

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

Laboratory Comments

Submitter: XYZ Water Facility

Report Date: January 10th, 2010

SAMPLE

In order to provide a greater understanding of sources of animal fecal contamination in the environment, the Source Molecular Corporation has developed and implemented novel analytical tools for determining specific sources of contamination by fingerprinting the DNA of *E. coli* bacteria and statistically comparing those fingerprints to a reference database of over 5,000 *E. coli* from various known animal sources. This statistical comparison has been shown to be effective in making an initial determination as to whether or not an unknown *E. coli* isolate is from a human or an animal source.

Although statistical analysis is an excellent analytical tool for initially assessing the probable human or animal source of an *E. coli* isolate, it is often desirable to increase the specificity of the classification to include specific animal sources. One way to make this determination is to compare exactly DNA fingerprints from unknown *E. coli* in a water sample to fingerprints produced from *E. coli* isolated from client-submitted fecal samples from known animal sources. This approach is called a direct DNA fingerprint comparison analysis and has been effective in many studies within defined watersheds with defined probable sources of fecal pollution.

Although direct comparison analysis (i.e.: exact matches of DNA fingerprints) is an excellent analytical tool for determining sources of animal contamination, previous studies that have used this type of analysis have shown in some cases to be too restrictive. Direct comparison analysis does not allow any leeway in isolates that demonstrate small variations yet are very similar statistically to the reference isolates.

Often one or two specific facets of a particular fingerprint will be indicative of a specific animal source regardless of whether or not the fingerprint is a direct match to a known isolate. Therefore, by focusing on exact DNA fingerprint matches between isolates of water and fecal references, likely sources of contamination might be overlooked because of the severe restrictions imposed by such an analysis. Of course this type of analysis remains a viable and valuable tool, and it continues to play a leading role in analyzing clonal bacterial strains and pinpointing precise sources of pollution, particularly in monitoring facility specific diffusions.

To circumvent these restrictions, we have combined the power of our statistical fingerprint analysis tools with a visual assessment of each individual band of every DNA fingerprint generated throughout the study. This approach was used to analyze the *E. coli* isolates of the water samples received on April 10, 2004 with the *E. coli* isolates of the fecal reference database # 1. The entire project resulted in the successful generation of 65 DNA fingerprints from the *E. coli* isolated from the water samples. These fingerprints were then compared to the 100 DNA fingerprints from the fecal reference database #1.

The first method that was used was the Bionumerics statistics program from Applied Maths, Inc. (www.applied-maths.com).¹ The program assigned for each isolate from the water samples a probable match with the isolates from the fecal reference database. This program was used to gain a preliminary indication of possible matches.

Afterwards, the Bionumeric results were compared with the previous results of the discriminant analysis that was done between the *E. coli* isolates of the water samples and the library of *E. coli* strains from Source Molecular.² The two statistical results had to be in agreement before a matched classification was accepted. Small variances to this rule were nonetheless accepted for *E. coli* isolates that were classified as indeterminate by the discriminant analysis program. This is logical since further analysis with other fecal samples was necessary to help remove this ambiguous classification. Lastly, the isolate that was classified as from a human source by the discriminant analysis was also re-verified by Bionumeric analysis for any conflict. No conflict was detected therefore this isolate retained its human classification.

Only isolate matches with a stringent confidence level of 90% or more were accepted as probable matches. A Unique Match designation was given for water *E. coli* isolates found in only one animal category of the client's fecal reference samples. Isolates that had a confidence level of less than 90% or did not agree between the matched categories were classified as a No Match. This conservative cutoff criterion was preferred rather than risking misclassification errors. The results of the statistical analysis are presented in the following pages.

The overall result of the study was the successful classification of more than 60% of the submitted unknown *E. coli* isolates. This is a high degree of classification using this type of analysis and indicates good correlation between the submitted fecal samples and those isolated from the environment. Because any watershed will likely contain *E. coli* from a variety of sources, it is highly unlikely that all of the isolated *E. coli* will be successfully classified. With that in mind, it is important to realize that any study such as this will identify many likely sources of *E. coli*. Therefore, it is important to concentrate on the predominant sources identified by the fingerprint comparison analysis.

The current results (barring the submission of additional known fecal isolates) indicate that the predominant sources of fecal contamination in the sampled watershed include raccoons, chicken and deer. It is therefore suggested that these sources be considered as a primary source. Source Molecular offers additional analytical tests to confirm the presence or absence of specific sources of fecal contamination such as human or dog. By specifically targeting sampling upstream and downstream of suspected sources (such as a septic drain field or a dog park), point and non-point sources of contamination can be identified without the interference from minor contributing sources present in environmental water samples.

