# Quantitation using informative zeros (QUIZ) part 2 - application for testing GMOs

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## Background

The Food Standards Agency is the competent authority for the Genetically Modified (GM) Food and Feed Regulation (EC) 1829/2003 which lays down labelling requirements for GMOs and products containing GM material. This stipulates that any food or feed product containing a GM ingredient must declare this on the label. A threshold of 0.9% is in place for the unintentional and technically unavoidable presence of authorised GM material below which labelling is not required. Unauthorised GM food or feed is not permitted at any level.

To determine whether the labelling regulations are working in practice it is necessary that robust high throughput, low cost methods for the detection of GMOs are available. This project aimed to determine the potential of a new approach for the quantification of GM material in food by further development and testing of the methodology established in a previous proof of concept study (G03025). Ultimately this may provide a method that is more accessible to Public Analysts for enforcement.

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## Research Approach

The Quantitation Using Informative Zeros (QUIZ) technique involves a solution of DNA being subject to serial dilution until either zero or more target molecules are present in each individual sample. The polymerase chain reaction (PCR) will then only amplify DNA when present in a sample and give either a positive or negative result. As it is not possible to know how many target molecules are present with a positive result, it is the negative results that are ‘informative’ and allow the concentration of GM DNA to be calculated. The aim of the project is to provide a high throughput method for robust and sensitive quantification of relative amounts of GM to non-GM DNA in a food sample.

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## Results

The successful demonstration of QUIZ was achieved through measurements of DNA samples extracted from certified reference materials (CRMs) for 2 GM events, RoundUp ReadyTM soya

(RRS) and MON810 maize, using event-specific primers and corresponding reference genes for soya and maize. The percentage DNA concentrations measured showed a good correlation with expected values but systematic bias was observed between the two assays for RRS and MON810. This bias was attributed to the different lengths of markers used for quantification which could result in different PCR efficiency for the two targets and differential DNA degradation, which would result in a relatively higher concentration of the smaller amplicon. This was demonstrated experimentally by reversing the relative lengths of the GM and reference target amplicons for RRS which reversed the observed bias. Using primer sets that resulted in amplicons of the same length gave the best estimates of GM content. In addition it was concluded that primers should be designed that do not span reference gene polymorphisms as this can also affect estimations of GM content.

The project has fulfilled its primary objective of demonstrating accurate QUIZ estimations of the GM content of CRMs and food matrices for two GM events (RRS and MON810), although considerable effort was necessary to achieve this. Software has been developed to allow greater access to the statistical evaluation of practical data, although there are certain aspects of the software that could be improved, such as the ability to highlight ‘data deficiencies’. Even though there is a move to produce commercial reference materials that provide calibration of GM DNA content per haploid genome unit, QUIZ already does this so long as the markers used have been carefully assessed for their copy number and are of equivalent lengths to remove bias in the testing. With further development and refinement QUIZ has the potential to be an alternative method of GM quantification and would be extremely useful when certified reference materials for a particular GMO are not available.

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## Published Papers

1. La Mura, M., Allnutt, T.R., Greenland, A., Mackay, I. & Lee, D. (2010) Application of quiz for GM quantification in food. *Food Chemistry*, published online doi: 10.1016/j.foodchem.2010.10.002