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In the last couple of decades the pinniped population in the waters surrounding Cape Cod has exploded. In parallel, beach closures are on the rise due to high levels of fecal indicator bacteria (FIB). Few studies have determined the link between seal scat and beach closures. Quantitative PCR is widely used to track the source of FIB. Accordingly, we developed primers specific for strains of *Bacteroides* species that abound in seal scat. The primers were evaluated with endpoint PCR using template DNA extracted from seal scat and, for comparison, domesticated dogs. The primers generate a product of the appropriate size with fecal matter from two locations: a seal pen at Moody Garden aquarium and a in the national seashore. The primers did not generate a PCR product from feces collected from dogs.

Since mammals can have similar gut microbiota, testing the primer against dog fecal matter will eliminate the outcome of the primer not being specific to seals. Antibiotic resistant bacteria associated with FIB threaten the public health (Leonard et al, 2017). Seals are the numerically abundant mammals in many coastal system and could contribute a significant amount of scat. To determine if the hypothesis is correct, the first step is to develop and test a primer specific to seals. Since mammals can have similar gut microbiota, testing the primer against dog fecal matter will eliminate the outcome of the primer not being specific to seals.

Fecal matter from two different sources for seal scat; Moody Gardens, Galveston (fresh sample) and NOAA (frozen sample, 2007 & 2017) were used to validate primer design. A dog feces sample was used for a negative control. DNA was recovered from each sample with a fecal DNA extraction kit (IBI SCIENTIFIC). Nanodrop was performed to check for clean DNA to continue. A PCR primer specific to seal bacteroidetes was developed. Gradient PCR with the seal primer was performed to optimize PCR conditions.

The quality of DNA was assessed by UV spectra and qPCR. Nanodrop results showed the fresh dog and seal scat samples yielded DNA suitable for PCR. DNA from frozen samples showed spectra indicating contamination and were not considered suitable. DNA from the remaining seal and dog preps was suitable for PCR as assessed by qPCR (Fig. 1). Gradient PCR with seal-specific primers only generated a product from the seal sample. This suggests the primers can differentiate scat from seals from dog feces.

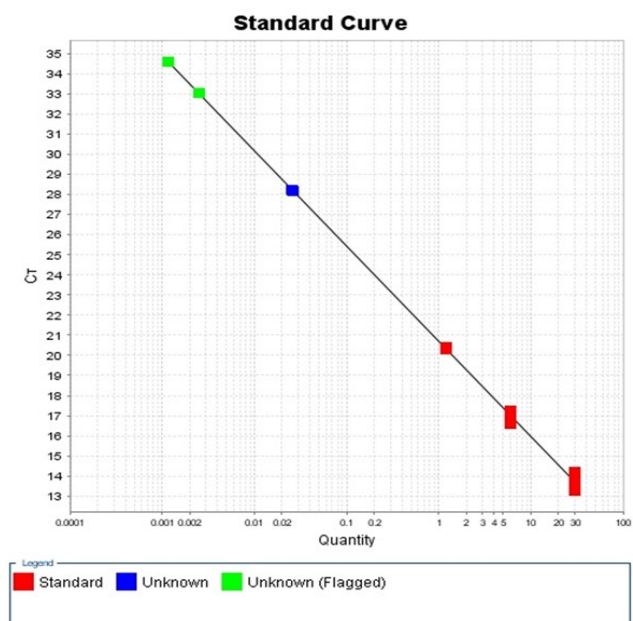


Figure 1: Standard Curve of Concentration for Seal, Dog, and E Coli obtained from qPCR using 16s primers.

Leonard AFC, Zhang L, Balfour AJ, et al. (2018) Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment International* 114:326.