RICE CRC

FINAL RESEARCH REPORT

Title of Project :	Construction of user-friendly plant expression vectors using rice promoters
Project Reference number :	3403A
Research Organisation Name :	CSIRO Plant Industry
Principal Investigator Details :	
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RICE CRC

Final report – project number: 3403A

This project (3 months duration) was embedded within our ongoing projects on “Rice Functional Genomics”. A PhD student, Andrew Eamens was employed in this project to continue work on the development of user friendly plant expression vectors based on rice promoters. This work was started towards the end of Andrew’s PhD studentship. Using reporter genes containing a minimal promoter (enhancer trap) or intron splice acceptors (gene trap) in T-DNA or transposon tagging systems, several promoter sequences were identified by Andrew during his doctoral research and were used to produce plant expression vectors with tissue specific expression. The previously developed double right boarder (DRB) vector technology was used to construct a small group of user-friendly plant expression vectors with tissue-specific expression promoters.

A new base binary vector construct (PDRB12dn) was constructed during this project period. The binary vector contained a promoterless reporter gene (*sgfpS65T*) mounted between the second right border (RB2) and the T-DNA left border (LB). The reporter gene is flanked upstream by a multiple cloning site (MCS) containing several unique restriction enzyme (RE) cleavage sites for easy cloning of putative promoter fragments. A total of 12 promoter fragments were also amplified by the polymerase chain reaction (PCR), ready for addition to the base vector. Cloning of individual promoter fragments is now in progress.

The plant expression constructs being produced will enable the production of selectable marker free transgenic plants expressing GOIs in specific cells, tissues or organs.

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1. Background to the Project

This project was primarily funded by Rice CRC to support Andrew's last three month research period at CSIRO Plant Industry. This period followed the conclusion of Andrew's PhD fellowship, while he was waiting for an overseas post-doctoral fellow (PDF) placement. The project was embedded within our ongoing projects on "Rice Functional Genomics" and Andrew was employed to continue to work on the development of user-friendly plant expression vectors using rice promoters. This project continued on from work that Andrew started towards the end of his PhD fellowship. Using reporter genes containing a minimal promoter (enhancer trap) or intron splice acceptors (gene trap) in T-DNA or transposon tagging systems, several putative rice promoter sequences with tissue and organ specific expression patterns have been identified. Some of these were identified by Andrew during his doctoral research work. Several promoter sequences were selected by Andrew to produce plant expression vectors conferring tissue specific expression. In this project Andrew used the previously developed double right boarder (DRB) vector technology (Lu et al. 2001), to prepare a set of user-friendly plant expression vectors with tissue-specific expressing promoters.

2. Objectives

Using reporter genes containing a minimal promoter (enhancer trap) or intron splice acceptors (gene trap) in T-DNA or transposon tagging system we have identified several putative rice promoter sequences with tissue and organ specific expression patterns. Several of these were identified by Andrew during his doctoral research. Andrew selected several promoter sequences to produce plant expression vectors conferring tissue specific expression. During this project Andrew will use the DRB vector technology, which will allow for enable the production of selectable marker free transgenic plants.

3. Introductory technical information concerning the problem or research need

Several existing rice CRC projects are using transformation breeding approach to improve agronomic traits. Plant expression promoters are crucial for desirable level and timing of introduced gene expression. Furthermore, incorporation of marker-free technology is highly desirable in view of current public concerns regarding the release of selectable markers genes into the environment through genetically modified organisms (GMO's).

