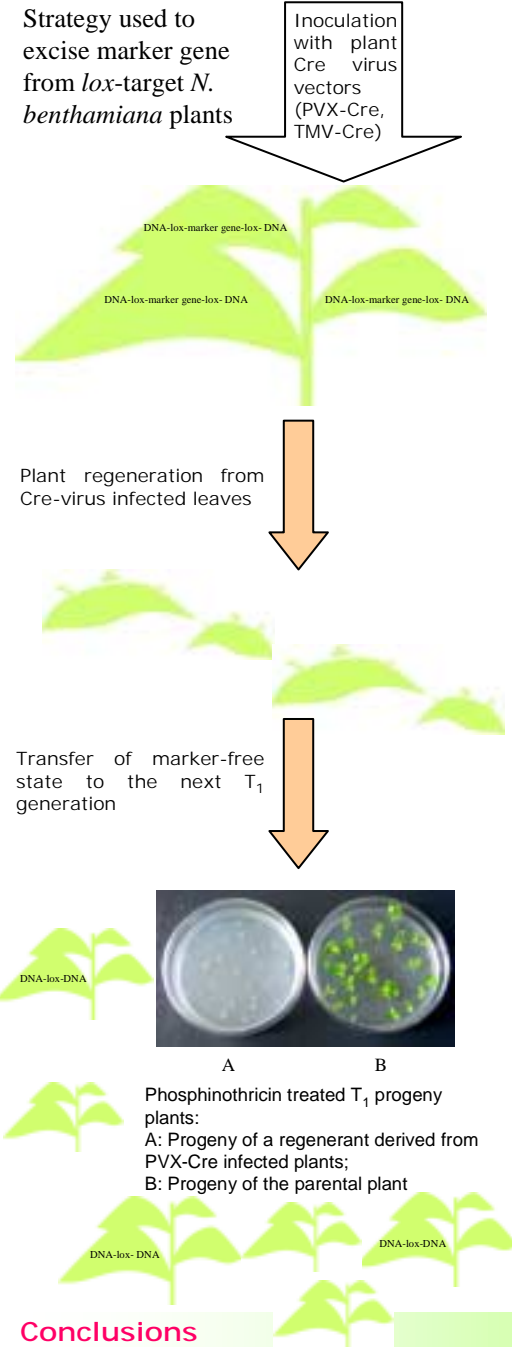


# Marker gene elimination mediated by transient expression of bacteriophage P1 Cre recombinase in plants

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## Introduction

Incorporation of a selectable marker is essential to identify transgenic cells and regenerate transgenic plants. However, once transformation is accomplished, the marker gene becomes superfluous. The Cre-*lox* recombination system from bacteriophage P1 was shown to be useful for marker gene elimination in higher plants. Cre-*lox* recombination system includes two *lox* sites and a 38 kDa Cre recombinase protein that mediates recombination reaction. When two *lox* sites are directly repeated the flanked marker gene is deleted. The future employment of the Cre-*lox* recombination system for practice will need a greater control of the recombinase activity. Limiting recombinase activity can help to minimise possible negative effects from constitutive recombinase expression.

## Objective

The aim of this project is developing an alternative strategy to excise selectable markers from transgenic plants by site-specific recombination via transient expression of the *cre* recombinase gene.

## Results

We utilised plant Cre virus vectors (TMV-Cre and PVX-Cre) for transient expression of *cre* recombinase in *lox*-target *N. benthamiana* plants. These plants contain the *bar* gene which confirms resistance to phosphinothricin flanked by two directly oriented *lox* sites. Cre-mediated site-specific recombination results in deletion of the *bar* sequence and phosphinothricin sensitivity. Our strategy includes several steps:

1. Efficient *cre* transient expression in *lox*-target *N. benthamiana* plants.
2. Selection of plants with precise marker gene excision. PVX-Cre and TMV-Cre systemically infected leaves were taken as explants for subsequent plant regeneration.
3. Transfer of the recombined state to the next  $T_1$  generation.

Transgenic plants containing *lox* sites and *bar* gene were inoculated with PVX-Cre and TMV-Cre recombinant viruses. PVX-Cre and TMV-Cre systemically infected leaves were allowed to regenerate without selection pressure. Western blot and PCR analysis showed that selectable marker gene (*bar*) was eliminated in PVX-Cre systemically infected leaf tissue. To remove the virus from plant tissue the nucleoside analogue ribavirin was used in the regeneration medium. The frequency of Cre-mediated *bar* gene excision was evaluated by PCR analysis. This approach allowed us to separate regenerants with complete *bar* gene excision from chimeric plants. 50-82% of the regenerants from TMV-Cre and PVX-Cre infected explants were marker free. Self progeny of virus free and marker free plants was examined for the inheritance of the recombined state. Seeds were grown on medium with 6 mg/l phosphinothricin. No phosphinothricin-resistant seedlings for all examined lines were observed on the selective medium, confirming that excision of the *bar* sequence has been transmitted to the next generation. Molecular analysis proved that  $T_1$  progeny plants did not contain selectable marker gene (*bar*) in their genome.

## Conclusions

An alternative method for the production of marker-free transgenic *N. benthamiana* plants has been developed. We used plant Cre virus vectors to express *cre* recombinase transiently. The frequency of recombination expressed as a percentage of regenerated plants without marker gene varied from 50 to 82 % for TMV-Cre and PVX-Cre virus vectors, respectively. The  $T_1$  progeny of these regenerants did not contain the marker gene (*bar*) in their genome. The strategy can be applied to plant species that depend on organogenesis or somatic embryogenesis for regeneration, particularly, potato and woody plants.