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Characterization of a novel mitochondrial plasmid in *Brassica*

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Introduction

Plants in the genus *Brassica* contain an independent, linear mitochondrial plasmid of approximately 11.6 kilobases in length (Figure 1). The *Brassica* plasmid is characterized by terminal inverted repeats and covalently bound proteins at its 5' termini (Erickson, Beversdorf, & Pauls 1985). The plasmid also contains six genes that encode a DNA and RNA polymerase and several unknown proteins (Handa 2008).

Currently, both the function of the *Brassica* plasmid and the mechanisms by which it is transported into and replicated within the mitochondria are largely unknown. The primary objectives of this study are to

1) **insert the plasmid into a cloning vector**
2) **monitor the plasmid's movement using a fluorescent protein tag**

If we can determine how the *Brassica* plasmid moves between the cytoplasm and mitochondria, it has the potential to be used as a mitochondrial-targeting vector in plant cells, which would allow for direct manipulation of the genetic and biochemical environment of plant mitochondria.

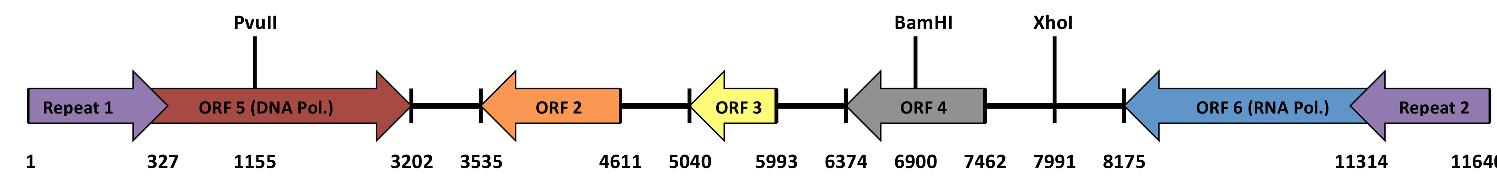

Figure 1. *Brassica* linear mitochondrial plasmid

Materials and Methods

The *Brassica* plasmid was extracted from *B. rapa*, amplified by the polymerase chain reaction (PCR), and cloned into the vector pCR-XL-2-TOPO™ (Figure 2) using the TOPO XL-2 Cloning Kit (Invitrogen). Once the *Brassica* plasmid was successfully inserted into pCR-XL-2-TOPO™ , the construct was digested with restriction enzymes AvrII and XhoI. The red fluorescent protein gene eqFP611 was amplified by PCR and also digested with AvrII and XhoI, and the two fragments were assembled using Rapid DNA Ligation (Thermo Scientific).

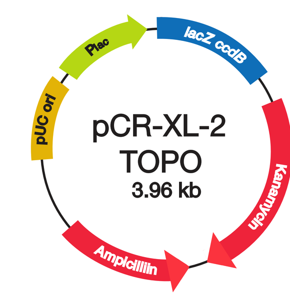

Figure 2. pCR-XL-2-TOPO™ vector

Results

Insert plasmid into cloning vector

Following transformation, the vectors were isolated from *E. coli* and digested with restriction enzymes SalI and BamHI to confirm the presence of the *Brassica* plasmid. The fragments were run on an agarose gel to verify their sizes (Figure 3).

