

Quantitation Using Informative Zeros (QUIZ) proof of concept

Maes o ddi-ddordeb ymchwil: [Novel and non-traditional foods, additives and processes](#)

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Cod prosiect: G03025

Cynhaliwyd gan: National Institute of Agricultural Botany (NIAB)

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Background

The Food Standards Agency is the competent authority for Genetically Modified (GM) Food and Feed Regulation 1829/2003 which lays down the labelling requirements for GM food and feed.

To determine whether the labelling regulations are working in practice it is necessary for GM DNA detection methods in foods to be available using robust methodologies. This study seeks to demonstrate the potential of a new approach to DNA detection of GM material in food. Ultimately this may provide a method that is more accessible to Public Analysts for enforcement work.

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Research Approach

This proof of concept study was designed to demonstrate the utility of Quantitation Using Informative Zeros (QUIZ) before development as a high throughput method for robust and sensitive quantitation of relative amounts of genetically modified (GM) material to non-GM material in food products.

A set of defined standards was made by mixing two certified reference materials containing GM RoundUp Ready (RR) soya (0 and 5%). Samples were produced to contain approximately 0, 0.5, 1, 3 and 5% RR soya based on the declared GM content. DNA was extracted from samples, the purified DNA quantified and appropriate dilutions used to determine the GM content of each sample using QUIZ. QUIZ must demonstrate a linear relationship between the estimated GM content and the proportion of RR soya in the samples.

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Results

The results of the study show a linear relationship between the estimated GM content and the proportion of RR soya in the samples, despite a general under estimation of the GM content. This indicates that QUIZ maybe a realistic alternative for GM quantification. Improvements to the application of QUIZ will be investigated within a second stage of the project (G03030).

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Additional Info

Detailed results

The number of positive reactions for both lectin and GMO targets using extended numbers of PCR cycles and fluoroscein-labelled primers, for detection in automated genotypers, show successful amplification from single molecules. Notably, most peaks observed on the gel runs were oversized, even though only 0.5 μ L of reactions were loaded. Given the DNA concentrations of the samples, it was statistically unlikely for all reactions to contain more than one target amplicon.

Quantitation obtained using QUIZ compares favourably with those of the RT-PCR assay: the correlations between the measured and nominal values are 0.9901 and 0.9878, respectively. However, the dilutions used for all the samples, except the 0% CRM, should contain approximately one amplicon per reaction. At this concentration, two-thirds of the reactions are expected to be positive. The actual percentage positives varied between 21 and 64 (with a mean of 44). The starting DNA concentrations (approximately 20 μ g μ L⁻¹) were corroborated by the Ct values of the RT-PCR reactions for the reference gene. The lower than expected number of positives may reflect the impact of organellar DNA in the samples, which would lead to an overestimation of the expected number of amplicons. Potential loss of DNA during the preparative stages could also be a contributing factor.

These results show that QUIZ may be a realistic alternative for GMO quantification; however clearly the disadvantage at present is the number of reactions that need to be performed for accurate determination of the quantity of DNA present in a sample. The generation of this data set required 5 individual 384 well plates to be run in duplicate, resulting in 3840 PCR reactions. However, as already stated above, QUIZ allows testing at different DNA concentrations. Replicates of a single dilution series may provide sufficient confidence to condemn or accept samples with a given threshold/tolerance GMO level. Furthermore, there are improvements that can be made to the procedure; for example, PCR reactions could be performed in a reduced volume thus reducing reaction costs.

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Published Papers

1. Lee D., La Mura M., Greenland A. & MacKay (2008) Quantification Using Informative Zeros: application for GMO detection and quantification without recourse to certified reference material, DOI: 10.1016/ J. Food Chem. 2008.05.118

Research report

England, Northern Ireland and Wales

PDF

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