¹ Example of the use of Bionumerics analysis:

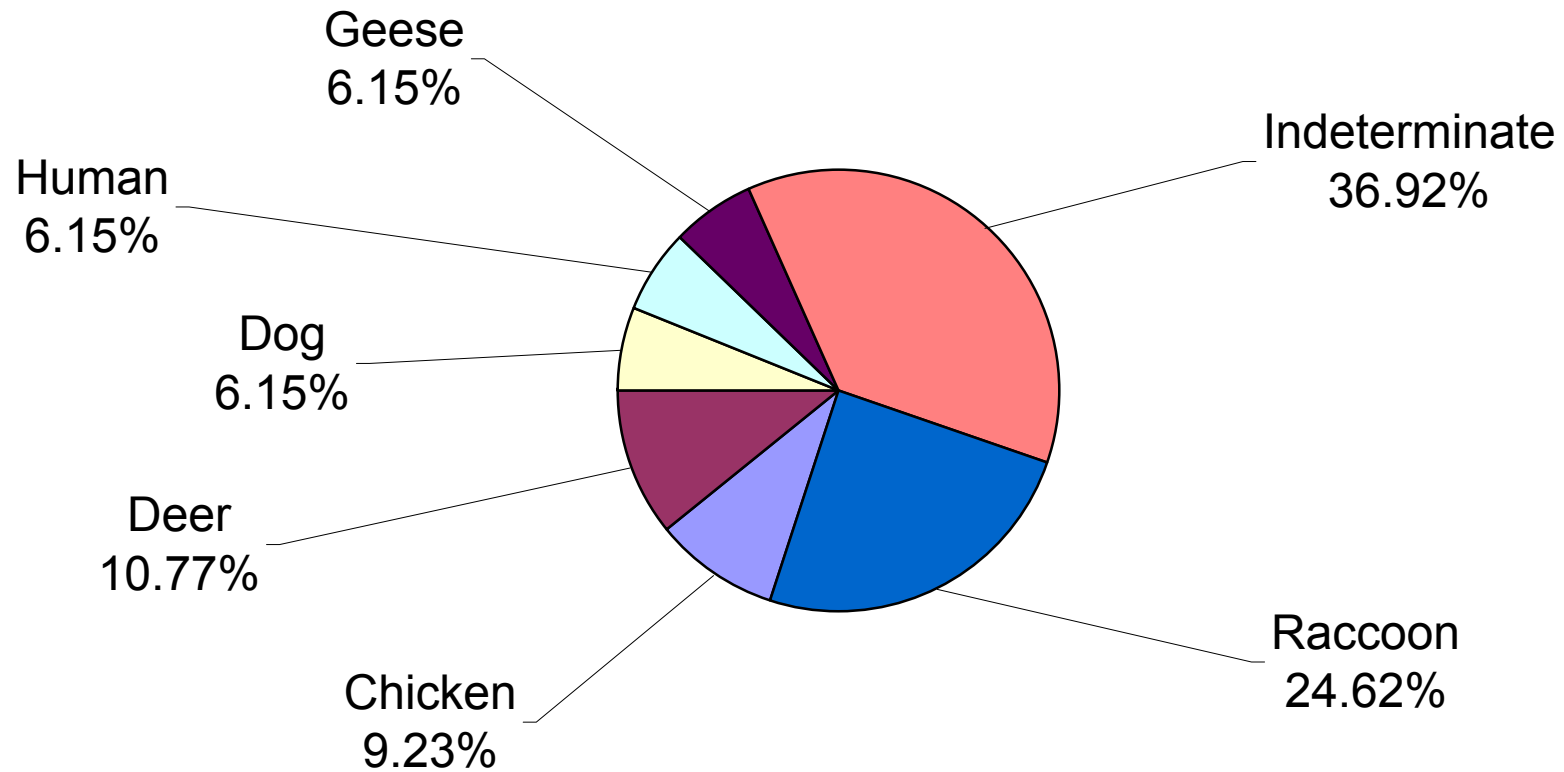
McLellan, Sandra L., Daniels, Annette D., Salmore, Alissa K. **Genetic Characterization of Escherichia coli Populations from Host Sources of Fecal Pollution by Using DNA Fingerprinting** Appl. Environ. Microbiol. 2003 69: 2587-2594.

² Example of the use of discriminant analysis:

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.

