GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Qualitative PCR method for detection of E. coli K-12 event AG3139 (Mazzara et al., 2009)

Event specific

Last updated 23/11/2017

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of E. coli K-12 event AG3139 and the bacterial host genome
PCR Assay	Simplex Real Time
Detection	TaqMan®
Compendium Reference	QL-EVE-EC-001

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	feed sample PT73 (TM) and DNA
Materials used for calibration/controls	Positive control extracted from the PT73 (TM) biomass, plasmid pUC-TMD

Tested GM Events

Event Name	Unique Identifier	Species
AG3139	Not applicable	*Escherichia coli*

Collaborative Trial Description

The participants received a PT73 (TM) biomass sample made from dried killed cells of *Escherichia coli* K-12 event AG3139 and a genomic DNA sample extracted from the same bacterial strain. The laboratories received in addition a DNA positive control, reactions reagents and primers and probes (TMD system) for the event-specific detection of the bacterial event AG3139. In the first phase of the collaborative study the laboratories performed four independent DNA extractions on the bacterial biomass PT73 (TM) sample. The partecipants estimated the DNA concentrations and prepared a 12-point dilution series from each DNA extraction starting with a DNA working concentration of 10ng/µl. The serial dilutions were tested using the PCR TMD specific system. Four replicates for dilution point were analysed in two runs with two dilution series loaded per PCR plate. In the second phase of the collaborative study, the laboratories tested the method for detecting event AG3139 in DNA purified from bacterial strain E. coli K-12 event AG3139. The laboratories prepared from the delivered AG3139 DNA stock solution four independent 12-point dilution series. The serial dilutions were tested using the PCR TMD specific system. Four replicates for dilution point were analysed in two runs with two dilution series loaded per PCR plate. The laboratories submitted the raw data to the EURL-GMFF.

Method Performance

LOD Relative	Not applicable	**LOD Absolute**	≤32 genomic copies

Values determined in the collaborative trial

	GMO Target
Mean Slope	-3.6
Mean PCR Efficiency %	91
Mean R2	0.99

	Test Level							
Specificity (%)	262144	65536	16384	4096	1024	256	64	16
Sensitivity (%)	100%	100%	100%	100%	100%	100%	100%	98%

Unit of Measurement Test Level Genome copy N.

Comment

The data reported in the table refers to the performance of the method of detection for event AG3139 on DNA from control sample E.coli K-12 event AG3139. The identification and removal of outliers through Cochran and Grubbs tests was based on the slope datasets, in particular the mean value of the slope of the regression lines.

3. REFERENCES

Mazzara M, Foti N, Savini C, Bonfini L, Van den Eede G. Event-specific method for the detection of dried-killed bacterial biomass PT73 (TM) derived from E. coli GM strain AG3139 using real-time PCR - Validation Report and Protocol. EUR 24236 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56610 (ISBN 978-92-79-14981-8).

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of E. coli K-12 event AG3139 and the bacterial host genome
Primer Forward	5'-AATACCGTTAAACGTAAATTCTTTTTCTTT-3'
Target element	5'-host genome
Primer Reverse	5'-TCCTCCCGGTTTTTTTCGTA-3'
Target element	insert
Amplicon length	90 bp
Probe	5'-FAM-AGATCGAGTATTCATTCGGTGTATTGATTCACTTGA-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan Universal PCR Master Mix (2x)	0.96x
Primer Fw	0.439 µmol/L
Primer Rev	0.439 µmol/L
Probe	0.146 µmol/L
Template DNA	maximum 100 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
Decontamination (UNG)	50°C	120”	1
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45

The Ajinomoto Eurolysine S.A.S company producing the product PT73 (TM) stated that to their knowledge no conventional counterpart to PT73 (TM) existed on the EU market at the time of method submission, i.e. no bacterial biomass by-product of threonine production, consisting of conventional strain of E. coli K-12 used as feed material. Consequently, if the bacterial biomass PT73 (TM) is present in feeding stuff it should always be 100% GM and not a potential mixture of GM E. coli K12 biomasss plus wild-type E. coli K-12 biomass: thus no relative quantification is needed for such product. As a result a real-time PCR based qualitative method was proposed by the applicant and validated by the EURL GMFF for the detection of PT73 (TM) product.

Considering the confidential nature of the strain AG3139, as a positive control in the protocol, it can be used the plasmid pUC-TMD containing the entire amplicon DNA sequence necessary for the detection and identification of the biomass PT73 (TM). The plasmid pUC-TMD was inserted by transformation into an E.coli K-12 strain named DH5alpha to result in the strain DH5alpha /pUC-TMD.