2nd Canadian Peptide and Protein Community Virtual Meeting

Abstract: Directed Evolution using a Deaminase Mutator in T7 Bacterial Systems

Protein engineering is an important tool for modifying biomolecules and can be classified into two categories: rational design and directed evolution. Combined with rational design, we use Phage Assisted Non-Continuous Evolution (PANCE), in which the protein of interest (POI) is subjected to cycles of mutagenesis and selection to improve its structure and function. Due to lack of specificity and low mutation rates observed with other mutagenesis plasmids, we shifted to the eMutaT7 mutagenesis plasmid in our viral PANCE system to mutate genes. Since the cytidine deaminase in eMutaT7 is fused to T7 RNA Polymerase, the addition of the T7 promoter and terminator encourages accumulation of mutations in the POI only. We will present the application of our viral eMutaT7 Phage Assisted Evolution (eMPAE) system to evolve proteins that bind specifically to large DNA target ligands. In this way, we are optimizing our system for phage-assisted evolution toward evolving DNA-binding proteins by circumventing the limitations of other bacterial mutagenesis plasmids.