The use of potato (*Solanum tuberosum*) suspension cells for rapid screening of chloroplast transformation vectors

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ABSTRACT

Chloroplast genetic engineering is unique in that it allows for high levels of gene expression while providing a means of natural bio-confinement. Although the chloroplast genome sequence of over 800 plant species is known, the elements that naturally regulate chloroplast gene expression are poorly understood. Recently, our lab has developed a 264 part modular cloning kit that contains known chloroplast regulatory elements. This kit can be used for the construction of novel chloroplast transformation cassettes; however, functional testing of these cassettes is currently hindered by standard chloroplast transformation technologies. Therefore, the aim of this experiment was to develop a rapid cell-based screening method that can be used for analyzing chloroplast transformation vectors. Golden Gate cloning was used to assemble a cassette from our kit that contained the native chloroplast *Prrn* promoter driving expression of a spectinomycin resistance/green fluorescent protein gene fusion. The cassette was introduced into potato (*Solanum tuberosum*) suspension cells via particle bombardment and the cells were screened as early as 48 hours for GFP expression. The results of this study could significantly enhance chloroplast genetic engineering efforts by allowing for rapid testing of chloroplast regulatory elements and by accelerating the screening time of novel chloroplast transformation vectors.

INTRODUCTION

Genetic engineering of the chloroplast genome offers several benefits over nuclear transformation such as maternal inheritance of the transgene(s), which lends to a natural means of bio-confinement, and the ability to produce recombinant proteins up to 46% total soluble leaf protein [1]. The standard method for chloroplast transformation is particle bombardment of plant cells and incorporation of the transgene cassette into the chloroplast genome through homologous recombination. Transplastomic plants are then regenerated through successive rounds of either direct organogenesis or somatic embryogenesis, two processes that are laborious and time-consuming. To balance the time requirements for generating transplastomic plants, standard chloroplast transformation vectors have utilized a limited number of regulatory elements, such as the plastid rRNA operon promoter (Prrn) and the psbA 3' untranslated region (UTR), and integration sites that have been shown to be successful for high levels of transgene expression [1]. For expression of multiple genes in a pathway, however, additional regulatory elements are needed that can coordinate gene output and promote efficient chloroplast genetic engineering. To meet this need, our lab has developed a chloroplast modular cloning kit that can be used for the creation of novel chloroplast transformation vectors. This kit is based on Golden Gate cloning [2] and contains 84 promoters, 49 5' UTRs, ten 3' UTRs, and integration sites for corn, tobacco, and potato. Some of the components of this kit have only been reported in the literature and have not been functionally tested. Therefore, the objectives of this project are to 1) assemble a chloroplast transformation construct using the kit and 2) develop a rapid assay to screen functionality of the construct in potato suspension cells.

METHODS

Use Golden Gate cloning to assemble a chloroplast transformation cassette.

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Introduce chloroplast transformation cassette into *Solanum tuberosum* cv. Desiree suspension cells via particle bombardment.

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Screen bombarded cells as early as 48 hours after bombardment for fluorescent protein expression.

RESULTS

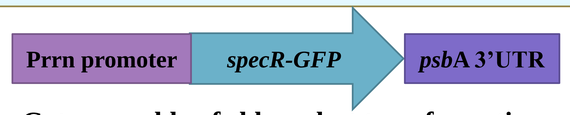

Figure 1. Golden Gate assembly of chloroplast transformation cassette
Golden Gate cloning was used to assemble a chloroplast transformation cassette from parts of our modular cloning kit. The cassette contains the Prrn promotor driving expression of a spectinomycin resistance/green fluorescent protein gene fusion and the *psb*A 3' UTR.

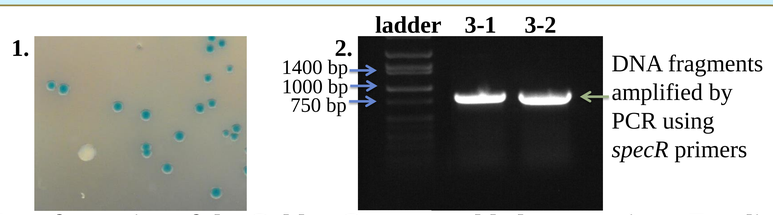

Figure 2. Transformation of the Golden Gate-assembled cassette into *E. coli* and colony PCR to detect transgene cassette
1) Bacteria plated on selection media after transformation. White colonies indicate removal of the lacZ reporter gene and insertion of the assembled cassette. 2) Colony PCR of two putative positive colonies using *specR* primers (For: 5' ATGAGGGAAGCGGTGATCGC 3', Rev: 5' TTATTTGCCGACTACCTTGGTGATCTC 3') to detect presence of the cassette.

