

SOURCE MOLECULAR CORPORATION

4985 SW 74th Court, Miami, FL 33155 USA

Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

E. coli ID™ – DNA Fingerprinting of *E. coli* (Discriminant Analysis of Ribotype Profiles of *E. coli*)

Submitter: XYZ Municipal Water Plant

Submitter #: ABCDE

Source Molecular #: SM 0111

Sample Received: January 3rd, 2011

Date Reported: January 10th, 2011

SAMPLE

Fecal Coliform ⁵ mpn*/100 ml	<i>E. coli</i> Isolate # (5 colonies of cultured <i>E. coli</i> were analyzed)	Probable Source
> 2,400	1 2 3 4 5	Animal Animal Indeterminate Animal Human

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

Laboratory Comments

Five *E. coli* DNA fingerprints were compared with an internal library of *E. coli* strains to determine whether they were from an animal or a human source (or both). The information provided should only be considered a preliminary source indicator. The client must run additional tests such as the *E. coli* Comparison ID™ test to confirm animal / indeterminate sources and the Human Enterococcus ID™ test to confirm the presence of human sources.

As shown in the above table, the DNA fingerprints of 4 colonies of *E. coli* cultured from the water sample statistically matched both human and animal sources when compared to a database of known source DNA fingerprints. One of the *E. coli* isolate DNA fingerprints was classified as indeterminate. The probable source of this *E. coli* isolate can either be human or animal.

DNA Fingerprinting Method Explanation

E. coli were enumerated by taking plates that are positive for fecal coliforms, transferring the membrane filter to EC with MUG media (Difco), and incubating for an additional 24 hours at 37°C. Colonies that fluoresced under UV light were counted as *E. coli* and isolated for ribotyping.

Ribotyping of *E. coli* isolates was accomplished by the method of Parveen *et al* (1999)¹. Chromosomal DNA was extracted from *E. coli* isolates and digested with *Hind*III. Fragments were separated by agarose electrophoresis. The DNA was then transferred and fixed to a Zeta -probe membrane. A cDNA probe complementary to the *E. coli* 16S and 23S rDNA was labeled with digoxigenin-dUTP and was used to probe the membranes. The resulting genetic fingerprint was translated to a binary code based on the presence and absence of predetermined bands. The resulting binary code was then analyzed by discriminant analysis using Bionumerics software against a library of known source isolates- similar to the method elaborated in Scott *et al* (2003)³.

DNA Fingerprinting Theory Explanation

After cultivating *E. coli* from the submitted sample, one or more *E. coli* isolates are selected. Isolates are clusters of *E. coli* colonies on an agar plate. A DNA fingerprinting analysis called ribotyping is performed on each *E. coli* isolate selected. This genetic fingerprint comes from genes that code for ribosomal ribonucleic acids (rRNA) of *E. coli*. Ribosomal RNA together with various proteins makes up the cell structure called a ribosome.

The ribosome is the cell structure where proteins are manufactured. In order to produce proteins, the messenger RNA and the amino acids are transferred to the ribosome. As the ribosome moves down the messenger RNA, it places the correct amino acid in the growing protein. It has been shown that looking at small differences in the DNA that code for these 16S and 23S rRNA's help identify different strains of *E. coli*.

Ribosomal genes are also known to be highly conserved in microbes, meaning that the genetic information coding for rRNA will vary much less within bacteria of the same strain than it will between bacterial strains. This characteristic allows for a greater ability to distinguish between different bacterial strains.

In ribotyping, restriction enzymes are used to cut the genes coding for rRNA into pieces, and electrophoresis separates the pieces by size through a gel.² Genetic probes then visualize locations of different size fragments of DNA in the gel, which appear as bands. The banding pattern of DNA fragments corresponding to the relevant rRNA is known as the ribotype. The banding patterns are compared to a database of other *E. coli* strains and matched for each determined strain. If the client submits fecal samples, then banding patterns are also investigated between the fecal samples and blind samples submitted.⁴

¹ Parveen, Salina, Portier, Kenneth M., Robinson, Kevin, Edmiston, Lee, Tamplin, Mark L. **Discriminant Analysis of Ribotype Profiles of Escherichia coli for Differentiating Human and Nonhuman Sources of Fecal Pollution** Appl. Environ. Microbiol. (1999) 65: 3142-3147

² Carson, C. Andrew, Shear, Brian L., Eilersieck, Mark R., Asfaw, Amha **Identification of Fecal Escherichia coli from Humans and Animals by Ribotyping** Appl. Environ. Microbiol. (2001) 67: 1503-1507

³ Scott, Troy M., Parveen, Salina, Portier, Kenneth M., Rose, Joan B., Tamplin, Mark L., Farrah, Samuel R., Koo, Andrew, Lukasik, Jerzy **Geographical Variation in Ribotype Profiles of Escherichia coli Isolates from Humans, Swine, Poultry, Beef, and Dairy Cattle in Florida** Appl. Environ. Microbiol. (2003) 69: 1089-1092

⁴ Scott, T.M., J. Caren, R. Nelson, T.M. Jenkins, and J. Lukasik. 2004 **Tracking sources of fecal pollution in a South Carolina watershed by ribotyping Escherichia coli: A case study.** Environ. Forensics. 5: 15-19.

⁵ Standard methods for the Examination of Water and Wastewater Method 9223 A1 (APHA, 1998)

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of the Source Molecular Corporation, as well as its agents or representatives, the liability of the Source Molecular Corporation shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to the Source Molecular Corporation. The Source Molecular Corporation shall not be liable for any damages, either direct or consequential. The Source Molecular Corporation provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request.