name shown is unique to the MicroSEQ ID software.

   Note: You can complete the remaining setup information for the MicroSEQ ID Run at a later time.

4. Click **Save Run**.

5. Click **OK**. By default, the MicroSEQ ID Run template is saved in the MicroSEQ ID Manager as a MicroSEQ ID Run.

6. Click **Close** to exit the MicroSEQ ID Run Wizard.

Workflow 2: Using Autoanalysis with 3130 Series Genetic Analyzers

This workflow covers:

- Integrating MicroSEQ ID and 3130 Series Data Collection Software 58
- Create required files in the Data Collection Software. 59
- Schedule and start an autoanalysis run. 66
- Launching Autoanalysis Manager 67

For more information on software features, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

Integrating MicroSEQ ID and 3130 Series Data Collection Software

Overview

You can automatically analyze sequencing data generated on 3130/3130*xl* Genetic Analyzers using the MicroSEQ ID software without requiring user interaction.

Autoanalysis uses three software packages automatically installed by the installer program:

- 3130/3130*xl* Analyzer Data Collection Software v3.0
- Autoanalysis Manager
- A hidden version of MicroSEQ ID software that the Autoanalysis Manager uses to analyze the data in the projects

Note: Autoanalysis can be performed only on the same instrument computer that collected the sample files.

IMPORTANT! The data collection software must be running when installing MicroSEQ ID Microbial Identification Software Version 3.0 on a computer that is connected to a 3130 Series Genetic Analyzer. If data collection software is not running, MicroSEQ ID software does not register with the Data Service.

Requirements

Note: This workflow applies to installation of MicroSEQ® ID Microbial Identification Software Version 3.0 on 3130 Series Genetic Analyzers only (System installation); for information on:

- Running a complete versus a simple analysis, see "System vs. Lite" on page 8
- Performing autoanalysis in 3500 Series Genetic Analyzers, refer to the *Help system*

Autoanalysis requires that:

- Data Collection is running when MicroSEQ ID software is installed.
- You have launched MicroSEQ ID software at least once, registered the software, and created the appropriate user names.
- A project is created but not open in the MicroSEQ ID software, and the MicroSEQ ID software is closed during the autoanalysis run..
- Autoanalysis Manager software is running.

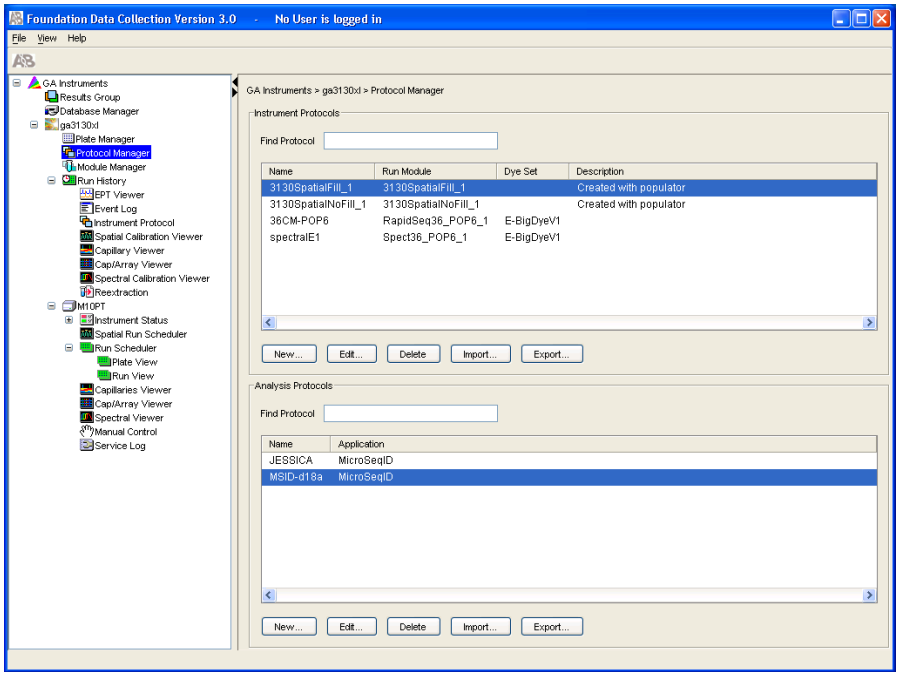
For more information

For information on setting up and using the 3130/3130*xl* Genetic Analyzer and the 3130 Data Collection software, see the *Applied Biosystems 3130/3130xl Getting Started Guide* (Part no. 4352715).

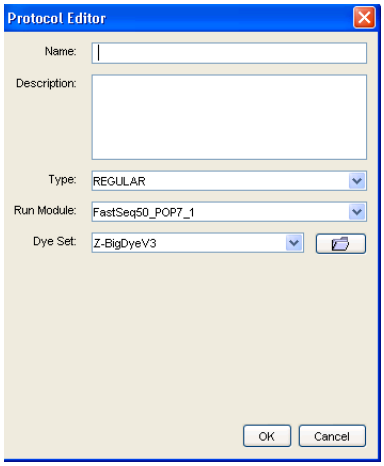
Create required files in the Data Collection Software

Create an instrument protocol

1. In the Data Collection navigation pane, click the **Protocol Manager** icon to display the Protocol Manager.



2. In the Instruments Protocols section, click **New** or select an existing instrument protocol file, then click **Edit** to open the Protocol Editor.



3. Complete the Protocol Editor:
   a. Type a name for the protocol.
   b. (*Optional*) Type a description for the protocol.
   c. Select **Regular** in the Type drop-down list.
   d. Select the correct run module for your run. (See the appropriate instrument user guide for information on selecting the correct run module.)

e. Select **Dye Set E** for your run. (See the appropriate instrument user guide for information on selecting the correct Dye Set.)

f. Click **OK**.

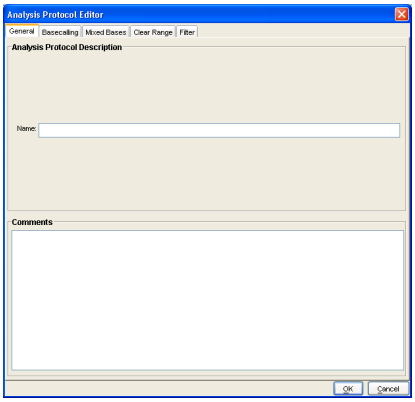
Create an analysis protocol

Note: If you created an appropriate analysis protocol in MicroSEQ ID software, you can use it in data collection software. You must have Administrator or Scientist privileges.

1. In the Analysis Protocols section of the Protocol Manager, click **New**.

   Note: If more than one analysis application is installed on the data collection computer, the Analysis Applications dialog box opens. Select **MicroSeqID**, then click **OK**.

   The Analysis Protocol Editor dialog box opens.



2. In the **General** tab, enter a unique name and comments for the new protocol.

3. Select the Basecalling tab, then:

   a. Select the appropriate basecaller and dye set/primer file.

   Note: Make sure that the basecaller and the Dye Set/Primer file types match. Do not rename the files. Refer to your instrument user guide for basecaller and dye set/primer files.

   b. If desired, select one or more stop points for data analysis. Base your selection on the basecaller being used, as indicated below.

Basecaller	Stop Point
KB	At PCR Stop check box
KB	After __ Bases check box

   c. For the KB basecallers only, select True Profile or Flat Profile for processed data, then select a quality threshold.

4. For the KB Basecaller only, select the Mixed Bases tab, then:

   a. For mixed bases only, select Use Mixed Base Identification.

   b. Use the default setting of 25% or change the detection level by entering a new value or dragging the % line up or down.

5. Select the Clear Range tab, then, if desired, select one or more stop points for data analysis.

   Note: The clear range is the region of the sequence that remains after excluding the low-quality or error-prone sequence at both the 5′ and 3′ ends.

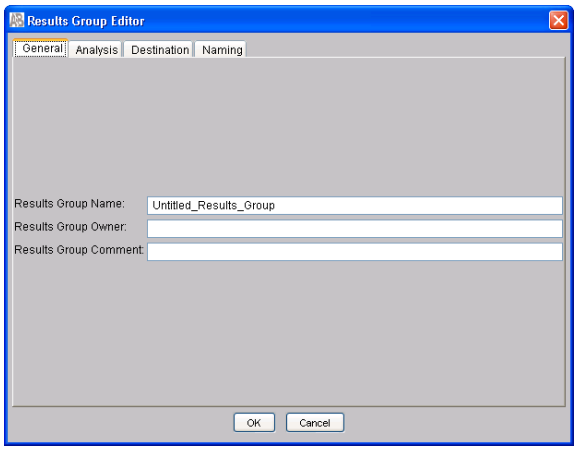
6. Select the **Filter** tab, then, if desired, change one or more of the settings.

7. Select **OK** to save the protocol and close the Analysis Protocol Editor.

Create a results group

To create a results group:

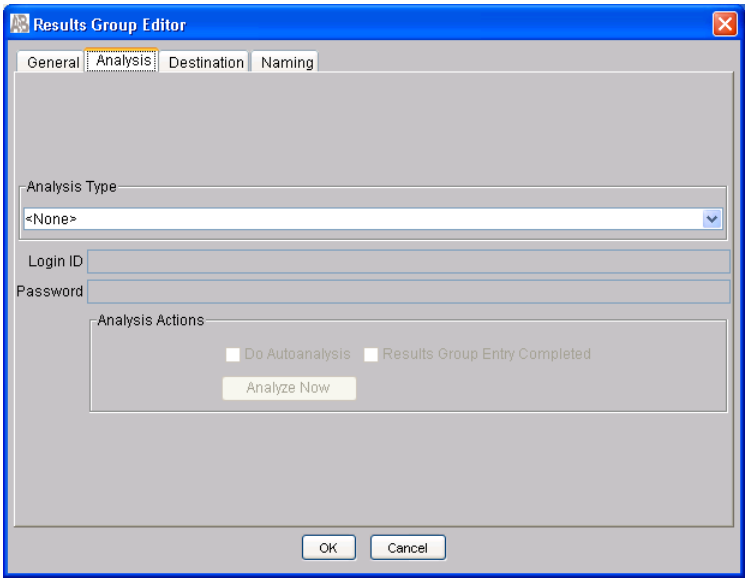
1. Click the **Results Group** icon in the navigation pane to open the Results Group Editor.



2. Click **New**, or select an existing group, then click **Edit**.

3. Make the following entries in the **General** tab:
   a. Type a Results Group Name. The name can be used in naming and sorting sample files and must be unique.
   b. (*Optional*) Type a Results Group Owner. The owner name can be used in naming and sorting sample files.
   c. (*Optional*) Type a Results Group Comment.

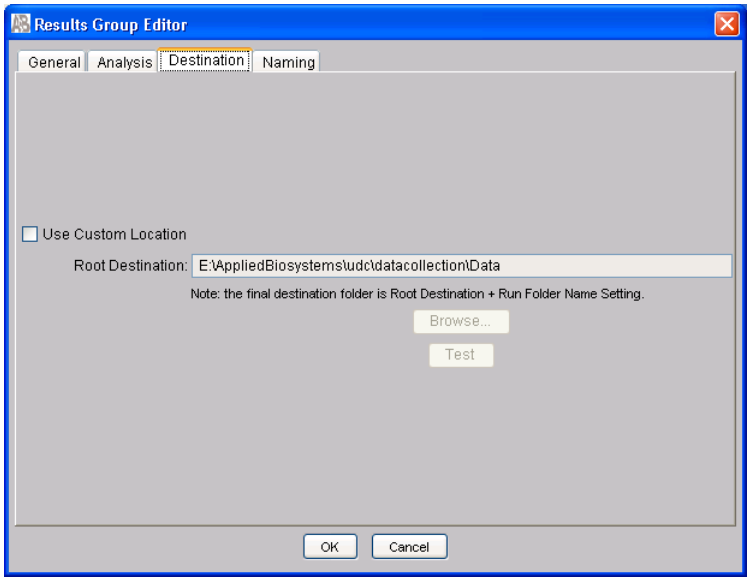
4. Select the **Analysis** tab.



5. Make the following entries in the Analysis tab:
   a. In the Analysis Type drop-down list, select **MicroSeq**_*YourInstrumentName*.
   b. In the Analysis Actions section, select **Do Autoanalysis**.
   c. In the text boxes, type a valid MicroSEQ ID Login ID and Password.

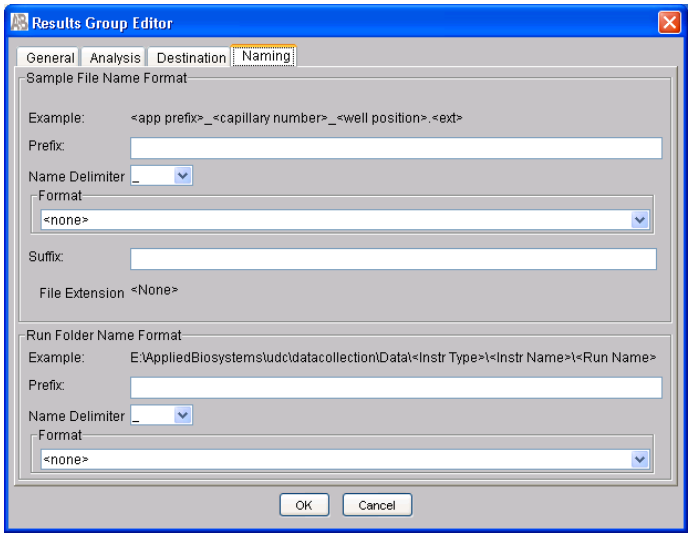
Note: Failure to use the proper login and password causes your project to fail being transferred into the MicroSEQ ID software or to be analyzed. Use reextraction and try again. If the MicroSEQ ID software password entered here is set to expire (see page 31), update this field as needed

6. Select the **Destination** tab to use the default location or define a new location.



7. To define a new location:
   a. Select **Use Custom Location**, then click **Browse** to navigate to a different save location.
   b. Click **Test** to test the Location path name connection.
      If the path name is valid, a message box displays "Path Name test successful."
      If the path name is not valid, a message box displays "Could not make the connection. Please check that the Path Name is correct." Click and retry to establish a connection.
   c. Click **OK**.

