Promega

Product Application

Amplification of Inhibitor-Rich Fecal and Environmental DNA Samples

Amplify inhibitor-rich fecal and environmental DNA samples via qPCR using the GoTaq® Endure qPCR Master Mix.

Kit: GoTaq® Endure qPCR Master Mix (Cat.# A6220)

Analyses: qPCR

Sample Type(s): Fecal and Environmental DNA Samples

Input: 2µl or 5µl

Materials Required:

- GoTaq® Endure qPCR Master Mix (Cat.# A6220)

Protocol:

1. Amplify DNA samples via qPCR according to instructions from the GoTaq® Endure qPCR Master Mix Technical Manual (TM752).

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM752, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Results:

The GoTaq® Endure qPCR Master Mix can be used to amplify 2µl or 5µl of inhibitor-rich DNA samples with little to no inhibition as compared to competitor master mixes.

Table 1: Summary of inhibition for the amplification of fecal and environmental DNA samples with different qPCR master mixes. Fecal and environmental DNA samples were amplified according to the methods described in Figure 1. A summary of the samples that were amplified with less than 1 cycle inhibition is shown. Inhibition was assessed using an Internal Positive Control (IPC) or by comparing the amplification of undiluted and 1:10 diluted samples. A green "✓" indicates there was less than 1 cycle of inhibition for the given master mix and metric. A red "X" indicates that there was more than 1 cycle of inhibition for the given master mix and metric.

Master Mix	Metric	2µl Sample Input				5µl Sample Input			
		Human Feces	Soil	Water	Dog Feces	Human Feces	Soil	Water	Dog Feces
Supplier A Universal	IPC	X	X	X	X	X	X	X	X
	1:10 Dilutions	X	X	X	X	X	X	X	X
Supplier A Environmental	IPC	✓	✓	✓	✓	X	X	X	X
	1:10 Dilutions	X	X	✓	✓	X	X	X	X
GoTaq® Endure	IPC	✓	✓	✓	✓	✓	✓	✓	✓
	1:10 Dilutions	✓	✓	✓	✓	✓	✓	✓	✓

