Detection of cotton derived materials by Real-Time PCR — cotton detection method using sinapis Arabidopsis homolog 7 (sah7)

Contents

1 Purpose and Scope 1

2 Principle 1

3 Reagents and materials 2

3.1 General 2

3.2 PCR reagents 2

4 Apparatus 2

5 Procedure 2

5.1 PCR setup 2

5.2 Thermocycling parameters 3

6 Citations 3

1 Purpose and Scope

This document describes a taxon-specific quantitative Real-Time PCR method for the determination of the presence of cotton species-specific DNA. The method amplifies a 115 and a 123 base pairs sequences of *sinapis Arabidopsis homolog 7* gene (*sah7*) (GenBank accession number FN610856.1) present in the A and D subgenomes, respectively. This method is intended for use by an operator trained in performing Real-Time PCR analyses.

The method, when validated, may be applicable to DNA isolated from seed and grain containing mixtures, plant material, and food and feed. Its primary use is as a comparator in quantitative determination of transgenic cotton in seed and grain, but it also can be used to show that DNA is of suitable quality or quantity for a PCR reaction. DNA can be extracted using a suitable DNA extraction method if the method is validated for the matrix before use. The DNA to be analyzed should be tested for quality and quantity prior to the use in the Real-Time PCR assay. Before use, the DNA extraction and PCR method should be subjected to an in-house validation procedure (ISO 17025).

2 Principle

Detection of the cotton species-specific DNA sequence of the *sah7* gene is performed in a Real-Time PCR using a forward and reverse primer and a *sah7* gene-specific probe labeled with labeled with a VIC® fluorescent reporter and a TAMRA™ fluorescent quencher. The *sah7* gene is present in two copies per haploid genome.

The use of this method is subject to the terms and conditions as specified at http://www.detection-methods.com

3 Reagents and materials

3.1 General

Only chemicals and water of analytical grade, appropriate for molecular biology, should be used. Solutions should be prepared by dissolving the corresponding reagents in water and be autoclaved unless otherwise indicated. For all operations for which gloves are used it should be ensured that these are powder-free. The use of aerosol protected pipette tips (for protection against cross contamination) is recommended.

3.2 PCR reagents

Ready-to-use PCR reagent mixtures or mixtures of individual components are typically used. It is important to avoid contamination of all buffers with DNA or DNAse enzymes. Oligonucleotides used for this method are listed in Table 1.

Table 1 — Oligonucleotides

Name	Sequence
Forward Primer	AGTTTGTAGGTTTTGATGTTACATTGAG
Reverse Primer	GCATCTTTGAACCGCCTACTG
Probe [a]	VIC®-AAACATAAAATAATGGGAACAACCATGACATGT-TAMRA™
[a]The fluorescent reporter is VIC® and the fluorescent quencher is TAMRA™. Equivalent reporter dyes and/or quencher dyes may be used for the probe if it can be shown to yield similar or better results.	

4 Apparatus

The usual laboratory equipment is required, and a Real-Time PCR device suitable for the excitation of fluorescent molecules and the detection of fluorescence signals generated during PCR.

5 Procedure

The DNA used for the reaction should be of sufficient quality to avoid inhibition of the PCR reaction.

5.1 PCR setup

The method described applies for a total reaction volume of 25 µl per PCR (Table 2). Method has been previously validated, and changes to the method would need validation.

Thaw the reagents, if necessary, and handle as recommended. Prepare a PCR master mix which contains all the components except for the sample DNA. Aliquot master mix and add control or sample DNA just prior to running the PCR reaction.

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Table 2 — PCR Reaction Setup[a]

Reagents	Final Concentration	Volume per Reaction (µl)
Nuclease-free water	Not applicable	5.562
Master mix (2X)[b]	1X	12.50
Forward primer (10 µM)	350 nM	0.875
Reverse primer (10 µM)	250 nM	0.625
probe (10 µM)	175 nM	0.438
Control/Sample DNA	30 ng/µl	5.0

[a]Different stock concentration for primers and probe may be used, as long as final concentration and total reaction component ratios are maintained.

[b]TaqMan® Universal PCR Master Mix

5.2 Thermocycling parameters

Run the PCR with general cycling conditions listed below in the Real-Time PCR detection platform. Check manufacturer product bulletin for specific PCR platform compatibility and cycling parameters.

Table 3 — Cycling Program[a]

Step	Stage	Temperature (°C)	Time (seconds)	Fluorescence measurement	Number of Cycles
1	A[b]	50	120	No	1
2	B	95	600	No	1
3	C	95	15	No	40
4		60	60	Yes	

[a] Changes to the conditions included in this table are not recommended and must be validated.

[b] UNG step (uracil-N-glycosylase enzyme).

6 Citations

Method provided by Syngenta Crop Protection, LLC, Durham NC, USA.

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