ACCESSING BACTERIAL DARK MATTER FOR IMPROVED ENZYME DISCOVERY AND ENGINEERING

David F. Ackerley, Victoria University of Wellington
david.ackerley@vuw.ac.nz
Michelle H. Rich, Victoria University of Wellington
Hannah R. Lee-Harwood, Victoria University of Wellington
Sarah R. Messenger, Victoria University of Wellington
Jenni J.B. Francis, Victoria University of Wellington
Thomas W. Skurr, Victoria University of Wellington
Edward M. McGuinniety, Victoria University of Wellington
Alexandra J. Linton-de Boer, Victoria University of Wellington
Jeff S. Mumm, Johns Hopkins University
Abigail V. Sharrock, Victoria University of Wellington
Alistair S. Brown, Victoria University of Wellington
Mark J. Calcott, Victoria University of Wellington

Key Words: New technologies for enzyme engineering, Enzyme discovery, Antibiotic resistance, Nitroreductases, Non-ribosomal peptide synthetases.

Functional screening of environmental DNA libraries is a powerful strategy to recover enzymatic 'unknown unknowns' from the dark web of bacteria that occupy complex environments such as soil. However, it can also be heavily biased toward the tiny subset of genes that are preferentially transcribed and translated by the screening strain. To overcome this bias, we have developed generally applicable methods to precision clone captured genes downstream of a strong host promoter and ribosome-binding sequence. Our approach greatly improves screening for weak phenotypes and also permits direct recovery of the corresponding enzymes, providing rapid access to a broad diversity of previously unexplored biocatalysts. We have also shown that environmental DNA can be a rich source of enzymatic domains or sub-domains to boost the throughput of non-rational engineering strategies.