8. Select the **Naming** tab, then define custom names for the sample file and the run folder, if desired. As you select from the drop-down list in the format fields in either the Sample File Name Format or Run Folder Name Format panes, another item field appears.

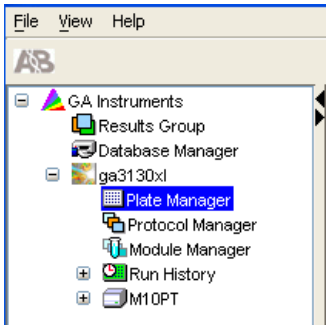


9. Click **OK** to save the Results Group and close the Results Group Editor.

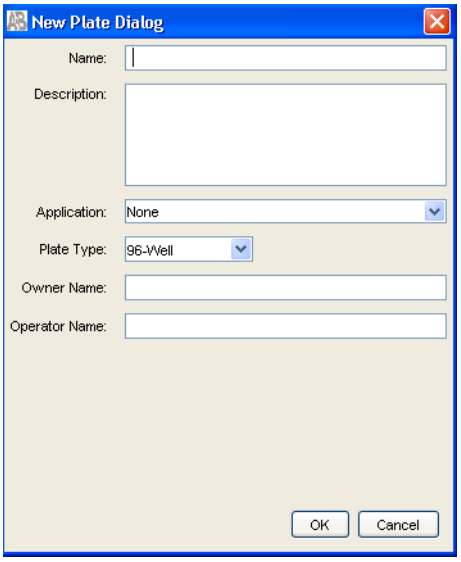
Create a plate record

To create a new plate record:

1. Click the **Plate Manager** icon in the navigation pane.



2. Click **New** to open the New Plate Dialog dialog box.



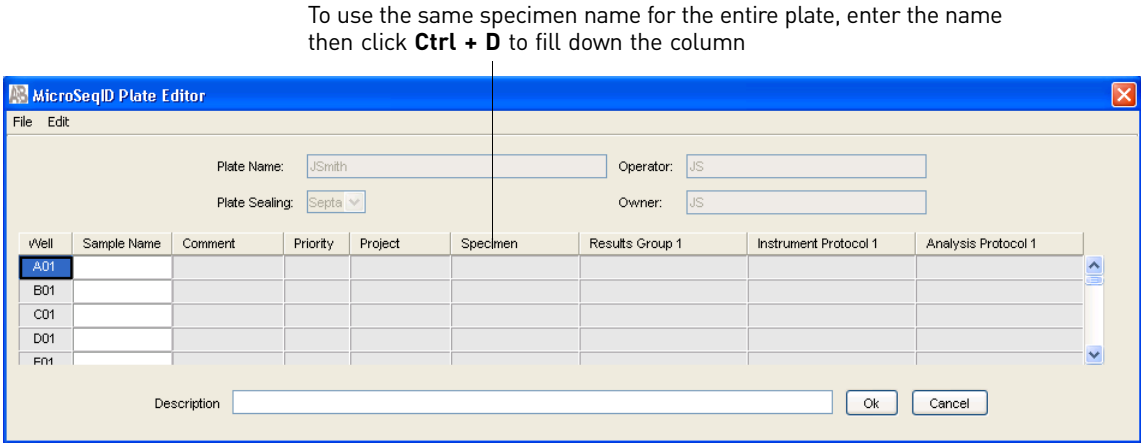
3. Complete the information in the New Plate Dialog box:
   a. Type a name for the plate.
   b. (*Optional*) Type a description for the plate.
   c. Select **MicroSeq_***YourInstrumentName* in the Application drop-down list.
   d. Select **96-well** or **384-well** in the Plate Type drop-down list.
   e. Type a name for the owner and operator.
   f. Click **OK** to open the MicroSeq Plate Editor.

Sample sheet template

By creating a sample sheet template you can save time because you can create a plate editor or use a text editor. For more information on using a sample sheet template in the autoanalysis feature, refer to the *Help system*. Press **F1**, or select **Help** ▸ **Search**, then type **sheet**.

Complete a plate record

The MicroSeq Plate Editor dialog box below, allows you to fill out a plate record with the run conditions, sample information, and analysis conditions.



1. Type sample names.

   Note: You can not use duplicate sample names in the same specimen.

2. (*Optional*) Add comments.

3. (*Optional*) Change the priority of the sample. (The default value 100 automatically displays.)

4. Select a project or create a new one by clicking in the project column cell and selecting from the drop-down list. The project's analysis protocol will be copied into the analysis protocol column.

5. Select a specimen or create one by clicking in the specimen column cell and selecting from the drop-down list.

   Note: If the specimen has an assigned analysis protocol, that analysis protocol will be copied into the analysis protocol column, replacing the project analysis protocol.

6. Select a results group, duplicate one, or create one by clicking in the Results Group 1 column cell and selecting from the drop-down list.

7. Select an instrument protocol, edit one, or create one by clicking in the Instrument Protocol 1 column cell and selecting from the drop-down list.

8. To add additional runs to the plate record:

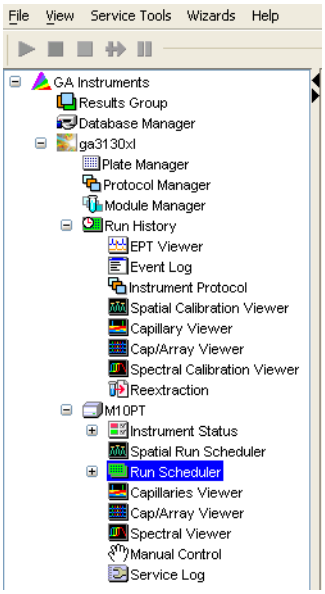
   a. Select Edit ▸ Add Sample Run.

   b. Select a results group, and instrument protocol.

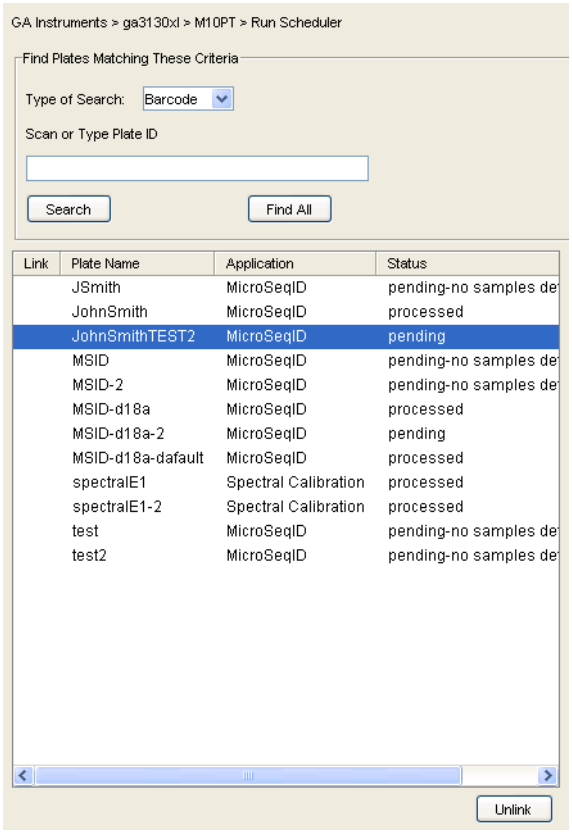
9. Click **OK**.

Schedule and start an autoanalysis run

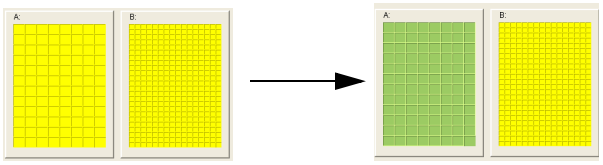
1. Click the Run Scheduler icon in the Data Collection software navigation pane.



2. Click **Find All** in the Plate View to find your plate record. You can also select **Barcode** or **Advanced** in the Type of Search drop-down menu to find your plate record, enter matching criteria and click **Search**.



3. Link the plate record to a plate layout:
   a. Select your plate record.
   b. Click the appropriate plate layout, A or B. The selected plate turns green.

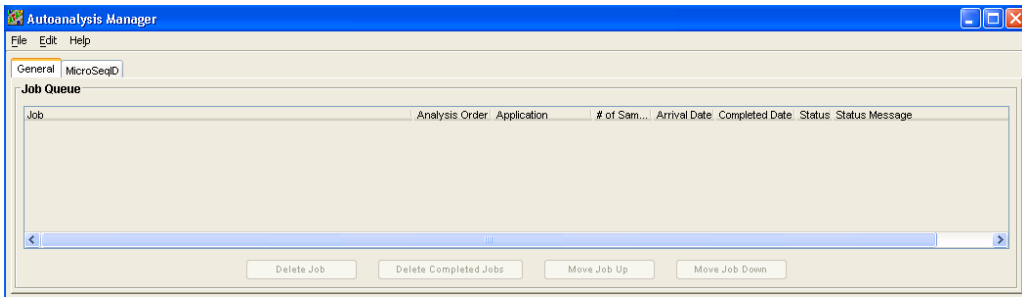


4. Click  (**Run**) to start the run.

Launching Autoanalysis Manager

Launch Autoanalysis Manager

To launch the Autoanalysis Manager, select **Start ▸ Applied Biosystems ▸ Autoanalysis Manager ▸ Autoanalysis Manager**.

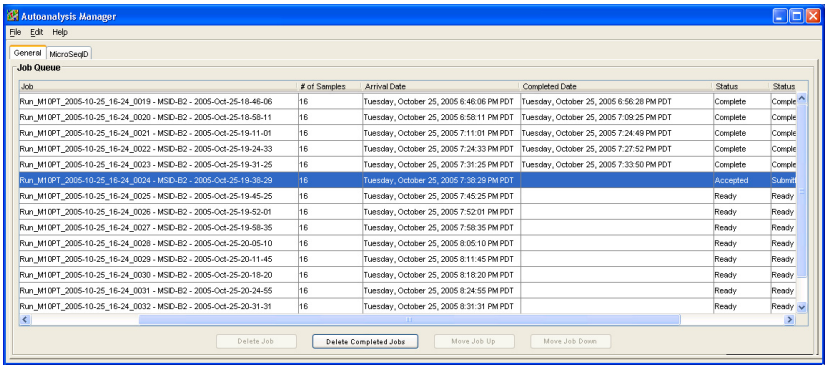


IMPORTANT! Do not run other applications when the Autoanalysis Manager and the data collection software are running. Never open a project while it is in use by the Autoanalysis Manager. Otherwise, the Autoanalysis Manager will not analyze the project.

General tab

The General tab shows the jobs that have been submitted and their statuses. For more information on the General tab, refer to the *Help system*. Press **F1**, or select **Help ▸ Search**, then type **autoanalysis** and select Autoanalysis Manager Window.

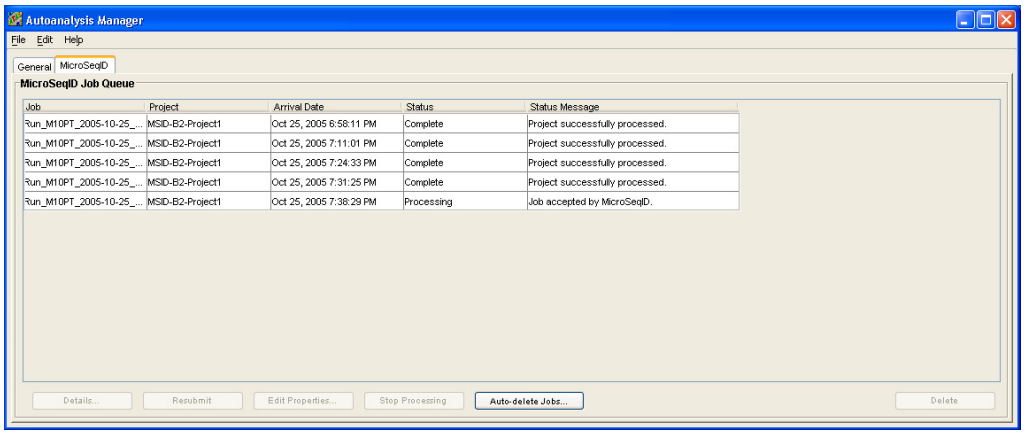
Note: Each Job in a row represents one run.

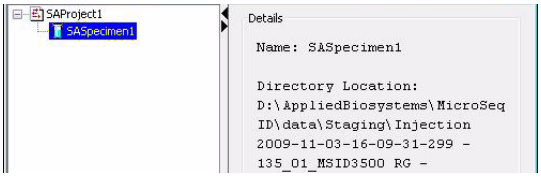


MicroSeqID tab

The MicroSeqID tab shows the jobs, project, and status information.

Note: Each Job in a row represents one project.



