E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester 1824 The University of Manchester · Biotechnology and Biological Sciences Research Council

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Northumbria University NEWCASTLE · Imperial College London · PROZOMIX

Conserving the position of rare codons for the optimised production of commercially-important, cofactor-containing enzymes

***"This project demonstrated a very significant increase in the number of expressed soluble enzymes. This increase would lead to clear commercial benefits if Prozomix employed such advanced expression optimisation technology,"* Prozomix.**

PROJECT AIMS: Iron cofactors, such as such cytochrome P450 reductases (CYPs) are used in industrial biotechnology to perform difficult oxidative reactions. Yet producing CYPs in new host organisms is challenging. This project investigated the use of coding sequences containing rare codons to improve protein synthesis. The presence of rare codons likely slows translation, leading to correct protein folding that may result in higher enzyme yields.

The project aimed to: 1. analyse iron-containing enzyme families for conserved clusters of rare codons | 2. construct software to rank the abundance of each codon in a coding sequence in the original host organism | 3. test if the coding sequences generated with this tool improved enzyme yields and activities in Escherichia coli.

OUTCOMES & NEXT STEPS:

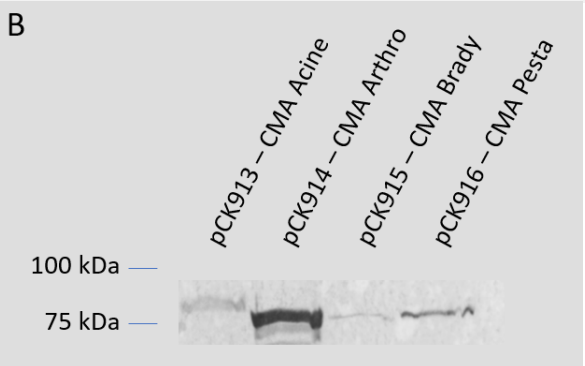
- **An M.Res. student is conducting biochemical analysis of CYPs and ferredoxins, and is working on tool for conserved rare codons**
- **Prozomix have asked the PI to generate a new sequence for another iron-containing enzyme of great interest to their customers**
- **A new CYP is now available to Prozomix customers**
- **A manuscript describing the bioinformatics tools and rare-codon cluster conservation is in preparation**
- **Software tools will be published on GitHub and a web interface built**
- **The generated sequences are forming the basis of a larger BBSRC application**

RESULTS: <u>Bioinformatics tools and analysis</u>: Software tools to rank of each codon in a coding sequence were generated, and a sequence matching each ranked codon was produced. One tool facilitated the expression of any prokaryotic gene in any other prokaryote; and another allowed for expression of any prokaryotic or eukaryotic gene in human cells. A tool to analysis protein families for conserved rare codons is ongoing.

<u>Experimental validation of novel sequences</u>: Four class VII CYPs and five ferredoxins from a wide variety of bacteria were tested in E. coli; these proteins had not been previously produced in E. coli. Sequences encoding these proteins were generated using a commercially available codon optimisation tool, then sequences encoding the same proteins were produced using the newly developed software. After E.coli culture, harvesting and lysis, all four tested CYPs were detected in the soluble fractions of the cell lysate. All five of the tested ferredoxins were produced and purified, and had excellent iron cofactor incorporation. Biochemical characterisation of these proteins is ongoing.

KEY MESSAGE: Rare codons can be used to optimise CYP enzyme expression in E. coli.

Change in technology readiness level: 2 to 3/4



Western blot analysis shows that all four target CYPs can be produced and are soluble in Escherichia coli using the 'ported' coding sequence.