Spring 4-2016

Is a Mitochondrial Plasmid Really a Virus?

Mackenzie Strehle

University of Nebraska - Lincoln, mackenziestrehle@gmail.com

Follow this and additional works at: http://digitalcommons.unl.edu/ucareresearch

Part of the Genetics Commons, Molecular Genetics Commons, Plant Biology Commons, and the Plant Breeding and Genetics Commons


This Poster is brought to you for free and open access by the UCARE: Undergraduate Creative Activities & Research Experiences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in UCARE Research Products by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Is a Mitochondrial Plasmid Really a Virus?
Mackenzie Strehle
University of Nebraska–Lincoln, School of Biological Sciences

Introduction
In addition to containing a large and complex mitochondrial genome, the mitochondria of several species of plants have been shown to contain an independent, self-replicating DNA molecule in the form of a plasmid. Plants in the Brassica genus contain a linear plasmid that is approximately 11.6 kilobases in length. The plasmid is characterized by the presence of terminal inverted repeats and covalently bonded proteins at its termini (Handa 2008). The plasmid also contains six ORFs that encode DNA and RNA polymerases and a number of unknown proteins (Figure 1). Currently, both the function of this plasmid and the mechanisms by which it is transported into and replicated within the mitochondria are largely unknown. Our current hypothesis is that these mitochondrial plasmids were originally acquired as a virus by a subspecies of Brassica and have since become an integrated component of the plant’s mitochondrial machinery. During the course of our research, we hope to discern the genetic basis and overall nature of the Brassica mitochondrial plasmid, as well as develop practical methods for targeting specific gene sequences and protein products into the mitochondria through the use of this plasmid.

Objectives
Our research has been primarily focused on the Torch cultivar of Brassica napus, an oilseed plant in the turnip family, which has been shown to contain the linear mitochondrial plasmid. The objectives for this project include:

- Cloning the plasmid into a stable vector
- Determining the identity of the proteins covalently attached at the plasmid’s termini
- Transforming a plant species that does not normally contain the plasmid, such as Arabidopsis thaliana

Methods
Cloning plasmid into stable vector.
NEBuilder HiFi DNA Assembly was used to clone the Linear mitochondrial plasmid into pUC19. According to the DNA Assembly protocol, the general steps for cloning include:
1. Creation of construct using online assembly tool
2. Amplification of overlapping fragments via PCR
3. NEBuilder HiFi Assembly reaction
4. Transformation of electrocompetent or chemically competent cells
5. Construct verification via junction PCR

Determing identity of terminal proteins.
Mitochondrial extraction from the Torch plant and pre-electrophoresis sample treatments were performed according to the method of Erickson, Beversdorf, and Pauls (1985). Following mitochondrial isolation, the Torch sample was suspended in lysis buffer composed of 0.05M Tris and 0.02M EDTA. The mitochondrial DNA was then fractionated by electrophoresis in a 0.7% agarose gel with 0.01% SDS. After electrophoresis, the gel was washed with 0.5x TAE and stained with ethidium bromide for UV imaging.

Results
Following initial extraction of the mitochondria from the Torch plant, PCR was used to verify the presence of the linear mitochondrial plasmid. The entire plasmid was amplified using primers that selectively anneal to the terminal inverted repeats (Figure 3).

Discussion
Qualitative analysis of the Brassica linear mitochondrial plasmid has presented a number of unique challenges to traditional molecular genetics techniques. The plasmid’s large size, as well as the presence of inverted repeats and covalently bonded proteins at its termini, have largely inhibited the progress of effectively examining its behavior by the aforementioned strategies.

Conclusion
Plant mitochondrial genomes are notorious for both their size and complexity, and the Brassica linear mitochondrial plasmid has proven no exception to this trend. Given that previous attempts at isolating the plasmid and cloning it into pUC19 have seen marginal success, goals for subsequent research currently include:

- Developing a more efficient means by which to extract plant mitochondria
- Modifying the NEBuilder HiFi DNA Assembly protocol so as to produce positively transformed colonies
- Utilizing mass spectrometry to analyze the plasmid’s terminal proteins

Once we are able to successfully clone the plasmid into an optimized vector, we will begin investigating the role of its terminal proteins in mitochondrial transport and the mechanisms by which it replicates itself. By the end of this study, we hope to be able to present an argument for the viral origin of the Brassica linear mitochondrial plasmid, as well as use the practical applications of this information in the context of plant biology.

References

Acknowledgments: This project was made possible by the academic guidance of Dr. Alan Christensen and Dr. Richard Prentice. Financial support was provided by the University of Nebraska–Lincoln’s Creativity Incentive and Grant Program and the University of Nebraska–Lincoln Research Foundation (RIF-14-I12).