Apr 12th, 2:00 PM - 3:00 PM

Expression of Gene Product 126 from Phage Gizmo and Creation of a Substrate

Manish Mandava
Illinois Wesleyan University

David Bollivar, Faculty Advisor
Illinois Wesleyan University

Expression of Gene Product 126 from Phage Gizmo and Creation of Substrate

Manish Mandava and David Bollivar
Department of Biology, Illinois Wesleyan University Bloomington, IL

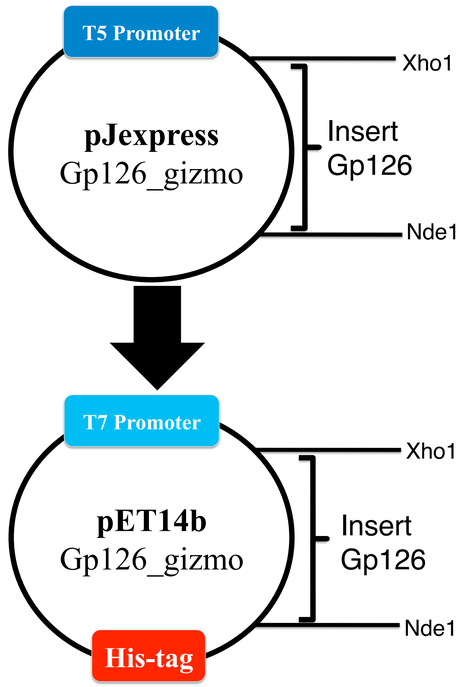
Introduction

Endonucleases are enzymes which cleave phosphodiester bonds in the backbone of nucleic acids such as DNA. Homing endonucleases(HEase) are a group of endonucleases that are site-specific, mobile, and persistent genetic elements[1]. HEases are a large and diverse class of nucleases found in the genomes of Archaea, Bacteria, Eukarya, and viruses. As a result of the Homing process, HEases are able to propagate their self-splicing genes into recipient alleles that lack such genes[2]. This process is initiated by the expression of a homing endonuclease gene(HEG) which results in a HEase. The HEase goes on to cleave a target site in a homolog of the hosting gene to induce a homologous recombination event, and thereby transforming the vacant homolog into a HEG(Figure 1)[3]. The ability of HEases to initiate and target specific sequences along a genome and effectively propagate its HEG throughout a genome allows for HEases to be possible reagents for gene targeting, DNA modification, and genome editing.

It is predicted gene product 126(gp126) from the mycobacteriophage Gizmo encodes a HEase. To test whether the gene encodes a HEase the phage sequence of gp126 Gizmo was transferred into the his-tag containing plasmid(PET14b). The protein was expressed and purified. The mycobacteriophage Shrimp was utilized as a substrate to test the predicted function and evaluate possible target sequence of the HEase derived from gp126. A long term goal is to define the recognition sequence for this homing endonuclease.

Results

Transfer of gp126 into pET14b



Gp126 Expression

1) Creation of Expression plasmid-
2) Transformation of pET14b::gp126 into Tuner™(DE3) via heat shock method
3) Growth of *E.coli* colonies grown in LB media
- Cells induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG)

4) Protein gel electrophoresis (Figure 2)

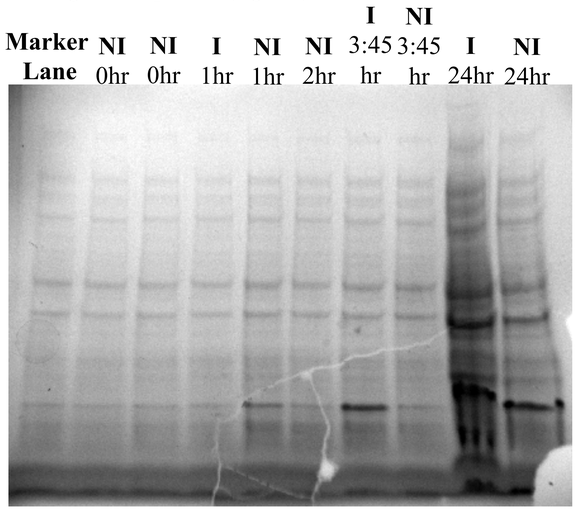


Figure 2. I, induced with IPTG. **NI**, not induced.

