



# Source Molecular Corporation

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## Ruminant Fecal ID™

Detection of Ruminant-Associated Fecal Indicator Bacteria by Real-Time Quantitative Polymerase Chain Reaction (qPCR)

**Submitter:** ABC Company

**Date Received:** October 3, 2011

**Date Reported:** October 11, 2011

SM #	Client #	Analysis Requested	DNA Analytical Results
SM 16300	01012011A	Ruminant Fecal ID	<b>Negative</b>
SM 16298	01012011E	Ruminant Fecal ID	Positive

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

## **Laboratory Comments**

Each submitted water sample was filtered for *Bacteroidetes* and the DNA was extracted and purified for DNA analysis. All reagents, chemicals and apparatus were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminants, including PCR inhibitors.

Samples 276 (Our Ref: SM 0226) and 278 (Our Ref: SM 0228) tested negative for the fecal *Bacteroidetes* ruminant gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have ruminant contamination. The biomarkers serve as an indicator of the targeted fecal pollution, but the absence of the biomarker does not signify conclusively the absence of that form of fecal pollution. Only repeated sampling events (both during wet and dry events) will enable you to draw more definitive conclusions.

Samples 275 (Our Ref: SM 0225) and 277 (Our Ref: SM 0227) tested positive for the fecal *Bacteroidetes* ruminant gene biomarker suggesting that ruminant fecal contamination is present in these water samples. Nonetheless only repeated sampling events (both during wet and dry events) will enable you to draw more definitive conclusions.

## **DNA Analytical Method Explanation**

Each submitted water sample was filtered through 0.45 micron membrane filters. Each filter was placed in a separate, sterile 5ml disposable tubes containing a unique mix of beads and lysis buffer and then bead beaten for 5min. DNA extraction was prepared using the MoBio Power Water DNA Isolation kit (MoBio, Carlsbad, CA), as per manufacturer's protocol.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOne real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul containing sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays were run in duplicate.

For quality control purposes, a positive control consisting of ruminant fecal DNA and a negative control consisting of PCR-grade water, were run alongside the sample(s) to ensure a properly functioning reaction and to reveal any false negatives or false positives. The accumulation of PCR product was detected and graphed in an amplification plot. If the fecal indicator organism was absent in the sample, this accumulation was not detected and the sample was considered negative. If accumulation of PCR product was detected, the sample was considered positive

## Ruminant Fecal ID™ Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabit the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.<sup>1</sup> Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Ruminant Fecal ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.<sup>2,3,4,5,6</sup> Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in ruminants. Within these *Bacteroidetes*, certain genetic sequences in the *Bacteroides* and *Prevotella* genus have been found in ruminants.<sup>7</sup> As such, these bacterial strains can be used as indicators of ruminant fecal contamination.

One of the advantages of the Ruminant Fecal ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve indicates that the ruminant *Bacteroidetes* gene biomarker is not present.

### References

- <sup>1</sup> Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions**. Appl. Environ. Microbiol. (2002) 68: 5796-5803.
- <sup>2</sup> Bernhard, A.E., and Field, K.G. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes**. Appl. Environ. Microbiol. (2000a) 66: 1,587-1,594.
- <sup>3</sup> Bernhard, A.E., and Field, K.G. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA**. Appl. Environ. Microbiol. (2000b) 66: 4,571-4,574.
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- <sup>5</sup> Fogarty, Lisa R., Voytek, Mary A. **Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. (2005) 71: 5999-6007.
- <sup>6</sup> Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification** Appl. Environ. Microbiol. (2005) 71: 3184-3191.
- <sup>7</sup> Reischer, Georg H., Kasper, David C., Steinborn, Ralf., Mach, Robert L., Farnleitner, Andreas H. **Quantitative PCR Method for Sensitive Detection of Ruminant Fecal Pollution in Freshwater and Evaluation of This Method in Alpine Karstic Regions**. Appl. Environ. Microbiol. (2006) 72: 5610-5614