Amplification of Inhibitor-Rich Soil Samples

Amplification of soil DNA samples using the inhibitor-resistant GoTaq® Endure qPCR Master Mix.

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

Analyses: End-point, qPCR

Sample Type(s): DNA purified from soil samples

Materials Required:

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

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

Contact Technical Services at: techserv@promega.com

Protocol:

1. Amplify ≤250ng of soil DNA according to the technical manual (TM752) with the GoTaq® Endure qPCR Master Mix.
2. An example of an end-point PCR reaction set-up is given in Table 1 and recommended cycling conditions are given in Table 2.

Table 1. PCR Setup

Reagent	Volume per reaction (µl)
GoTaq® Endure Master Mix (2X)	10
Forward Primer 10µM	1
Reverse Primer 10µM	1
Nuclease-Free Water	6.5
Template DNA	1.5
Total	**20**

Table 2. Thermal cycling conditions for PCR Assay

Step	Number of Cycles	Temperature, Time
GoTaq® Activation	1	95°C, 2 minutes
Denaturation	30	95°C, 15 seconds
Annealing		60°C, 15 seconds
Extension		72°C, 45 seconds
Final Extension	1	72°C, 5 minutes

3. An example of a qPCR reaction set-up is given in Table 3 and recommended cycling conditions are given in Table 4.

Table 3. qPCR Setup

Reagent	Volume per reaction (µl)
GoTaq® Endure Master Mix (2X)	10
Forward Primer 10µM	1
Reverse Primer 10µM	1
Hydrolysis probe	1
Nuclease-Free Water	5
Template DNA	2
Total	**20**

Table 4. Thermal cycling conditions for qPCR Assay

Step	Number of Cycles	Temperature, Time
GoTaq® Activation	1	95°C, 2 minutes
Denaturation	40	95°C, 15 seconds
Annealing/ Extension (Read)		60°C, 15 seconds

Results:

The GoTaq® Endure qPCR Master Mix can be used to amplify inhibitor-rich soil DNA samples with little to no inhibition compared to other systems.

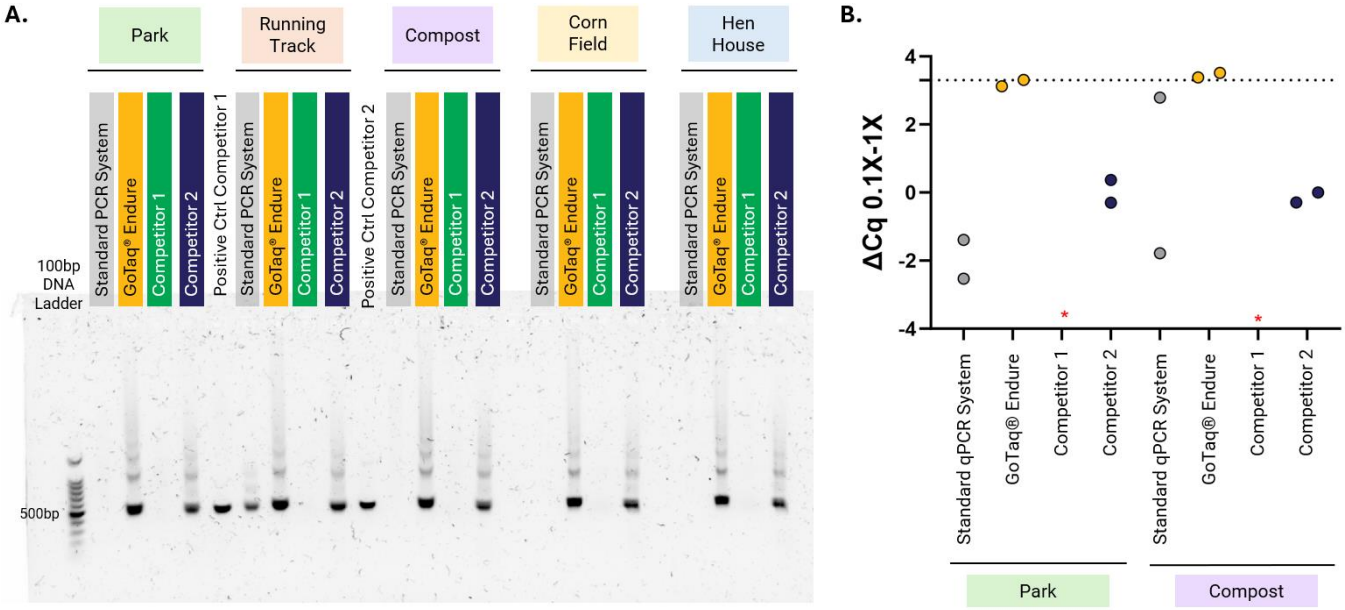

Figure 1. Amplification from soil DNA samples. Soil DNA was purified with the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600) on the Maxwell® RSC Instrument (Cat.# AS4500). Eluates were amplified using universal bacterial primers[1] and probe (amplicon ~470bp). **A.** Bacterial amplicons were visualized on a 1% agarose gel. End-point amplification was carried out with a standard PCR system (grey), with the GoTaq® Endure Master Mix (yellow), or with two different competitor systems designed for inhibitor-rich samples, the SsoAdvanced™ Universal Probes Supermix (green) and the TaqMan™ Environmental Master Mix 2.0 (blue). *E. coli* DNA was used for positive control. **B.** qPCR was performed using a standard probe qPCR System (grey), the GoTaq® Endure qPCR Master Mix (yellow), or two different competitor systems designed for inhibitor-rich samples, Competitor 1 (SsoAdvanced™ Universal Probes Supermix, green) or Competitor 2 (TaqMan™ Environmental Master Mix 2.0, blue). Soil DNA eluates were used neat or diluted 0.1X in Nuclease-Free Water. $\Delta Cq_{0.1X\text{-}1X}$ values ($Cq_{0.1X}$-Cq_{1X}), for duplicate purifications amplified in technical duplicate are shown. Red asterisk indicates no amplification of undiluted and diluted samples (strong inhibition). The dashed line represents a ΔCq value of 3.3, which is the expected ΔCq value assuming 100% amplification efficiency.

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.