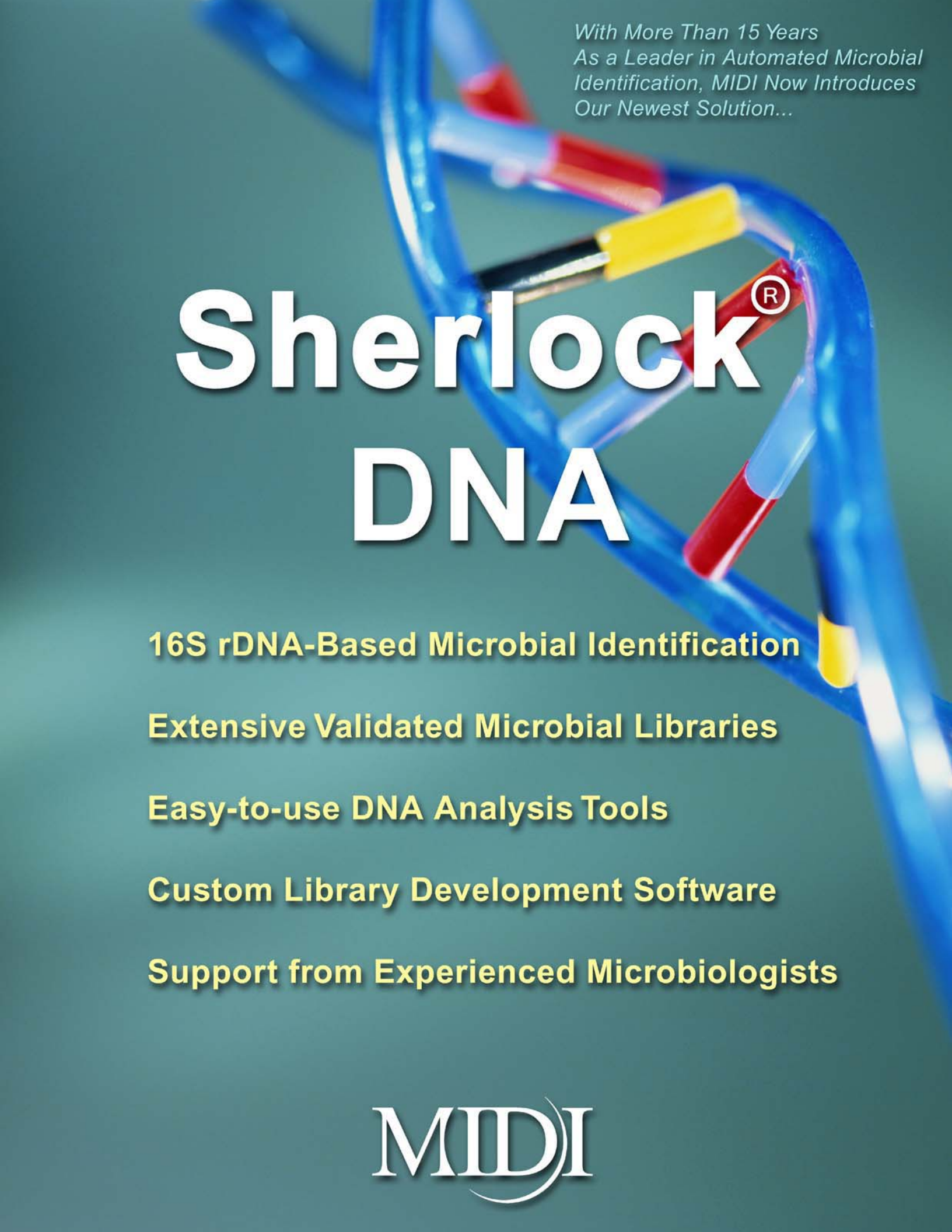

With More Than 15 Years
As a Leader in Automated Microbial
Identification, MIDI Now Introduces
Our Newest Solution...
Sherlock®
DNA
16S rDNA-Based Microbial Identification
Extensive Validated Microbial Libraries
Easy-to-use DNA Analysis Tools
Custom Library Development Software
Support from Experienced Microbiologists
MIDI

Sherlock® DNA

Product Overview

Sherlock® DNA offers an array of features for bacterial/fungal identification and analysis.

