

MicroSEQ® ID Analysis Software v2.2

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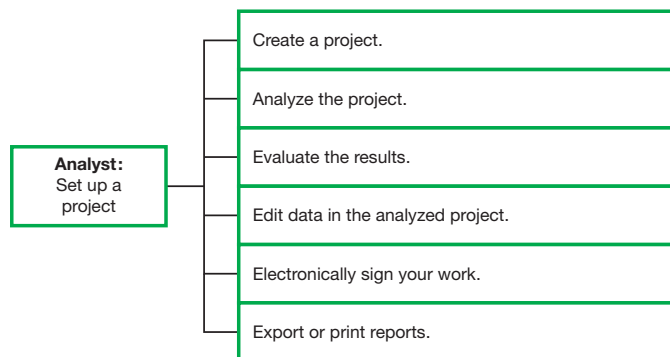
MicroSEQ® ID Analysis Software

MicroSEQ® ID Analysis Software Version 2.2 is a tool for microbial identification of bacteria and fungi. The software analyzes data generated using a MicroSEQ® chemistry kit and an Applied Biosystems capillary-based genetic analyzer.

For information on this product, go to <http://www.microseq.com>.

Analyst Workflow

This card provides the Analyst with a brief description of the tasks required to create and analyze a project in the MicroSEQ ID Analysis software. These tasks are shown in the following workflow:



Use this card when you need quick reminders to help you create and analyze your projects. For more detailed information, refer to the MicroSEQ ID Analysis Software Online Help. Launch the MicroSEQ ID Analysis Software and press **F1** or select **Help ▶ Contents & Index**.

To access context sensitive help in the software application, click located on selected windows and dialog boxes.

Terms Used in This Card

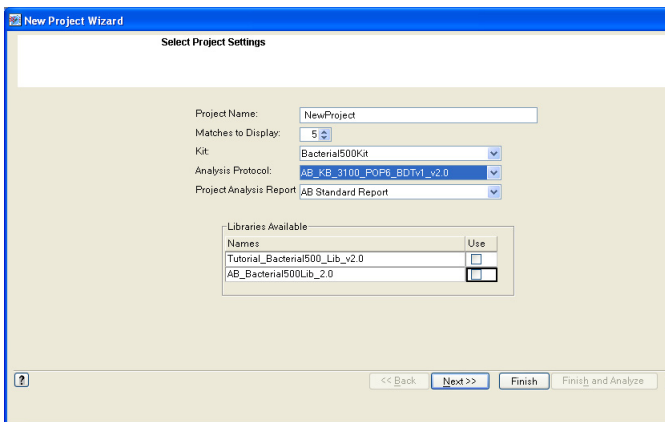
Term	Definition
Analysis protocol	Specifies the parameters for basecalling and data analysis.
Consensus quality value (consensus QV)	A per-base estimate of the accuracy of the consensus-calling algorithm.
Consensus sequence	The sequence assembled by the MicroSEQ® ID Analysis software from all the specimen's sample sequences that meet the clear range and filtering thresholds. The consensus sequence is compared with a library of sequences to generate the closest matches.
Kit	One of the MicroSEQ chemistry kits supported by the software: <ul style="list-style-type: none"> • MicroSEQ® 500 16S rDNA Bacterial Identification Kit • MicroSEQ® Full Gene 16S rDNA Bacterial Identification Kit • MicroSEQ® D2 rDNA Fungal Identification Kit
Project	A container for related specimens. All analysis in the MicroSEQ ID Analysis software occurs in a project.
Sample file	A data file generated by a sequencer. It is part of a set of data files that are assembled by the MicroSEQ ID Analysis software to generate the consensus sequence.
Sample quality value (QV)	A per-base estimate of basecaller accuracy.
Specimen	A container that holds the sample data (sample files) from a single bacterial or fungal unknown isolate.
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 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.