Comparison E. coli ID™ – DNA Fingerprinting of E. coli
(Statistical analysis of ribotype profiles of water sample E. coli isolates
with Database # 1 E. coli isolates)

Submitter: XYZ Water Facility
April 20, 2004



Comparison E. coli ID™ – DNA Fingerprinting of E. coli

(Statistical analysis of ribotype profiles of water sample E. coli isolates
with Database # 1 E. coli isolates)

Submitter: XYZ Water Facility

Report Date: April 20, 2004

Submitted Scat Samples (Database # 1)

Source Molecular #	Client Scat Sample Reference	Isolate #	General Grouping	Submittal Date of Scat Sample	Classification
SM 0355	Chicken Fecal Dropping #1	1	Chicken	20-Nov-03	Animal
SM 0355	Chicken Fecal Dropping #1	2	Chicken	20-Nov-03	Animal
SM 0355	Chicken Fecal Dropping #1	3	Chicken	20-Nov-03	Indeterminate
SM 0355	Chicken Fecal Dropping #1	4	Chicken	20-Nov-03	Animal
SM 0355	Chicken Fecal Dropping #1	5	Chicken	20-Nov-03	Animal
SM 0356	Chicken Fecal Dropping #2	1	Chicken	20-Nov-03	Animal
SM 0356	Chicken Fecal Dropping #2	2	Chicken	20-Nov-03	Animal
SM 0356	Chicken Fecal Dropping #2	3	Chicken	20-Nov-03	Animal
SM 0356	Chicken Fecal Dropping #2	4	Chicken	20-Nov-03	Animal
SM 0356	Chicken Fecal Dropping #2	5	Chicken	20-Nov-03	Animal
SM 0357	Chicken Fecal Dropping #3	1	Chicken	20-Nov-03	Animal
SM 0357	Chicken Fecal Dropping #3	2	Chicken	20-Nov-03	Animal
SM 0357	Chicken Fecal Dropping #3	3	Chicken	20-Nov-03	Animal
SM 0357	Chicken Fecal Dropping #3	4	Chicken	20-Nov-03	Animal
SM 0357	Chicken Fecal Dropping #3	5	Chicken	20-Nov-03	Animal
SM 0358	Chicken Fecal Dropping #4	1	Chicken	20-Nov-03	Animal
SM 0358	Chicken Fecal Dropping #4	2	Chicken	20-Nov-03	Animal
SM 0358	Chicken Fecal Dropping #4	3	Chicken	20-Nov-03	Animal
SM 0358	Chicken Fecal Dropping #4	4	Chicken	20-Nov-03	Animal
SM 0358	Chicken Fecal Dropping #4	5	Chicken	20-Nov-03	Animal
SM 0359	Chicken Fecal Dropping #5	1	Chicken	20-Nov-03	Animal
SM 0359	Chicken Fecal Dropping #5	2	Chicken	20-Nov-03	Animal

