*Application Note* A09-005A

**Prime Pro reagents: GMO detection**

PrimePro 48

## Introduction

A wide range of qPCR reagent kits are available for use on Prime Pro 48 and other real-time thermal cyclers. In this application note we demonstrate the use of the TKIT06010 kit for detection of Cauliflower mosaic virus (CaMV) 35S promoter and NOS (nopaline synthase) terminator, derived from *Agrobacterium tumefaciens,* in genetically modified maize.

Since both CaMV and *A. tumefaciens* are naturally found in the soil, the kit provides control primers and probes to detect these organisms in regions of their genome which is not used in the genetic modification of plants. This mitigates against false positives due to natural infection of a sample. The kit also provides primers and probes to detect the endogenous wild-type of the maize host plant. This demonstrates successful extraction of DNA from the sample and absence of PCR inhibitors. A positive control for all assays is also provided.

## Method

In order to test the kit, DNA extractions were performed from a number of foods containing maize as the main or major ingredient (Table 1).

| Product | Approximate DNA yield (µg/ml) |
|---|---|
| Corn–based snack | 30.7 |
| Pet food (for small rodents) | 91.5 |
| Frozen sweet corn | 49.2 |
| Corn flour | 2.0 |

***Table 1**: List of food products from which DNA extractions were made.*

Each of the samples was ground to a powder or paste in a mortar and pestle. Triplicate samples of approximately 200mg were weighed into 2ml microcentrifuge tubes. DNA was extracted using the DNeasy *mericon* Food Kit from Qiagen which is designed to extract DNA from raw or highly processed foods. Due to the likelihood that the DNA may have degraded during processing of some samples, the protocol for small fragments was followed. Following extraction, The DNA yield was measured using the Jenway Genova Nano micro volume spectrophotometer then diluted to a concentration of 1ng/µl ready for amplification.

The GMO detection kit requires five separate assays to be run:

1. CaMV-GM detection.
2. CaMV-WT detection.
3. NOS-GM detection.
4. NOS-WT detection.
5. Maize-WT detection.

Each assay uses a specific hydrolysis probe which is detected on the Green (FAM) channel. The master mix used was the lyophilised 2x qPCR Mastermix, Prime Pro reagent part code TKITMM001. 20µl reactions (with 5µl of template) were prepared in duplicate for each sample and assay, including positive and no template controls. Reactions were amplified for 50 cycles according to the instruction manual in the Prime Pro 48, as set out in Table 2. Note that selecting Pro Reagents from the home screen automatically sets up the required thermal profile; only changes to the polymerase activation step may be required depending on the master mix used. Also, due to the number of samples

and assays, the reactions were split across two plates. The results were combined and analysed using the Techne Pro Study software to determine the Cq values for each sample.

| | Step | Time | Temperature |
|---|---|---|---|
| | Enzyme activation | 15 min | 95°C |
| Cycling x 50 | Denaturation | 10 sec | 95°C |
| | Extension/data collection | 60 sec | 60°C |

***Table 2**: Thermal cycling program for the maize GMO detection kit. Fluorescence data was collected through the FAM channel.*

## Results

The maize GMO detection kit is based on the PCR amplification of the highly active CaMV promotor and the NOS terminator from *A. tumefaciens*. To interpret the results, the following criteria must be met:

1. The negative control wells should give no signal and the positive control wells should give a strong signal at a Cq value of around 19±2 cycles.
2. A positive signal in CaMV-GM and NOS-GM reactions indicates the presence of GM material provided that there is no amplification in the respective WT control reactions.
3. If there is a positive reaction in the CaMV-WT or NOS-WT assays this indicates that the naturally occurring organisms are present in the samples. The data must be examined to check if the results from the GM samples give earlier Cq values, in which case the sample may contain both GM and WT organisms.
4. The maize WT assay should give a positive reaction to indicate successful amplification of DNA and also to show absence of inhibitors.