Button Name	Function
Details	Displays the project in the navigation pane. 
Resubmit	Resubmits a failed job for analysis.
Edit Properties	Edits the name and password (active only if analysis failed).
Stop Processing	Interrupts the processing of the current job. The job is marked "failed" and the Autoanalysis Manager goes to the next job.
Auto-delete Jobs	Automatically deletes the completed jobs from the Autoanalysis Manager. This option allows you to select when to delete (in number of days) the completed jobs. This option is a convenient way to save disk space.
Delete	Deletes a job from the Autoanalysis Manager.

Workflow 3: Using the New Project Wizard to Create a Project

Note: This workflow applies to installation of MicroSEQ® ID Microbial Identification Software Version 3.0 (Lite and System installations).

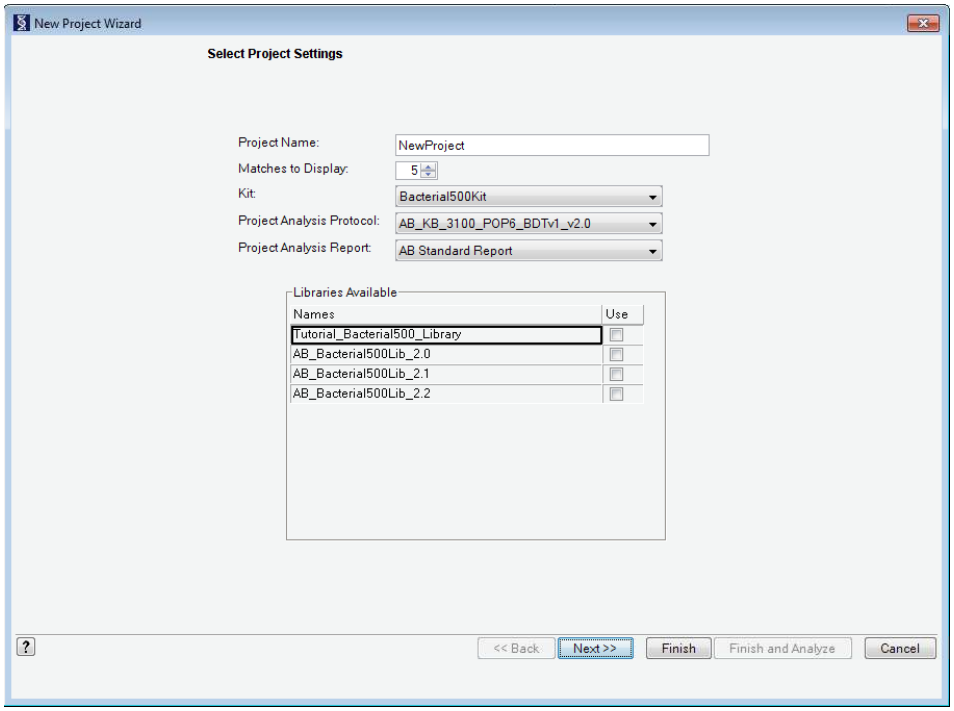
This workflow covers:

- Select project settings 69
- Creating a new specimen and import sample files 70
- View the project setup summary 72

Select project settings

1. In the MicroSEQ ID software main window, select **File ▸ New Project**. The New Project Wizard opens with the Select Project Settings dialog box displayed.

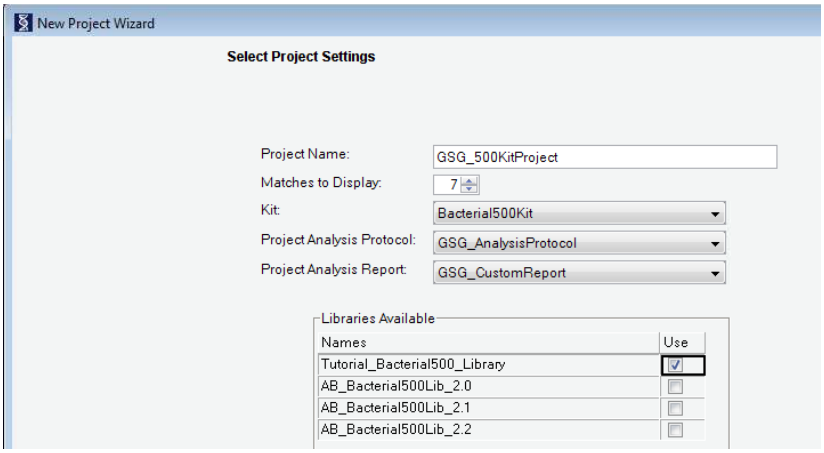
2. In the New Project Wizard dialog box, enter the settings shown in the table below.



Parameter	Description
Project Name	Type **GSG_500KitProject**. Do not use illegal characters; for more information, see page 18.
Matches to Display	Select **7** as the number of matches to display in the Library Results Project view. **Note:** Whenever Auto-ID is enabled for the kit in the project, as is the case here, the minimum number of matches must be set to 7 for Auto-ID to occur. Administrators can change the Auto-ID state for all projects by selecting **Tools ▸ Options**.

Parameter	Description
Kit	Select **Bacterial500Kit** as the chemistry kit used to generate the sample data in the project. The Bacterial500Kit is the MicroSEQ® 500 16S rDNA Bacterial Identification Kit.
Project Analysis Protocol	Select **GSG_AnalysisProtocol** as the analysis protocol. This is the master analysis protocol created earlier in the tutorial (see page 38).
Project Analysis Report	Select **GSG_Custom_Report** as the analysis protocol used for the analysis. This is the custom report created earlier in the tutorial (see page 39).
Libraries Available	Select the **Tutorial_Bacterial500_Library** check box to select this library for analysis. Only libraries that specify the selected kit are listed. **Note:** When you create your own projects, you may search the proprietary libraries and any of your own custom libraries at the same time. Do not, however, use the tutorial libraries for your projects for other than analyzing tutorial project data. For optimal performance, we recommend searching the pre-installed libraries first.

3. Check that the completed Select Project Settings dialog box of the New Project Wizard looks as shown below:



4. Click **Next >>** to continue with "Creating a new specimen and import sample files," below. Do not click **Finish** at this time.

Creating a new specimen and import sample files

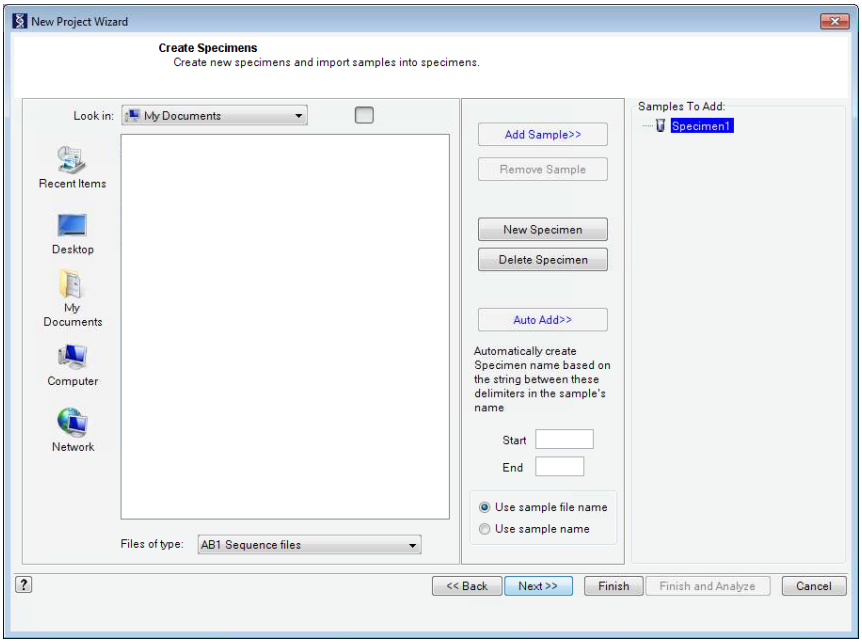
In the MicroSEQ ID software, a specimen refers to a container that holds the sample data (sample files) from a single bacterial or fungal unknown isolate.

Create a new specimen, then import sample files into the specimen. You can import sample files individually or import an entire sample folder.

Sample files imported into a specimen are in the .ab1 file format and are unassembled. The following tutorial sample files are for use with this *Getting Started Guide*:

Specimen	3500 tutorial data
MicroSEQ Fungal (*S. cerevisae*)	Specimen1_F_3500xl.ab1
	Specimen1_R_3500xl.ab1
MicroSEQ Full Gene (*B. cereus*)	Specimen1_1F_3500.ab1
	Specimen1_1R_3500.ab1
	Specimen1_2F_3500.ab1
	Specimen1_2R_3500.ab1
	Specimen1_3F_3500.ab1
	Specimen1_3R_3500.ab1
MicroSEQ Bacterial 500 (*S. aureus*)	Specimen1_F_3500xl.ab1
	Specimen1_R_3500xl.ab1

1. In the Create Specimens dialog box of the new Project Wizard, click **New Specimen**.

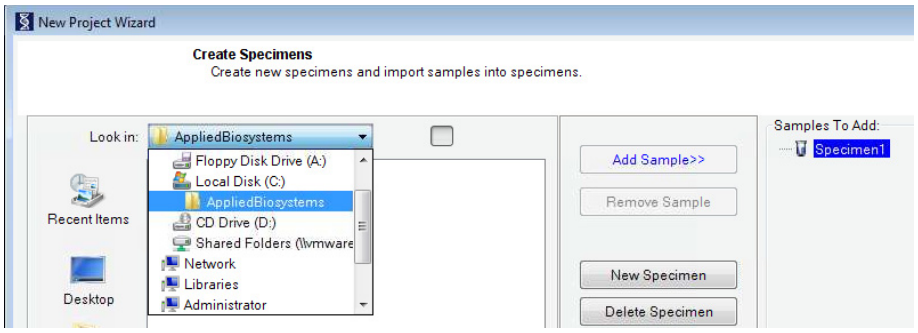


   A new specimen (Specimen1) is added to the "Samples to Add" section (at right). You will rename the specimen after project analysis; see step 2 on page 80.

   Note: The default specimen name (Specimen1) can only be changed in the Project Navigator pane after the project has been created.

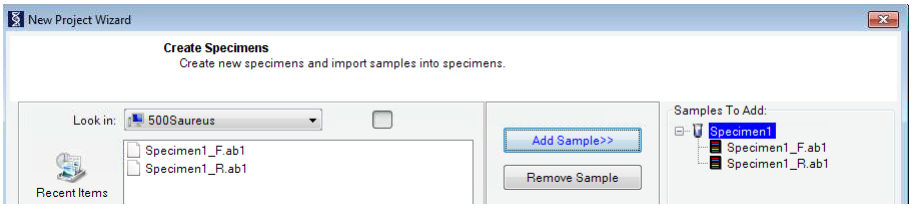
2. In the Look in field drop-down list (top left), navigate to and select the **500Saureus** sample folder:

   drive letter:\AppliedBiosystems\MicroSeqID\Tutorial Data\Bacterial500Samples\500Saureus



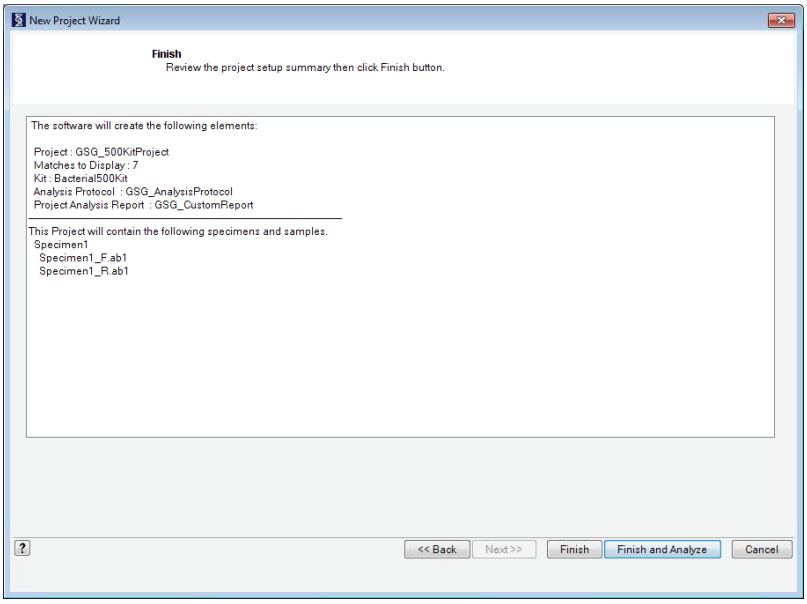
3. Add the samples by selecting:
   - **Specimen1_F.ab1,** then click **Add Sample**.
   - **Specimen1_R.ab1,** then click **Add Sample**.

   The sample files display under Specimen1 in Samples To Add (right pane).



View the project setup summary

1. In the Create Specimens dialog box of the New Project Wizard, click **Next** >>. The Finish dialog box appears, showing the project setup summary:

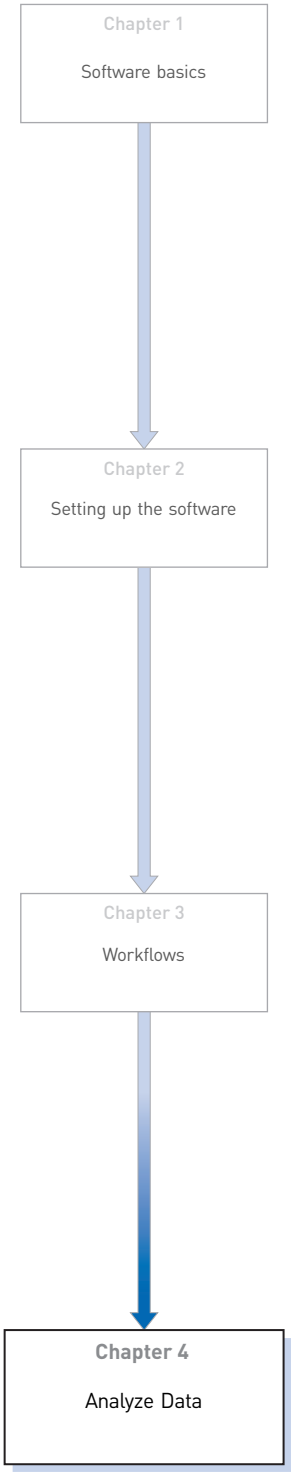


2. Review the summary to make sure all information has been entered correctly.

3. If you need to make any changes:
    a. Click **<< Back** to return to the appropriate dialog box (Create Specimens or Select Project Settings).
    b. Make your corrections.
    c. Click **Next >>** to open the Finish dialog box again.