Term	Definition
% Match	<p>How closely the unknown isolate matches the top library sequence (Top Match).</p> <p>$\%Match = (1 - (\text{The sum of mismatch penalties}) / (\text{The sum of the quality values for all the bases in the aligned consensus sequence})) \times 100$</p> <p>Mismatch penalty = (The mismatch factor) X (The quality value of each mismatched base in the aligned consensus sequence)</p> <p>Mismatch factors are assigned:</p> <p>0.5 for half-match of a mixed base aligned with a pure base and the mixed base contains the pure base</p> <p>1.0 for mismatch</p> <p>The % match calculation uses quality values to weight the likelihood of a true mismatch.</p> <p>For example, a low quality mismatched base has less weight than a high quality mismatched base when the two are at the same position.</p>

Setting Up a Project

Creating a project using the New Project Wizard

1. Select **File** ► **New Project** to open the Select Project Settings dialog box in the New Project Wizard.



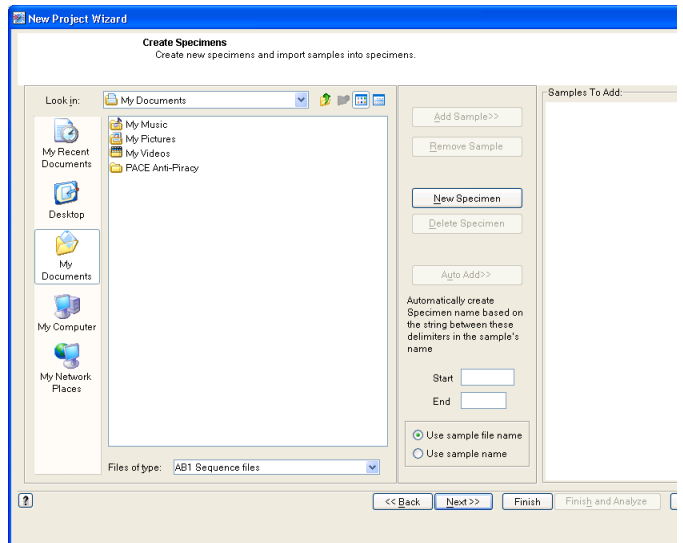
2. Complete the fields in the dialog box:
 - **Project Name:** Type a name for the project.
 - **Matches to Display:** Select the number of library matches to display in the Library Search Results Project View. You can choose to display from 3 to 20 of the closest matches.
 - **Kit:** Select the kit used to generate the sample data in the project.
 - **Analysis Protocol:** Select the analysis protocol to use for the analysis.
 - **Custom Report:** Select either the AB Standard report or a custom report type to display your data.

- **Libraries Available:** Select the libraries to use for the analysis. The drop-down menu lists only those libraries that specify the kit you selected above.

Note: Search the Applied Biosystems libraries and any of your own custom libraries at the same time. For optimal performance, Applied Biosystems recommends searching the Applied Biosystems libraries first.

Note: Do not search or select the tutorial libraries – they are specific for tutorial data only.

3. Click **Next** to open the Create Specimens dialog box.



4. Navigate to the directory and folder containing the sample(s) of interest by clicking (folder) icon.
5. Add samples to the project automatically or manually.


Note: To add samples automatically, the sample files must be located in the same folder and have a common string in their sample name or sample file name. If sample files are not located in the same folder and do not have a common string, manually add samples.

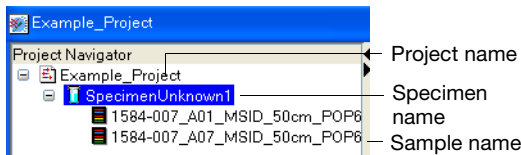
If you are adding samples...	Then...
Automatically	<ol style="list-style-type: none"> 1. Select the sample file(s) of interest. 2. Type the Start and End delimiters for the specimen name. 3. Select Use Sample File Name or Use File Name. 4. Click Auto Add.
Manually	<ol style="list-style-type: none"> 1. Click New Specimen. 2. Select the sample file(s) of interest. 3. Click Add Sample.

- Click **Next** to view the project setup summary. To make any changes to the project settings, click **Back**.

Analyzing the project

- Select when you want to analyze:
 - Now** – Click **Finish and Analyze** to save and analyze the project.
 - Later** – Click **Finish** to save the project and display the Project View.

