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Is a Mitochondrial Plasmid Really a Virus?

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Is a Mitochondrial Plasmid Really a Virus?

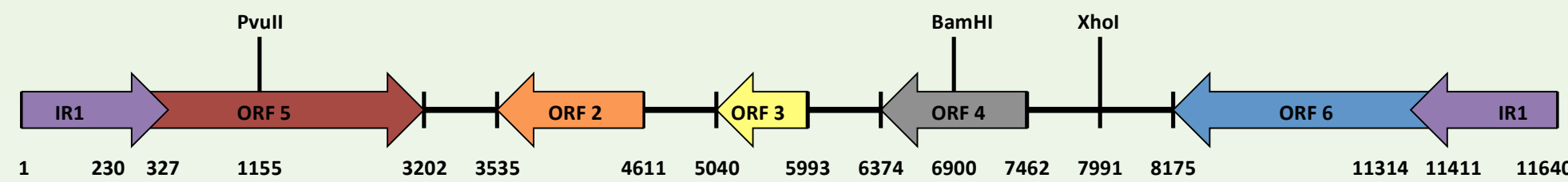
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Introduction

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.

Figure 1. *Brassica* Linear Mitochondrial Plasmid



Objectives

Our research has been primarily focused on the Torch cultivar of *Brassica rapa*, 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*

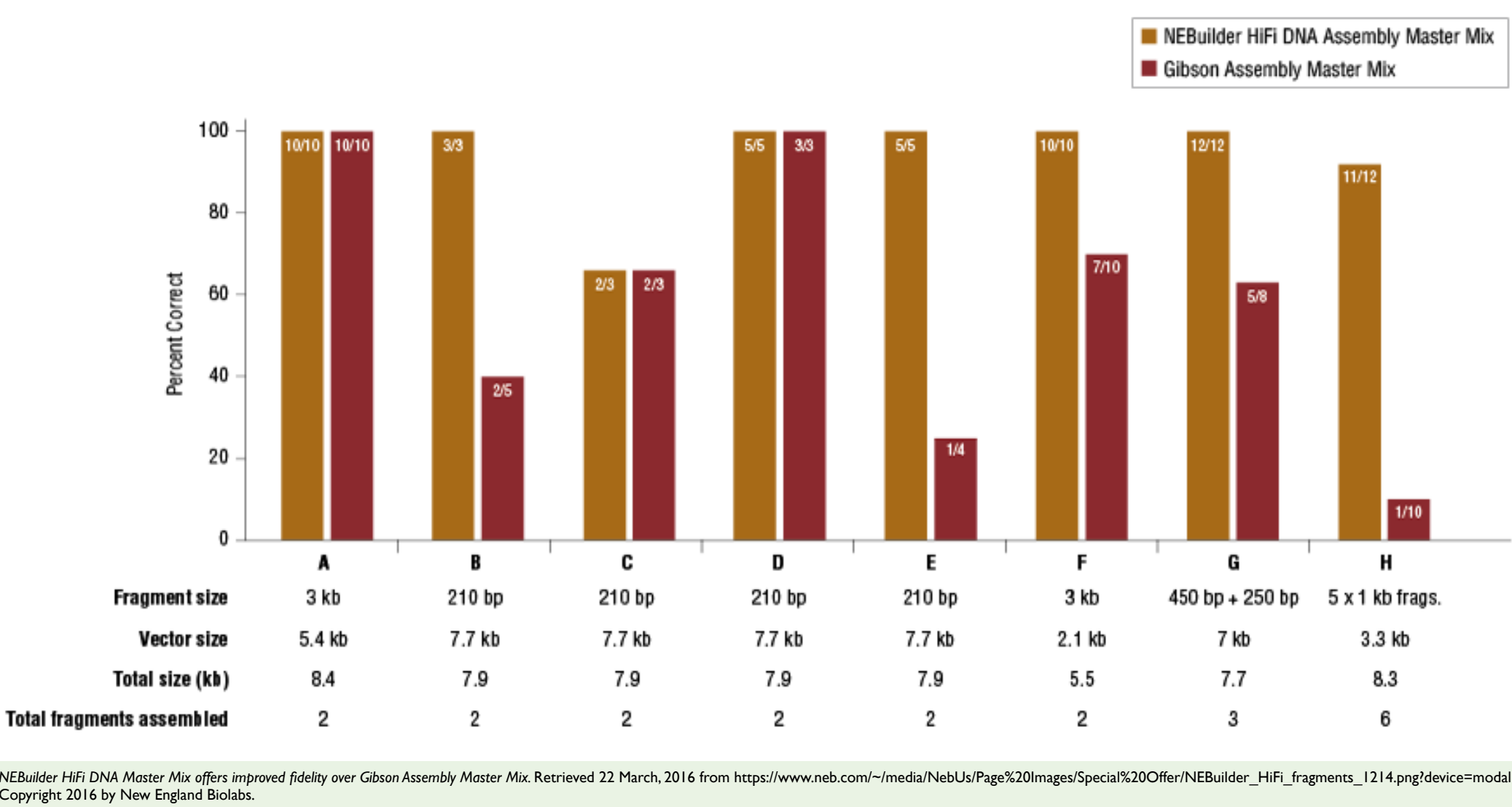
Methods

Cloning plasmid into stable vector.