Source Molecular #	Client Scat Sample Reference	Isolate #	General Grouping	Submittal Date of Scat Sample	Classification
SM 0359	Chicken Fecal Dropping #5	3	Chicken	20-Nov-03	Animal
SM 0359	Chicken Fecal Dropping #5	4	Chicken	20-Nov-03	Animal
SM 0359	Chicken Fecal Dropping #5	5	Chicken	20-Nov-03	Animal
SM 0360	Raccoon Fecal Dropping #1	1	Raccoon	20-Nov-03	Animal
SM 0360	Raccoon Fecal Dropping #1	2	Raccoon	20-Nov-03	Indeterminate
SM 0360	Raccoon Fecal Dropping #1	3	Raccoon	20-Nov-03	Animal
SM 0360	Raccoon Fecal Dropping #1	4	Raccoon	20-Nov-03	Animal
SM 0360	Raccoon Fecal Dropping #1	5	Raccoon	20-Nov-03	Animal
SM 0361	Raccoon Fecal Dropping #2	1	Raccoon	20-Nov-03	Indeterminate
SM 0361	Raccoon Fecal Dropping #2	2	Raccoon	20-Nov-03	Animal
SM 0361	Raccoon Fecal Dropping #2	3	Raccoon	20-Nov-03	Animal
SM 0361	Raccoon Fecal Dropping #2	4	Raccoon	20-Nov-03	Animal
SM 0361	Raccoon Fecal Dropping #2	5	Raccoon	20-Nov-03	Indeterminate
SM 0362	Raccoon Fecal Dropping #3	1	Raccoon	20-Nov-03	Animal
SM 0362	Raccoon Fecal Dropping #3	2	Raccoon	20-Nov-03	Animal
SM 0362	Raccoon Fecal Dropping #3	3	Raccoon	20-Nov-03	Animal
SM 0362	Raccoon Fecal Dropping #3	4	Raccoon	20-Nov-03	Animal
SM 0362	Raccoon Fecal Dropping #3	5	Raccoon	20-Nov-03	Animal
SM 0363	Raccoon Fecal Dropping #4	1	Raccoon	20-Nov-03	Animal
SM 0363	Raccoon Fecal Dropping #4	2	Raccoon	20-Nov-03	Indeterminate
SM 0363	Raccoon Fecal Dropping #4	3	Raccoon	20-Nov-03	Indeterminate
SM 0363	Raccoon Fecal Dropping #4	4	Raccoon	20-Nov-03	Indeterminate
SM 0363	Raccoon Fecal Dropping #4	5	Raccoon	20-Nov-03	Animal
SM 0364	Raccoon Fecal Dropping #5	1	Raccoon	20-Nov-03	Animal
SM 0364	Raccoon Fecal Dropping #5	2	Raccoon	20-Nov-03	Indeterminate
SM 0364	Raccoon Fecal Dropping #5	3	Raccoon	20-Nov-03	Animal
SM 0364	Raccoon Fecal Dropping #5	4	Raccoon	20-Nov-03	Animal
SM 0364	Raccoon Fecal Dropping #5	5	Raccoon	20-Nov-03	Animal
SM 0365	Deer Fecal Dropping #1	1	Deer	20-Nov-03	Animal
SM 0365	Deer Fecal Dropping #1	2	Deer	20-Nov-03	Animal
SM 0365	Deer Fecal Dropping #1	3	Deer	20-Nov-03	Animal
SM 0365	Deer Fecal Dropping #1	4	Deer	20-Nov-03	Animal
SM 0365	Deer Fecal Dropping #1	5	Deer	20-Nov-03	Animal
SM 0366	Deer Fecal Dropping #2	--	NA	20-Nov-03	Cancelled - No E. coli
SM 0367	Deer Fecal Dropping #3	1	Deer	20-Nov-03	Animal
SM 0367	Deer Fecal Dropping #3	2	Deer	20-Nov-03	Animal
SM 0367	Deer Fecal Dropping #3	3	Deer	20-Nov-03	Animal

Source Molecular #	Client Scat Sample Reference	Isolate #	General Grouping	Submittal Date of Scat Sample	Classification
SM 0367	Deer Fecal Dropping #3	4	Deer	20-Nov-03	Animal
SM 0367	Deer Fecal Dropping #3	5	Deer	20-Nov-03	Animal
SM 0368	Deer Fecal Dropping #4	1	Deer	20-Nov-03	Indeterminate
SM 0368	Deer Fecal Dropping #4	2	Deer	20-Nov-03	Animal
SM 0368	Deer Fecal Dropping #4	3	Deer	20-Nov-03	Indeterminate
SM 0368	Deer Fecal Dropping #4	4	Deer	20-Nov-03	Animal
SM 0368	Deer Fecal Dropping #4	5	Deer	20-Nov-03	Animal
SM 0369	Deer Fecal Dropping #5	1	Deer	20-Nov-03	Animal
SM 0369	Deer Fecal Dropping #5	2	Deer	20-Nov-03	Animal
SM 0369	Deer Fecal Dropping #5	3	Deer	20-Nov-03	Animal
SM 0369	Deer Fecal Dropping #5	4	Deer	20-Nov-03	Animal
SM 0369	Deer Fecal Dropping #5	5	Deer	20-Nov-03	Animal
SM 0370	Dog Fecal Material #1	1	Dog	20-Nov-03	Animal
SM 0370	Dog Fecal Material #1	2	Dog	20-Nov-03	Animal
SM 0370	Dog Fecal Material #1	3	Dog	20-Nov-03	Animal
SM 0370	Dog Fecal Material #1	4	Dog	20-Nov-03	Animal
SM 0370	Dog Fecal Material #1	5	Dog	20-Nov-03	Animal
SM 0371	Dog Fecal Material #2	1	Dog	20-Nov-03	Animal
SM 0371	Dog Fecal Material #2	2	Dog	20-Nov-03	Animal
SM 0371	Dog Fecal Material #2	3	Dog	20-Nov-03	Animal
SM 0371	Dog Fecal Material #2	4	Dog	20-Nov-03	Animal
SM 0371	Dog Fecal Material #2	5	Dog	20-Nov-03	Animal
SM 0372	Dog Fecal Material #3	1	Dog	20-Nov-03	Animal
SM 0372	Dog Fecal Material #3	2	Dog	20-Nov-03	Animal
SM 0372	Dog Fecal Material #3	3	Dog	20-Nov-03	Animal
SM 0372	Dog Fecal Material #3	4	Dog	20-Nov-03	Animal
SM 0372	Dog Fecal Material #3	5	Dog	20-Nov-03	Animal
SM 0373	Geese Fecal Dropping #1	1	Geese	20-Nov-03	Animal
SM 0373	Geese Fecal Dropping #1	2	Geese	20-Nov-03	Animal
SM 0373	Geese Fecal Dropping #1	3	Geese	20-Nov-03	Animal
SM 0373	Geese Fecal Dropping #1	4	Geese	20-Nov-03	Animal
SM 0373	Geese Fecal Dropping #1	5	Geese	20-Nov-03	Animal
SM 0374	Geese Fecal Dropping #2	1	Geese	20-Nov-03	Animal
SM 0374	Geese Fecal Dropping #2	2	Geese	20-Nov-03	Animal
SM 0374	Geese Fecal Dropping #2	3	Geese	20-Nov-03	Animal
SM 0374	Geese Fecal Dropping #2	4	Geese	20-Nov-03	Animal
SM 0374	Geese Fecal Dropping #2	5	Geese	20-Nov-03	Animal

