

Increasing identification accuracy with the MicroSEQ ID 16S rDNA 500 Supplemental Library

The Applied Biosystems™ MicroSEQ™ Microbial Identification System provides accurate species-level identification (ID) using the Applied Biosystems™ MicroSEQ™ ID 16S rDNA 500 Library v2013 (Cat. No. 4398220), which contains 2,100 bacterial reference sequences. The standard acceptance criteria used for percent-match ID are as follows:

Percent match	Result
<97	Below genus-level ID
97 ≤ x < 99	Genus-level ID
≥99	Species-level ID

Ordinarily, the MicroSEQ ID system–validated database will return species ID results with ≥99% ID matches. However, in some circumstances, an ID match of <99%, which is below the acceptance criterion for high-confidence species ID, may be delivered.

There are two primary reasons for an ID match of <99%:

- The relevant species is not included in the most current version of the validated library
- The species is either unknown, new, or not well described; or no reference sequence is available in public repositories



In cases of <99% matches for species ID, public databases with vast numbers of bacterial sequences may provide better resolution. However, in the absence of a validated library, these results would not be meaningful in a regulated laboratory.

The Applied Biosystems™ MicroSEQ™ ID 16S rDNA 500 Supplemental Library (Cat. No. A35993) contains ~7,100 curated public sequences. These entries were subjected to the same rigorous validation process as the MicroSEQ ID libraries. The supplemental library will expand the coverage of species and increase the resolution of microbial species–level ID.

To illustrate how the MicroSEQ ID 16S rDNA 500 Supplemental Library can increase the resolution of species-level ID when the MicroSEQ ID 16S rDNA 500 Library v2013 returns a <99% match, environmental samples were collected and analyzed with the two libraries. Table 1 summarizes the results.

Overall, 35% of the samples improved to a high-confidence ($\geq 99\%$) species ID match to the supplemental library compared with the v2013 library. However, as shown in Table 1, there are samples that resulted in a genus-level match ($97\% \leq x < 99\%$). This may be explained by four primary reasons:

- The isolate has not been previously described.
- The isolate has not yet been described as a valid species.
- The species is not in the MicroSEQ ID 16S rDNA 500 Supplemental Library or represented in the v2013 library.
- Library entries already included in the MicroSEQ ID 16S rDNA 500 Library v2013 were not included in the supplemental library. Consequently, in some cases, the best-match result for an isolate will be in the v2013 library, and the supplemental library will return a result with a lower-percent match.

The use of the MicroSEQ ID 16S 500 Supplemental Library, with its increased number of sequence entries, improves species-level ID by offering:

- An *in silico* validated library with over 7,000 types of strains that are quality-checked for:
 - Sequence
 - Taxonomy
 - Nomenclature
 - Phylogeny
- Reliable entries from public resources, converted to a simple format for routine validation of unique environmental isolates
- The use of the same acceptance criteria for analysis reporting, and regular updates for entries and information supported by our technical experts
- User ownership of the database and minimal need to outsource

Table 1. The MicroSEQ ID 16S rDNA 500 Supplemental Library can increase the resolution of ID when the MicroSEQ ID 16S rDNA 500 Library v2013 returns a <99% match.

MicroSEQ 16S rDNA 500 Library v2013	Total number tested	Species ID with MicroSEQ ID 16S rDNA 500 Supplemental Library	Genus ID with MicroSEQ ID 16S rDNA 500 Supplemental Library	Below genus ID with MicroSEQ ID 16S rDNA 500 Supplemental Library
Samples with genus-level ID match	40	14	19	7
Samples with below genus-level ID match	21	7	7	7

Ordering information

Product	Cat. No.
MicroSEQ ID 16S rDNA 500 Supplemental Library	A35993

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