Note: MicroSEQ ID projects (*prj.ctf) contain a copy of *.ab1 files, analysis results, project audit trails, and project electronic signature records.

4 Analyze Data



This chapter covers:

- Before you begin 76
- Analyze the project data 77
- Evaluate results: Analysis QC Report 80
- Evaluate results: Library Search Report 85
- Export or print reports 89
- Manage your audit records 91
- Electronically sign your work 91
- About electronic signatures for users 91

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

Before you begin

Overview

User privileges

You may have Administrator, Scientist, or Analyst privileges to perform the tasks in this chapter.

While any user group can analyze a project in the MicroSEQ ID software, to get the most out of this *Getting Started Guide*, an Analyst or Scientist should perform the tasks in this chapter.

Analysis information

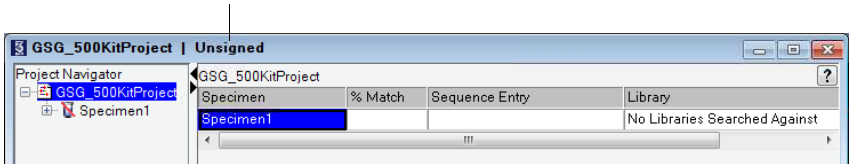
Before you can analyze the tutorial project for this *Getting Started Guide*, a Scientist must create a master analysis protocol (see page 38).

Audit and Electronic Signature information for users

During data analysis and review, if your Administrator has:

- Set the Audit Trail state to Prompt (see page 31), you will be prompted to select an Audit Reason after an auditable event occurs (see pages 91).
- Enabled the electronic signature (eSig) feature for the application (see page 33) and for your user account (see page 34), you will be prompted to authenticate or "sign" the project after an eSig event occurs by typing the user name and password of your account (see pages 93). The eSig status of a project is shown in the Project View and Report Manager title bars and is updated after each successful eSig event.

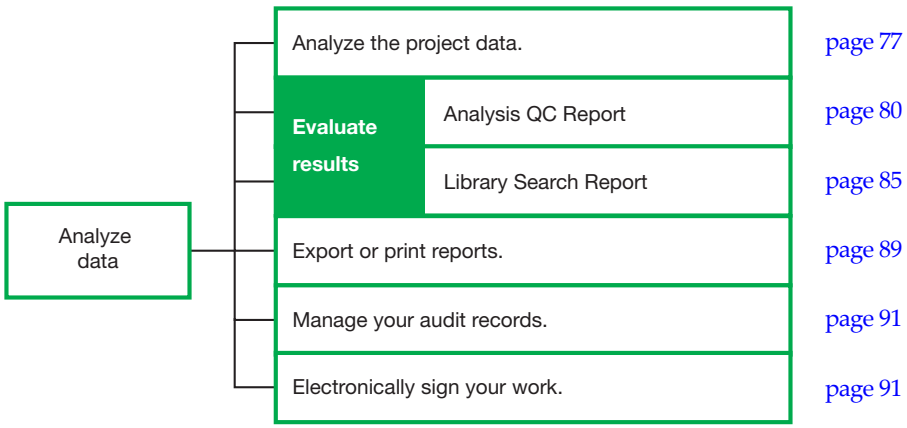
eSig status shown in Project View title bar



Data analysis workflow

The workflow below provides an overview of the data analysis tasks presented in this chapter.

IMPORTANT! Perform each task in the order given.



Analyze the project data

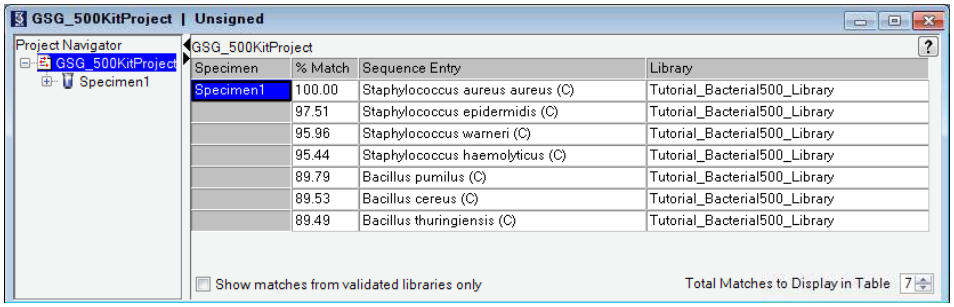
After using the New Project Wizard to specify the tutorial project settings (see page 69) and create a new specimen (see page 70), you are ready to analyze the project data.

Analyze Genetic Analyzer sample files

The 3500 and 3130 Series Genetic Analyzers apply a basecalling protocol to the .ab1 (sample) files it generates. When you analyze these sample files, the MicroSEQ ID software applies an analysis protocol (which uses the same KB Basecaller) and basecalls the data again. For information on creating an analysis protocol, see page 60.

Finish and analyze the project data

1. In the Finish dialog box of the New Project Wizard, click **Finish and Analyze**. An Analysis Status message appears as the software analyzes the data. Once the analysis you should see the following view:



   To view the projects and reports at the same time, from the toolbar, select **Window** ▸ **Tile**, then **Vertical** or **Horizontal**.

2. In the Project Navigator pane, change the default specimen name:
   a. Double-click the default specimen name (**Specimen1**).
   b. Type **Unknown_1**.
   c. Press **Enter**.

   IMPORTANT! If you do not press **Enter** after changing the specimen name, the change is not applied.

3. When you finish viewing the project analysis report, click  to close the Report Manager window. The project is automatically saved.

4. *(Optional)* Import a sequence for analysis by importing a text specimen (a .fsta file that contains a specimen name and a sequence) into the project.

Set the display

Editing the default display settings

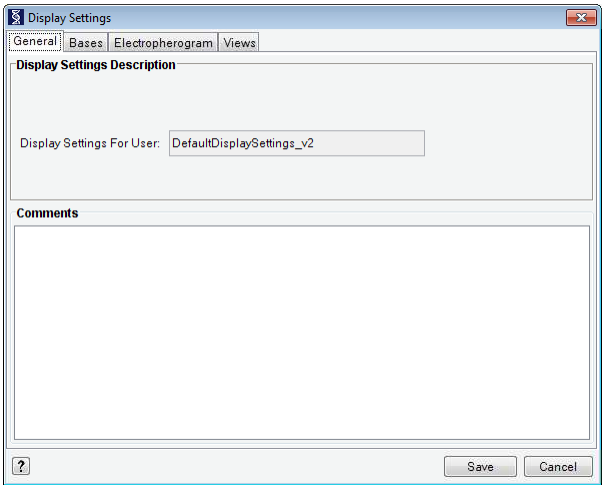
Display settings control how projects are visually displayed when they are first opened after analysis is complete.

You can set display settings to:

- Change the scaling of the sample electropherograms (Electropherogram tab)
- Select the items displayed by default in the Specimen and Sample Views (Views tab); for example, (Confidence QV), (Electropherograms), and (Column Selector)
- Change the color associated with each base (Bases tab)
- Change the range of QV values associated with each color (Views tab)
- Set the default QV values that will be available in the Tab Key Jumps to Next Function drop-down list in the project view (Views tab)

Users can customize their own display settings.

1. In the Project window, select **Analysis ▸ Display Settings** or click (**Display Settings**) from the Display Toolbar.



2. In the Comments field of the General tab, type **GSG tutorial: my customized display settings**.
3. Update the display settings in the remaining tabs as needed.
4. Click **Save** to save your display settings and close the Display Settings window.

The saved display settings are applied each time the current user logs in. You may change your display settings at any time.

Setting the displays in the project view

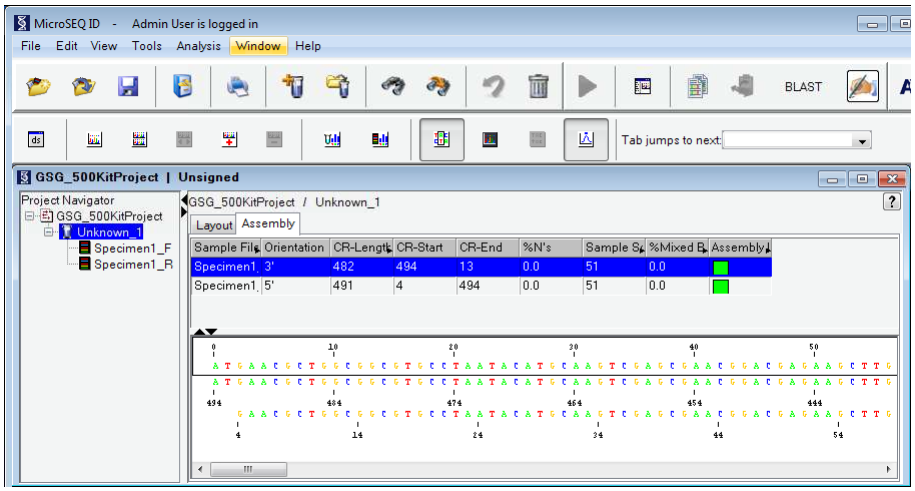
You can also customize the display of an open project:

- Set the Tab Key Jumps to Next function to advance to a Low, Medium, or High QV when you view results in the Specimen View
- Select the items to display in the Specimen and Sample Views; for example, (Confidence QV), (Electropherograms), and (Column Selector)

Set the tab function

The "Tab Key Jumps to Next" function is active when the Specimen View Assembly tab is selected.

1. Open the project of interest.
2. In the Project Navigator pane, click **Unknown_1**, then click the **Assembly** tab.



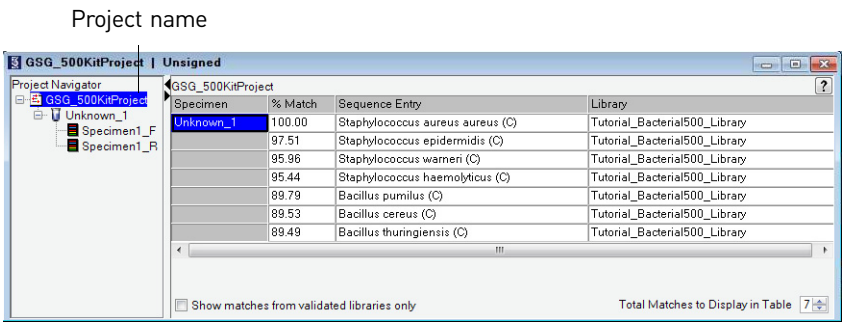
3. In the tool bar, click the down arrow next to Tab Key Jumps to Next.

4. Select one or more options:

Option	Description
Multiple	Select **Multiple**, select the **Low QV Base, User Edit,** and **Discrepancy** check boxes in the Multiple Tab Jump Settings dialog box, then click **OK**. Allows you to assign multiple items to the Tab Key Jumps to Next function.

View the Tutorial Report

1. In the Project Navigator pane of the Project window, select the project name (**GSG_500KitProject**).



2. Select **Analysis ▸ Report Manager** or click (**Report Manager**) to open the Report Manager for the GSG_500KitProject.

3. Click each section under the GSG_CustomReport folder to view the report items in the right-side preview pane.

4. (*Optional*) Change the report settings; see page 40.

Evaluate results: Analysis QC Report

To verify that the sample files passed each step of the analysis process, evaluate the information provided in the Analysis QC Report.

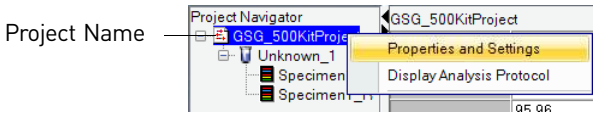
Note: The information shown below is not representative of the tutorial data and should be considered an example only.

View the Analysis QC Report

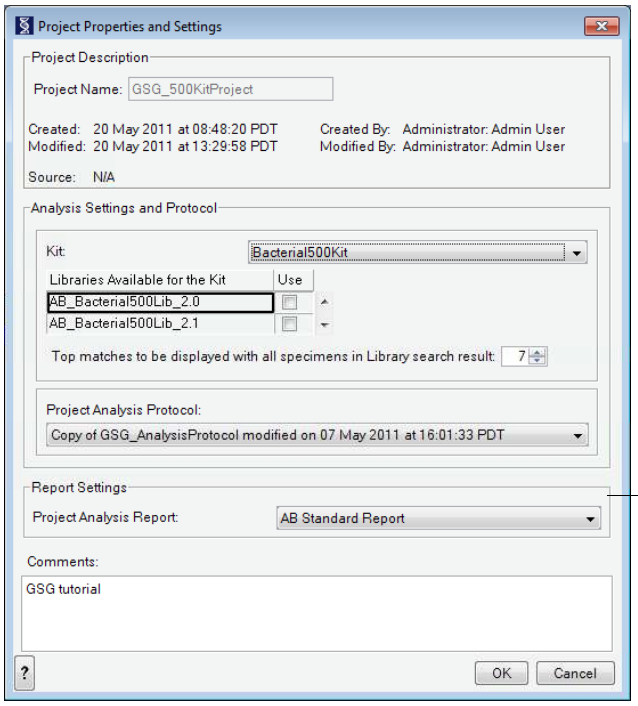
To change from a custom report setting to the AB Standard Report setting, use the Properties and Settings dialog box in the following tutorial procedure.

