

Dye-based Amplification of Inhibitor-rich Plant DNA Samples

Amplify inhibitor-rich plant DNA samples using the GoTaq® Enviro BRYT-Green® MasterMix.

Kit: GoTaq® Enviro BRYT-Green® MasterMix (Cat.# CS317427)

Analyses: qPCR

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

Materials Required:

- GoTaq® Enviro BRYT-Green® MasterMix (Cat.# CS317427)

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, contact Technical Services at:
techserv@promega.com

Protocol:

1. Prepare the reaction mix (minus DNA template) as described in Table 1. For more information refer to the Kit Instruction Manual (EA014), available on demand.

Table 1- qPCR Setup*

Reagent	Final Concentration
GoTaq® Enviro BRYT-Green® Master Mix (2X)	1X
Forward Primer (20X)	200nM–1µM
Reverse Primer (20X)	200nM–1µM
Nuclease-Free Water	to a final volume of 20µl

** Some instruments may require the addition of a CXR Reference Dye, which is provided with the kit.*

2. Add the DNA template (or water for no-template control reactions).
3. Cycle the reaction according to the parameters in Table 2 with the optical settings established for SYBR® Green I assays.

Table 2- Thermal cycling profile for qPCR Assay

Step	Number of Cycles	Temperature, Time
GoTaq® Activation	1	95°C, 2 minutes
Denaturation	40	95°C, 3 seconds
Annealing/Extension		60°C, 30 seconds

Results:

The GoTaq® Enviro BRYT-Green® MasterMix can be used to amplify up to 250ng of inhibitor-rich plant DNA samples with little to no inhibition compared to standard dye-based qPCR systems.

Table 1. Summary of inhibition for the amplification of plant DNA samples in dye-based qPCR.

Plant DNA samples, purified from leaves or from fruits, were amplified via dye-based qPCR according to the method described in Figure 1, using either a standard system or the GoTaq® Enviro BRYT-Green® MasterMix. Inhibition was assessed by determining the difference in Cq values between DNA eluates processed neat or diluted 1:10. For an amplification reaction with 100% amplification efficiency, $\Delta Cq = 3.3$ is expected between 1:10 dilutions. A green checkmark indicates the absence of qPCR inhibition, an orange cross indicates low to medium inhibition and a red cross is indicative of strong qPCR inhibition. Rose bush samples presented amplification inhibition with the GoTaq® Enviro BRYT-Green® MasterMix however, inhibition was significantly reduced when compared to a standard qPCR system.

Master Mix	Plant samples										
	Lemon tree	Olive tree	Peach tree	Rose bush	Zucchini	Wheat	Rice	Strawberry	Raspberry	Strawberry (fruit)	Grape (fruit)
Standard qPCR system	✓	✗	✓	✗	✗	✓	✗	✗	✗	✓	✓
GoTaq® Enviro BRYT-Green® MasterMix	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓

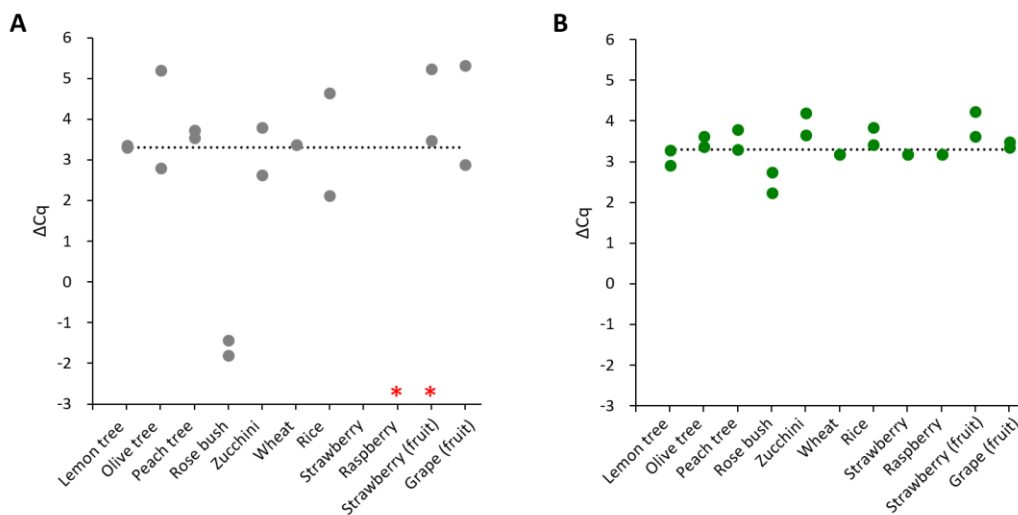


Figure 1. Plant DNA dye qPCR inhibition analysis by ΔCq calculation. ≤ 250 ng of plant DNA was amplified using either a **A)** standard dye-based qPCR system (grey) or **B)** the GoTaq® Enviro BRYT-Green® MasterMix (Cat.# CS317427, green). Samples were amplified undiluted and diluted 1:10 in Nuclease Free Water. Reactions were performed in a 20 μ l final volume, the final concentration of forward and reverse primers was 500nM (universal plant primers were 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). ΔCq values (1:10-neat) are shown. For an amplification reaction with 100% amplification efficiency, $\Delta Cq = 3.3$ is expected between 1:10 dilutions and is indicated by the dotted line. The red asterisk indicates no amplification of the neat samples (strong inhibition).