



Source Molecular Corporation

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Preliminary Interpretation of Gull Fecal "Quantification" ID™ Results

Detection and Quantification of the Fecal Gull Gene Biomarker for Gull Fecal Contamination by Real-Time Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Company

Submitter Reference: 01012011A, 01012011B, 01012011C, 01012011D

Source Molecular #'s: SM 16294, SM 16295, SM 16296, SM 16297

Date Received: October 3, 2011

Date Reported: October 11, 2011

SM #	Client #	Approximate Contribution of Gull Fecal Pollution in Water Sample	Comment
SM 16294	01012011A	Negative	Negative for the gull biomarker
SM 16295	01012011B	Negative	Negative for the gull biomarker
SM 16296	01012011C	Major Contributor	High levels of gull biomarker detected
SM 16297	01012011D	Major Contributor	High levels of gull biomarker detected

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Gull Fecal Quantification ID™

Detection of Gull Fecal Contamination by Quantitative Polymerase Chain Reaction (qPCR) DNA Analytical Technology

Submitter: ABC Company

Date Received: October 3, 2011

Date Reported: October 11, 2011

SM #	Client #	Analysis Requested	Gull Specific Marker Quantified*	DNA Analytical Results
SM 16294	01012011A	Gull Fecal ID		Negative
SM 16295	01012011B	Gull Fecal ID		Negative
SM 16296	01012011C	Gull Fecal ID	5.97E+02	Positive
SM 16297	01012011D	Gull Fecal ID	4.57E+03	Positive

*Numbers reported as copy numbers per 100 mL of water

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.

Laboratory Comments
Submitter: ABC Company
Report Date: October 11, 2011

Each submitted water sample was filtered for *Catelliboccus marimammalium* and 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.

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-Summary and Evaluation of Client Results

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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 sample extract, forward primer, reverse primer, probe and an optimized buffer. The following thermal cycling parameters were used: 95°C for 10 min and 40 cycles of 95°C for 15 s and 62°C for 1 min. All assays were run in duplicate. Absolute quantification was achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control consisting of *Catelliboccus marimammalium* genomic DNA and a negative control consisting of PCR-grade water, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

C. marimammalium Theory Explanation

C. marimammalium are shown to be ubiquitous in the gull gastrointestinal tract for multiple species of the gull genus *Larus* found throughout North America.¹

Classified as a novel genus and species in 2006, *C. marimammalium* is a Gram-positive, catalase-negative, facultatively anaerobic, coccus-shaped bacterium, related to, although distinct from, other catalase-negative genera which include *Enterococcus*, *Melissococcus*, *Tetragenococcus* and *Vagococcus*².

As a novel bacterium species, the pathogenesis of *C. marimammalium* is relatively unknown. However, there are increasing public health concerns regarding avian fecal contamination in the environment due to the potential spread of microbial avian pathogens to humans, domesticated animals, and human food sources¹. Studies have shown also that waterfowl carry human pathogens such as *Campylobacter spp*³, *Salmonella spp*⁴, and *E. coli*⁵, as well as being reservoirs of influenza viruses⁶.

The Gull Fecal ID™ service is designed around the principle that *C. marimammalium* is highly specific and sensitive to numerous gulls of the genus *Larus*¹. This *C. marimammalium* bacterium can be used as an indicator of gull fecal contamination. Use of real-time (quantitative) Polymerase Chain Reaction (qPCR) allows for the rapid amplification of the gene biomarker to demonstrate the presence of gull feces and allow for the real-time visualization of the target.

Accuracy of the results is possible because the method uses real-time (quantitative) PCR DNA technology. Real-time (quantitative) PCR allows small DNA sequences to be amplified exponentially and detected in real-time via fluorescent probes.

DNA amplification is accomplished with small pieces of DNA called primers that are specific to the genomes of interest. Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers. The DNA is replicated to create exact copies of the desired DNA fragment (i.e. the gene biomarker). This process is repeated rapidly many times ensuring an exponential growth 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 for detection. With real-time (quantitative) PCR, the desired DNA fragments are also bound by fluorescent reporter probes. Consequently, the more copies of the desired DNA fragments that are made, the stronger the fluorescent signal, thus allowing for a straightforward detection and quantification of the targeted gene in real-time via the real-time PCR associated software. Nonetheless, as with all analytical tests, in order to strengthen the validity of the results, the Gull Fecal ID™ service should be combined with other DNA analytical services such as the *E. coli* ID™ service.

References

¹**Phylogenetic Diversity and Molecular Detection of Bacteria in Gull Feces** Lu, Jungrang, Santo Domingo, Jorge W., Lamendella, Regina, Edge, Thomas, Hill, Stephen; *Appl. Environ. Microbiol.* **2008**, 74: 3969-3976.

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³**Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in Different Ecological Guilds and Taxa of Migrating Birds** Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R.P., Wagenaar, J.A., Olsen, B.; *Appl. Environ. Microbiol.* **2002**, 68: 5911-5917.

⁴**Diversity of *Salmonella* Strains Isolated from the Aquatic Environment as Determined by Serotyping and Amplification of the Ribosomal DNA Spacer Regions** Julia Baudart, Karine Lemarchand, Anne Brisabois, and Philippe Lebaron.; *Appl. Environ. Microbiol.*; **2002**, 66: 1544-1552.

⁵**Detection and Characterization of Shiga-toxin Producing *E. coli* from Seagulls** Makino, S., Korbi, H., Asakura, H., Watarai, M., Shirahata, T., Ikeda, T., Takeshi, K., Tsukamoto, T.; *Epidemiol. Infect.* **2000**, 125: 55-61.

⁶**Influenza in Migratory Birds and Evidence of Limited Intercontinental Virus Exchange** Krauss, S., Obert, C.A., Franks, J., Walker, D., Jones, K., Seiler, P., Niles, L., Pryor, S.P., Obenauer, J.C., Naeve, C.W., Widjaja, L., Webby, R.J., Webster, R.G.; *PLoS Pathog.*; **2007**, 3: 167.