1. Open the project **GSG_500KitProject**.

2. In the Project Navigator pane of the Project window, select **GSG_500KitProject**, then right-click to select **Properties and Settings**. This will bring up the Project Properties and Settings dialog box.



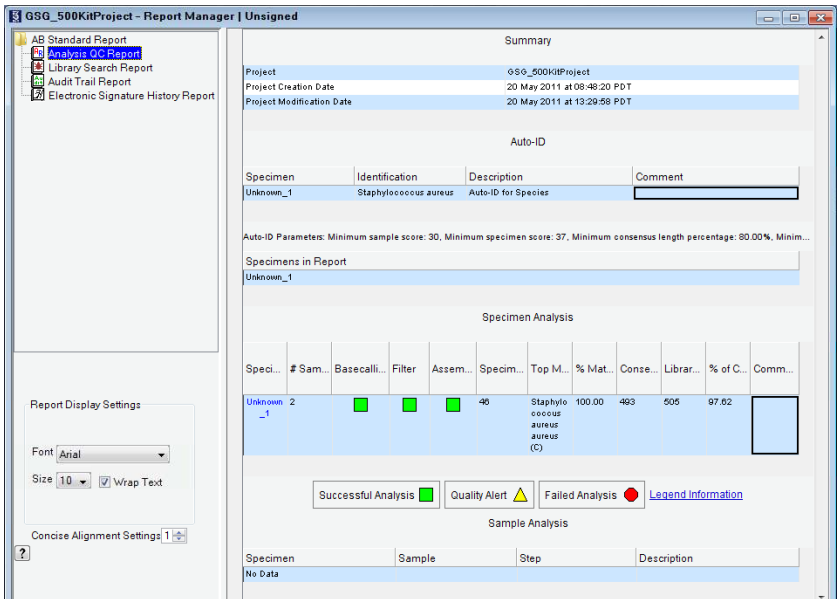
3. In the Report Settings pane of the Properties and Settings dialog box, select **AB Standard Report** as the Project Analysis Report.



4. Click **OK**.

5. Select **Analysis ▸ Report Manager** or click  (**Report Manager**).

6. In the Reports navigation pane, select **Analysis QC Report**.



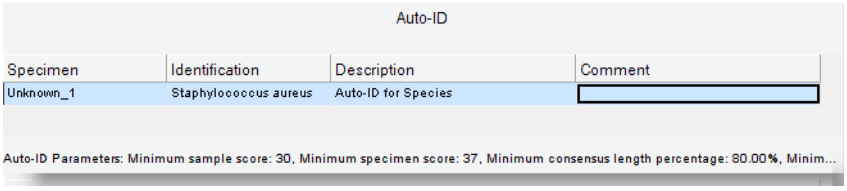
Note: After analyzing the project and evaluating the results in the Analysis QC Report, you can edit the data as needed and reanalyze the project.

Interpret the Auto-ID section

For the purposes of this tutorial, the Auto-ID state was set to "on" your kit (Bacterial500Kit) in the project (GSG_500KitProject; see page 37). In the Auto-ID portion of the above image you will see a table that lists for each specimen:

Column	Description
Specimen	The name of the specimen.
Identification	The genus or species name of the microorganism (library sequence) identified as the exact match. **Note:** "See description" indicates no identification.
Description	More information regarding the species or genus identification, or troubleshooting information for an unidentified specimen.
Comment	Any comments entered for this specimen. You can use this column to manually enter a specimen identification, if needed. This text is included in the printed or exported report. See "Export or print reports" on page 89. **Note:** This column is not shown if you view the Analysis QC Report from an open MicroSEQ ID Run.

The Auto-ID parameters for the kit, as edited by your Administrator, are listed immediately below the Auto-ID table:



Administrators can change the Auto-ID state for all projects by selecting **Tools ▸ Options**.

Interpret the Specimen Analysis section

Review Specimen Analysis, which displays a table that lists for the selected specimen:

Column	Description
Specimen	The name of the specimen. The specimen name is hyperlinked to the Specimen View.
# Samples	The number of sample files in the specimen.
Basecalling, Filter, and Assembly	The status of these analysis steps. See ""Analysis quality" on page 83.
Specimen Score	Average consensus quality value (QV). See "Specimen score" on page 83.
Top Match	The name of the identified microorganism (library sequence) with the highest % Match.

Column	Description
% Match	Indicates how closely the unknown isolate matches the top library sequence (Top Match). See "% Match" on page 84.
Consensus Length	The number of bases of the consensus sequence.
Library Entry Length	The number of bases of the library sequence entry.
% of Consensus Length	The amount of overlap between a consensus sequence and a library entry sequence. See "% of Consensus Length" on page 111.
Comments	(*Optional*) Enter your comments about the specimen.

Analysis quality

The quality of the analysis for each analysis step (basecalling, filtering, and assembling) is indicated by the icons displayed in the Basecalling, Filter, and Assembly columns.

Icon	Description	Symbol Linked to...
Green square – 	Successful Analysis: All of the sample files in the specimen successfully completed the analysis step.	—
Yellow triangle – 	Quality Alert: At least one of the samples in the specimen did not successfully complete the step.	The Sample Analysis section of the Analysis QC Report.
Red octagon – 	Failed Analysis: Check error messages. None of the sample files in the specimen successfully completed the analysis step.	

If any specimen displays the  or  icon, clicking the icon links you to the affected sample files in the Sample Analysis section.

In this tutorial you will see the Sample View of a  sample file.

1. In the Project Navigator pane, select:
    a. The project name (**GSG_500KitProject**) to view the Library Search Results (that is, the % Match results for all the specimens).
    b. The specimen name (**Unknown_1**) to view the Layout and Assembly Views for that specimen (see page 79).
    c. Click  (**sample QV**) to view the QV bars.

Specimen score

The information shown below is not representative of the tutorial data and should be considered informative only.

The Specimen Score is the average consensus QV (that is, the average QV of all base QVs in the specimen). To obtain the Specimen Score, per-base sample QVs are calibrated on a scale corresponding to:

$QV = -10\log_{10}(P_e)$; where P_e is the probability of error of the basecall

The range of a QV is 1 (low confidence) to 50 (high confidence). Values above 30 indicate high-quality results.

Note: Mixed bases are common in many bacterial species with multiple copies of the rRNA gene. Some bacterial species have mixed bases and the QV may be lower even though the sequences are of high quality.

The following table shows the probability of basecall errors for several QVs. This table provides an abbreviated list only. To view the probability of basecall errors for all QVs (ranging from 1 to 50), refer to the *Help system*.

QV	P_e	QV	P_e
10	10%	35	0.032%
20‡	1%	40‡	0.01%
25	0.31%	45	0.0032%
30‡	0.1%	50	0.001%

‡ Denotes commonly used cutoff values for Low Confidence, Medium Confidence, and High Confidence ranges.

% Match

The % Match indicates how closely the unknown isolate matches the top library sequence (Top Match). The % Match is determined as follows:

% Match = (1 – (The sum of mismatch penalties) / (The sum of the quality values for all the bases in the aligned consensus sequence)) X 100

Mismatch penalty = (The mismatch factor) X (The quality value of each mismatched base in the aligned consensus sequence)

Mismatch factors are assigned:

- 0.5 for half-match of a mixed base aligned with a pure base and the mixed base contains the pure base
- 1.0 for mismatch

The % match calculation uses quality values to weight the likelihood of a true mismatch. For example, a low quality mismatched base has less weight than a high quality mismatched base when the two are at the same position.

Interpret the Sample Analysis section

Note: The information shown below is not representative of the tutorial data and should be considered an example only.

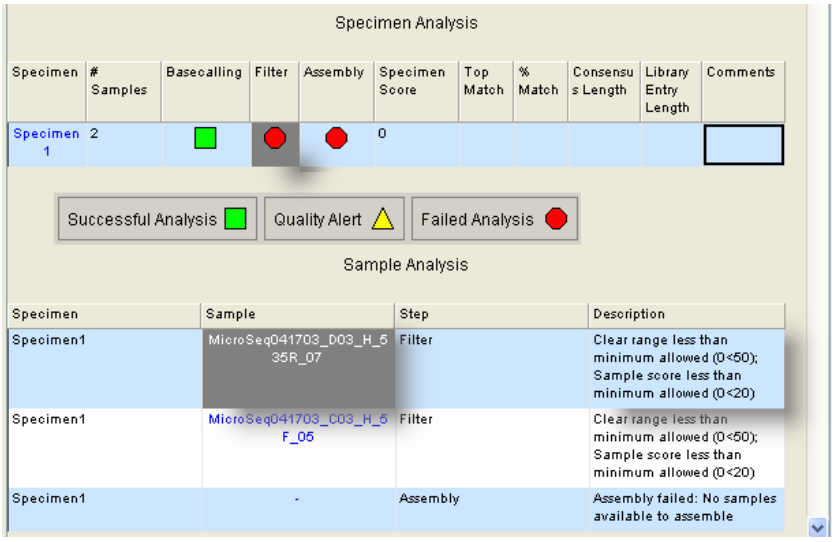
If the Specimen Analysis section displays the  or  icon for any specimen, clicking the icon links you to the affected sample files in the Sample Analysis section.

In the Sample Analysis section, the report specifies for each failed sample file:

Column	Description
Sample	The name of the failed sample file
Step	The analysis step at which the failure occurred: basecalling, filtering, or assembling

Column	Description
Description	A detailed description of the particular analysis failure

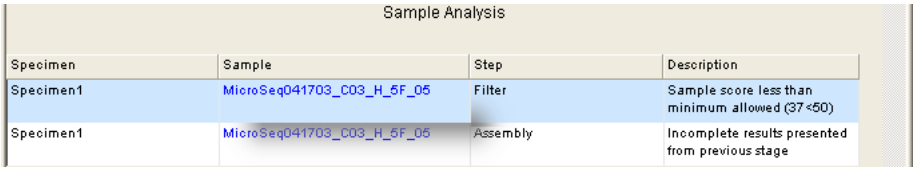
In the figure below for example, clicking (System Error icon) under the Filter column for Specimen1 links you to the Sample Analysis section. The Sample Analysis section displays the failed sample file (MicroSeq041703_D03_H_535R_07) and a description of the failure.



Hyperlinks to view sample files

The specimen name is hyperlinked to the Specimen View.

You can view the data for a failed sample file by clicking its hyperlink to open the Sample View for the selected sample.



Evaluate results: Library Search Report

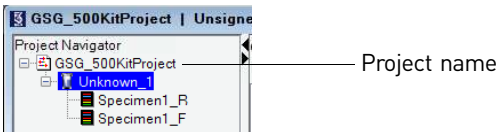
The next step in the evaluation process is to check the library matches against each specimen consensus sequence.

Use the Library Search Report to:

- Identify the unknown organism.
- Assess the accuracy of the identification.
- Examine results for all matches.
- View phylogenetic relationships between the unknown and the top matches in a phylogenetic tree.
- Determine if a match was made against a validated (proprietary) library or a custom library.

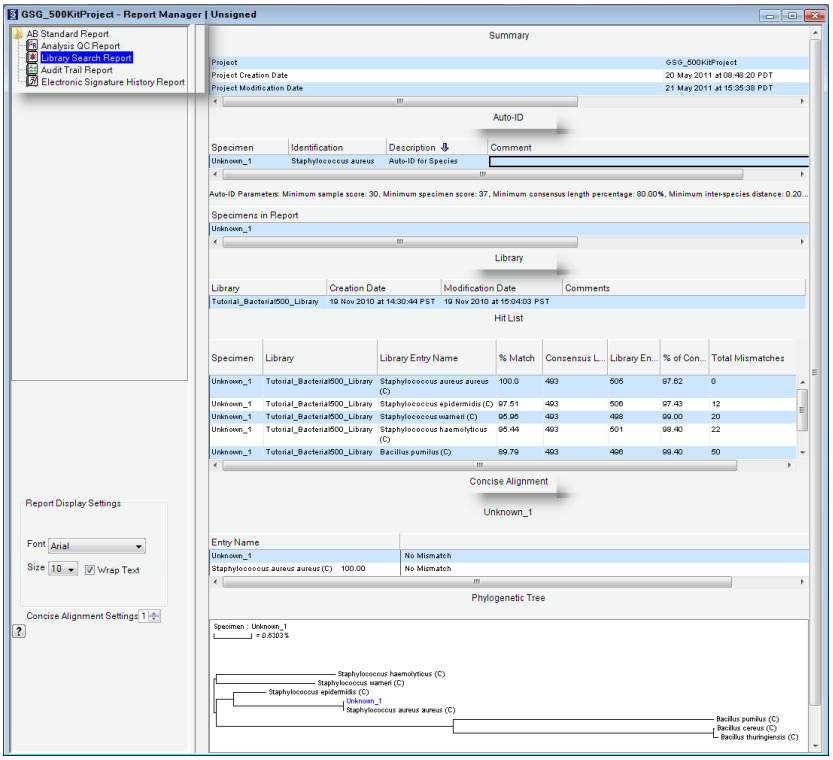
View the Library Search Report

1. In the Project Navigator pane of the Project window, select **GSG_500KitProject**.



2. Select **Analysis** ▸ **Report Manager** or click (**Report Manager**).

3. In the Reports navigation pane, select **Library Search Report**.



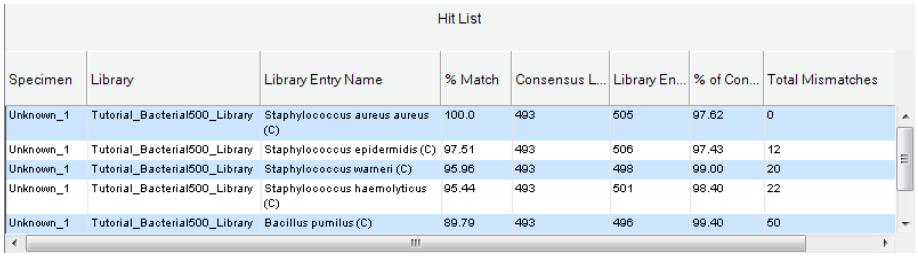
4. *(Optional)* Export or print reports (see page 89), or export the project (see page 90).