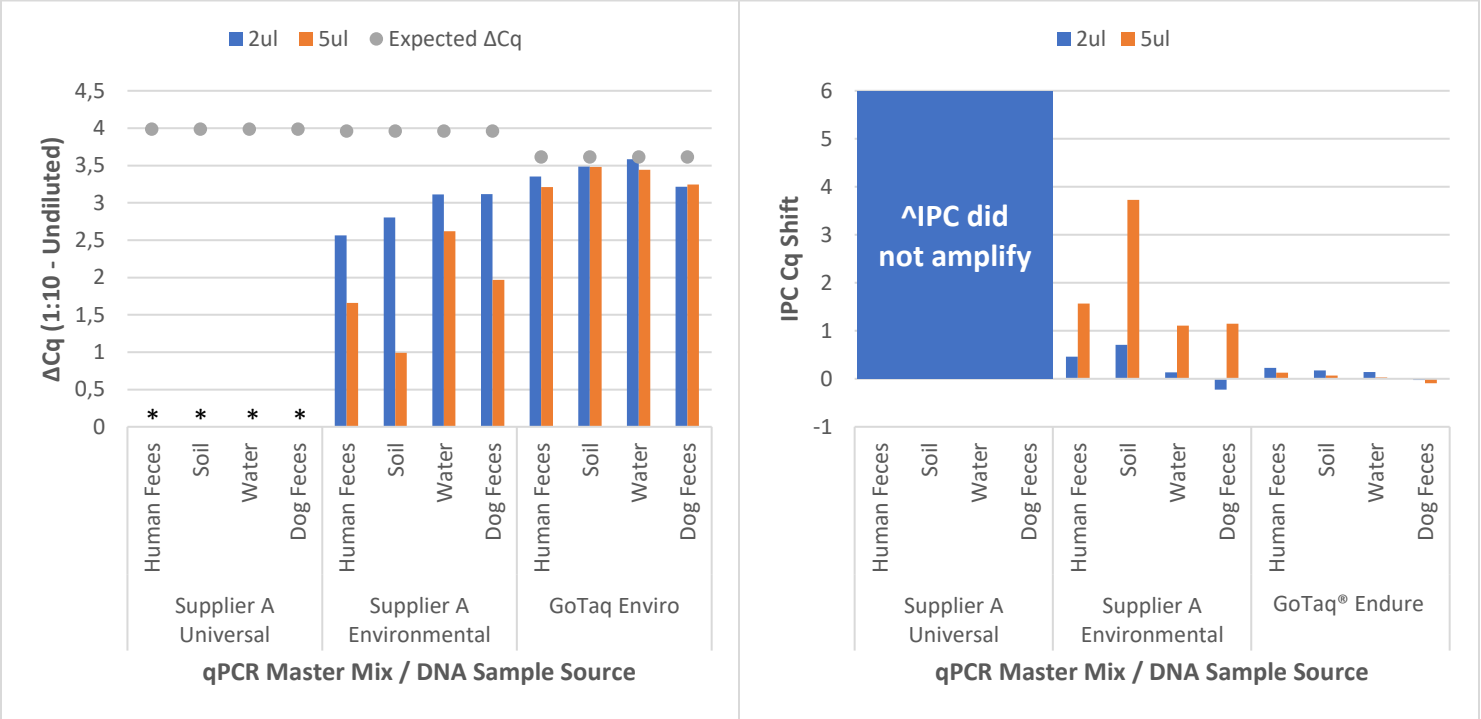

Figure 1: (Left) ΔCq values from amplification of undiluted and 1:10 diluted fecal and environmental DNA samples. 2µl or 5ul of inhibitor-rich DNA samples purified from human feces, soil, environmental water runoff (water), or dog feces were amplified in duplicate using three different master mixes – Supplier A Universal Master Mix, Supplier A Environmental Master Mix, or the GoTaq® Endure qPCR Master Mix (Cat.# A6220). Samples were amplified undiluted or diluted 1:10 in Nuclease Free Water using primers specific to the 16S rRNA gene[1]. 20µl reactions were prepared with a final primer concentration of 500nM and a final probe concentration of 250nM. Reactions were cycled on a QuantStudio™ 6 Pro Real-Time PCR Instrument (Applied Biosystems™) according to each respective technical manual with an annealing temperature of 62°C. ΔCq values were calculated between the average Cq value of undiluted and 1:10 diluted samples. An expected ΔCq value between 1:10 dilutions for each master mix was calculated using the amplification efficiency and indicated no inhibition. *No ΔCq values are reported for Supplier A Universal master mix as undiluted samples did not amplify for this master mix, except for 1 replicate of the 2µl dog feces sample. **(Right) Average Internal Positive Control (IPC) Cq shift in the presence of fecal and environmental DNA samples.** 2µl or 5ul of the samples listed above were also amplified in duplicate with each master mix using the IPC qPCR Inhibition Control Assay, CAL Fluor®560 (Cat.# AM2030). 20µl reactions were prepared with a final concentration of 1X IPC qPCR Inhibition Control Assay and were amplified according to the conditions listed above. For each sample, 16S rRNA amplification was compared to the standard curve to determine which standard most closely matches the DNA concentration of that sample. The IPC amplification from that standard was then used to calculate the IPC shift for that sample. An IPC shift closer to zero indicates less inhibition. ^IPC did not amplify for these samples.

References:

1. Zucol F, Ammann RA, Berger C, Aebi C, Altwegg M, Niggli FK, Nadal D. Real-time quantitative broad-range PCR assay for detection of the 16S rRNA gene followed by sequencing for species identification. J Clin Microbiol. 2006 Aug;44(8):2750-9.