Development of a standard, validated procedure for the isolation of transgene flanking regions in GM crops and detailed analysis of transgene insertion

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Introduction

The isolation and analysis of transgene flanking regions forms a key component of the molecular analysis and safety assessment of GM plants. The determination of flanking regions may identify the position of the transgene in the host genome to provide information on whether functional or regulatory genes have been disrupted, new open reading frames created, and unique identification sequences for traceability.

There are currently a number of PCR-based protocols that have been used to isolate the genomic regions flanking transgenes. However, there is no standard, validated method that has been shown to be appropriate for a range of different GM plants and a range of different types of insertion.

Objectives

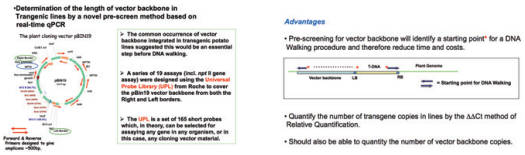
The aim of this project is to develop standard, validated procedures for the isolation of transgene flanking regions in a range of GM crops, with a range of different transgene insertion patterns.

Materials & Methods

Over ten DNA extraction methods were compared to determine the optimal procedure for potato and barley leaf material including:

- Commercial Spin Column kits (DNeasy®, NucleoSpin®, PowerPlant™, E.N.Z.A™ Plant DNA);
- Magnetic Resins (ChargeSwitch®);
- Four Manual CTAB- or SDS-based methods;
- Proprietary Reagents (DNAzol®, AquaGenomic™).

A novel pre-screen method based on real-time qPCR and the Universal Probe Library (Roche) for determination of the length of vector backbone was developed due to its common integration in transgenic potato lines. Real-time qPCR was also used to quantify the copy number of transgenes in the potato and barley lines under investigation.



Three commercial DNA Walking kits were compared with the Adaptor-Mediated PCR technique previously used successfully for barley (Harwood *et. al.,* 2004).

Kits:

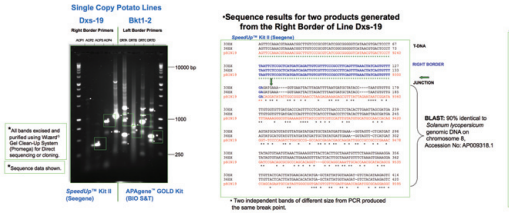
- DNA Walking *SpeedUp*™ Kit II (Seegene)
- APAgene™ GOLD Genome Walking Kit (BIO S&T)
- Universal Vectorette™ System UVS1 (Sigma)

Transgene-specific primers were designed according to the kit specifications to determine flanking regions from the right border (RB) and left border (LB) in single insert, *Agrobacterium*-derived lines of transgenic potato (vector pBin19; Morris *et. al.,* 2006a,b), single and double transgenic copy barley lines (vector pAL135; Travella *et. al.,* 2005), and methods compared in terms of success and reliability.

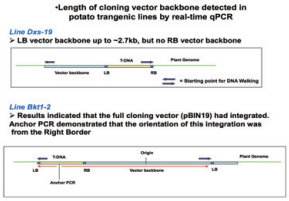
Results

There was no significant difference between DNA extraction methods for potato and barley leaf material in terms of quality based on PCR amplification and restriction enzyme digestion, but DNA yield was significantly higher using the manual methods (i.e., CTAB- or SDS-based; 25 µg DNA/100mg) or the AquaGenomic™ solution (15 µg DNA/100mg).

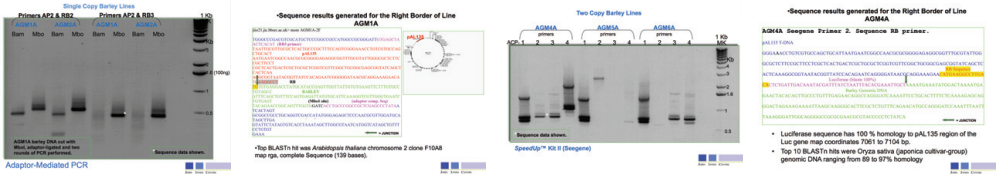
Both the SpeedUp ™ and APAgene™ GOLD DNA Walking kits were successful in isolating transgene flanking regions in potato transgenic lines.



There were considerable amounts of backbone at either the left border (LB) and/or right border (RB) and some unusual rearrangements detected in single copy transgenic lines of potato under investigation.



In transgenic barley lines, the Adaptor-Mediated method was as successful as the SpeedUp™ kit, although the commercial kit was less time consuming and had the advantage that it could be used to isolate two independent transgene junctions in one reaction.



Conclusions

- In terms of success, throughput, and reliability, the DNA Walking SpeedUp™ Kit II (Seegene) emerged as the preferred method for analysis of single copy transgenic lines of potato and barley. Work is currently underway to extend the methods to more complex lines with multiple transgene copies and to a range of other GM crops.
- It is anticipated that the recommendations made in this proposal will be relevant to emerging regulations for the safe development of transgenic crops.

References

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Acknowledgments

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