STUDY REPORT

Preparation of positive control DNA to be used as a reference material in *Komagataella phaffii* MxY0541 detection

Batch 1

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EXECUTIVE SUMMARY

***Scope*:** The report described the preparation of pDNA reference material (RM) to be used as a positive control in qualitative qPCR detection of *Komagataella phaffii* strain MxY0541 as part of the EFSA GMO Dossier (EFSA-GMO-NL-2019-162). The test strain is genetically modified to produce leghemoglobin from soybean (*Glycine max*).

***Methods*:** The target DNA was cloned into a plasmid. The insert was confirmed by Sanger sequencing. The indicative target copy number was quantified by digital PCR. The stability and homogeneity were confirmed from ten replicate samples analysed by dPCR.

***Results and conclusion*:** The indicative copy numbers of the pDNA dilutions measured equal to starting concentrations of 1,319,990,400 to 1,861,017,600 copies/µL. The pDNA stock will be delivered to authorities performing the GM monitoring as 1/100 dilution which contains 13 to 19 x 10^6 indicative copies/µL target DNA.

Biosafe will store the DNA and sell it to authorities performing GMO monitoring activities upon request.

COMMON ABBREVIATIONS/DEFINITIONS USED IN THIS REPORT

bp	Base pair
DNA	Deoxyribonucleic acid
dPCR	Digital PCR (dPCR) is a technique where the sample is partitioned into many individual reactions so that either zero, one or more target molecules are present in each reaction. The absolute number of molecules present in the sample can be calculated based on the presence/absence of the positive signal in each partition.
EFSA	European Food Safety Authority
JRC	Joint Research Centre
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction, a thermal cycling method used to amplify a specific region of a DNA strand (the DNA target)
Qualitative PCR	A method of analysis whose response is either the presence or absence of the target DNA sequence(s) in a sample
qPCR	Quantitative polymerase chain reaction, used to estimate the relative amount of target sequence in a sample
pDNA	plasmid DNA
QPS	Qualified presumption of safety
RM	reference material
µL	microliter

1 INTRODUCTION

Biosafe Ltd prepared plasmid DNA (pDNA) to be used as a positive control in qualitative qPCR detection of *Komagataella phaffii* strain MxY0541, hereafter the test strain, due to request received from Joint Research Centre (JRC) as part of the EFSA GMO Dossier (EFSA-GMO-NL-2019-162). The test strain is genetically modified to produce leghemoglobin from soybean (*Glycine max*).

In this work, the PCR target fragment was cloned into a plasmid, and the DNA isolated in maxiprep scale. The target copy number in the preparate was confirmed using digital PCR. The plasmid DNA will be used as a reference material (RM) in monitoring the GM strain.

2 MATERIALS AND METHODS

The bioinformatics analysis if the test strain has been completed in Biosafe report T1345R1207.4/2021. The qualitative PCR detection method has been developed and described in Biosafe report T1318R1189.1/2021.

The target DNA was cloned into a plasmid. The insert was confirmed by Sanger sequencing. The indicative target copy number was quantified by digital PCR. The stability and homogeneity were confirmed from ten replicate samples analysed by dPCR.

3 RESULTS

3.1 Indicative copy number

The copy number of pDNA preparate was evaluated from a serial dilution series of the pDNA using QIAcuity® Digital PCR system (Qiagen) using the primers and probe as described in Biosafe report T1318R1189.1/2021.

The indicative copy numbers of the original pDNA preparate (1x) concentration was 1,319,990,400 to 1,861,017,600 copies/µL. The pDNA will be delivered as 1/100 dilution, which equals 13,199,900 to 18,610,180 copies/µL, i.e. 13 to 19 x 10^6 copies/µL.

3.2 pDNA homogeneity

Once the material was processed and divided into vials, ten (10) samples were randomly selected and analysed with event-specific, qualitative PCR analysis. The data confirms the presence and homogeneity of the targeted DNA fragment in the test samples. The samples show positive PCR result in the range of 10x10^6 copies / µL.

3.3 pDNA stability

Stability of the reference DNA is guaranteed for one year from the processing date. The materials are sealed and stored in -20 ºC, therefore not exposed to air and are expected to be stable for longer than the estimated expiration date. The stability of the pDNA extract material will be re-evaluated annually. If the samples are still on the requested level 10^6 target copies per µL, the certificates will be extended for one year.

4 CONCLUSION

Biosafe has prepared pDNA as reference material for qualitative PCR detection of genetically modified *Komagataella phaffii* strain MxY0541. The sample homogeneity and stability has been tested and the indicative copy number determined using digital PCR as copies/µL. The indicative copy numbers of the pDNA dilutions measured equal to starting concentrations of 1,319,990,400 to 1,861,017,600 copies/µL.

The pDNA stock will be delivered to authorities performing the GM monitoring as 1/100 dilution which contains 13 to 19 x 10^6 indicative copies/µL target DNA. Biosafe will store the DNA and sell it to authorities performing GMO monitoring activities upon request.