Source Molecular #	Client Scat Sample Reference	Isolate #	General Grouping	Submittal Date of Scat Sample	Classification
SM 0375	Geese Fecal Dropping #3	1	Geese	20-Nov-03	Animal
SM 0375	Geese Fecal Dropping #3	2	Geese	20-Nov-03	Animal
SM 0375	Geese Fecal Dropping #3	3	Geese	20-Nov-03	Animal
SM 0375	Geese Fecal Dropping #3	4	Geese	20-Nov-03	Animal
SM 0375	Geese Fecal Dropping #3	5	Geese	20-Nov-03	Animal

Comparison E. coli ID™ – DNA

Fingerprinting of E. coli

(Statistical analysis of ribotype profiles of water sample E. coli isolates
with Database # 1 E. coli isolates)

Submitter: XYZ Water Facility

Report Date: April 20, 2004

Glossary of Terms

No Match - No direct DNA fingerprint matches were found among the *E. coli* isolates from the water sample and all the submitted fecal samples.

Unique Match - A unique DNA fingerprint match was found between one of the fecal sample *E. coli* isolates and one of the water sample *E. coli* isolates. The general animal group is given. This DNA fingerprint was found in no other animal group of the submitted fecal samples.

Source Molecular #	Client Water Sample Reference	Isolate #	Submittal Date of Water Sample	Classification	Isolate Match	Previous Classification
SM 0550	Water Sample # 1	1	10-Apr-04	Unique Match	Deer	Animal
SM 0550	Water Sample # 1	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0550	Water Sample # 1	3	10-Apr-04	Unique Match	Geese	Animal
SM 0550	Water Sample # 1	4	10-Apr-04	Unique Match	Geese	Animal
SM 0550	Water Sample # 1	5	10-Apr-04	No Match	Indeterminate	Animal
SM 0551	Water Sample # 2	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0551	Water Sample # 2	2	10-Apr-04	Unique Match	Raccoon	Animal
SM 0551	Water Sample # 2	3	10-Apr-04	No Match	Indeterminate	Animal
SM 0551	Water Sample # 2	4	10-Apr-04	No Match	Indeterminate	Animal
SM 0551	Water Sample # 2	5	10-Apr-04	Unique Match	Raccoon	Animal
SM 0552	Water Sample # 3	1	10-Apr-04	Unique Match	Raccoon	Animal
SM 0552	Water Sample # 3	2	10-Apr-04	Unique Match	Raccoon	Animal
SM 0552	Water Sample # 3	3	10-Apr-04	Unique Match	Raccoon	Animal
SM 0552	Water Sample # 3	4	10-Apr-04	Unique Match	Raccoon	Animal
SM 0552	Water Sample # 3	5	10-Apr-04	Unique Match	Raccoon	Animal
SM 0553	Water Sample # 4	1	10-Apr-04	Unique Match	Raccoon	Animal