Interpret the Auto-ID section

For information, refer to "Interpret the Auto-ID section" on page 82.

Interpret the Hit List section

In the Hit List section (shown below), the report lists for each specimen:



Column	Description
Specimen	The name of the specimen.
Library	The name of the library searched for possible matches.
Library Entry Name	An entry found in the library that possibly matches the consensus sequence.
% Match	Indicates how closely the unknown isolate matches the top library sequence (Top Match). See "% Match" on page 84.
Consensus Length	The number of bases of the consensus sequence.
Library Entry Length	The number of bases of the library sequence entry.
% of Consensus Length	The amount of overlap between a consensus sequence and a library entry sequence. See "% of Consensus Length" on page 111.
Total Mismatches	The number of bases in the consensus sequence that do not match the library sequence.

Interpret the Concise Alignment section

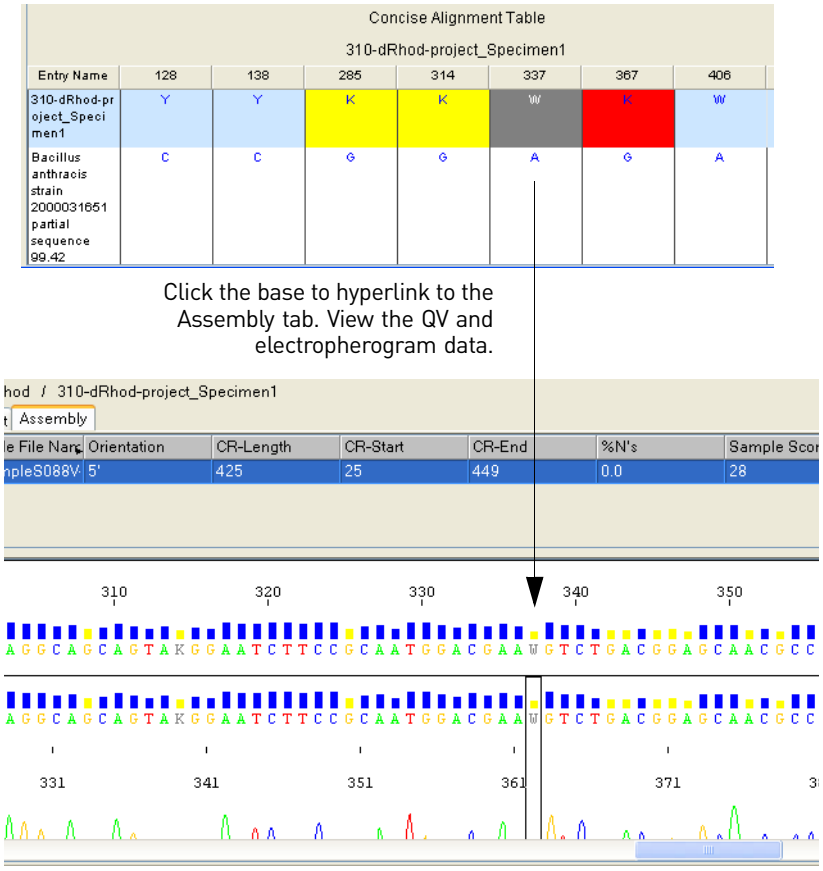
In the Concise Alignment section (shown in image above), the report lists for each specimen with a % Match <100:

- The position at which each mismatch occurred. The position numbers displayed are the internal index numbers assigned to the consensus sequence bases in the MicroSEQ ID software. Position 0 is the first base in the consensus sequence.
- The base called in the consensus sequence for that position. Base letter colors correspond to their Quality Value colors as set in the Quality Values pane of the Display Settings dialog box.

Note: Your Administrator can configure the number of top matches to display.

The Concise Alignment table shown below displays several positions that highlight low quality values. To display both the Project view and the Library Search concurrently, select **Window ▸ Tile**.

Click the base of interest; it turns grey, then the Assembly tab opens. For the example shown in the figure on the following page, the Assembly tab displays position 337, W's quality value location, and is shown tiled with the Concise Alignment table.



Interpret the Phylogenetic Tree section

For information on creating a phylogenetic tree and using the tree diagram, see page 95.

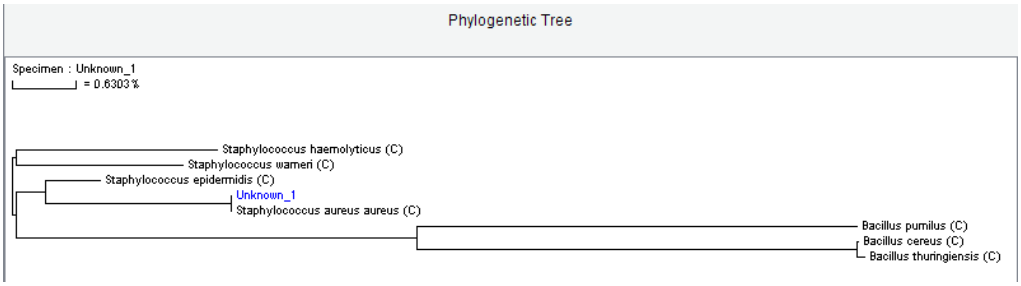
In the Phylogenetic Tree section (shown in image above), for each specimen in the Hit List, the unrooted phylogenetic tree displays:

- **Specimen** – Name of the specimen; a MicroSEQ ID software component that contains the sample data from a single colony or pure culture and a part of the project that describes the relationship of individual samples to each other.
- **Scale bar** (⌊_____⌋ = X.XX%) – The scale bar represents the genetic distance % between the two closest library matches, fit to the viewable area of the tree. The scale length updates if you zoom the tree diagram or print the Library Search report (see page 99).
- **Genetic Distance (%)** – The Genetic Distance represents how closely one species in the tree is related to another species. Genetic Distance is calculated based on the branch lengths between the species. You can view the Genetic Distance between individual species in the tree (see page 99).
- **Phylogenetic Tree diagram** – The phylogenetic tree is a graph composed of nodes and branches, where the external nodes represent the species. The unrooted tree used in the MicroSEQ ID software represents the evolutionary relatedness between organisms based on the sequence of the 16S rRNA Gene. Using the

neighbor-joining distance matrix method, the tree is constructed by successive clustering of lineages, setting branch lengths as the lineages join. Entries with synonyms in the proprietary bacterial libraries are denoted with *. Matches made against custom libraries are flagged in the tree diagram with "(C)" (see page 99).

Note: There are examples where two different species have the same rRNA gene sequence and where two different rRNA gene sequences belong to two different strains of the same species.

For example, in the unrooted Phylogenetic Tree shown below, the specimen Unknown_1 is the same species as its closest library match, but the specimen may be a different serotype, biovar, strain, and so on.



Note: The ordering of the specimens may vary between the Phylogenetic Tree Viewer and the Library Search Report, though the relative distances will be the same.

Export or print reports

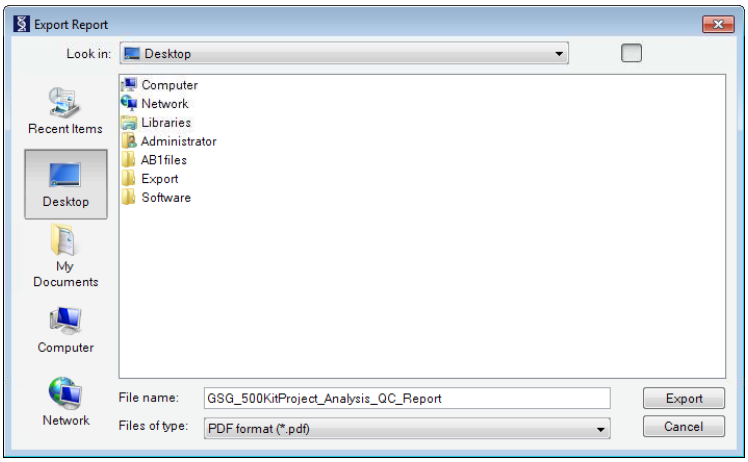
After analyzing the project and evaluating the results, you can export or print the following reports:

- Analysis QC Report
- Library Search Report
- Audit Trail Report
- Electronic Signature History Report
- A custom report designed by a Scientist

For this *Getting Started Guide*, follow the tasks below to export and print the Analysis QC Report for the GSG_500KitProject.

Export the report

1. Open the report you would like to export. For the purposes of this tutorial, with the Analysis QC Report open, go to the file menu bar and select **File ▸ Export ▸ Report** to open the Export Report browser.



2. Navigate to a destination for the exported file.

   Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed.

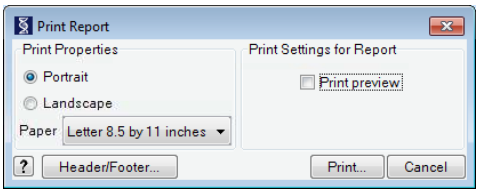
3. Type a name for the exported file (GSG_500KitProject_Analysis_QC_Report). Do not use illegal characters.

4. Select a file type from the drop-down menu:
   - PDF
   - HTML
   - XML
   - TXT

5. Click **Export**.

Print the report

1. Open the report you would like to export. For the purposes of this tutorial, with the Analysis QC Report open, go to the file menu bar and select **File ▸ Print** to open the Print Report dialog box.



2. Select the paper orientation and size.

3. (*Optional*) Click **Header/Footer** to customize the header and footer.

4. (*Optional*) Click **Print preview** to see the report before printing.
5. Click **Print**.
6. Select a printer, specify the page range, then click **OK**.

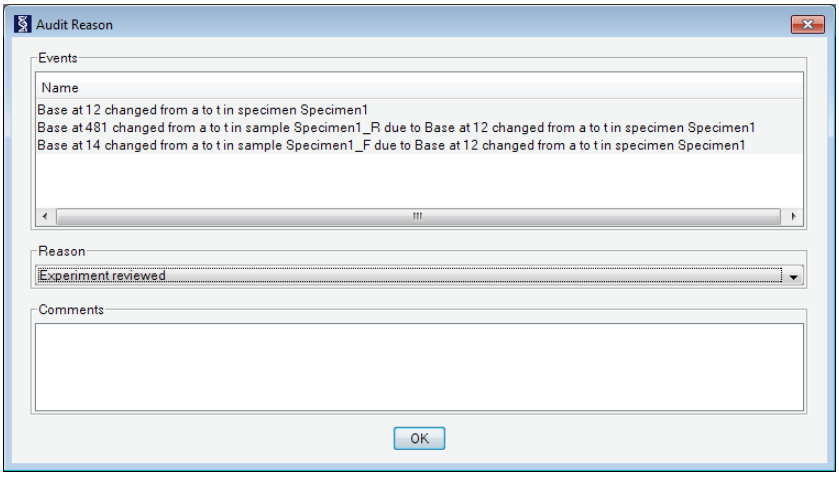
Manage your audit records

Select an audit reason

You are prompted in an Audit Reason dialog box to select a reason whenever an auditable event occurs, such as the creation of a MicroSEQ ID Run (see page 49). This action is recorded in the Audit Trail Report. In the event you do not receive a prompt, check with your Administrator to see whether your audit has been set to silent rather than prompt. For information on setting up the audit trail, see page 31.

For a list of auditable events or for information on archiving and restoring application level audit records, refer to the *Help system*. Press **F1**, or select **Help ▸ Search**.

1. After performing an auditable event, select the **Enable** check box to enable the Action List.
2. Select a reason for the event from the Reason drop-down list in the Audit Reason dialog box. (In this example, the event is "Experiment Reviewed".)
3. *(Optional)* Enter a comment.
4. Click **OK**. Your action is recorded in the Audit Trail Report.



Electronically sign your work

About electronic signatures for users

Note: The information shown below is not representative of the tutorial data and should be considered an example only.

Note: The feature is turned off by default. For details on how your Administrator sets up electronic signature, see page 33.

