Procedure for the isolation of transgene flanking regions in GM crops

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Background

The isolation and analysis of transgene flanking regions forms a key component of the molecular analysis and safety assessment of GM plants.

It is important to identify exactly where the additional DNA (transgene) has been inserted in a GM plant as this may highlight whether a plant gene has been disrupted or whether there are rearrangements of the DNA at the site of insertion that may be of concern; for example if a new open reading frame has been created that may be expressed in the plant. Although there are a number of methods available that allow the transgene junction region to be isolated, in general these methods are complex and have been used only with the insertion of specific transgenes or with specific plant species.

The aim of this project was to develop standard and validated PCR-based methods to allow the routine isolation and characterisation of the transgene junction regions in a range of different GM crops with diverse transgene insertions of different complexity.

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Research Approach

Suitable methods were developed for the isolation of transgene junction regions in relatively simple GM lines of barley and potato. This was done by comparing a number of commercially available DNA sequence walking kits with standard laboratory protocol developed under the G02 programme (project G02002). The methods developed were extended and tested in more complex GM potato lines and also in a range of other crops. The GM barley lines used were produced using two different methodologies for introducing transgenes (biolistic and Agrobacterium mediated transformation). The methods developed were subsequently validated thorough the analysis of a standard set of GM samples in two different laboratories.

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Results

During the project it emerged that two commercially available kits were appropriate for the isolation of transgene junction regions. The DNA Walking SpeedUpTM Kit II (manufactured by Seegene) was found to be suitable for use in all GM crop types tested where the number of transgene insertions was low. This kit produced reliable and rapid results from small amounts of DNA. The APAgeneTM GOLD Walking Kit (manufactured by BIO S&T) was found to be

appropriate for the analysis of potato lines with both simple and more complex transgene insertions and gave reliable results.

As a result of the work carried out in this project, it is now possible to recommend standard operating procedures (SOPs) using the Seegene kit for the molecular analysis and safety assessment of a wide range of GM plant material, including potato, barley, maize, pea, wheat and Brassica. For more complex material (especially in potato), the SOP using the APAgeneTM GOLD kits, together with additional analysis steps, is appropriate.

The results obtained during the project identified some DNA rearrangements at the transgene insertion site in older experimental GM lines not intended to be grown commercially. Rearrangements were less frequent in GM lines produced more recently. Additional analysis is required to identify the reason for such rearrangements and in some cases to determine the origin of the additional sequences and potential consequences of such rearrangements.

The current guidelines for the molecular characterisation of GM crops specify that the junctions of the transgene and the plant genome should be identified and the DNA sequence determined to ensure that there are no rearrangements or other unintended effects at the site of insertion. The methods developed in this project will speed up this process and allow rapid screening and selection of candidate lines for commercialisation that lack such rearrangements or other unintended effects.

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Published Papers

1. Cullen, D., Harwood, W. A., Smedley, M. A., Davies, H., & Taylor, M. (2011) Comparison of DNA walking methods for isolation of transgene-flanking regions in GM potato. *Molecular Biotechnology*, 49(1), 19-31, published online doi: 10.1007/s12033-010-9371-5

Research report

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PDF
Gweld Development of a standard, validated procedure for the isolation of transgene flanking regions in GM crops and detailed analysis as PDF(Open in a new window) (1.19 MB)
WORD
Gweld Plant genomic DNA from potato leaf material research report as Word(Open in a new window) (60 KB)
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Gweld SOP Genomic DNA extraction from fresh leaf material research report as Word(Open in a new window) (54.5 KB)

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