RESULTS

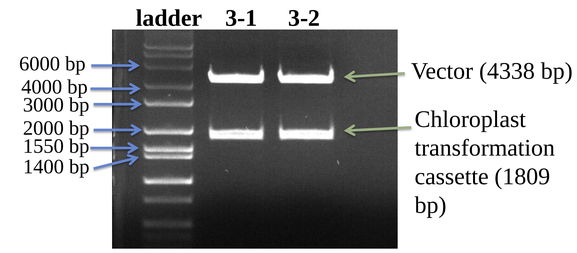

Figure 3. Restriction enzyme digestion to confirm cassette integration and size
Digested plasmid DNA of putative positive colonies with BbsI to determine proper integration and size of the chloroplast transformation cassette.

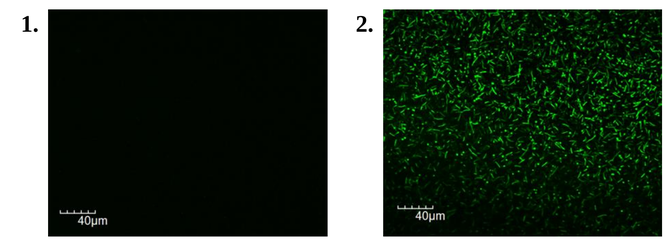

Figure 4. Screening bacteria to confirm functionality of chloroplast transformation cassette
1) Bacteria that do not contain the chloroplast transformation cassette. 2) Bacteria that do contain the cassette

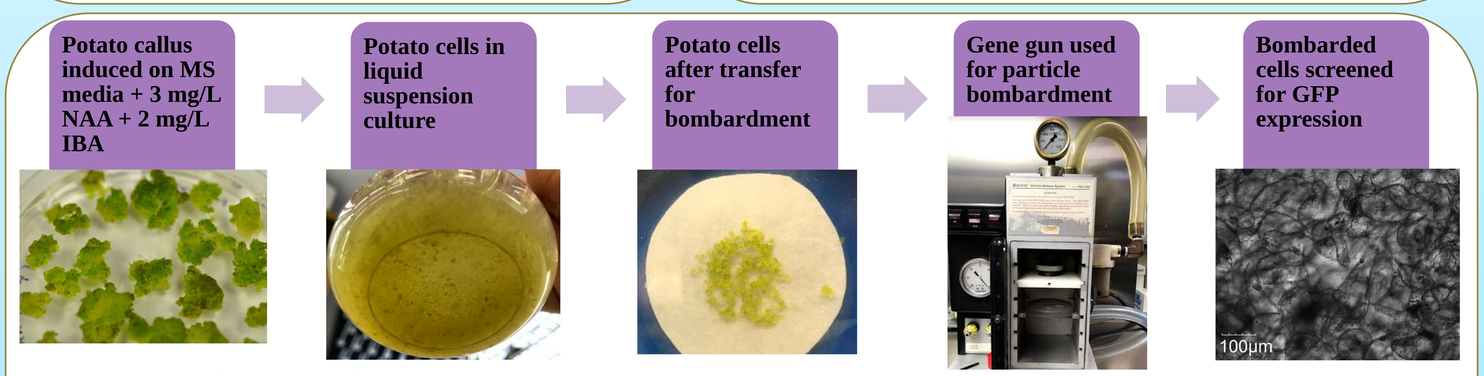

Figure 5. Transformation of *Solanum tuberosum* chloroplasts via particle bombardment
From *Solanum tuberosum* cv. Desiree callus, cell suspension cultures were established. After establishment, the cells were plated on bombardment media. The chloroplast transformation cassette was introduced to the cells via particle bombardment. After bombardment, confocal microscopy was used to screen cells for potential transformation.

DISCUSSION

A rapid screening technique for chloroplast transformation is necessary in order to functionally test novel transformation vectors before undertaking the laborious process of creating stable transplastomic plants. In this study, we aimed to detect GFP expression in potato chloroplasts as early as 48 hours after bombardment. Despite our efforts, GFP expression in the chloroplasts was not observed. This could be attributed to the low efficiency of plastid transformation. In early studies involving potato, only one transplastomic shoot was obtained per 15-18 bombardments [3]. Recently, however, researchers have been able to improve efficiency by one shoot per bombardment when transformation vectors contained potato-specific chloroplast integration sequences [3]. Therefore, to facilitate screening in future work, the cassette could be transformed into a vector containing potato flanking sequences that would allow for integration once introduced into the chloroplasts [3, 4]. Chloroplast size could be a factor as well. A comparison of Arabidopsis light-grown cell suspension cultures and leaf cells found that the chloroplasts of the suspension cells were on average smaller than the chloroplasts found in the leaf cells [5]. If this is also true for potato cells, the smaller chloroplast targets for bombardment could have contributed to a reduced transformation efficiency. Thus, further optimization of this protocol is necessary in order to develop a rapid screening method for chloroplast transformation.

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