- 16S rRNA DNA-based microbial identification for bacteria.
- 28S rRNA DNA-based microbial identification for fungi and yeast.
- Validated libraries available for 500 base-pair and full-gene 16S bacterial identification, as well as 28S fungi/yeast identification.
- Full integration with Sherlock's Microbial Identification System for fatty acid methyl ester (FAME) analysis of bacterial and yeast/fungal samples.
- Combined reporting of DNA-FAME results.
- DNA analysis tools for direct comparison of DNA samples.
- Custom library development tools for creating additional libraries.

The Sherlock DNA system begins where the sequencer leaves off: with a textual consensus sequence. The advantage of this approach is that Sherlock DNA can be used with any sequencer from any vendor.

Identification using Sherlock DNA

To identify a DNA sample, one simply creates a Sherlock sample and attaches a consensus sequence from the sequencer and then generates the identification report.

Typically the sequencer will create two or more DNA electropherograms for a given sample. These electropherograms need to have base-calling completed and then need to be assembled into a consensus sequence. Sherlock DNA does not require any particular program for base-calling and assembly; one may use software that comes with the sequencer or industry packages such Phred from the University of Washington and Sequencher™ from Gene Codes. Once the DNA is available in a textual consensus sequence it can be attached to a Sherlock sample.

Note: the Sherlock system accepts the full range of IUB characters for DNA consensus sequences.

Figure 1: Example DNA consensus sequence

CTTAACACATGCAAGTCGAWCGATGAAG.....

The sequence can simply be copied and pasted directly into a Sherlock sample in the Sherlock's DNA Entry mode. Further, if the assembly program generates FASTA files, these files can be imported directly into Sherlock.

Once the DNA consensus sequence is added to a Sherlock data file, the full reporting capabilities of Sherlock may be used to generate a DNA report. Sherlock's Sample mode includes a wide range of reporting options.

The following print options are supported:

- General information including user comments.
- The DNA consensus sequence
- DNA matches against the DNA library
- Concise alignment versus the top choice or choices.
- A Neighbor Joining Tree or a Rooted Neighbor Joining Tree

General information describes the sample and can include a variety of information.
The DNA consensus sequence itself can also be included.

DNA Matches

The list of matches is sorted by the percent difference. In Figure 2, the DNA of the sample is a near perfect match to Oligella-urethralis in the Sherlock library, demonstrated by a match with 0.28% difference. The second closest match shows a 4.43% difference, so this sample is an excellent match to Oligella-urethralis. Typically, the top ten matches are shown for a sample.

Figure 2: DNA Matches

Match	%Diff	Length	Library Entry Name
1	0.28	528	Oligella-urethralis
2	4.43	528	Oligella-ureolytica
3	11.27	528	Alcaligenes-faecalis
4	11.46	522	Achromobacter-xylosoxidans-xylosoxidans
5	11.74	522	Achromobacter-piechaudii
6	11.89	524	Herbaspirillum-rubrisubalbicans
7	11.93	522	Achromobacter-xylosoxidans-denitrificans
8	12.03	522	Bordetella-avium
9	12.08	524	Pseudomonas-huttiensis
10	12.15	524	Paucimonas-lemoignei

Concise alignment

When the percent difference displayed in the DNA match is above 0.0%, the concise alignment can be used to see exactly which bases differ from the library entry. Figure 3 shows the two base pairs that are different for the Oligella example.

