# An inexpensive extraction method to perform GM cotton detection in cotton fiber and greige yarn according to IWA 32:2019

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## 1. Introduction

Organic cotton is a claim that genetically modified organisms (GMOs) are not deliberately or knowingly used and that organic producers take far-reaching steps to avoid GMO contamination along the organic cotton value chain, from farmers to spinners, to brands. To manage this, it is essential that organic cotton stakeholders can reliably test their products for the potential presence of GM cotton.

In 2019, Global Organic Textile Standard (GOTS), the Organic Cotton Accelerator (OCA) and Textile Exchange partnered to develop the *ISO IWA 32:2019 protocol* (now on IWA32) to create a common language among laboratories worldwide to screen for the potential presence of GM cotton along the organic cotton value chain. Since the publication of this globally accepted reference protocol, qualitative GM cotton screening as per the IWA 32 is mandatory within the GOTS and OCS (Organic Content Standard) supply chain and OCA's Farmer Engagement and Development program.

The IWA 32 document provides requirements and recommendation to laboratories that perform genetically modified organism analysis in cottonseed, leaf, cotton fiber and cotton fiber-derived materials. The screening of six typic genetic modification elements is based on real-time PCR methods which depends on obtaining good quality amplifiable DNA. Good quality DNA samples (those fit for purpose) are defined as those where the amplification of an endogenous cotton gene is observed. Experimental results showed that good quality DNA can be isolated from the production stages of cottonseed up to greige yarn and greige fabric, while it showed no amplifiable DNA can be isolated from processed yarn or fabric.

While for cottonseed and leaf the official ISO 21571 CTAB based extraction method works perfectly, for cotton lint fiber and greige yarn, IWA 32 suggests as DNA isolation method the Qiagen QIAamp® Fast DNA Stool Mini Kit. However, IWA 32 specifies that any other suitable DNA isolation method can be applied, provided that this method has been proven by means of to perform equally well or better compared to the Qiagen kit.

The use of Qiagen kit is high cost, is not scalable and requires trained personnel. At Generon we validated the use of FASTfood buffer to extract DNA of good quality from cotton lint fiber and greige yarn. The buffer is inexpensive, and the protocol is easy, requires minimal hands-on and basic equipment to be executed. Moreover, the protocol is scalable allowing more representative samples to be extracted.

## 2. Materials and methods

Four different organic cotton yarns (A, B, C and D) and one cotton lint (E) samples were used for the validation. For each sample, three independent extractions were performed: according to IWA 32, DNA was extracted from 100 mg of cotton lint and 100 mg of cotton yarns cut into small parts of approximately 0.5 cm, weighed in a 2 ml centrifuge tube.

The DNA was extracted using FASTfood Universal DNA extraction buffer (Cat.# EXD009), according to the following protocol: 1 ml of FASTfood was added to each sample and then the mix was incubated 30 minutes at 95° C, followed by a centrifugation at 13000 rpm/5 min. It was not necessary to separate the supernatant for the subsequent dilution of the extract.

DNA concentration of the 10-fold diluted extracts was measured using a fluorometric technique (Quantus Fluorometer - Promega) and the DNA (undiluted extract and 2-fold, 5-fold and 10-fold water diluted extracts) was eventually amplified in two replicates using MODIfinder Real-Time PCR kit for the detection of Cotton SAH7 endogenous reference gene (Cat.# PGE21AIWA32-50).

## 3. Results and discussion

To confirm the presence, the quality and the amplifiability of the DNA, each extract was tested in qPCR amplifying SAH7 reference gene sequence as indicated by IWA 32. The table below shows: the mean Cq values obtained from each sample extract and its standard deviation; the fluorimetric quantification of the 10-fold diluted DNA and the reference Cq. The two different reference Cq are the average Cq obtained from the 10-fold diluted DNA of ten cotton lint extracts amplified in duplicate, and from the 10-fold diluted DNA of fifteen greige yarn and/or greige fabric samples, as reported in IWA 32 Annex C.

The results show that FASTfood buffer can be used to isolate good quantity and good quality DNA from cotton lint and cotton yarn; the Cq are equivalent or better to those obtained using the method suggested by IWA 32 (Qiagen QIAamp® Fast DNA Stool Mini Kit). Moreover, the use of the FASTfood buffer allows to solve the problem of possible hygroscopicity of the samples under analysis; the 1 ml volume guarantees complete wettability of the sample even when not finely cut.

It is worth noting there is no correlation between the amount of DNA extracted from cotton yarn samples measured by fluorimetry and the Cq obtained on PCR. This indicates the presence of a high percentage of DNA degraded in small fragments which cannot be amplified by the PCR system due to processing.

| MATRICE | DNA quantification ng/µl (of 10X DNA) | 1000 µl Endo SAH7 | | Average Cq from the 10x | Reference Cq |
|---|---|---|---|---|---|
| | | Cq 5X | Cq 10X | | 10X |
| COTTON YARN A1<br>sd | 0,173 | 35,77<br>0,11 | 34,47<br>0,07 | 36,15 | 38.4 ± 1.37 |
| COTTON YARN A2<br>sd | | 36,17<br>0,32 | 36,74<br>0,51 | | |
| COTTON YARN A3<br>sd | | 35,2<br>0 | 37,25<br>0,4 | | |
| COTTON YARN B1<br>sd | 0,622 | NA | 37,19<br>0,41 | 37,32 | |
| COTTON YARN B2<br>sd | | NA | 37,27<br>0,33 | | |
| COTTON YARN B3<br>sd | | NA | 37,49<br>0,33 | | |
| COTTON YARN C1<br>sd | 0,44 | 37,21<br>0,08 | 35,38<br>0,04 | 36,04 | |
| COTTON YARN C2<br>sd | | 38,78<br>1,55 | 36,27<br>0,06 | | |
| COTTON YARN C3<br>sd | | 39,02<br>0,64 | 36,48<br>0,25 | | |
| COTTON YARN D1<br>sd | 0,193 | NA | 35,46<br>0,15 | 35,25 | |
| COTTON YARN D2<br>sd | | 39,12 | 35,28<br>0,36 | | |
| COTTON YARN D3<br>sd | | 37,99<br>0,26 | 35,02<br>0,14 | | |
| COTTON LINT E1<br>sd | 0,338 | 34,32<br>0,06 | 35,58<br>0,31 | 35,83 | 36.87 ± 1.84 |
| COTTON LINT E2<br>sd | | 35,95<br>2,29 | 36,29<br>0,47 | | |
| COTTON LINT E3<br>sd | | 34,52<br>0,29 | 35,62 | | |

## 4. Conclusions

In this application study, we demonstrated the fit-for-use of a convenient buffer to extract DNA from cotton lint and yarn by PCR amplification of cotton reference gene with a qPCR kit developed according to IWA 32. The method is fast and simple and provides DNA in a sufficient quantity and quality to detect, in case, the presence of any GM-related target sequence. The method present multiple advantages when compared to those suggested by IWA 32 including a better practicability and a higher elution volume supporting multiple replicate tests on the 6 GM target markers indicated by IWA 32.