Promega

Product Application

Probe-based Amplification of Inhibitor-rich Plant DNA Samples

Amplify inhibitor-rich plant DNA samples using the GoTaq® Endure qPCR Master Mix.

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

Analyses: qPCR

Sample Type(s): DNA extracted from plant leaves or fruit.

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, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol:

1. Amplify ≤250ng of plant DNA according to instructions from the Technical Manual (TM752) with the GoTaq® Endure qPCR Master Mix.

2. Results:

The GoTaq® Endure qPCR Master Mix can be used to amplify up to 250ng of inhibitor-rich plant DNA samples with little to no inhibition compared to standard probe qPCR systems.

Table 1. Summary of inhibition for the amplification of plant DNA samples in probe qPCR.
Plant DNA samples, purified from leaves or from fruits, were amplified via probe qPCR according to the method described in Figure 1, using either a standard system or the GoTaq® Endure qPCR Master Mix. Inhibition was assessed by adding an Internal Positive Control (IPC) (Cat.# AM2030) to the reaction and calculating IPC shift (see details in Figure 1 legend). An IPC shift greater than 0.3 flags a sample for amplification inhibition. A green tick indicates the absence of qPCR inhibition, a red cross is indicative of qPCR inhibition. Note: For lemon tree, olive tree and rice samples 1 out of 2 replicates showed inhibition with a standard qPCR system.

Master Mix	Plant samples											
	Mint	Lemon tree	Olive tree	Peach tree	Rose bush	Zucchini	Wheat	Rice	Strawberry	Raspberry	Strawberry (fruit)	Grape (fruit)
Standard qPCR system	✗	✗	✗	✗	✗	✗	✓	✗	✗	✗	✓	✓
GoTaq® Endure qPCR Master Mix	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

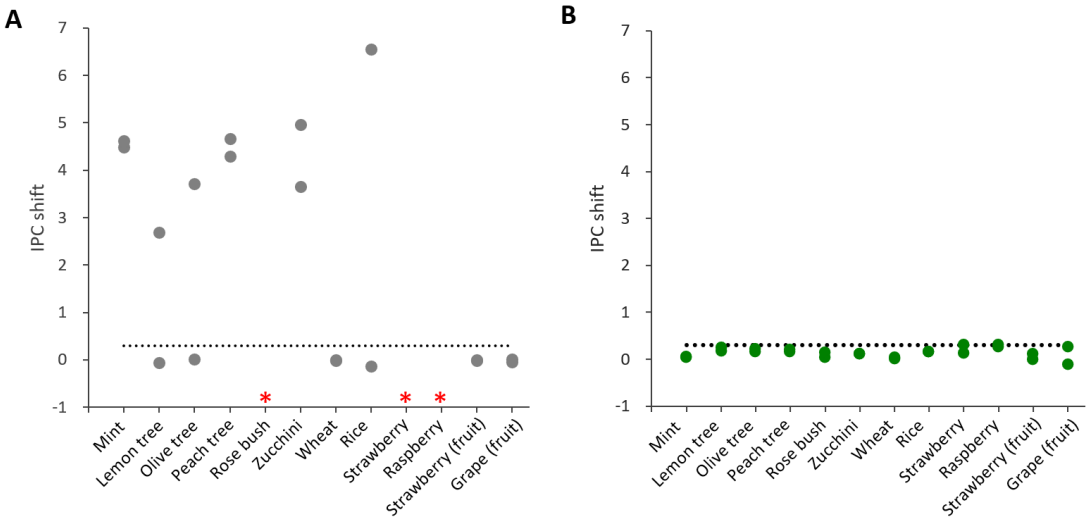

Figure 1. Plant DNA probe qPCR inhibition analysis by IPC shift calculation. ≤250ng of plant DNA was amplified using either a standard probe qPCR system (grey) or the GoTaq® Endure qPCR Master Mix (Cat.# A6220, green). An exogenous internal positive control (IPC) (Cat.# AM2030) was added to the reaction. Reactions were performed in a 20µl final volume, the final concentration of forward and reverse primers was 500nM, the final concentration of the probe was 150nM (a universal plant primer/probe set was used). Samples were processed using a CFX96 Touch™ Real-Time PCR Detection System and according to the following conditions: 95°C for 2 minutes, 40 x (95°C for 15 seconds and 60°C for 1 minute). The amplification of the assay IPC was compared to the Cq value of the closest standard curve sample to calculate IPC shift. An IPC shift greater than 0.3 flags a sample for amplification inhibition and is indicated by the dotted line. The red asterisk indicates no amplification of the IPC (strong inhibition).