



REPORT INTERPRETATION GUIDE

Example: Alignment Report – 500 BP Identification

Library used		16S DNA: 504 base pairs			Size of DNA sequence
D16S2 DNA Match Report					
% Genetic Difference	Match	%Diff	Length	Library Entry Name	
	1	0.20	504	Micrococcus-luteus	Database entries
	2	2.58	503	Micrococcus-lylae	
	3	2.97	504	Arthrobacter-nicotianae	
	4	3.17	504	Arthrobacter-pascens	
	5	3.37	505	Arthrobacter-woluwensis	
	6	3.55	506	Arthrobacter-uratoxydans	
	7	3.67	504	Arthrobacter-mysorens	
	8	3.77	504	Arthrobacter-protophormiae	
	9	4.14	504	Arthrobacter-oxydans	
	10	4.16	502	Arthrobacter-histidinolorans	

Top ten matches to the Sherlock® DNA database

Genetic relationships are expressed in the form of Percent Genetic Differences (**%GD**). This is calculated as the percentage of positions that differ when two sequences are aligned in a way to minimize sequence gaps.

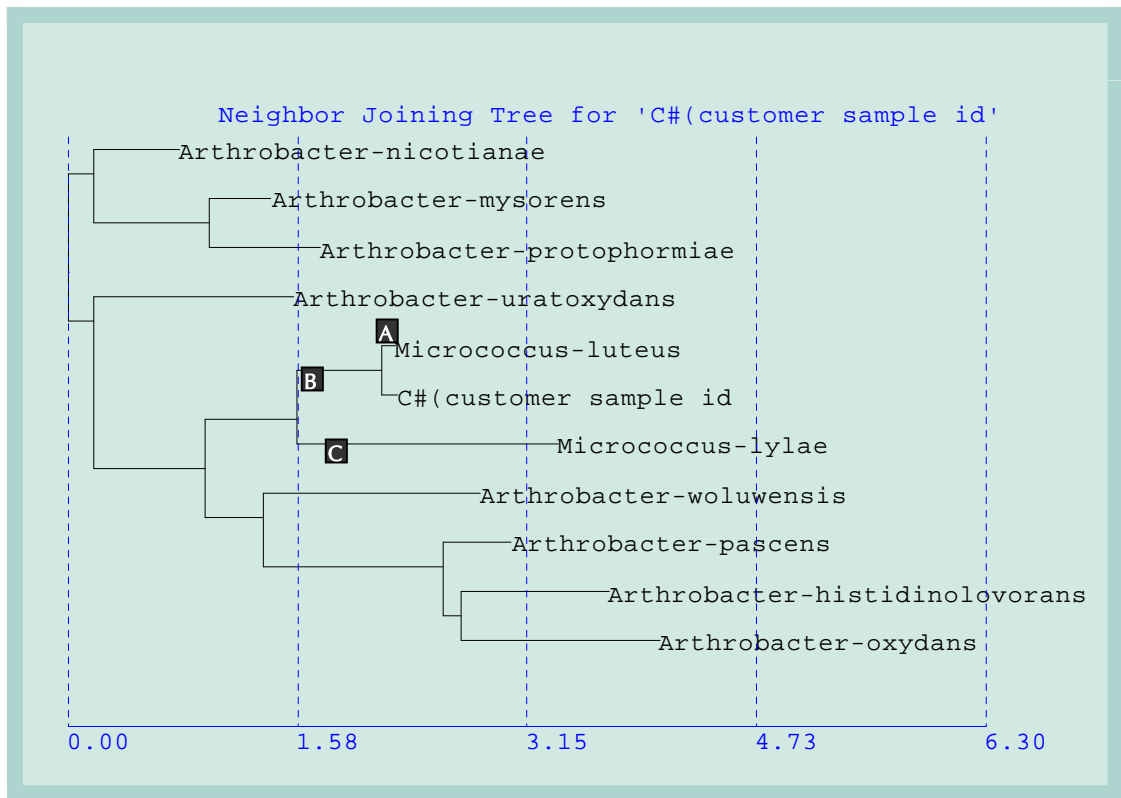
A **Species level match** may be assigned if the %GD between the unknown and the closest match is less than the approximate average %GD between species within that particular genetic family, which is usually 1%.

We utilize phylogenetic trees to approximate the average inter-species %GD and have found through our extensive database building experience (10+ years) that it ranges from 0.5% to 3.0%, with an average of about 1%. This is in agreement with the literature reports (1, 2).

Thus, we will assign a species level match only if the %GD between the unknown and a database entry is less than 3% **AND** is less than the average %GD between species related to it.

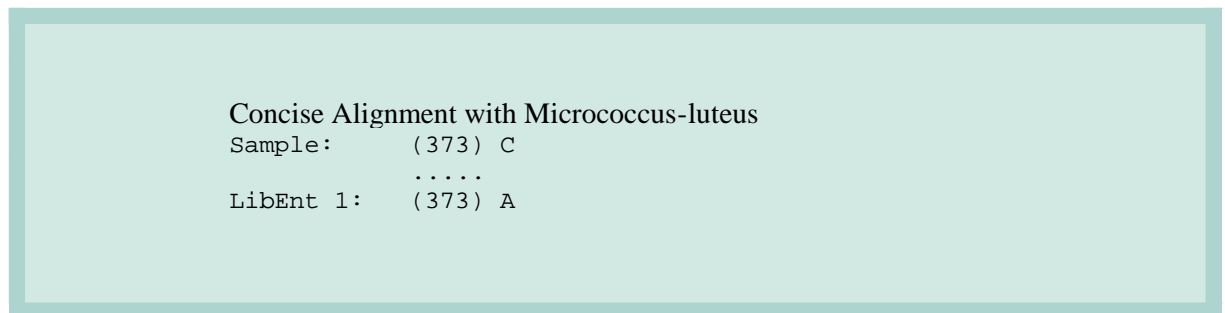
A **Genus level match** will be assigned when the sequence does not meet the requirements for a species level match, but still clusters within the branching of a well-defined genus. This is generally between 1% and 5%.