Figure 1 shows the amplification curves for each assay and Table 3 summarises the Cq results.

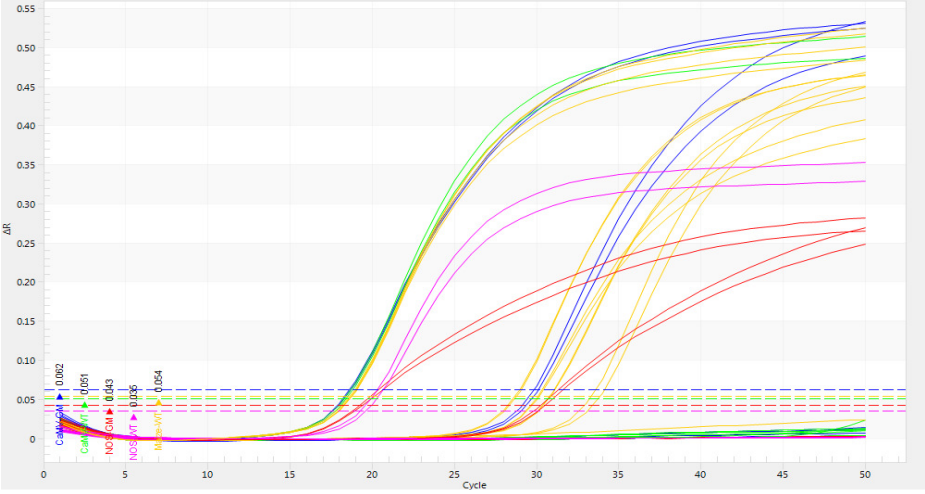


***Figure 1**: Amplification of test samples and controls colour coded by assay. Blue: CaMV-GM; Green: CaMV-WT; Red: NOS-GM; Pink: NOS-WT; Yellow: Maize-WT.*

| Assay | Corn snack | Pet food | Sweet corn | Corn flour | NTC | Positive |
|---|---|---|---|---|---|---|
| Maize-WT | 28.7 | 30.1 | 30.8 | 33.6 | - | 18.7 |
| CaMV-GM | - | 29.9 | - | - | - | 18.6 |
| CaMV-WT | - | - | - | - | - | 18.3 |
| NOS-GM | - | 30.4 | - | - | - | 19.4 |
| NOS-WT | - | - | - | - | - | 19.4 |

***Table 3**: Cq values for each sample within each assay. "–" indicates the sample was negative.*

The results show that all no template control samples were negative and all the positive controls gave a strong signal in the range 18.3 to 19.4, in agreement with the kit instructions. All samples were also positive for maize-WT indicating that DNA had been extracted successfully.

One of the samples did test positive for CaMV-GM and NOS-GM. This was the sample of pet food which contained flakes of dried maize. The regulations governing pet food are much less stringent than those for foods for human consumption and even for feed for farmed livestock. GM material is commonly used in animal feed, so it is therefore unsurprising to find it in this sample.

## Conclusions

Using the GMO DNA detection kit together with the Techne Prime Pro 48 real-time thermal cycler, we have demonstrated successful detection of GM maize extracted from pet food and positive reactions for all maize-based food samples tested.

This kit is one of a range of 400 qPCR detection kits available for use with Prime Pro 48 which include the following: human pathogen testing kits, veterinary and agricultural pathogen testing kits, food and water testing kits and bio-threat detection kits.

Every assay is expert designed, involving in-depth literature review followed by extensive bioinformatics analysis. The kits are optimised via a strict biochemical validation on synthetic oligos to ensure optimal PCR performance. Every kit operates with the same standardised protocol and cycling conditions, which allows multiple kits to be used on the same plate in the same PCR run. Techne Prime Pro detection kits are lyophilised to allow shipping at ambient conditions. All kits are designed and manufactured in the UK to work with Pro 48 and all other qPCR machines. Kits are sold with or without master mix, with sufficient reagent for 150 tests. The shelf life is 18 months.

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