EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

EURL
European Union Reference Laboratory
for GM Food & Feed

Evaluation of a real-time PCR method from Sciensano targeting the pUB110 shuttle vector and a GMM protease gene fragment, under the provision of Reg. (EU) No 2017/625

29/03/2021

1. BACKGROUND

- On 24/02/2021, Sciensano informed DG SANTE and the JRC-EURL GMFF that a case of contamination from a GMM strain producing protease had been detected. Sequence information on the recombination region (the 'junction') was obtained and a real-time PCR method was developed and in-house validated;

- On 05/03/2021, DG SANTE agreed with Sciensano to receive the in-house validation dossier for the EURL GMM to carry out an evaluation of the performance characteristics of the method to its intended scope under the provisions of Reg. (EU) No 2017/625;

- On 09/03/2021, DG SANTE and the EURL GMFF received from Sciensano an internal quality document used for accreditation, entitled "Validation of TaqMan screening qPCR method for detection of a GMM overproducing protease (GMM protease2)". Sciensano also enclosed a manuscript for publication entitled "Development of a real-time PCR marker targeting a new unauthorized genetically modified microorganism producing protease identified by DNA walking". These two documents have been provided under confidentiality clause;

- The EURL GMFF reviewed the submitted documents with the aim to evaluate the performance of the method for the detection of the GMM overproducing protease (so-called protease 2);

- On 16/03/2021, the EURL GMFF requested Sciensano to complement the submitted file with additional clarifications on the method;

- On 23/03/2021, Sciensano provided the requested clarifications on the method in a revised document entitled "Validation of TaqMan screening qPCR method specific to the GMM protease2 in targeting the junction between the pUB110 shuttle vector and a Bacillus gene coding for a protease (GMM protease2 marker)"[1].

[1] Ares(2021)2075568

2. EVALUATION OF THE REAL-TIME PCR BASED METHOD TARGETING THE JUNCTION PUB110-PROTEASE 2 MARKER

This note refers to the EURL GMFF evaluation of the method described in the document "Validation of TaqMan screening qPCR method specific to the GMM protease2 in targeting the junction between the pUB110 shuttle vector and a Bacillus gene coding for a protease (GMM protease2 marker)". The EURL GMFF has focused on the parameters applicable for a qualitative use of real-time PCR methods reported in the ENGL guidance "Definition of minimum performance requirements for analytical methods of GMO testing" (2015)[2] and in the "Guidelines for validation of qualitative real-time PCR methods"[3].

3. OVERALL CONCLUSION(S)

Based on the available information, the method, developed and in-house validated by Sciensano for the detection of unauthorised GMM presence in food and feed microbial fermentation products, targets the junction between the sequence of a plasmid pUB110, according to supporting evidence provided in a manuscript in preparation by Sciensano, and a fragment of the sequence of a protease gene. Therefore, based on current evidence, the method is construct-specific.
The method is sufficiently specific as it does not react with a considerable number of DNA extracts from microorganism species and strains and does not react with DNA extracted from food enzyme products or GMM strains coding for alpha-amylase, vitamin B_2 or protease marker. In *silico* analyses performed by the EURL GMFF did not show any cross-reactivity with GMOs in the molecular database and no perfect match of the two primers against more than 80 plant genomes.
The method sensitivity as determined at Sciensano and at a second laboratory on a plasmid carrying one copy of the target sequence is within the ENGL acceptance criterion (i.e. below 25 copies). Additionally different master mix and thermocycler brands were used at the two facilities, thus providing an indication of method transferability.

Therefore, the method is considered as suitable for the detection of the junction between the pUB110 shuttle vector, according to supporting evidence provided in a manuscript in preparation by Sciensano, and a *Bacillus* gene coding for a protease marker in DNA extracts.

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2 https://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

3 Broeders S., Huber I., Grohmann L., Berben G., Taverniers I., Mazzara M., Rossens N., Morisset D. Trends in Food Science & Technology 37 (2014) 115e126