N. Joining Tree



Neighbor joining tree displays the inter-species relationships between the top ten matches and the unknown.

Distances can be calculated by summing horizontal differences. For example, the estimated %GD between *M. luteus* and *M. lylae* is the sum of segments A, B, and C.



Concise alignment displays the regions in a sequence where there are differences.

Each region is labeled by its starting base number. When 3 or more samples are compared an asterisk shows which bases have differences. When more than two consecutive positions have no difference, the concise alignment skips printing until another area with differences is encountered.

In this example, the only difference between the sample and *M. luteus* is at position 373; the sample contains a “C” at this site, whereas *M. luteus* has an “A” at this position.

IUB ambiguity nucleotide codes

R	=	A or G (puRine)	B	=	C, T, or G
Y	=	C or T (pYrimidine)	H	=	A, C, or T
K	=	G or T (Keto)	D	=	A, G, or T
M	=	A or C (aMino)	V	=	A, C, or G
S	=	G or C (Strong)			
W	=	A or T (Weak)			

Occasionally, a sequence obtained from MIDI Labs will contain an ambiguity nucleotide call. These types of calls occur in situations where there appears to be more than one type of nucleotide present at a particular position. Ambiguity codes in sequences obtained from MIDI Labs are not the result of low quality sequence data, but rather are often due to polymorphism events occurring within the multiple copies of the rRNA genes.

Taxonomy

We strive to keep pace with the ever-changing current taxonomy with respect to naming organisms within our database. We use multiple taxonomic resources to check current names and changes frequently. If there is a name change or an organism that has a basonym or synonym that will better explain the results, we will note that on the report. Should you discover a case where we have failed to do so, please contact us.

Due to complex taxonomy, confidence levels are not made on Yeast and Fungi reports. General guidelines apply to all Alignment Reports.

References

- Montgomery, S. O., S. Anderson, M. G. Waddington, J. G. Bartell, G. Nunn, and Foxall, P.** 1999. Variation in Bacterial Interspecific Distances – New Rules for interpretation of 16S rDNA Sequences? Abstract, 1999 International Union of Microbiological Societies Meeting, Sidney.
- Palys, T., L.K. Nakamura, and F. M. Cohan.** 1997. Diversity in the Bacterial World: the Role of DNA Sequence Data. IJSB. 47:1145-1156.



16S Microbial Identification System Evaluation Papers

Woo, et al. 2003. Usefulness of the MicroSeq 500 16S Ribosomal DNA-Based Bacterial Identification System for Identification of Clinically Significant Bacterial Isolates with Ambiguous Biochemical Profiles. *J. Clin. Microbiol.* 41:1996-2001.

Patel, J. B., D.G.B. Leonard, X. Pan, J. M. Musser, R. E. Bergman and I. Nachamkin. 2000. Sequence-Based Identification of *Mycobacterium* Species Using the MicroSeq 500 16S rDNA Bacterial Identification System. *J. Clin. Micro.* 38:246-251.

Kolbert, C. P. and D. H. Pershing. 1999. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *C. Opinions in Microbiol.* 2:299-305.

Selected Papers using 16S as an Identification Tool

(ML = sequence and/or identification data generated at MIDI Labs)

Levi, M.H., J. Bartell, L.M. Gandolfo, S.C. Mole, S.F. Costa, L.M. Weiss, L.K. Johnson, G. Osterhout, and L.H. Herbst. 2003. Characterization of *Mycobacterium montefiorensis* sp. nov., a novel pathogenic mycobacterium from moray eels. *Journal of Clinical Microbiology.* 41:2147-2152.

Vreeland, R.H., W. D. Rosenzweig and D. W. Powers. 2000. Isolation of a 250 Million -Year -Old Halotolerant Bacterium From a Primary Salt Crystal. *Nature.* 407:897-900. (ML)

Kilbane, J. J. II, R. Ranganathan, L. Cleveland, K. J. Kayser, C. Ribiero, and M. M. Linhares. 2000. Selective Removal of Nitrogen from Quinoline and Petroleum by *Pseudomonas ayucida* IGTV9m. *Appl. Envir. Microbiol.* 66:688-693. (ML)

Pitulle, C., D. M. Citron, B. Bochner, R. Barbers, and M. D. Appleman. 1999. Novel Bacterium Isolated from a Lung Transplant Patient with Cystic Fibrosis. *J. Clin Micro.* 37:3851-3855.

Solano, F. and A Sanchez-Amat. 1999. Studies on the phylogenetic relationships of melanogenic marine bacteria: proposal of *Marinomonas mediterranea* sp. nov. *Int J Syst Bacteriol.* 49:1241-1246. (ML)

Parsonnet J, H. Shmuelly and T. Haggerty. 1999. Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults. *JAMA* 282:2240-5. (ML)

Dutta, T., S. A. Selifonov, and I.C. Gunsalus. 1998. Oxidation of Methyl-Substituted Naphthalenes: Pathways in a Versatile *Sphingomonas paucimobilis* Strain. *Appl. Envir. Microbiol.* 64:1884-1889. (ML)