METAGENOMIC DISCOVERY AND DIRECTED EVOLUTION OF GENES THAT DEFEND AGAINST CHEMOTHERAPEUTICS

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Numerous cell and gene therapies could potentially benefit from labelling with defensive genes that provide resistance to chemotherapy selections. However, most forms of chemotherapy resistance that arise spontaneously in the clinic either impact other aspects of cellular function or are not readily transferrable to other cell types. To provide portable chemotherapy resistance genes we are seeking to identify genes that encode enzymes able to detoxify a range of chemotherapeutics. For this, we have constructed an environmental DNA library in *Escherichia coli,* using a bespoke method that places captured genes immediately downstream of a strong *E. coli* promoter, ribosome binding domain, and hexahistidine tag. We have shown that this approach provides access to promiscuous activities that manifest as weak and otherwise undetectable phenotypes. Proof-of-principle was demonstrated using the well-characterized chemotherapeutic methotrexate. In addition to the expected recovery of dihydrofolate reductases, ubiquitous genes already known to defend against methotrexate, we also discovered oxidoreductases, a phosophoribosylanthranilate isomerase, and numerous genes of unknown function. In ongoing work, we are moving into more clinically relevant chemotherapy agents that are also toxic to *E. coli,* validating the defensive activities of promising 'hit' genes in human cell and using directed evolution to enhance resistance further.