The Summer Undergraduate Research Fellowship (SURF) Symposium
4 August 2016
Purdue University, West Lafayette, Indiana, USA

Design and Development of a Plasmid Vector for Protein Expression and Purification

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ABSTRACT

Production and isolation of proteins are difficult, costly and time-consuming processes. The aim of this project is for the development of plasmids, which allow for streamlined production and isolation of proteins. To allow for modular insertion of varying segments of DNA we are using 'recursive directional ligation by plasmid reconstruction'. This technique uses type II restriction endonucleases, which cut downstream from their recognition site allowing multiple insertions without losing a restriction site. Using this process, we can ligate multiple DNA sequences together and express them to be able to construct a scar less fusion protein. In order to accomplish this, we have used techniques such as restriction digestion, ligation, dephosphorylation and transformation in order to ligate our vector, pET 25B+, and different DNA inserts together and inserted them in competent cells. To screen whether proper ligation has occurred we have used techniques such as colony PCR and running diagnostic restriction digestions on agarose gels. Sanger sequencing was used to confirm the successful insertion of individual sequences in our plasmid, which will allow for different modes of purification of any protein of choice.

KEYWORDS

Colony PCR, Recursive directional ligation, Plasmid, Protein expression