When you are ready to analyze the project, click  in the Project window toolbar.
- In the Project Navigator pane, select the way you want to view results. Select:
 - The project name to view the Library Search Results (that is, the % Match results for all the specimens).
 - The specimen name to view the Layout and Assembly Views for that specimen.



Interpreting Sample Quality Values

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). The table below lists the probability of basecall errors for several QVs.

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.

This table provides an abbreviated list. To view the probability for all QVs (ranging from 1 to 50), refer to the Online Help. Press **F1**, or select **Help ▶ Contents & Index**. Go to the Index tab and type **quality value**.

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%

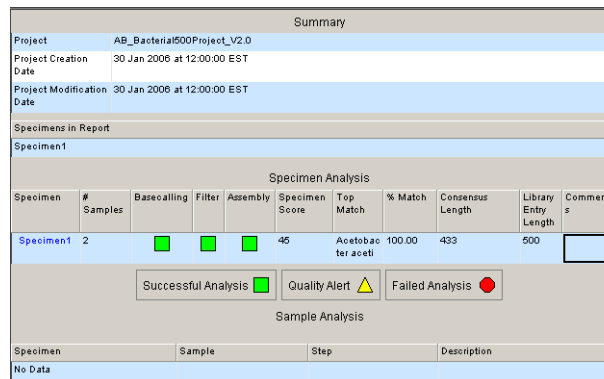
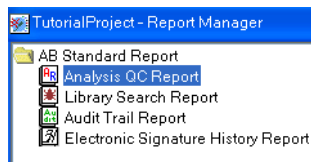
[‡] Denotes commonly used cutoff values for Low Confidence, Medium Confidence, and High Confidence ranges.




Evaluating the Results

Evaluating the Analysis QC Report

The Analysis QC Report provides the following information:

- Summary:** Displays project information and the specimens in the report.
 - Specimen Analysis:** Displays specimen name, number of samples in the specimen, status of the analysis steps (basecalling, filtering, and assembling), specimen score (average consensus QV), the top library match, and the % Match.
 - Sample Analysis:** Displays sample analysis errors and details.
- Select **Analysis ▶ Report Manager**, then select **Analysis QC Report**.



- Check the analysis step columns (Basecalling, Filter, and Assembly) for analysis results (the , , or  icons).
- Check the values displayed in the Specimen Score column. Values above 30 indicate high-quality values.
- Check the values displayed in the % Match column.
- Check the consensus and library entry length.
- Add comments to the comments field.

Evaluating the Library Search Report

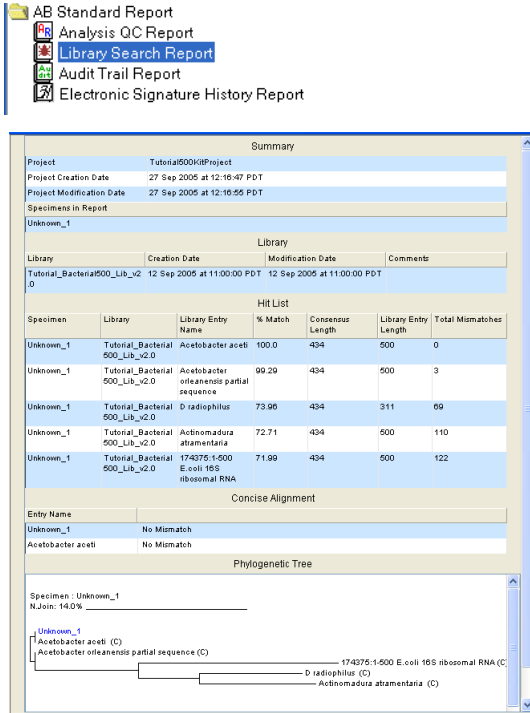
The Library Search Report provides the following information:

- Summary:** Displays project information and the specimens in the report.
- Library:** Displays the names of the libraries searched.
- Hit List:** Displays the library matches found, their % Match, the number of bases searched, and the number of bases in the consensus sequence that did not match the library sequence.

