EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

European Union Reference Laboratory for GM Food & Feed

Evaluation of two real-time PCR methods from Sciensano (BE) targeting respectively the right and the left transgene flanking region between the pUB110 shuttle vector and a *Bacillus velezensis* gene coding for a protease (GMM protease1), under the provision of Reg. (EU) No 2017/625

20/09/2021

1. BACKGROUND

- On 24/06/2021, Sciensano (BE) submitted to the EURL GMFF two dossiers for evaluation.
- The dossiers describe two Taqman real-time PCR methods targeting DNA fragments corresponding to the right and left border between the shuttle vector pUB110 and a DNA sequence codifying for a protease in a food enzyme (protease) product originating from a microbial fermentation process; the product was the subject of a notification in the EU alert system[1]. The methods have been already published and the GMM characterised by whole genome sequencing[2].

- The EURL GMFF reviewed the information submitted with the aim to evaluate the performance of the methods for the detection of the GMM producing protease (so-called protease 1).

- On 09/08/2021, the EURL GMFF requested Sciensano to complement the information with additional clarifications on the methods.

- On 23/08/2021, Sciensano provided the requested clarifications on the methods and submitted revised documents[3].

- Further to a request from the EURL GMFF, additional clarification was provided on 07/09/2021 with the documents entitled "Validation of TaqMan screening qPCR method specific to the GMM protease1 in targeting the right transgene flanking region between the pUB110 shuttle vector and a *Bacillus velezensis* gene coding for a protease (GMM protease1 right border)" and "Validation of TaqMan screening qPCR method specific to the GMM protease1 in targeting the left transgene flanking region between the pUB110 shuttle vector and a *Bacillus velezensis* gene coding for a protease (GMM protease1 left border)"[4].

[1] RASFF 2019.3332
[2] Fraiture et al. (2021) Scientific Reports 10:7094| https://doi.org/10.1038/s41598-020-63987-5
[3] Ares(2021)5231097
[4] Ares(2021)5543872

2. EVALUATION OF THE TWO REAL-TIME PCR METHODS

This note refers to the EURL GMFF evaluation of the methods described respectively in the documents "Validation of TaqMan screening qPCR method specific to the GMM protease1 in targeting the right transgene flanking region between the pUB110 shuttle vector and a *Bacillus velezensis* gene coding for a protease (GMM protease1 right border)" and "Validation of TaqMan screening qPCR method specific to the GMM protease1 in targeting the left transgene flanking region between the pUB110 shuttle vector and a *Bacillus velezensis* gene coding for a protease (GMM protease1 left border)", received on 07/09/2021[4].

In its evaluation the EURL GMFF focused on the parameters applicable for a qualitative use of real-time PCR methods and outlined in the ENGL guidance "Definition of minimum performance requirements for analytical methods of GMO testing" (2015) [5] and in the "Guidelines for validation of qualitative real-time PCR methods"[6].

Based on the available information, the analytical procedures developed and validated in-house by Sciensano consist of two TaqMan real-time PCR methods targeting the right and left flanking regions between the pUB110 vector and a gene sequence coding for a protease (so-called protease 1); therefore, the methods are construct-specific.

Data from experimental testing show that the methods are sufficiently specific as they do not react with a considerable number of DNA extracts from microorganism species and strains. Moreover, the methods did not react with DNA from a GMM strain coding for vitamin B2 and DNA extracted from a food enzyme product, labelled as containing protease, previously reported as being contaminated by the GMM producing protease2 (RASFF2021.1641).

In *silico* analyses performed by the EURL GMFF did not show any cross-reactivity of the two methods with sequences of GMOs present in the molecular database and no perfect match of the two primers against more than 80 plant genomes.

The sensitivity of the two methods, tested on respective plasmids carrying one copy of the target sequence, is within the ENGL acceptance criterion (i.e. below 25 genome copies), as tested at Sciensano and at a second laboratory. Different master mixes and thermocycler brands were used at the two facilities, thus providing an indication of method transferability[7].

The gDNA from *B. velezensis* strain 2019-3332 is available at Sciensano.

In conclusion, the construct-specific methods are considered suitable for the detection of the right and left-flanking region between the pUB110 vector and a gene sequence coding for a protease.

[5] https://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

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

[7] Sciensano used the Taq®Man 2x Master Mix (Diagenode BE). The corresponding catalogue number cannot be found in the Diagenode website (last visited on 05/08/2021). Sciensano informed that to purchase this master-mix, the customer service of the company needs to be contacted directly.