NEBuilder HiFi DNA Assembly was used to clone the *Brassica* 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

Figure 2. Comparison Between NEBuilder HiFi DNA Assembly and Gibson Assembly



NEBuilder HiFi DNA Assembly was used for cloning rather than the more traditional Gibson Assembly technique due to its improved fidelity in terms of both construct size and number of fragments used (Figure 2). Following linearization of pUC19 with PCR, the *Brassica* plasmid was amplified in three 3.8 kb pieces for a total construct size of approximately 14 kb.

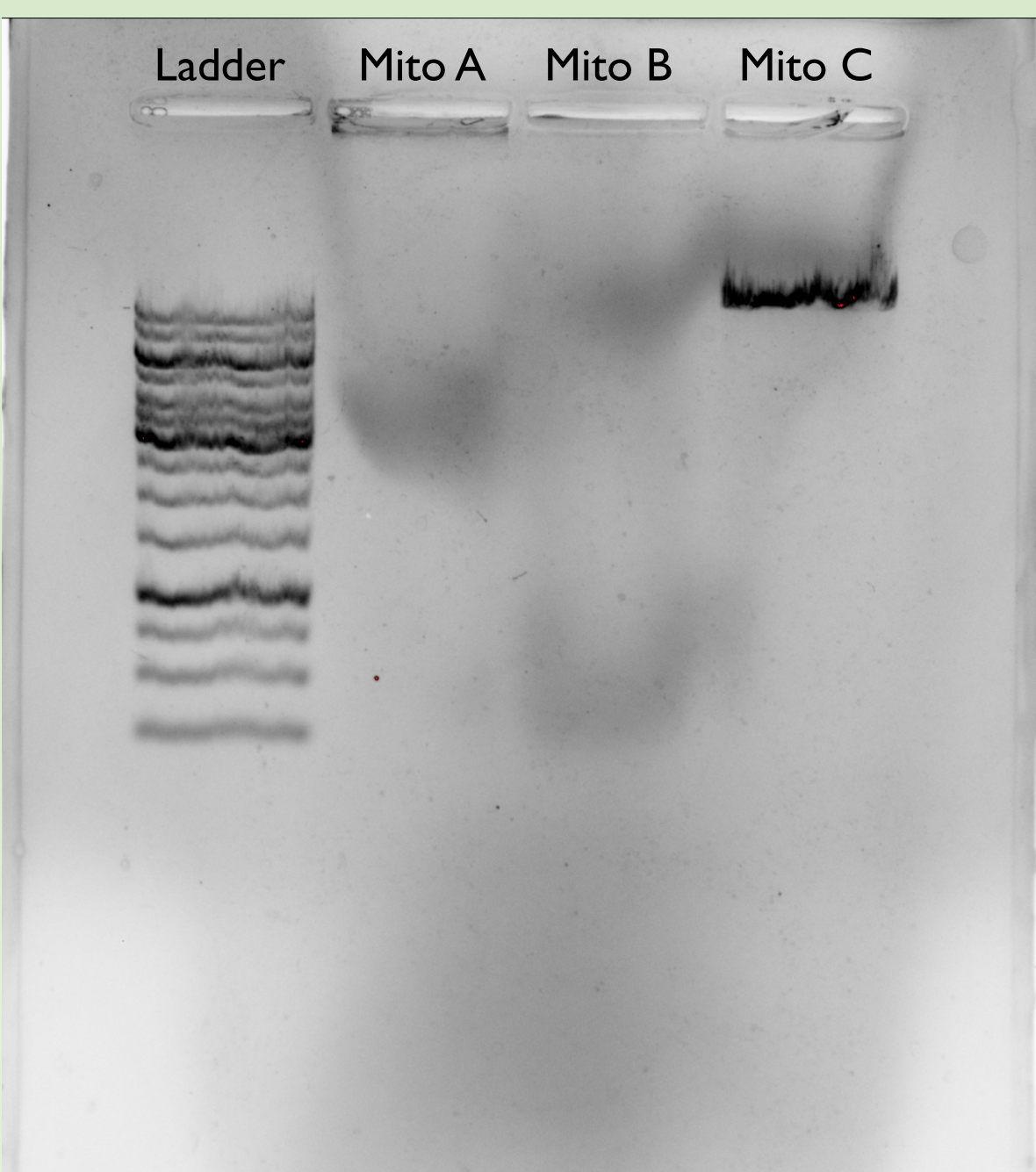
Determining identity of terminal proteins.

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).