- **Concise Alignment:** For each specimen with a % Match <100, displays the position at which each mismatch occurred, the base called in the consensus sequence for that position, and the base present in the library for that position.
- **Phylogenetic Tree:** For each specimen listed in the Hit List, displays a phylogenetic tree diagram.

To evaluate results using the Library Search Report:

1. Select **Analysis ▶ Report Manager**, then select **Library Search Report**.



2. Examine results for all matches.
3. Determine if a match was made against a validated (proprietary) library or a custom library (c).
4. View concise alignment base variants by selecting **Window ▶ Tile** to display both the Concise alignment table and the Project view. Double-click the base in the Concise Alignment table to open the Assembly tab quality value location.
5. View phylogenetic relationships between the unknown isolate and the top matches in a Neighbor Joining Tree.

Editing Data in the Analyzed Project

After the project has been analyzed, edit the data if:

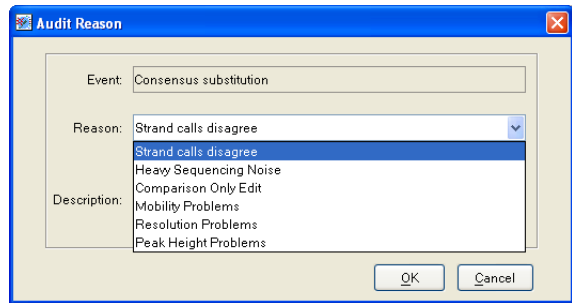
- An analysis step (basecalling, filtering, or assembling) fails.
- A Specimen Score value is below 30.
- Consensus sequence needs to be edited. For example, if a base is incorrectly called or there are missing bases.

Editing the consensus sequence

1. To open the Specimen View, click the specimen name in the:
 - Specimen Analysis pane in the Analysis QC report or
 - Project Navigator pane
2. Click the **Assembly** tab.
3. In the Project window toolbar, click and to display QV bars and click to display all electropherograms.
4. Check for medium (yellow) or low (red) QV bars. Medium or low values can decrease the Specimen Score.
5. Check for incorrectly called bases (for example, a noise peak called as a base or a true base peak not labeled).
6. Change, insert, or delete bases as needed.

Using the Audit Reason dialog box

If your administrator has enabled the audit trail function, then an event such as editing the consensus sequence prompts the Audit Reason dialog box to open.




1. Select a reason for the event (consensus substitution) from the drop-down list.
2. (Optional) Enter a description.
3. Click **OK**. Your action is recorded in the Audit Trail Report.

Viewing assigned analysis protocols

1. Open a project to access the Project Navigator tree pane, then select a level to view:

Level	Right-click on the...
Project	Project name. Select Display Analysis Protocol to view the analysis protocol settings.
Specimen	Specimen name. You will either: See a message that the Specimen is using the project level analysis protocol. or Select Display Analysis Protocol to view the analysis protocol settings.
Sample	Sample name. Select Display Last Successful Analysis Protocol.

2. Select another analysis protocol at the project level:
 - a. In the Project Navigator pane of the Project window, select the project name, then right-click to select **Properties and Settings**.
 - b. In the Project Analysis Protocol pane, select an analysis protocol from the drop-down list.
 - c. Click **OK**.
3. Select another analysis protocol at the specimen level:
 - a. In the Project Navigator pane of the Project window, select the specimen name, then right-click to select **Properties and Settings**.
 - b. Select:
 - **Use Specimen Analysis Protocol below**
 - An analysis protocol from the drop-down list
 - c. Click **Apply**.
4. Reanalyze the project: Click  in the Project window toolbar.

Creating a phylogenetic tree

Create a specific type of Phylogenetic tree called a neighbor joining tree.

1. Select **Tools ▶ Create Phylogenetic Tree** to open the Phylogenetic Tree Viewer.
2. Click **New Search**.
3. Click **Search Libraries** and/or **Search Projects** to search for specimens to add to the tree.
4. Click **New Search** (or the appropriate search type).
5. Select search criteria. If you are searching a:
 - Library, select a criterion from the Library Entry Name drop-down list.
 - Project, select a criterion from the Specimens Name drop-down list.

