4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ACCREDITED
Certificate 4126.01
ISO 17025 Accredited
Testing Laboratory

Gull Fecal Quantification ID

Detection of the fecal associated Gull gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Company A
Date Received: January 2, 2017
Report Generated: January 8, 2017

SM #	Sample ID	Analysis Requested	DNA Analytical Results
SM-6A00001	Sample 1	Gull Fecal ID	**Detected**
SM-6A00002	Sample 2	Gull Fecal ID	Not Detected
SM-6A00003	Sample 3	Gull Fecal ID	Not Detected
SM-6A00004	Sample 4	Gull Fecal ID	**Detected**
SM-6A00005	Sample 5	Gull Fecal ID	**Detected**
SM-6A00006	Sample 6	Gull Fecal ID	Not Detected

Revision 1.2
Effective Date 11/2/17

Laboratory Comments

Submitter: Company A

Report Generated: January 8, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus 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. All assays are run in duplicate. Quantification is 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 and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

C. marimammalium Gull Fecal "Quantification" ID™

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 sources[1]. 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 viruses[6].

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* baterium 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] **Phylogenic 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.

[2] ***Catellicoccus marimammalium* gen. nov., sp. nov., a novel gram-positive, catalase-negative, coccus-shaped bacterium from porpoise and grey seal** Lawson, P.A., Collins, M.D., Falsen, E., Foster, G.; *Int J Syst Evol Microbiol.* **2006**, 56: 429-432.

[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 Shinga-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.