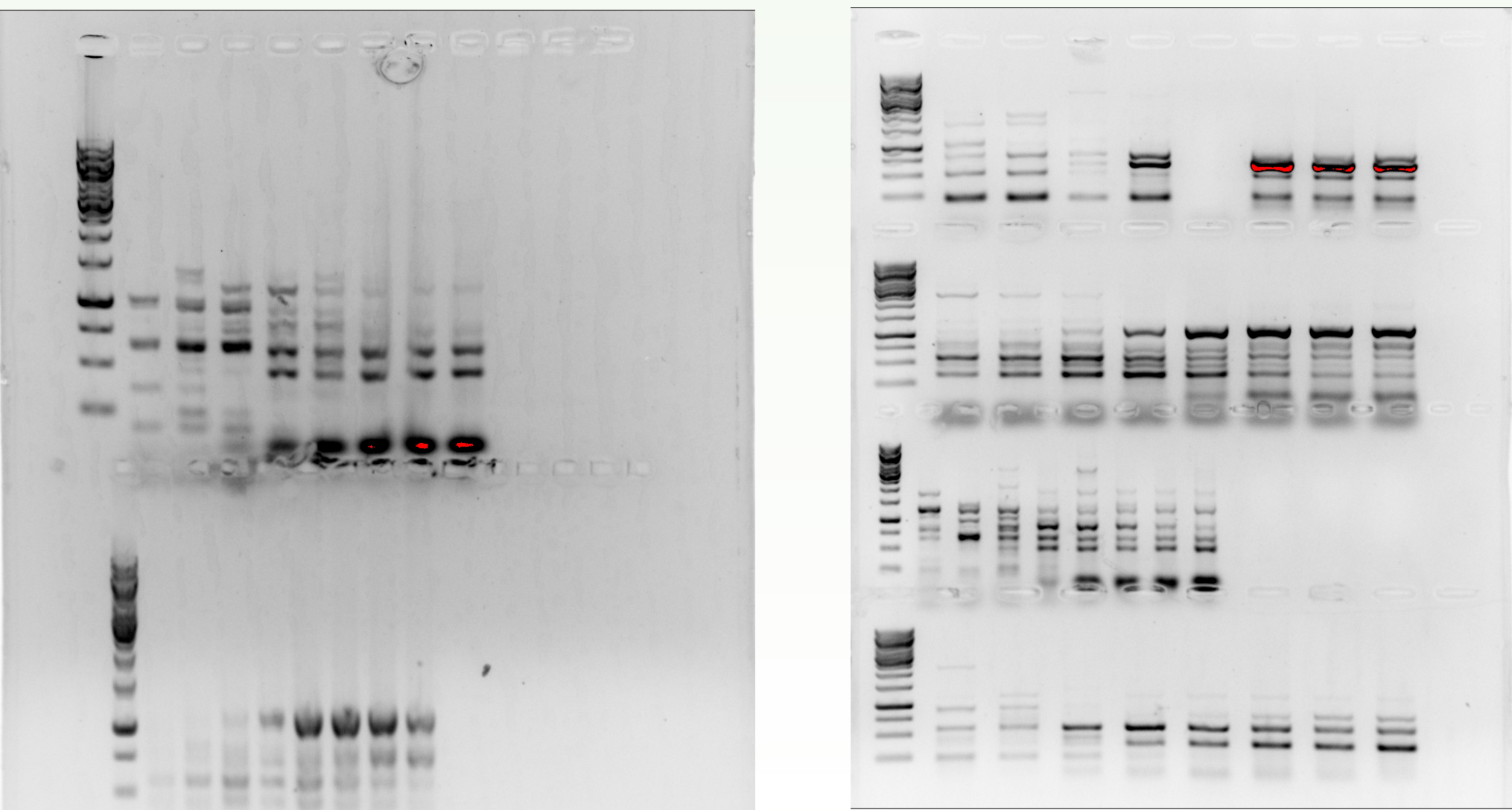
Figure 3. *Brassica* Linear Plasmid Amplification



Imaging of the mitochondrial plasmid as per the method outlined by Erickson et al. yielded an indiscernible streak on the gel, likely as a result of inefficient mitochondrial isolation.

Transformation of chemically competent *E. coli* cells with the NEBuilder HiFi DNA Assembly reaction mixture produced colonies on ampicillin plates. The colonies showed amplification during PCR with primers that overlapped each junction of the construct (Figures 4 & 5); however, the bands on the gel did not show an organized or predictable pattern, undermining the validity of these results. Furthermore, a restriction assay of the plasmid prepped colonies did not indicate that any DNA was present in the samples, further implying that the transformation had been unsuccessful.

Figures 4 & 5. Torch NEBuilder Junction PCR



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 the practical applications of this information in the context of plant biology.

References

Erickson L, Beversdorf WD, Pauls KR. 1985. Linear mitochondrial plasmid in *Brassica* has terminal protein. *Current Genetics*. 9:679-682.

Handa H. 2008. Linear plasmids in plant mitochondria: peaceful coexistences or malicious invasions? *Mitochondrion*. 8(1):15-25.

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