Catalog #: T0501S

GMO Test Kit

Research use only

01 INTRODUCTION

Genetically modified organisms are either of plant or animal origin, and contain an altered characteristic or trait. We will focus on genetically modified plants. One of the most common genetic modifications of plants is to include a gene encoding a toxin from a bacterium that confers resistance to various insect pests. GMO plants/plant products are characterized by the presence of the cauliflower mosaic (CaMV) 35S promoter and the terminator sequence of the nopaline synthase (tNOS) gene from Agrobacterium tumifaciens used to control gene expression of the foreign gene. The Chai GMO Educational kit uses primers to amplify and detect part of the tNOS terminator sequence and a conserved region of the chloroplast gene as an extraction control by real-time PCR. Amplification of the control ensures that the extract has been prepared correctly and the PCR Master Mix functions correctly. The amplification plot does not differentiate between the tNos and Chloroplast gene products. Melt Curve Analysis (MCA) is used to further differentiate between the tNos and chloroplast target sequence based on melting temperature of the amplified products.

02 APPLICATIONS

Identify GMO in plant and seeds based on the detection of the *Agrobacterium tumifaciens* tNOS gene sequences by real-time PCR followed by MCA.

03 SAMPLE TYPE

Crude extract from leaves or frozen corn.

04 USER PROVIDED MATERIALS

a. Thermocycler with capabilities for real-time PCR and melt curve analysis
b. Low speed mini centrifuge for microfuge tubes and PCR tubes
c. Vortexer
d. Tube racks for PCR tubes and microfuge tubes

05 PRODUCT CONTENTS

Kit Components	Volume/Quantity
Plant Extraction Buffer A	5 mL
Enzyme B	Lyophilized
Reconstitution Buffer "C"	0.05 mL
tNos DNA (control)	Lyophilized
Primers	Lyophilized
PCR Master Mix + Hot Start DRY	Lyophilized
Reconstitution Buffer	0.7 mL
Chai Green 20X Dye	1 mL
Water	5 mL
1.5 mL tubes	30
8-strip PCR Strip Tubes	15
8-strip PCR Strip Caps	15

06 STORAGE CONDITIONS AND REAGENT PREPARATION

Spin down all tubes to collect contents at the bottom of the tube before opening the caps. It is recommended to keep reconstituted reagents on ice. Store all dry components at -20 °C for long-term storage. Once the various components are reconstituted, store at -20 °C and avoid repeated freeze-thaw.

1. Transfer 50 µL Reconstitution Buffer "C" to lyophilized Enzyme B using the adjustable-volume micropipette provided in the biohacker kit. Mix gently by pipetting. Spin down the tube contents.
2. Transfer 50 µL of reconstituted Enzyme B using the adjustable-volume micropipette to 5 mL of Extraction Buffer A in the bottle and mix well by gently swirling the bottle. This is the Chai Plant Extraction Solution. Avoid repeated freeze-thaw. Store at 4 °C (short-term) or -20 °C (long-term). The product is stable for two years from date of receipt when stored at -20 °C without freeze-thaw. Make aliquots for use.
3. Reconstitute primers with 275 µL (5 x 50 µL + 1 x 25 µL) water using the adjustable volume micropipette and mix by pipetting. Reconstitute tNos DNA (control) with 50 µL water and mix by pipetting. Keep reconstituted primers and control DNA at room temperature for an hour so that all components go into solution.

4. Spin down the tubes briefly to collect tube contents at the bottom of the tubes. Reconstitute PCR Master Mix + Hot Start DRY with 0.660 mL (1 x 0.5 mL + 3 x 50 µL + 1 x 10 µL) of Reconstitution Buffer to give PCR Master Mix + Hot Start 2X. Mix gently by pipetting five to ten times.
5. Protect the Chai Green 20X dye from light.

07 PROTOCOL

1. Sample preparation: Transfer 0.1 mL (2 x 50 µL) of Chai Plant Extraction Solution using the adjustable volume pipette to a 0.1 mL PCR tube for each sample that you are testing. You can cut tubes from the strip and use them as needed. For leaves, cut a 5-10 mm diameter disc of the leaf and avoid the thick veins. You can use the cap of a PCR tube to obtain the sample. Transfer the sample to a 0.1 mL PCR tube containing the Chai Plant Extraction Solution. For frozen corn, thaw and homogenize the pulp with a spoon. Transfer a small portion of the pulp to a 0.1 mL PCR tube containing the Chai Plant Extraction Solution making sure that it is submerged in the solution. Adding excessive pulp to the tube will result in a very concentrated crude extract, causing PCR inhibition and poor amplification of target.