Figure 3: Concise Alignment

Concise Alignment with Oligella-urethralis

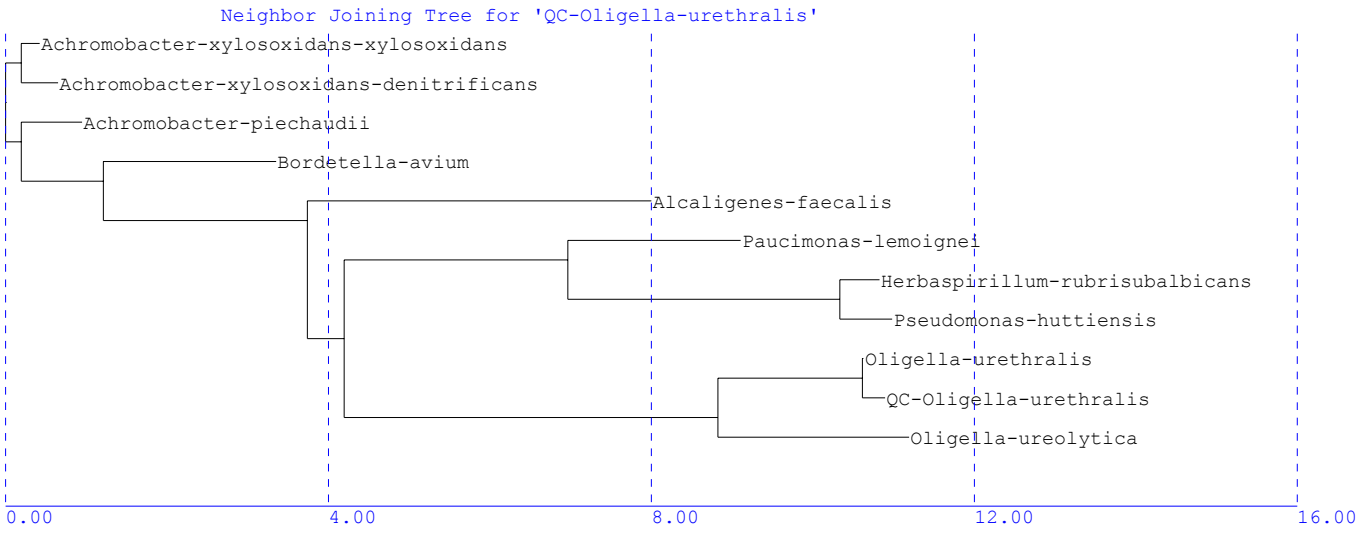
```
Sample:     (136) Y (246) G
            .....   .....
LibEnt 1:   (136) T (246) A
```

There are exactly two bases that differ. In position 136, the sample has a “Y” instead of a “T”. (“Y” is a consensus character indicating either a “T” or a “C”.) In position 246, the sample has a “G” instead of an “A”. The “Y” mismatch is only half a mismatch (because “Y” includes “T”); the “G” versus “A” is a full mismatch. So the system regards this match as 1½ base differences out of 528 base pairs, which is 0.28%.

Neighbor Joining Trees

When one wishes to see the taxonomic neighborhood for the sample, the Neighbor Joining Tree can be used to show the relationships between the organisms. Figure 4 shows the Neighbor Joining Tree for the Oligella example.

