ATTAQ of the Clones


Introduction:
Peptide libraries are a fundamental part of drug design in the pharmaceutical industry, however, very large libraries of peptides cost millions of dollars and are time consuming to produce. These extremely high costs ripple to the consumer resulting in the high retail price of peptide drugs and a large cost to researchers. Our project utilizes a novel platform technology for the rapid production of peptides at a low economic price.

Project:
This machine includes the use of a plasmid that incorporates a T7 promoter under control of lac elements to express a soluble thermostable protein carrier molecule. The carrier molecule facilitates sufficient production of the peptide and provides a simplified biosynthesis. The addition of peptide sequences to the vector is by a rapid and economic process termed Ligation-independent cloning (LIC).

Carrier Molecules

T7 Expression Vector
The pET vectors are arguably the best protein expression systems available; using a T7 promoter regulated by Lac operon elements. Our part contains a complete LacO/LacI binding site and CAP binding site as regulatory elements allowing for the controlled transcription of the chosen peptide and simplified cloning of the 220 bp fragment into any assembly standard.

Ligation Independent Cloning (LIC)
LIC is a new procedure that exploits the 3' 5' mononuclease activity of DNA polymerases to produce sequence-specific overhangs for target primer insertion. Two test peptides were chosen to explore the capacity of the peptide expression platform being Lisartan and angiotensin vasopressin.

Taq Mutations
Taq polymerase is a DNA-dependent DNA polymerase. Taq expression levels within E.coli will be limited by toxic effects the protein will have when binding to genomic DNA and disrupting cellular functions. Mutations that increase the thermostability and knock out catalytic activity of Taq were identified (H465V, S51S, K348R, R659I and K663L) to remove DNA binding and therefore toxicity (improving protein/peptide production). Mutations to enable LIC were added to the N and the C terminus, along with the prefix (P) and the suffix (S).

Conclusion
Thus far, the project is looking promising despite not all experiments being completed. The objective is to finish all aspects of the project upon arrival back to Australia.

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