2. Preparation of Crude Cell Extract: Set protocol in the Open qPCR instrument as shown in figure 1, making sure the gather data (red arrow) is turned off under step 2. Place the tubes containing the sample in the Open-qPCR, close the lid, and start the protocol. This will heat the sample at 65 °C for six minutes to digest cellular proteins, including nucleases that would otherwise degrade the DNA, further releasing the DNA into solution. Heating the sample at 98 °C for two minutes will inactivate the enzymes that would otherwise interfere with down-stream applications such as PCR or qPCR. Once the protocol completes, carefully remove the PCR tube (the lid and the tube holder will be hot) and cool the contents to room temperature. Centrifuge to collect the condensate and to separate the extract from the debris. You can leave the leaf sample in the crude extract and use the crude extract for PCR amplification. Keep on ice. The crude extract can be stored at -20 °C for at least two-four months and used for analysis. Best results are obtained with freshly prepared crude cell extracts.

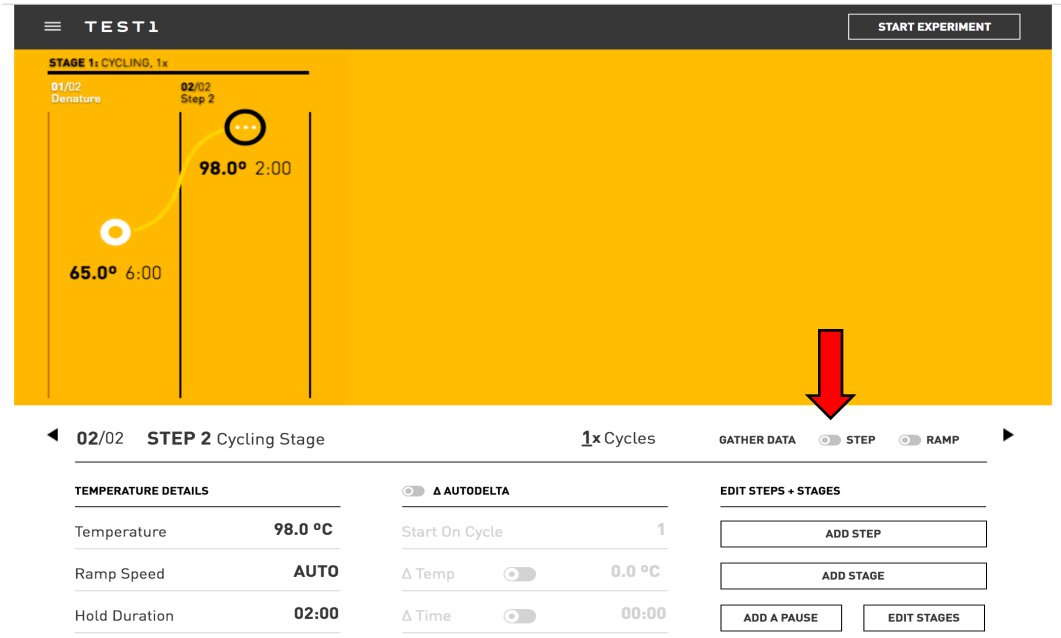

Figure 1. Open qPCR Protocol for preparation of Crude Cell Extract.

3. Preparing the qPCR reaction mix and setting up the real-time PCR protocol: Prepare a common qPCR mix for the total number of reactions that you plan to use. Table 1 shows the amount required for a single reaction. Make a common reaction mix without the DNA or crude cell extract. Determine the amount of each component required for the number of reactions that you plan to perform. Add an extra 10% of the volume for each component used. Add 20 µL of the reaction mix to each tube in the qPCR strip. Set aside one tube for DNA control.

   The PCR strip tubes and strip caps can be cut and used for each reaction. Dilute the sample 1:1 as follows in a PCR tube: add 50 µL crude extract to 50 µL water (provided) and mix well by gently pipetting. Use 5 µL of the 1:1 diluted crude extract or 5 µL of the control for qPCR in a 25 µL reaction volume.

   Note: You could also use 5 µL of a sample that has been diluted 10X (10 µL crude extract + 90 µL water) for the real-time PCR-MCA reaction.

08

Components	1 Rx (µL)
PCR Master Mix 2X	12.5
Chai Green 20X Dye	1.25
Primer Mix	5
Water	1.25
SUM	20

Table 1. Reaction setup

Use the protocol (figure 2) for real-time PCR followed by a melt curve analysis (stage 3).