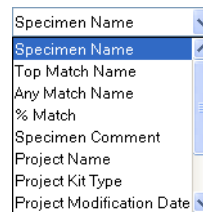
6. From the Contains drop-down list, select text to narrow the search, then enter an appropriate word in the right-side field.
7. (Optional) To refine your search, select:
 - **Case Sensitive**
 - **AND/OR** in the No More Criteria drop-down list
8. Click **Search**.
9. Select your results and click **Add Selected Specimens to Tree**.
10. Click **Compute Tree**.

You can clear the tree and results pane by clicking **Clear**.

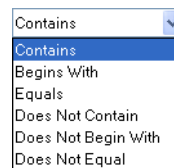
Searching for specimens to create new project

You can search for specimens from other projects to add to a new project using search criteria.

1. Select **Tools ▶ Search**.
 - a. Select a search criterion by scrolling the Specimen Name drop-down list and highlighting a criterion to select it.

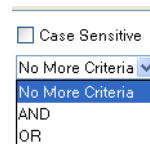


- b. Apply more search criteria:



Select text under **Contains** drop-down list and enter a value or text in the right-side field.

and/or



Select **Case Sensitive** and/or text in the No More Criteria drop-down list.

Electronically Signing Your Work

Your administrator activates the eSig feature for each user and events, prompting an eSig dialog box. Events that can be enabled:

- Save/Save As project
- Print
- Export Report
- Export Project

Activate the electronic signature system either of two ways:

- Perform an event that prompts an Electronic Signature Verification dialog box.
or
- Select a project, specimen, or sample in the Project navigator pane, then select **Tools ▶ Electronic Signature ▶ Sign** to open the Electronic Signature Verification dialog box.

Fill out 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. (Optional) Select **2 signatures** to have two people sign with their user ID and password. The Electronic Signature Verification dialog box adds another User ID and Password field for the second user.
4. Click **OK**. A message is displayed either stating you do not have electronic signature privileges or your verification was successful.
5. Click **OK** to close the message. If you do not have electronic signature privileges, another user who does have them may sign with his/her user ID and password, then click **OK**.
6. Click **OK** to close the Electronic Signature Verification dialog box.

Validating a Project

Validating a project

1. Select **File ▶ Open Project**.
2. Click **Tools ▶ Electronic Signature ▶ Verify**. A message states that the current electronic signature is **VALID** or **NOT VALID**.

Validating an invalid project

Your project is invalid if the project differs from the previous record of the project or lacks a signature.

If the project differs from the previous record, then save the project before you sign. A new eSig record is created for the saved project.

If the project lacks a signature, then sign the project:

1. Select **Tools ▶ Electronic Signature ▶ Sign** if you did one of the following:
 - Added or deleted specimens
 - Changed the type of library
 - Changed the number of matches to display
 - Edited bases
 - Reanalyzed the project
2. Click **OK**, then fill out the eSig Verification dialog box.
3. Click **OK**.

Exporting Projects

You may want to back up your projects by exporting them to a designated folder.

1. Close the project.
2. Select **Tools ▶ MicroSeqID Manager**, then select the **Projects** tab.
3. Select the project(s) to be exported, then click **Export**.
4. Browse to your designated folder.
5. Click **Export**, then click **Close**.

Selecting Reports to Export or Print

Viewing the report

1. In the Project window, select **Analysis ▶ Report Manager**.
2. To change to a different Project Analysis Report (AB Standard vs. a custom report):
 - a. Right-click on the project name, then select **Properties and Settings**.
 - b. In the Project Analysis Report drop-down list, select the desired report.
 - c. Click **OK**.
 - d. Select **Analysis ▶ Report Manager**.

Exporting the report

1. Select the report in the Report Manager navigation pane. Reports can be exported in the following formats:
 - html
 - pdf
 - xml
 - txt
2. Select **File ▶ Export ▶ Report**.
3. Navigate to and select the desired destination folder, then click **Export**.

Printing the report

1. Select **File ▶ Print**.
2. Select the desired print settings.
3. Click **Print ▶ OK**.

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