Source Molecular #	Client Water Sample Reference	Isolate #	Submittal Date of Water Sample	Classification	Isolate Match	Previous Classification
SM 0553	Water Sample # 4	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0553	Water Sample # 4	3	10-Apr-04	Unique Match	Deer	Animal
SM 0553	Water Sample # 4	4	10-Apr-04	Unique Match	Deer	Animal
SM 0553	Water Sample # 4	5	10-Apr-04	No Match	Indeterminate	Animal
SM 0554	Water Sample # 5	1	10-Apr-04	Unique Match	Dog	Animal
SM 0554	Water Sample # 5	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0554	Water Sample # 5	3	10-Apr-04	No Match	Classification Accepted	Human**
SM 0554	Water Sample # 5	4	10-Apr-04	Unique Match	Raccoon	Animal
SM 0554	Water Sample # 5	5	10-Apr-04	Unique Match	Chicken	Animal
SM 0555	Water Sample # 6	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0555	Water Sample # 6	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0555	Water Sample # 6	3	10-Apr-04	Unique Match	Geese	Animal
SM 0555	Water Sample # 6	4	10-Apr-04	Unique Match	Raccoon	Animal
SM 0555	Water Sample # 6	5	10-Apr-04	No Match	Indeterminate	Animal
SM 0556	Water Sample # 7	1	10-Apr-04	Unique Match	Raccoon	Animal
SM 0556	Water Sample # 7	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0556	Water Sample # 7	3	10-Apr-04	Unique Match	Deer	Animal
SM 0556	Water Sample # 7	4	10-Apr-04	Unique Match	Deer	Animal
SM 0556	Water Sample # 7	5	10-Apr-04	No Match	Classification Accepted	Human**
SM 0557	Water Sample # 8	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0557	Water Sample # 8	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0557	Water Sample # 8	3	10-Apr-04	Unique Match	Raccoon	Animal
SM 0557	Water Sample # 8	4	10-Apr-04	Unique Match	Raccoon	Animal
SM 0557	Water Sample # 8	5	10-Apr-04	Unique Match	Raccoon	Animal
SM 0558	Water Sample # 9	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0558	Water Sample # 9	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0558	Water Sample # 9	3	10-Apr-04	Unique Match	Deer	Animal
SM 0558	Water Sample # 9	4	10-Apr-04	Unique Match	Deer	Animal
SM 0558	Water Sample # 9	5	10-Apr-04	Unique Match	Geese	Animal
SM 0559	Water Sample # 10	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0559	Water Sample # 10	2	10-Apr-04	Unique Match	Chicken	Animal
SM 0559	Water Sample # 10	3	10-Apr-04	Unique Match	Chicken	Animal
SM 0559	Water Sample # 10	4	10-Apr-04	Unique Match	Chicken	Animal
SM 0559	Water Sample # 10	5	10-Apr-04	No Match	Indeterminate	Animal
SM 0560	Water Sample # 11	1	10-Apr-04	No Match	Classification Accepted	Human**
SM 0560	Water Sample # 11	2	10-Apr-04	No Match	Indeterminate	Animal
SM 0560	Water Sample # 11	3	10-Apr-04	No Match	Indeterminate	Animal
SM 0560	Water Sample # 11	4	10-Apr-04	Unique Match	Chicken	Animal

Source Molecular #	Client Water Sample Reference	Isolate #	Submittal Date of Water Sample	Classification	Isolate Match	Previous Classification
SM 0560	Water Sample # 11	5	10-Apr-04	Unique Match	Chicken	Animal
SM 0561	Water Sample # 12	1	10-Apr-04	No Match	Indeterminate	Animal
SM 0561	Water Sample # 12	2	10-Apr-04	Unique Match	Raccoon	Animal
SM 0561	Water Sample # 12	3	10-Apr-04	Unique Match	Raccoon	Animal
SM 0561	Water Sample # 12	4	10-Apr-04	No Match	Indeterminate	Animal
SM 0561	Water Sample # 12	5	10-Apr-04	No Match	Indeterminate	Animal
SM 0562	Water Sample # 13	1	10-Apr-04	Unique Match	Dog	Animal
SM 0562	Water Sample # 13	2	10-Apr-04	Unique Match	Dog	Animal
SM 0562	Water Sample # 13	3	10-Apr-04	Unique Match	Dog	Animal
SM 0562	Water Sample # 13	4	10-Apr-04	No Match	Indeterminate	Animal
SM 0562	Water Sample # 13	5	10-Apr-04	No Match	Classification Accepted	Human**

** Result should be confirmed with the Human Bacteroidetes ID and/or Human Enterococcus ID test(s).

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 codes 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., Ellersieck, 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.

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.