You, the user, can activate the eSig system two ways by:

- Performing an event that prompts an Electronic Signature Verification dialog box, as described above.
- Selecting **Tools ▸ Electronic Signature** or clicking the eSig icon (last icon on the right in the Analysis tool bar): 

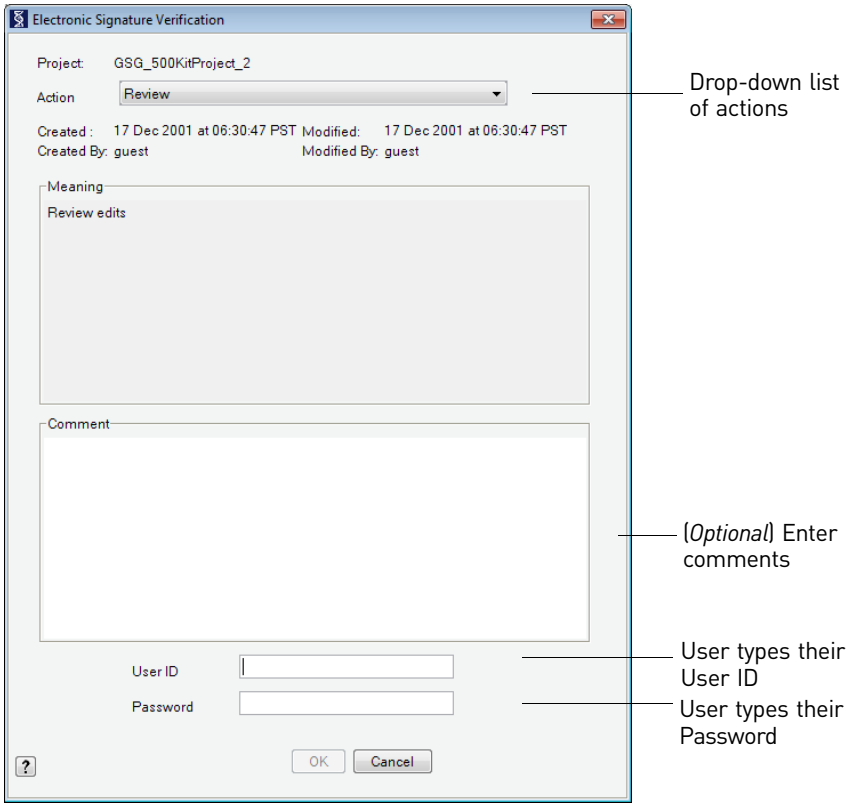
.

You must then authenticate or "sign" the project by typing the User ID and Password of your account.

- If you enter the correct User ID and Password, the software performs the requested eSig event, records the electronic signature transaction, saves the project, displays an Electronic Signature window informing you that the Electronic Signature verification was successful, and updates the project eSig status (shown in the title bar): 

.
- The eSig system tracks the number of failed login attempts. If you enter an invalid User ID or Password in the Electronic Signature Verification dialog in three attempts within a minute, the software locks you out for 3 minutes (default setting) or a time specified by your Administrator.

If you perform an action, such as saving a project, that requires an electronic signature, the following Electronic Signature Verification dialog box opens:

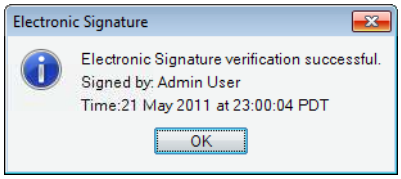


Note: The MicroSEQ ID software tracks the number of failed login attempts.

Sign Your Work

To complete the Electronic Signature Verification dialog box:

1. Select an action from the Action drop-down list.
2. Enter any comments, your User ID, and your Password.
3. Click **OK**. A message is displayed either stating your verification was successful or you that do not have electronic signature privileges.



4. Click **OK** to close the message. If you do not have electronic signature privileges, another user who does have them may sign with their User ID and Password, then click **OK**.
5. Click **OK** to close the Electronic Signature Verification dialog box.

A Create a phylogenetic tree

This appendix covers:

- Overview 96
- Search libraries to add sequences to tree 96
- Search projects to add specimens to tree. 97
- Create a phylogenetic tree. 98

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

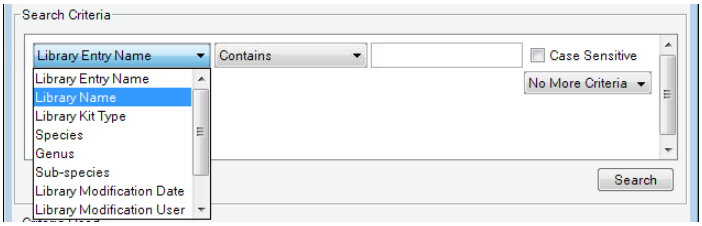
Overview

This tutorial procedure familiarizes you with the Phylogenetic Tree Viewer as you search for specimens and create a phylogenetic tree. For information on viewing a phylogenetic tree from the Library Search Report of an open project, see page 88.

Search libraries to add sequences to tree

In this tutorial you will create a specific type of unrooted phylogenetic tree using the neighbor-joining distance matrix method, which specifies the evolutionary relationships, and therefore sequence relationships, between neighboring species without defining the evolutionary path among all specifies.

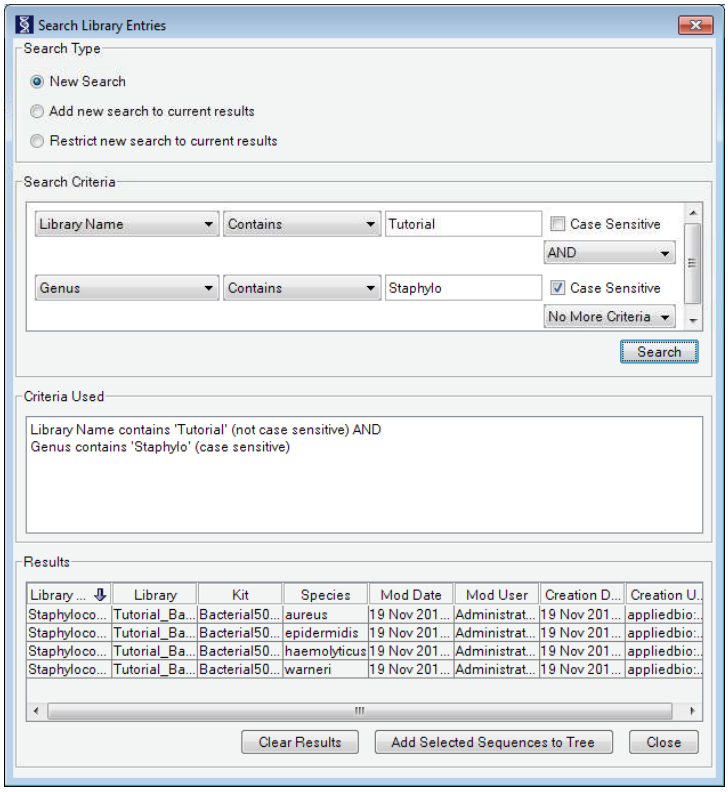
1. Select **Tools** ▸ **Phylogenetic Tree** to open the Phylogenetic Tree Viewer.
2. Click **Search Libraries** to open the Search Library Entries dialog box.
   a. Select **Library Name** from the Library Entry Name drop-down list.



   b. Select **Contains** in the Contains drop-down list, then in the search field, at right enter **Tutorial**.
   c. Select **AND** in the No More Criteria drop-down list.
   d. For the second line of criteria:

Parameter	Description
Library Entry Name	Select **Genus**.
Contains	Select **Contains**.
Search Field	Enter **Staphylo**.
Contains	Select **Case Sensitive**.

e. Click **Search** to view the criteria used and the results. Your criteria and search results should look like the one below:



f. Select all four results and click **Add Selected Sequences to Tree**.

Proceed to "Create a phylogenetic tree" on page 98 to evaluate the search results.

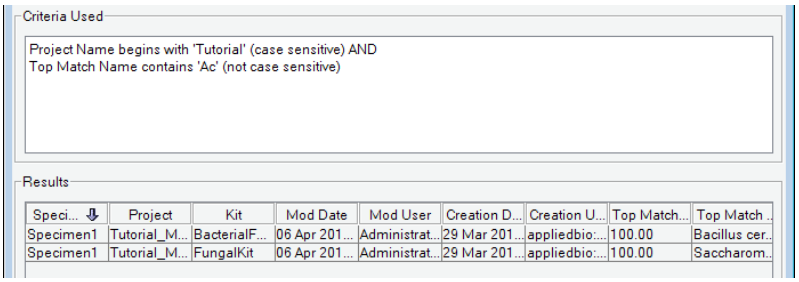
Search projects to add specimens to tree

1. Select **Tools ▸ Phylogenetic Tree** to open the Phylogenetic Tree Viewer.
2. Click **Search Projects** to open the Search Specimens dialog box with project search criteria.
3. Search for a specimen using project search criteria:
   a. Select **Project Name** from the Specimen Name drop-down list.
   b. Select **Begins With** in the Contains drop-down list, then in the search field at right, enter **Tutorial**.
   c. Select **Case Sensitive**.
   d. Select **AND** in the No More Criteria drop-down list.
   e. For the second line of criteria:

Parameter	Description
Library Entry Name	Select **Top Match Name**.

Parameter	Description
Contains	Select **Contains**.
Search Field	Enter **Ac**.

f. Click **Search** to view the criteria used and the results. Your criteria and search results should look like the one below:



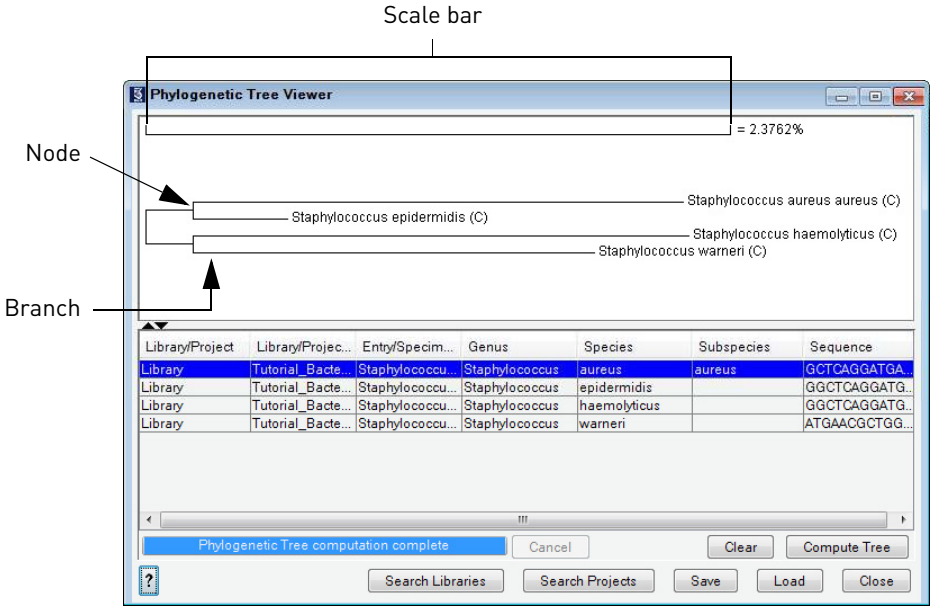
4. Select your result and click **Add Selected Specimens to Tree**.

Proceed to "Create a phylogenetic tree" below to evaluate the search results.

Create a phylogenetic tree

Create a phylogenetic tree by continuing the following tutorial procedure.

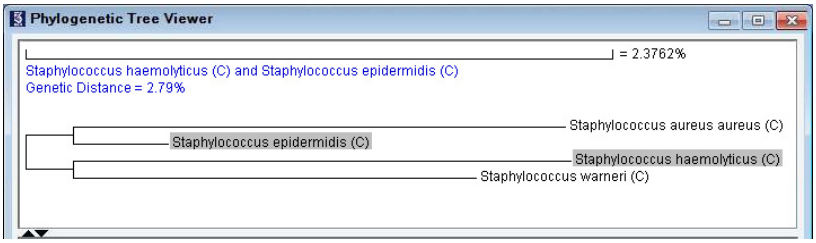
1. Click **Compute Tree**. The Tree Computation dialog box opens, then click **OK**.
   Your screen should look similar to the one below once the computation is complete:



The tree diagram includes:

- A scale bar, representing the genetic distance % (step 2 below) between the two closest sequence entries in the Search Results pane, fit to the viewable area of the tree.
- A graph composed of nodes and branches, where the external nodes represent the bacterial species.

2. Click the species name **Staphylococcus epidermidis** in the tree, then click **Staphylococcus haemolyticus** as your second selection to view the Genetic Distance % (see page 88) for the selected species in the tree diagram, calculated using the branch lengths between the two species.



Note: To change your selections, click a species name again to deselect it, then select another name. If you select a third species name in the tree, the first name you highlighted is automatically deselected so only a pair of names are active at any one time.

3. Experiment with opening and viewing the tree:
   - Zoom the tree diagram as needed (see *Help system* for more information).
   - Select **File** ▸ **Print** to print the tree diagram (see "Print the report" on page 90).

     Note: The scale bar length shown on the printed page depends on the viewable area of the tree that can fit on the page size selected. Any Genetic Distance % you select to view between individual species in the tree is not included in the printed tree.

   - Click **Save** to open the Save Phylogenetic Tree dialog box and save the tree (but not the results pane). You can name the file, browse to a folder, and save it as a .xml file.
   - Click **Clear** to clear the tree and the search results pane.
   - Click **Load** to open the Load Phylogenetic Tree dialog box. You can browse to your saved .xml file, select it, then click **Load** to display the phylogenetic tree in the Phylogenetic Tree Viewer.

B Maintain the Data and Software

This appendix covers:

- Maintenance schedule 102
- Data maintenance 102
- Manage the 3500 Series Data Collection software licenses. 106

For more detailed descriptions of the information and tasks contained in this *Getting Started Guide*, refer to the *MicroSEQ® ID Help system* supplied as part of the MicroSEQ ID software. Press **F1**, or select **Help ▸ Search**.

Maintenance schedule

This section lists common tasks required to maintain the MicroSEQ ID software on your system.

Review the recommended maintenance tasks listed below, then perform the tasks as scheduled.