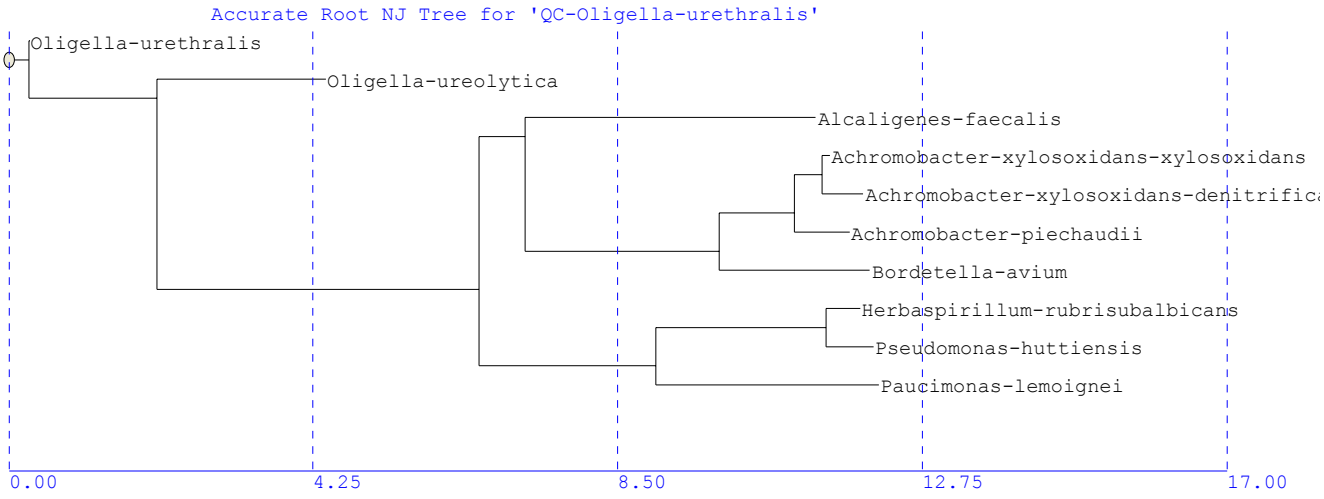
Figure 4: Neighbor Joining Tree



One can see in this diagram that the sample, QC-Oligella-urethralis, is extremely close to the library entry Oligella-urethralis. One can also see that the other Oligella, ureolytica, is linked to these two forming an Oligella neighborhood. The Achromobacter entries are grouped at the upper left corner of the tree.

Sherlock also includes a capability not found in any other system: Accurate Root Neighbor Joining Trees. When the tree is "rooted", the unknown sample is placed at the root of the tree and the picture is redrawn from this perspective. Here is the example rooted from the QC sample:

Figure 5: Accurate Root Neighbor Joining Tree



By displaying the sample at the root of the tree, and using the "Accurate Root" algorithm, developed by MIDI Inc., the exact distances from the sample to all organisms can be read directly off the scale at the bottom of the diagram.

