SUMMER RESEARCH 2024/25
PROJECT ABSTRACT

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

PROJECT # 71

SUPERVISOR/S: Dr Adele Williamson

PROJECT TITLE: Cut-&-Paste with an unnatural base: how to assemble Xeno Nucleic Acids for synthetic biology

FIELD: Molecular Biology

DIVISION/SCHOOL: HECS - Te Aka Mātuatua School of Science

PROJECT LOCATION: Hamilton

PROJECT ABSTRACT:

Xeno nucleic acids (XNAs) are synthetic analogues of naturally-occurring RNA and DNA, but have alterations in their nitrogenous bases, backbone sugar unit, or the phosphodiester linkage. XNAs with different nitrogenous bases are often referred to as Unnatural Base Pair (UBP) XNAs. UBPs can base pair only with themselves and not with the naturally-occurring base pairs A-T or G-C. This gives the possibility of expanding the genetic code and doing synthetic biology with these non-natural UBP bases; however, this relies on having a tool-kit of enzymes that will act on them.

In traditional molecular biology, we will often introduce new pieces of DNA into a genome or produce artificial pieces of DNA using 'restriction-ligation cloning', usually in combination with PCR. PCR, which uses a DNA polymerase enzyme, amplifies the gene of interest so we have many copies. Then we use a restriction endonuclease that recognises and cuts at particular sites in DNA, creating cohesive overhangs (also called 'sticky ends' informally) which can be re-joined. Finally, we use a DNA ligase to seal these sticky ends and the resulting DNA is introduced into a cell.

The problem with UBPs is that the enzymes do not always recognise and work with unnatural bases. To overcome this, we have tested a range of nucleases and ligases for their ability to cut and join UBP-XNA, and found two with suitable activities. These will be the topic of your research.

In this project, you will test these enzymes in combination to:

1. Use the nuclease to cut UBP-XNA pieces at a specific sites that create compatible sticky ends
2. Use the ligase to re-assemble these pieces into longer segments that contain XNA bases

This will be tested on a model substrate to optimise the reaction conditions and then used to assemble a large gene-length segment, suitable for producing XNA aptamers or XAN-tRNA by transcription.

STUDENT SKILLS:

- Have basic molecular biology lab skills such as those learned in 2nd and 3rd gear genetics/ microbiology/ biochemistry papers.
- Attention to detail in the lab and adherence to safety protocols
- An interest in doing molecular research!

PROJECT TASKS:

1. Optimise reaction conditions for cutting and re-joining XNA-DNA on a two-part model substrate. This will involve varying the concentrations of the two enzymes (the nuclease and ligase) in the reaction, as well as altering the length of time and temperature the reaction is run at. Analyse the results on a gel to determine which strategy gives the most product.
2. Based on the results from Task 1, optimise reaction conditions for cutting and re-joining XNA-DNA on a three-part model substrate which includes two fluorescent reporters. This might require further altering the variables (time, temperature) of the reaction. Use analysis on the gel to compare the yield of the two-part and three-part assemblies.
3. On the computer, design a strategy to synthesise a 'gene-length' assembly that includes a promoter, a gene encoding an RNA aptamer or tRNA which incorporates UBP-XNAs and a terminator. Describe how this could be assembled using your strategy and what you would expect the yield of this assembly to be, based on your previous experiments.

4. Test an additional 1-2 nucleases for their ability to cut UPB-XNA and compare these to the enzyme that you are using already. Consider factors such as: Do the type of UBP-XNAs in the sequence alter the efficiency? Do the number of consecutive XNAs alter the efficiency? What are the advantages of having multiple UBP-XNA cutting enzymes in a molecular toolkit?
5. Create a poster for the summer scholarship function
6. Write a summary of your results in the form of a short report (10-20 pages) and present your results at our group meeting in a short talk (~20 minutes)

EXPECTED OUTCOMES:

- Student's Research Poster (as per clause 6 of the Scholarship regulations)
- Determined optimal reaction conditions (i.e. time, concentration and temperature) for assembling UBP-XNAs
- Designed sequences for 'gene-length' assembly that can be ordered and tested in the future
- Communicated key results to the research group n the form of a written report and oral presentation
- Had a wonderful summer doing fun science with a highly motivated research team of academics and students!!!