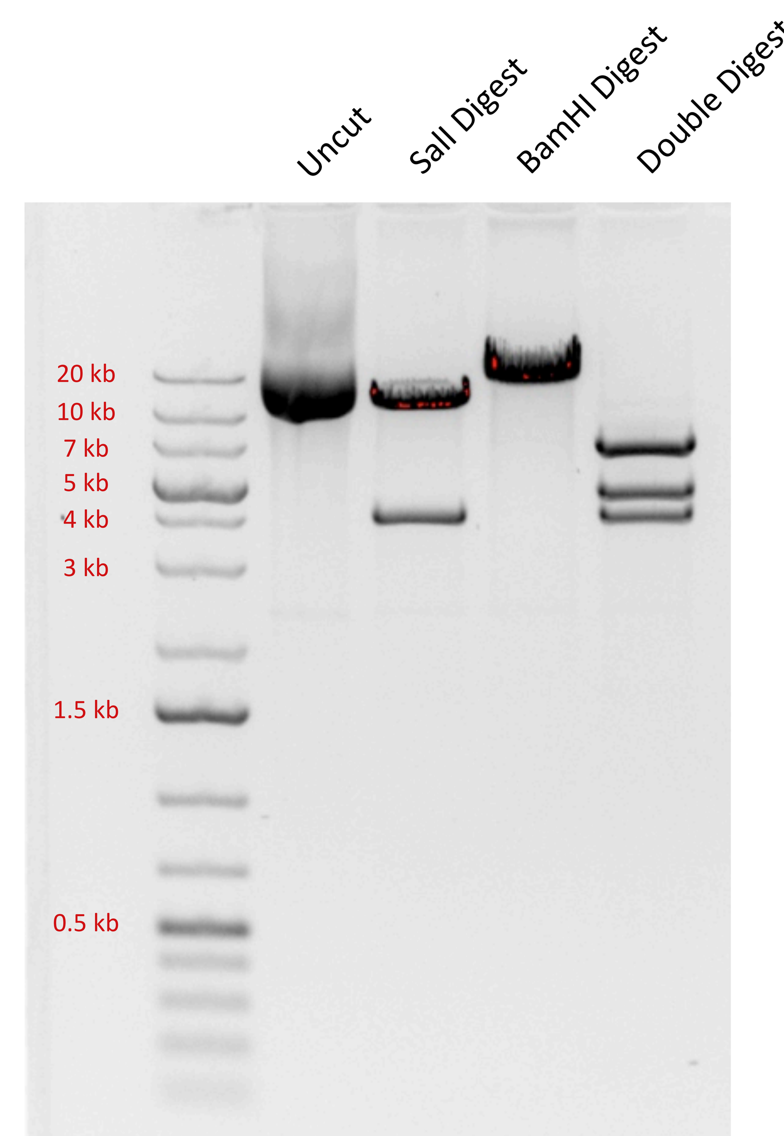

Figure 3. Plasmid restriction digestion with SalI and BamHI

Digestion with SalI yielded two fragments of approximately 4 kilobases and 11.6 kilobases in length. Digestion with BamHI gave a single fragment of approximately 15 kilobases, and digestion with both SalI and BamHI produced three fragments of approximately 4 kilobases, 4.7 kilobases, and 7 kilobases in length. These fragments correspond to the expected sizes of the plasmid.

Monitor plasmid's movement using a fluorescent protein

To verify successful insertion of eqFP611 between the AvrII and XhoI restriction sites of the *Brassica* pcR-XL-2-TOPO™ construct, PCR was performed using a pair of primers flanking the insert region. The *Brassica* pCR-XL-2-TOPO™ construct without eqFP611 was also amplified using the same pair of primers and the PCR products were run on an agarose gel to compare their sizes (Figure 4).

Results (cont.)

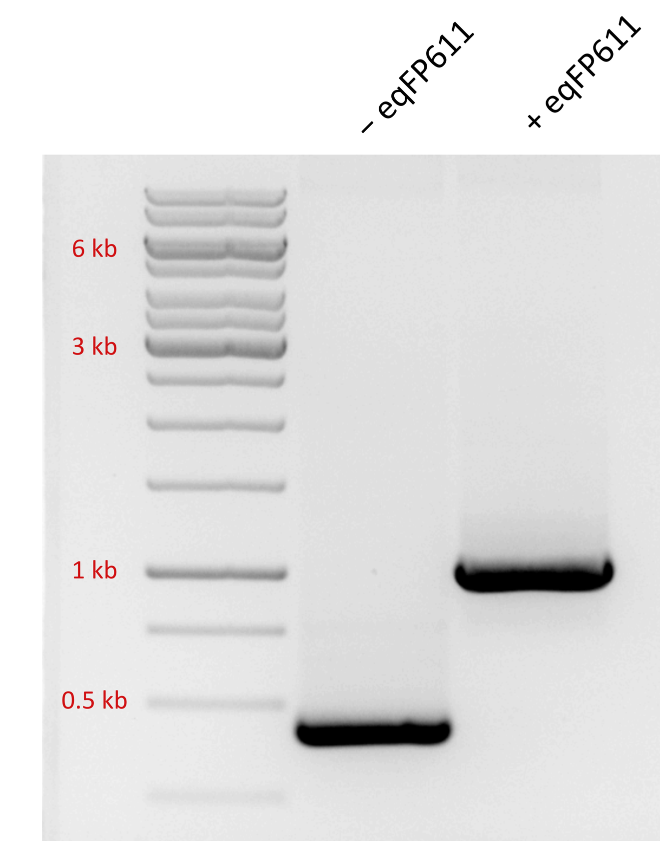

Figure 4. PCR of *Brassica* pCR-XL-2-TOPO with flanking primers

Brassica pCR-XL-2-TOPO™ without eqFP611 amplified a fragment of approximately 450 base pairs in length, while *Brassica* pCR-XL-2-TOPO™ with eqFP611 amplified a fragment of approximately 1000 base pairs in length. The presence of eqFP611 in the *Brassica* pCR-XL-2-TOPO™ construct was also confirmed by DNA sequencing.

Conclusions

Due to its length and terminal inverted repeats, the *Brassica* mitochondrial plasmid has been difficult to genetically manipulate, making the seemingly straightforward task of molecular cloning tedious and complicated. Nevertheless, its successful insertion into a cloning vector has now paved the way for further analysis, with goals for subsequent research currently including

- Infiltration of *Arabidopsis thaliana* with the *Brassica* pCR-XL-2-TOPO™ construct
- Monitoring of the movement of the plasmid in plant cells via eqFP611
- Transport of foreign DNA into plant mitochondria through use of the plasmid

Once the replicative and transitory behavior of the *Brassica* plasmid is better understood, its use as a mitochondrial-targeting vector in plant cells has the potential to revolutionize plant mitochondrial research by allowing scientists to directly alter the physical environment of the organelle.

Acknowledgments

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