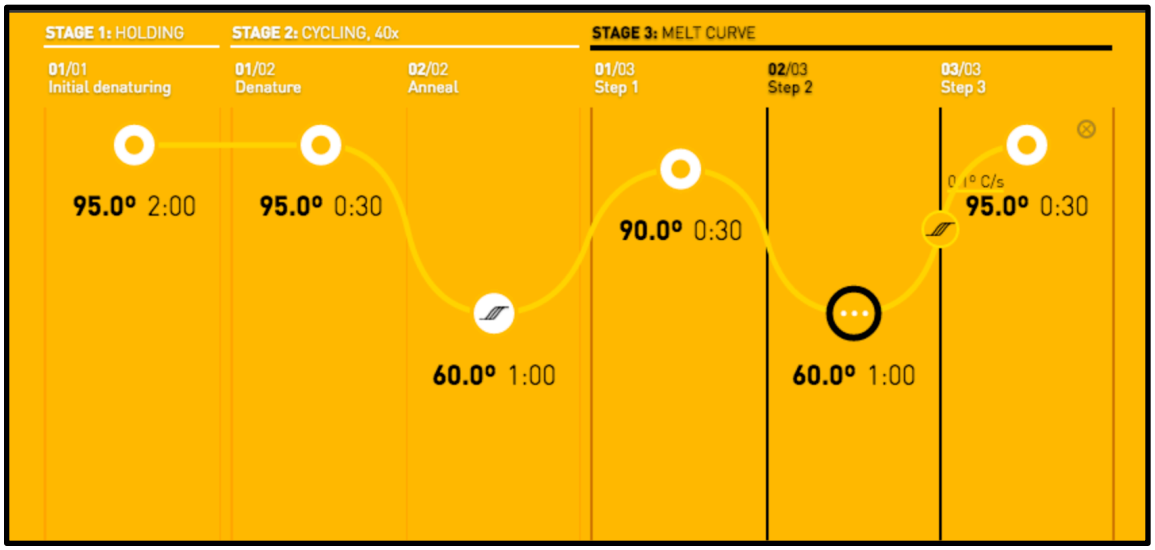

Figure 2. Real time PCR and Melt Curve Analysis Protocol

09 ANALYSIS OF RESULTS

The presence of an amplification curve indicates the presence of an amplification product.

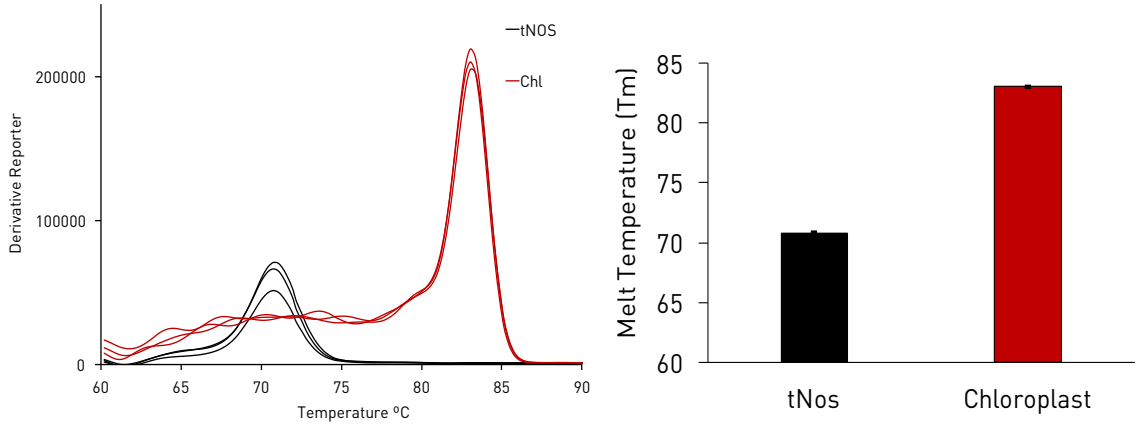

Figure 3: Melt curve analysis and melt temperatures of the chloroplast gene and the tNOS amplicon. A crude extract was prepared from frozen corn using the Chai Plant Extraction solution. 5 µL of the 1:1 diluted crude extract was used for qPCR to detect the presence of tNos and the chloroplast gene as an extraction control (ensures that the extraction was successful). A. Melt curve analysis of the sample showing the presence of two peaks, one for each target amplicon. B. The tNos and chloroplast gene products have a melt temperature of approximately 70 and 83, respectively. Melt temperature will change depending on the dye and master mix that is used.

10 SEQUENCES

a. Chloroplast FP: 5'-AGTTCGAGCCTGATTATCCC-3'
b. Chloroplast RP: 5'-GCATGCCGCCAGCGTTCATC-3'
c. tNOS FP: 5'-TTATCCTAGTTTGCGCGCTATATTT-3'
d. tNOS RP: 5'-GATTAGAGTCCCGCAATTATACATTTAA-3'
e. tNos DNA control:
TTATCCTAGTTTGCGCGCTATATTTTGTTTTCTATCGCGTATTAAATGTATAATTGCGGGACTCTAAT

11 USING THE MICROPIPETTE

Turn knob (figure 4a) for adjusting volume settings.

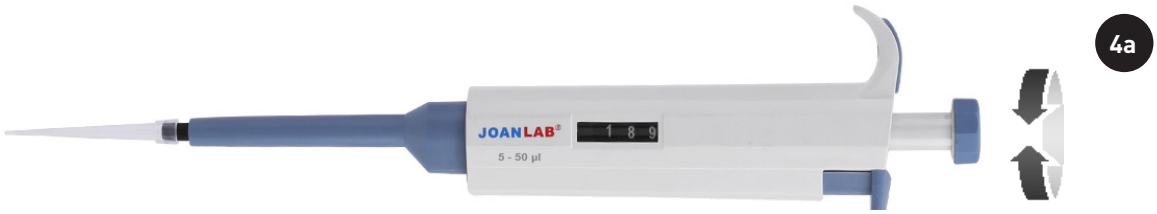

Figure 4a: Turn the knob on the top of the device in either direction to adjust volume

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