His tag purification

1) Collection of protein aliquots (Figure 3)

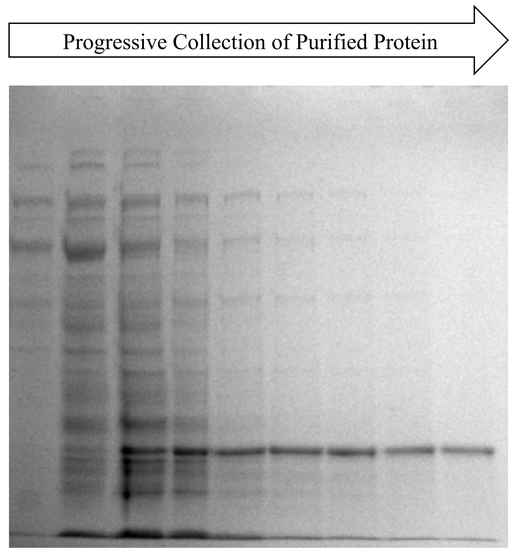


Figure 3. SDS-PAGE of collected HEase

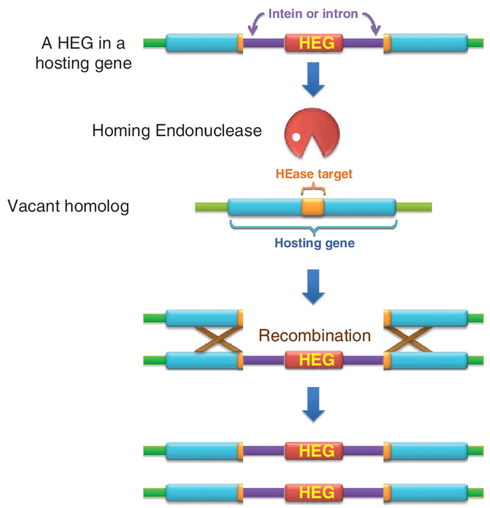


Figure 1[2] (Barzel A et al., 2011)

The Homing Process. The homing endonuclease (HEase) is expressed from the HEG (red), residing in an intron or as an in-frame domain of an intein (purple) in a hosting gene (cyan). It cleaves the target site (orange) in a vacant homolog of the hosting gene to induce homologous recombination (gene conversion or double crossover), turning the vacant homolog into a HEG-carrying one.

Results

Cloning of DNA target for HEG

1) Amplification of target DNA from phage Shrimp genome via PCR techniques
2) Ligation and Transformation of amplified DNA
3) Purification of Shrimp DNA via plasmid miniprep
4) Sequencing of DNA (*Eurofins*©)

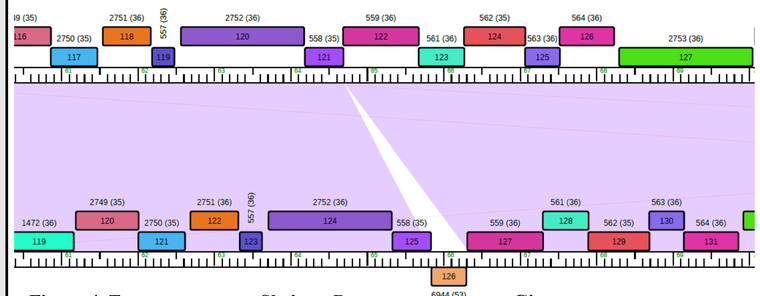


Figure 4. Top sequence map **Shrimp**. Bottom sequence map **Gizmo**

Conclusions

From our initial step we have established that the predicted homing endonuclease gene(gp126) from the bacteriophage Gizmo can be inserted into the plasmid vector (pET14b). With our second project we were able to understand the optimal time of induction and subsequent expression of gp126 inside the pET14b plasmid vector. Following expression we took advantage of the his-tag region on the pET14b vector in order to purify the HEase enzyme. The final step in our research was to clone a possible DNA target for the HEase. We believe that the nearly identical genome of the bacteriophage Shrimp will serve as a feasible target for the HEase that we have isolated.

Further Work

Further work would be to utilize the isolated homing endonuclease in order to determine binding and cleavage sites. This would involve determining the optimal conditions for the HEase enzyme(e.g- buffers, temperature, etc.). With knowledge of binding and cleavage sites, the goal would then be to understand the efficacy of the HEase to tolerate sequence degeneracy within the recognition site.

Literature cited

1= March 2014;5(1):1-31. Available from: Academic Search Premier, Ipswich, MA. Accessed April 10, 2014.
2= Shen B, Landthaler M, Shub D, Stoddard B. DNA Binding and Cleavage by the HNH Homing Endonuclease I-Hmul. *Journal Of Molecular Biology* [serial online]. September 3, 2004;342(1):43-56. Available from: Academic Search Premier, Ipswich, MA. Accessed April 10, 2014.
3= Barzel A, Privman E, Kupiec M, et al. Native homing endonucleases can target conserved genes in humans and in animal models.*Nucleic Acids Research* [serial online]. n.d.;39(15):6646-6659. Available from: Science Citation Index, Ipswich, MA. Accessed April 10, 2014.
4= Homing endonucleases: DNA scissors on a mission. *Genome* [serial online]. August 2012;55(8):553-569. Available from: Academic Search Premier, Ipswich, MA. Accessed April 10, 2014.

Acknowledgments

- Illinois Wesleyan University Ames Library
- Illinois Wesleyan Departments of Chemistry and Biology
- Cindy Honegger (Stock Room Manager, Center for Natural Sciences, IWU)