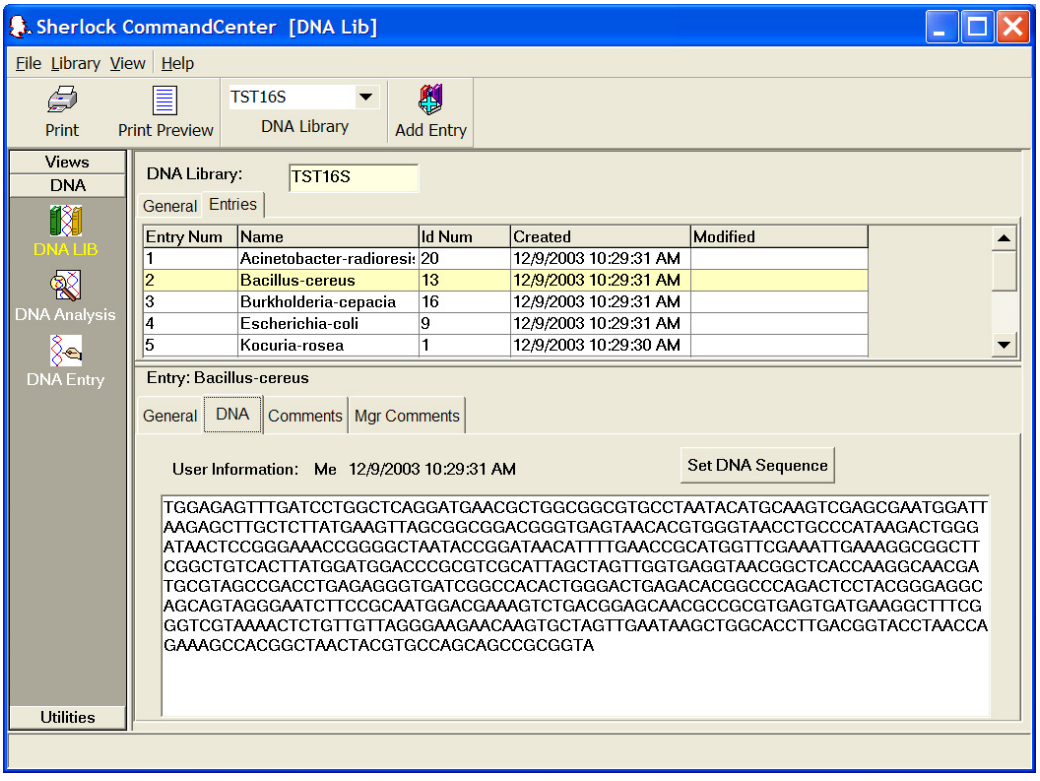
Sherlock DNA Libraries

Sherlock DNA includes three libraries curated by MIDI Inc. The bacterial libraries are based on sequencing the 16S gene, either full-length or the first ~500 base-pairs. The fungal/yeast library is based on sequencing the 28S gene. Details for the three libraries are in the following table.

Library	Application	#Genera	#Species	#Entries
D16S2	16S 500bp bacteria	260	1304	1369
MD16S2	16S 1500bp bacteria	251	1202	1264
FY28S2	28S fungal/yeast	331	1139	1277

Custom DNA Libraries

One can also create custom DNA libraries that can be searched instead of or along with MIDI's libraries. One can create library entries directly from samples that have been run. One can also import FASTA-formatted files to create a library.



Sherlock DNA includes validation reporting to ensure the quality of custom libraries.

Combined Reporting of Sherlock DNA-FAME

One of the unique features of the Sherlock DNA package is its tight integration with the Sherlock Microbial Identification System using Fatty Acid Methyl Ester (FAME) identification. A sample can be run using both techniques on this one system, the results can be stored together and reported in a single combined report. An example of the combined report is in Figure 6 below.

Figure 6: Combined FAME-DNA Report

Sample ID: DBLDIS-ENTC-FAECI-35667 (4402
Matches:

Library	Sim Index	Entry Name
RTSBA6 6.00	0.503	Enterococcus-faecalis-GC subgroup A
	0.464	Enterococcus-faecium-GC subgroup C
	0.458	Xanthobacter-agilis
	0.398	Enterococcus-faecium-GC subgroup E
	0.359	Enterococcus-faecalis-GC subgroup B

D16S2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.18	546	Enterococcus-faecium
2	0.37	546	Enterococcus-hirae
3	0.55	546	Enterococcus-durans
4	1.83	546	Enterococcus-mundtii
5	3.38	545	Enterococcus-pseudoavium
6	3.38	545	Enterococcus-avium
7	3.56	545	Enterococcus-malodoratus
8	4.20	545	Enterococcus-raffinosus
9	5.30	545	Enterococcus-casseliflavus
10	5.30	545	Enterococcus-flavescens