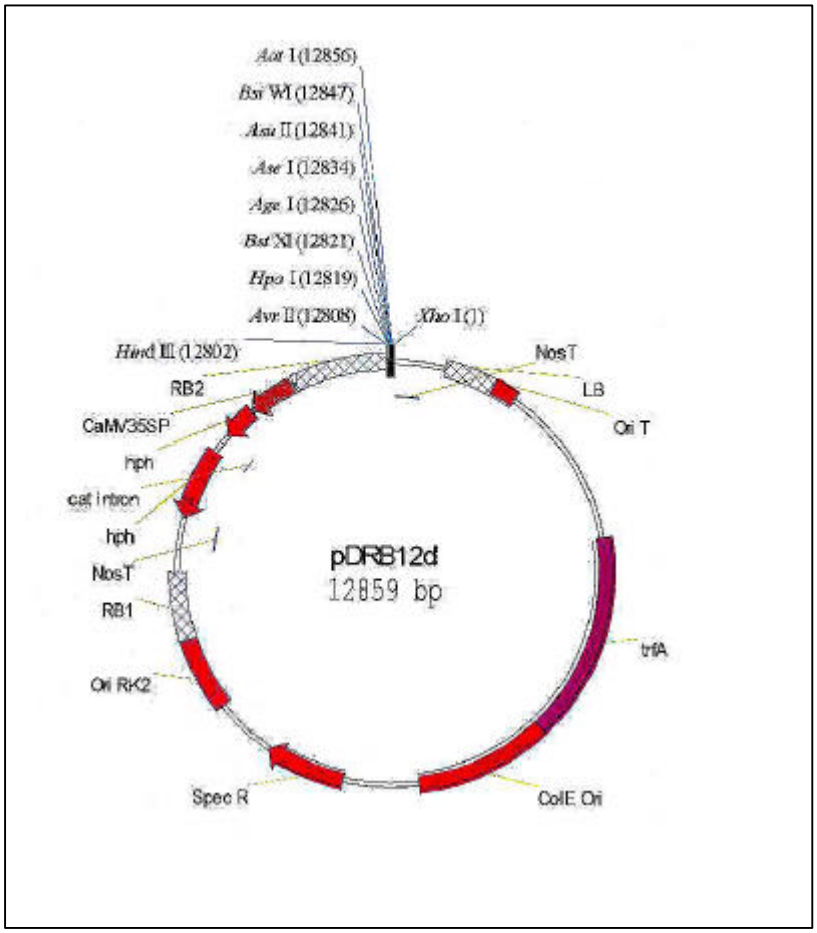
4. The Methodology - including a description and justification

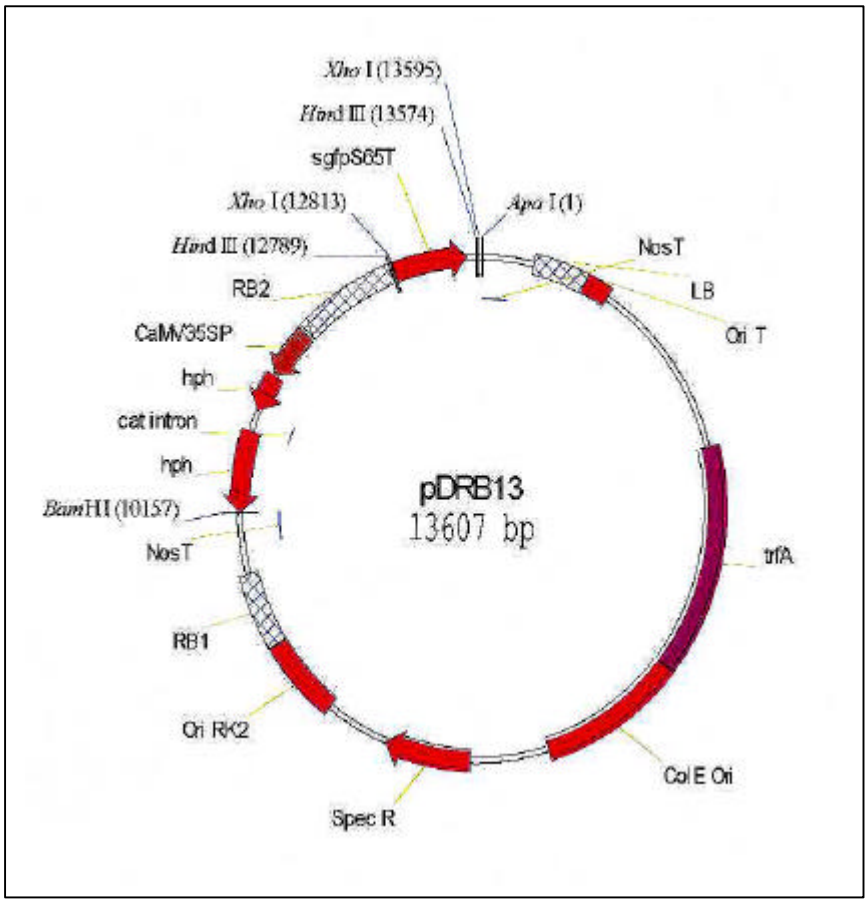
Predicted promoter region sequences upstream of the T-DNA and or *Ds* insertions in stable trap rice lines (Upadhyaya *et al*, 2002; Eamens *et al*., 2003) showing specific patterns of trap-

