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*

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**Introduction**

Endonucleases are enzymes which cleave phosphodiester bonds in the backbone of nucleic acids such as DNA. Homing endonucleases (HEases) are a group of endonucleases that are site-specific, mobile, and persistent genetic elements. 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. This process is initiated by the expression of a homing endonuclease gene (HEG) which results in a HEase. The HEase then 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). The ability of HEases to initiate and target specific sequences along a genome and effectively propagate their HEG throughout a genome allows for HEases to be possible reagents for gene targeting, DNA modification, and genome editing.

It is predicted that gene product 126gp126) 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 sequences 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**

- **T5 Promoter**
  - pJexpress
  - Gp126_gizmo
  - Xho1
  - Nde1
- **T7 Promoter**
  - pET14b
  - Gp126_gizmo
  - Xho1
  - Nde1

**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)

**His tag purification**

1) Collection of protein aliquots (Figure 3)

**Progressive Collection of Purified Protein**

**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 the homing endonuclease in order to determine the optimal conditions for the HEase to tolerate sequence degeneracy within the recognition site.

**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(s) and substrate. 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**


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