Cross Library Report:
DNA Library: D16S2 FAME Library: RTSBA6

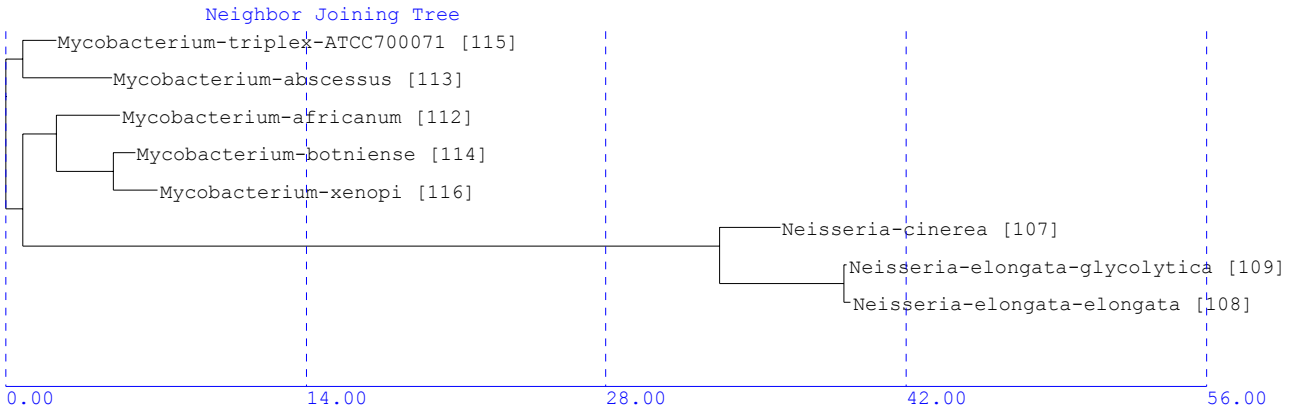
DNA Ind	%Diff	Genus	Species	FAME SI	FAME Ind
1	0.18	enterococcus	faecium	0.464	2
2	0.37	enterococcus	hirae	0.000	7
3	0.55	enterococcus	durans	0.150	4
4	1.83	enterococcus	mundtii	0.011	6
5	3.38	enterococcus	avium	0.138	5
6	3.38	enterococcus	pseudoavium	0.000	10
7	3.56	enterococcus	malodoratus	0.000	8
8	4.20	enterococcus	raffinosus	0.000	9
9	5.30	enterococcus	casseliflavus	0.000	11
10	5.30	enterococcus	flavescens	---	---
11	6.74	enterococcus	faecalis	0.503	1
12	28.75	xanthobacter	agilis	0.458	3

The FAME result is ambiguous among *Enterococcus-faecalis, E. faecium* and *Xanthobacter-agilis,* while the DNA result is ambiguous among *E. faecium, E. hirae* and *E. durans*. When evaluated using the combined results the correct answer, *Enterococcus-faecium*, is clear.

DNA Analysis Tools

The tools shown for identification can also be applied to direct analysis of sets of DNA samples. Even if the Sherlock DNA system is unable to give an identification, using DNA analysis one can determine which samples are closely related. Figure 7 shows a set of eight samples, five Mycobacteria and three Neisseria, displayed on a Neighbor Joining Tree.

Figure 7: Neighbor Joining Tree for Eight Samples



Note that the Mycobacteria cluster together as do the Neisseria.

Concise alignment is available as well as full alignment with color-coding. Figure 8 shows a section of the full alignment for the five Mycobacteria samples.

Portion of Full Alignment, Color-Coded - 5 Mycobacteria Species

```
    101|      110|       120|       130|       140|       150|       160|
absce GAAACTGGGTCTAATACCGGATAGGACCACGGGA-TGCATGTCTTGTGGTGGAAAGCG-
afric GAAACTGGGTCTAATACCGGATAGGACCAC-ACACTTCATGGTGAGTGGTGCAAAGC--
botni GAAACTGGGTCTAATACCGGATAGGACCACCCCA-TGCATGTGGTGTGGTGGAAAGCGT
tripl GAAACTGGGTCTAATACCGGATATGACCACGAGAC-GCATGTCCTGTGGTGGAAAGC--
xenop GAAACTGGGTCTAATACCGGATAGGACCATTCTG-CGCATGTGGGGTGGTGGAAAGTGT
                          *     ********     ****       *     ***
```

The range 101-123 shows an exact match between the five samples. In position 124, the fourth sample (M. triplex) differs from all the others, having a “T” instead of a “G”. Gaps are indicated with “-“. The row of asterisks (*) guides one to the columns where there are differences.

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