Next Generation Assay Optimisation for Drug Screening

Using design of experiments and automation for antibiotic adjuvant discovery

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Antimicrobial resistance (AMR) represents a major clinical healthcare challenge, causing 1.2 million deaths worldwide in 2019[1]. The rapid evolution of resistance to drugs by bacteria creates significant scientific and economic hurdles to developing new antibiotics. To overcome these hurdles, a novel strategy is to target the biochemical mechanisms that drive the evolution of resistance, such as the bacterial 'SOS response'. The conserved DNA repair enzymes RecBCD/AddAB initiate the SOS response in response to DNA-damaging antibiotics, and there are ongoing attempts to develop an appropriate inhibitor[2]. However, due to the limited financial market for new antibiotics, the search for novel antimicrobial agents is mostly conducted in academia, where resources are limited, and time efficiencies are valuable. More rapid, efficient, and cost-effective transferable methods to optimise biochemical assays could therefore have wide impact in accelerating academic drug discovery.

Currently there exists no high-throughput assay of RecBCD nuclease activity suitable for drug screening. We have developed such an assay and rapidly optimised it using space fill-based Design of Experiments (DoE) using automation and rapidly achieved assay conditions with a Z' of 0.7 in 3 rounds of experiments.

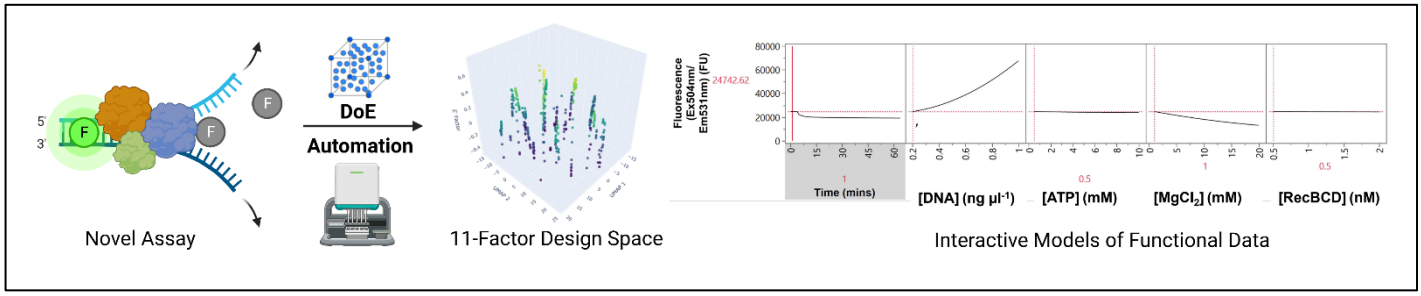

Fig 1. The RecBCD assay uses a dsDNA specific fluorescence dye to measure the activity of RecBCD. The combination of DoE and automation enables the interrogation of a complex design spaces. Functional DoE creates interactive models of the effect of variables on the shape of a curved response.

DoE provides a mathematical framework to effectively investigate the effect of multiple variables and their interactions on a particular experimental outcome by systematically varying these factors in an uncorrelated manner[3]. We introduce Functional DoE – a tool to model how changing different factors affects the shape of a functional curved response and use this to identify conditions giving more linear rates of reaction suitable for drug-screening. An industry-academia collaboration enabled the use of automation for rapid high content experimentation. A single DoE simultaneously evaluated 11 factors predicted to influence assay Z', mapping a complex multidimensional landscape. From this data, conditions yielding Z' scores above 0.7 were identified. Cost analysis was incorporated into optimisation to balance maximising Z' and minimising reagent expenses.

The optimised RecBCD assay enables identification of direct SOS pathway inhibitors and interrogate inhibitor Structure-Activity Relationships (SAR). Furthermore, the methods developed here have potential to accelerate antibiotic discovery and promote efficient biochemical assay development.

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