reporter activity will be cloned into DRB vectors (Lu *et al*., 2001) in front of a reporter gene (*sgfpS65T*) and MCS with unique RE cleavage sites for convenient exchange of any gene of interest (GOI). At least six individual constructs with unique rice promoter sequences will be constructed during this project.

5. Detailed results - including the statistical analysis of results

A new base binary vector construct (PDRB12d) was constructed during this project period. A binary vector having a promoterless reporter gene (*sgfpS65T*) mounted between the second RB and LB along with few unique restriction enzyme (RE) recognition sites for easy cloning of putative promoter fragments was also produced. A total of 12 promoter fragments have also been amplified by PCR, ready for addition to the base vector. Cloning of individual promoter fragments are now in progress.





6. Discussion of results including an analysis of research outcomes compared with the objectives

Several promoter sequences from rice have been amplified by PCR and the base construct for producing plant expression vectors with unique rice promoters have been produced. A further two-step cloning process will produce the final products.

7. Implications and recommendations - where possible, a statement of relevant costs and benefits to the Australian industry should be provided

Andrew's PhD research and what is proposed in this project is a part our on going research on building a "Rice Gene Machine" as a resource for cereal functional genomics. RIRDC and NSW Agricultural Genomics Centre are funding the "Rice Gene Machine" project. Useful gene expression control sequences are one of the deliverables in these projects.

8. A description of the Project Intellectual Property and of any commercially significant developments arising from the Project

None at this stage.

9. Recommendations on the activities or other steps that may be taken to further develop, disseminate, or to commercially exploit the results of the project

The plant expression constructs being produced will enable the production of selectable marker free transgenic plants expressing GOIs in specific cells, tissues or organs. This project can easily be continued as an honours project if suitable and interested candidate is available.

10. Relevant References.

Lu H-J, Zhou X-R, Gong Z-X and Upadhyaya NM (2001) Generation of selectable marker-free transgenic rice using double right-border (DRB) binary vectors. Australian Journal of Plant Physiology 28: 241-248.

Upadhyaya NM, Zhou X-R, Ramm K, Zhu Q-H, L.Wu, Eamens A, Sivakumar R, Kato T, Yun D-W, Kumar S, Narayanan KK, Thomas G, Peacock WJ and Dennis ES (2002). An *iAc/Ds* gene and enhancer trapping system for insertional mutagenesis in rice. Functional Plant Biology 29:815-826.

Eamens AL, Blanchard C, Dennis E, Upadhyaya NM (2003) A bidirectional gene trap construct suitable for T-DNA and *Ds* mediated insertional mutagenesis in rice (*Oryza sativa* L.) (submitted to Plant Biotechnology Journal)

11. Acknowledgements

Liz Dennis, Qianhao Zhu, Kerrie Ramm