

BCHM 421/422 – 2019/2020

Project Outline: Used as medicines, in agriculture and for the production and preservation of food; natural products are extremely important and valuable chemical commodities that are made by living organisms including bacteria. This project will focus on using genetic approaches for discovering new natural products made by marine bacteria. Techniques called Transformation-Associated Recombination (TAR) and Gibson Assembly will be used to facilitate the excision of a biosynthetic gene cluster of interest from Marine Bacteria. This technique takes advantage of homologous recombination in yeast between a designed pathway specific capture vector and fragmented Marine Bacterial genomic DNA. Upon capture the pathway will be introduced into a host bacterium (*Escherichia coli*) and its peptide products investigated by heterologous expression. In this project the student will use PCR reactions and gibbon cloning alongside cell culture, plasmid isolation and gel electrophoresis experiments to obtain the capture vector. Following the TAR experiments they will also have the opportunity to isolate and elucidate the structure of a naturally occurring peptide molecule.

Supervisor: Avena Ross

Project Title: Genome Guided Discovery of Marine Natural Products

Project Goals: To discover new natural product molecules made by marine bacteria using molecular cloning and heterologous expression approaches

Experimental Approaches:

- Bacterial genome analysis using webtool Antismash
- Cloning of biosynthetic gene clusters from marine bacteria using Transformation Associated Recombination/ Gibson Assembly
- Heterologous Expression of biosynthetic gene cluster in *E. coli*
- Purification and chemical characterization of new molecules produced

References:

- 1) Timmermans, M. L., Paudel, Y. P., Ross, A. C., Investigating the biosynthesis of natural products from marine proteobacteria, a survey of molecules and strategies, *Mar. Drugs*, **2017**, *15*, 235-272
- 2) Ross, A. C., Gulland, L., Dorrestein, P. C., Moore, B. S., Targeted Capture and Heterologous Expression of the *Pseudoalteromonas* Bromoalterochromide Gene Cluster in *Escherichia coli* Represents a Promising Natural Product Exploratory Platform, *ACS Syn. Biol.*, **2015**, *4*, 414-420.