Detection of Cotton derived materials by Real-Time PCR — Cotton detection method using *alcohol dehydrogenase* (*adhC*)

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1 Purpose and Scope

This document describes a taxon-specific quantitative Real-Time PCR method for the determination of the presence of cotton taxon-specific DNA. The method amplifies a 73 base pair sequence of the cotton *alcohol dehydrogenase* (*adhC*) gene (GenBank accession number AF403365.1). 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 taxon-specific DNA sequence of the *adhC* gene is performed in a in a real-time PCR using a forward and reverse primer and an *adhC* gene specific probe labeled with VIC fluorescent reporter and an QSY fluorescence quencher. The *adhC* gene is present in a single copy per haploid genome.

3 Reagents and Materials

3.1 General

Only chemicals and water of analytical grade, appropriate for molecular biology,

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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	5'-CACATGACTTAGCCCATCTTTGC-3'
Reverse Primer	5'-CCCACCCTTTTTTGGTTTAGC-3'
Probe [a]	5' VIC-TGCAGGTTTTGGTGCCACTGTGAATG-QSY-3'
[a] Equivalent reporter dyes and/or quencher dyes may be used for the probe if it can be shown to yield similar or better results. This VIC/QSY labeled probe is validated to work within a duplex assay with a target labeled with a 6-FAM reporter.	

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 (Corteva Agriscience), changes to method would need to be validated.

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	N_A	4.30
Master mix (2X) [b]	1X	12.50
Forward primer (10 µM)	600 nM	1.50
Reverse primer (10 µM)	600 nM	1.50
probe (10 µM)	80 nM	0.20
Control/Sample DNA		5.00

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

[b] For example, Bioline SensiFast Probe No-Rox

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	Time	Fluorescence Measurement	Number of Cycles
1	UNG	50°C	3 minutes	No	1
2	Initial denaturation	95°C	5 minutes	No	1
3	Anneal and Capture	95°C	3 seconds	No	40-45[b]
		60°C	30 seconds	Yes	

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

[b] Number of total cycles performed is dependent on PCR platform used.

6 Citations

Method provided by Corteva Agriscience, Johnston, IA, U.S.A.