User Role	Task	Frequency	For information, see...
Administrator	Export user accounts	After system validation/qualification, then per SOP	"Export or import user accounts" on page 102
	Export authentication settings		"Export or import authentication settings" on page 103
	Export the Application Audit Report	Weekly or per SOP	"Export the Application Level Audit Report" on page 104
	Archive and purge application audit records	Weekly or per SOP	"Manage application level audit records" on page 103
Administrator or Scientist	Export projects	Weekly or per SOP	"Export projects" on page 105
	Delete projects		"Delete projects" on page 106

Data maintenance

Export or import SAE parameters

IMPORTANT! You must have Administrator privileges to perform the following tasks.

Export or import user accounts

You can export or import user accounts from one computer to another. For example, an Administrator can set up user accounts (page 29) for many users, then select all the user account files and export them to other systems that use the MicroSEQ ID software.

1. Log in as Administrator.
2. Click **SAE Manager** on the MicroSEQ ID software main window.
3. In the SAE Manager dialog box, select the **User** tab.
4. Shift + Click or Ctrl + Click to select the user(s) to export, then click **Export**.
5. Complete the information in the Export User dialog box:
   a. Navigate to a destination for the exported file.

      Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed. To set up a default directory for importing/exporting, see page 36.

b. Type a name for the exported file using the .usr.ctf file extension. Do not use illegal characters.

c. Click **Export**.

Export or import authentication settings

You can export or import the authentication settings from one computer to another. For example, an Administrator may want to set up authentication information for many users (see page 31), then select all the files and export them to other systems using MicroSEQ ID software.

1. Log in as Administrator.
2. Click **SAE Manager** on the MicroSEQ ID software main window.
3. In the SAE Manager dialog box, select the **Authentication** tab.
4. Click **Export**, then complete the information in the Export Application Configuration dialog box:
   a. Navigate to a destination for the exported file.

      Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed. To set up a default directory for importing/exporting, see page 36.

   b. Type a name for the exported file using the .cfg.ctf file extension. Do not use illegal characters.
   c. Click **Export**.

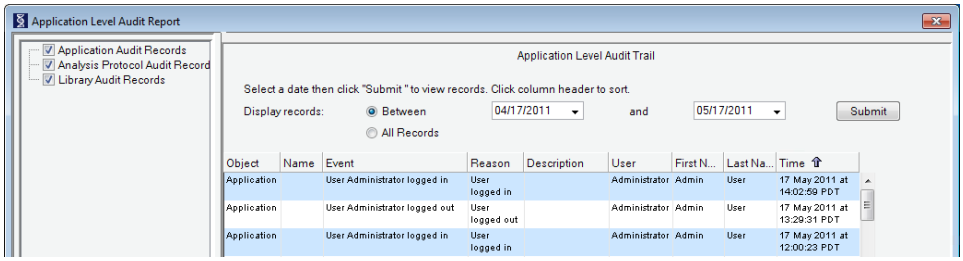
Manage application level audit records

You can use the Application Level Audit Report to view, export, archive, and restore audit records for all users at the application level, rather than from within a specific project.

For more information on the Application Level Audit Report, refer to the *Help system*.

View the Application Level Audit Report

1. Log in as Administrator.
2. Click **SAE Manager** on the MicroSEQ ID software main window.
3. In the SAE Manager dialog box, select the **Audit Trail** tab.
4. Click **Application Level Audit** to open the Application Level Audit Report window.



5. Do any of the following:
   - View audit records by date; enter date search criteria at top of right pane
   - View audit records by type; select the **Object** column heading
   - Customize the report display; change current Report Display settings in bottom left pane (not shown above)
   - Export (see below), archive (see page 104), or restore (see page 105) audit records

Export the Application Level Audit Report

As scheduled (see page 102), you can export application level audit records to a file for additional manipulation and reporting outside the MicroSEQ ID software.

1. Open the Application Level Audit Report (see page 103).
2. In the left pane of the report window, select the audit records to export:
   - Application Audit Records
   - Analysis Protocol Audit Records
   - Library Audit Records
3. Click **Export**, then complete the information in the Export Report dialog box:
   a. Navigate to a destination for the exported file.

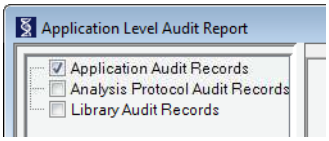
      Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed. To set up a default directory for importing/exporting, see page 36.

   b. Type a name for the exported file. Do not use illegal characters.
   c. Select the export format (*.pdf is the default).
   d. Click **Export**.

Archive application audit records

Archiving audit records saves the audit records to a .sma file and gives you the option to purge (delete) audit records. Archive and delete as scheduled (see page 102) to clear disk space. Save the archived audit record for future import back into the software as needed (see below).

1. Select **Tools** ▸ **SAE Manager**.
2. Select the **Audit Trail** tab, then click **Application Level Audit**.
3. To enable the archive function, select *only* the **Application Audit Records** check box in the left pane of the report window.



4. Click **Archive**, then complete the information in the Archive Application Level Audit Records to File dialog box:
   a. Navigate to a destination for the exported file.

      Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed. To set up a default directory for importing/exporting, see page 36.

   b. Type a name for the exported file using the .sma file extension. Do not use illegal characters.
   c. Click **Archive**. A message is displayed when the archive is complete.
5. Specify whether to keep or delete system audit records after archive. If you specified to delete records after archive, you are prompted to confirm deletion.

Restore application audit records

1. Open the Application Level Audit Report (see page 103).
2. To enable the restore function, select *only* the **Application Audit Records** check box in the left pane of the report window.
3. Click **Restore**, then complete the information in the Restore Application Level Audit Records from File dialog box:
   a. Navigate to the .sma file to import.
   b. Click **Restore**. A message is displayed when the import is complete.

   Note: The restored system audit records will display the name of the computer that originally generated the audit events.

Manage projects

IMPORTANT! You must have Administrator or Scientist privileges to perform the following tasks.

Export projects

Back up your projects as scheduled (see page 102) by exporting them to a designated folder. Save the projects for future import back in to the software as needed.

1. Close any open projects.
2. Select **Tools ▸ MicroSEQ ID Manager**. The view opens to the Projects tab by default.
3. Shift + Click or Ctrl + Click to select the project(s) to export, then click Export.

4. Complete the information in the Export Project dialog box:
   a. Navigate to a destination for the exported file.

      Note: Do not export to the AppliedBiosystems folder where the MicroSEQ ID software is installed. To set up a default directory for importing/exporting, see page 36.

   b. If you selected only one project to export, type a name for the exported file using the .prj.ctf file extension. Do not use illegal characters.
5. Click **Export**. If you selected multiple items to export, the software exports all selected items into separate files.

Delete projects

Delete your projects as scheduled (see page 102) to clear disk space.

1. Close any open projects.
2. Select **Tools ▸ MicroSEQ ID Manager**. The view opens to the Projects tab by default.
3. Shift + Click or Ctrl + Click to select the project(s) to delete, then click **Delete**. You are prompted to confirm deletion.

   Note: You cannot delete a project if it is associated with an open MicroSEQ ID Run (see page 47).

Manage the 3500 Series Data Collection software licenses

IMPORTANT! If you replace or add a network card in the computer running the software, add a network card, or relocate the software to a new computer, contact Applied Biosystems to update your license for the new network card or computer.

Note: This information applies to installation of MicroSEQ® ID Microbial Identification Software Version 3.0 on 3500/3500xL Genetic Analyzers only (System installation); for more information, see "System vs. Lite" on page 8.

For information on the MicroSEQ® ID registration number, see "Register the MicroSEQ ID software" on page 20.

Obtain and activate a software license

The 3500 Series Data Collection Software v1.1 requires a license to run.

This task is typically performed by the Applied Biosystems service representative during installation of the instrument.

1. Ensure that all network cards in the computer are enabled.

   IMPORTANT! You can run the 3500 Series Data Collection software using only the network cards enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

2. Display the Software Activation dialog box by starting the 3500 Series Data Collection software.
3. Obtain the license key. The license key is provided on the 3500 Series Data Collection Software v1.1 CD case, or in an email from Applied Biosystems. Make a record of it.
4. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen.
5. Contact your Life Technologies service representative for information on adding or renewing your 3500 Series Data Collection Software license.

Renew your license

The following information is needed at the time of license renewal:

- **Installation date** – You will need this date when you contact Life Technologies in order to obtain a new license file. The software license needs to be renewed each year.
- **Email address used during license activation** – You must use the same email address to renew the software license when it expires.

IMPORTANT! Be sure to record the requested information in the fields below.

Installation Date:
Email Address Used During License Activation:

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Instrument or Software	Document	Part number
MicroSEQ® ID Microbial Identification Software Version 3.0	MicroSEQ® ID Microbial Identification Software Version 3.0 *Quick Reference Card*	4465103
MicroSEQ® ID Microbial Identification System Workflow	MicroSEQ® ID *Microbial Identification System Workflow Quick Reference Card*	4465104
Applied Biosystems 3500 and 3500xL Genetic Analyzers	*Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*	4401661
Applied Biosystems 3130 and 3130xl Genetic Analyzers	*Applied Biosystems 3130/3130xl Genetic Analyzer Getting Started Guide*	4352715
MicroSEQ® ID Microbial Identification Software Version 3.0 Help	*MicroSEQ® Microbial Identification Software Version 3.0 Help*	4461037
MicroSEQ® ID Software v2.2 and KB Basecaller 1.4	*MicroSEQ® Microbial Identification System analysis and interpretation guidelines*	CO16970

Using the MicroSEQ® ID Microbial Identification Software v3.0 Help

The MicroSEQ ID software has a *Help system* that describes how to use each feature of the user interface. It is supplied on the software CD-ROM. When the MicroSEQ ID software is installed and running, access the *Help system* by doing one of the following:

- Click ? in the toolbar of the MicroSEQ ID software window.

  Note: The ? is not present in all windows and dialog boxes.

- Select **Help ▸ Contents & Index**.
- Press **F1**.

You can use the *Help system* to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.appliedbiosystems.com/sds**

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Glossary

% of Consensus Length	The amount of overlap between a consensus sequence and a library entry sequence. % of Consensus Length = (The consensus sequence length) / (The library entry length) X 100
% Match	Indicates how closely the unknown isolate matches the top library sequence (Top Match). % Match = (1 – (The sum of mismatch penalties) / (The sum of the quality values for all the bases in the aligned consensus sequence)) X 100 See "% Match" on page 84 for more information.
Aligned consensus sequence	The consensus sequence after pairwise alignment with the library sequence. May contain gaps.
Analysis protocol	A file that specifies the parameters for basecalling and data analysis.
Auto-ID	A scoring term that uses the quality values, scores, and % Match to automatically assign a specimen identification from a library (proprietary or custom).
Basecalling	A Data Collection software protocol that defines the analysis settings used for sequencing applications.
Clear range	The region of the sequence that is basecalled and used to generate the consensus sequence. The clear range is determined by settings in the analysis protocol.
Consensus quality value (consensus QV)	A per-base estimate of the accuracy of the consensus-calling algorithm.
Consensus sequence	The sequence assembled by the MicroSEQ ID software from all sample sequences in the specimen that meet the clear range and filtering thresholds. The consensus sequence is compared to a library of sequences to generate the closest matches.
Instrument Protocol	A Data Collection software protocol that defines the application type and instrument settings used in the MicroSEQ ID Run.
Kit	One of the MicroSEQ chemistry kits supported by the software: MicroSEQ® 500 16S rDNA Bacterial Identification Kit (Fast and standard chemistry) MicroSEQ® Full Gene 16S rDNA Bacterial Identification Kit MicroSEQ® D2 rDNA Fungal Identification Kit (Fast and standard chemistry)

Library	A collection of known sequences that are derived from known microorganisms.
Library, custom	A library created by the user.
Library match	A consensus sequence that agrees closely with a sequence in a library.
Library, proprietary	A library created and maintained by Applied Biosystems. Three proprietary libraries, validated for accuracy, are available for purchase from Applied Biosystems for use with the MicroSEQ ID software. Sequences in proprietary libraries are not viewable or editable. Proprietary libraries cannot be deleted.
MicroSEQ ID Run	A container for setup, data collection, and analysis of project data generated using Applied Biosystems 3500/3500xL (3500 Series) Genetic Analyzers.
MicroSEQ ID Run template	A type of MicroSEQ ID Run that is used to create new runs with similar properties. Save time by saving the setup data in an existing MicroSEQ ID Run as a run template and using the template to create future runs. **Note:** The template itself cannot be run.
Project	A container for specimen(s) where the specimen's sample files generate a single assembly and consensus sequence. This consensus sequence is then compared with the sequences in a library. All analysis in the MicroSEQ ID software occurs in a project.
Results group	A Data Collection Software setting that is used to name, sort, and customize the folders in which sample data files are stored.
Sample file	A .ab1 file that contains the output of a single capillary from a DNA capillary electrophoresis instrument.
Sample quality value (sample QV)	A per-base estimate of basecaller accuracy.
Specimen	A container for sample data from a single colony or pure culture and a part of the project that describes the relationship of individual samples to each other.
Specimen score	Average consensus QV.
Start and End delimiters	Project setting parameters that determine the portion of the sample or sample file name used to create the specimen name. Characters that follow the Start delimiter and precede the End delimiter are used for the specimen name. For Example, if the sample file name is MSID_A03_C_781F.ab1 and you specify _ (underscore) as the Start delimiter and _ (underscore) as the End delimiter, the specimen name created is A03.
Unknown isolate	Bacterial or fungal DNA isolated from a single colony or pure culture.

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www.lifetechnologies.com/support