An Alternative Selection System for Tobacco Transformation

Gemma Hayton, Jayne Green, Louise Jones and Juan-Pablo Sanchez Tamburrino.

Corresponding Author’s email gemma.hayton@atcbiotech.com


Presented at the SEB 2012, Salzburg, Austria 29th June to 2nd July

INTRODUCTION

The use of marker genes in the transformation process allows differential selection of transformed from non-transformed cells. At present, the marker gene NPTII, which confers kanamycin resistance is used extensively. To enable the stacking of genes into transformed plants, removal of the selectable marker and re-usage of the NPTII gene in the next round of transformation would be a valuable system. We aim to access the recyclable property of the NPTII marker by the use of the pCLEAN vectors (John Innes Centre) to enable the delivery of multiple transgenes from distinct T-DNA regions into the plant nuclear genome as well as facilitating the production of marker-free plants. In this work, we use the pSOUP-10B vector developed by JIC which confers kanamycin resistance with pCLEAN-G152 to demonstrate the co-transformation procedure in tobacco. We determine the transformation efficiency by PCR and GFP expression and determine future uses of this technology for tobacco transformation.

METHODS

2µg of plasmid DNA from pSOUP10B was co-transformed along with 2µg plasmid DNA from pCLEAN-G152 into Agrobacterium tumefaciens by electrot transformation. Transformants were selected on high salt Luria Broth supplemented with 100µg/ml kanamycin. Tobacco leaf discs were incubated with the co-transformed Agrobacterium and grown on MSBN C for 2 days before being transferred to MSBN C + kanamycin selection. Leaf discs were then transferred to fresh media every two weeks until shoots were formed. Shoots were cut and cultured on LSBN until the plants were large enough to sample. Transformation efficiency was determined by PCR using primers designed to the GFP region in pCLEAN-G152 and kanamycin resistance in pSOUP-10B. GFP was also visualised under a Olympus SZX12 fluorescent microscope using a GFP filter cube.

RESULTS

![Figure 1. Schematic representation of vectors pCLEAN-G152 and pSOUP-10B. pSOUP-10B is the 'helper plasmid' which has kanamycin resistance between the left and right borders. This can be segregated out of the plant in later generations. pCLEAN-G152 is the vector that will harbour the gene of interest, but in this instance contains green fluorescence protein so that the system can be evaluated in different varieties of N.tobacum.](image)

![Figure 2. Green fluorescent protein expression in tobacco plants. Tobacco plants co-transformed with pCLEAN-G152 (CaMV35Spromoter::mgfp5-ER::SPAtargeting) and pSOUP-10B (35Spromoter::kan::CaMVterminator) (A), PEAS-HT-DEST1 (positive control) (B) and wild-type (WT) plants (C). Images on the left were obtained with a standard GFP filter, with a longpass GFP filter in the middle and under white light on the right. Expression of GFP was clearly observed under a standard GFP filter cube and under a longpass GFP filter for pCLEAN/pSOUP plants and PEAS-HT-DEST1 (positive control) but not wildtype plants. The red colouration which was seen under the longpass GFP filter is due to autofluorescence of the chlorophyll.](image)

![Figure 3. PCR electrophoresis gel. DNA was extracted from 10 co-transformed tobacco plants and used in a PCR with NPTI-specific primers. All 10 plants produced a positive NPTI band of ~791bp. The positive control (+ve) was the pSOUP-10B plasmid DNA and the negative (-ve) control contained sterile distilled water instead of DNA.](image)

DISCUSSION

Preliminary data indicated that co-transformation of the pCLEAN system has been successful in tobacco. The co-transformed plants described here will be further evaluated in the subsequent generations in order to segregate out the kanamycin resistance gene, leaving the plant free of any antibiotic resistance marker. Figure 2A shows GFP fluorescence with very little autofluorescence (red colouration). The PCR results further confirmed that NPTI was also present in the transformed plants. Further analysis using GFP-specific primers will be carried out in future work. This study verifies the use of pCLEAN vectors in tobacco and can potentially be used as a system to transform commercial varieties of N.tobacum. Future work will include evaluating the system using a variety of promoters as well as stacking genes to deliver multiple traits in one transformation event.

www.bat-science.com