A Design of Experiments Toolbox for Assay Development and Optimization

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Synthace

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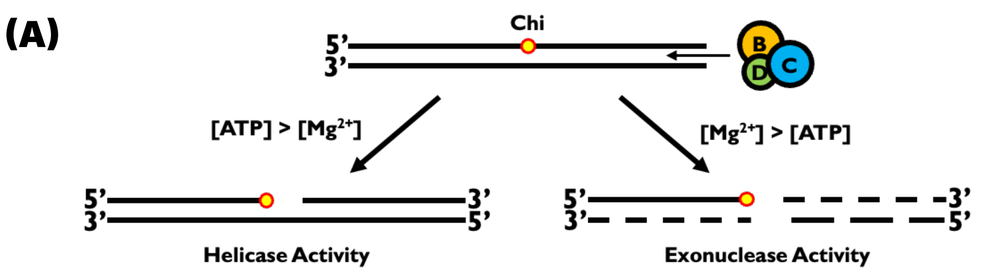
Adam's assay development challenge

Adam is a Master's student in the Department of Pharmacology at the University of Oxford investigating antimicrobial resistance. His research focuses on developing and optimising a novel biochemical assay for RecBCD, a DNA damage–repair enzyme central to the evolution of antibiotic resistance.

Starting with theoretical knowledge of the mechanism of action for RecBCD and a limited timeframe, he sought to acquire the experimental tools necessary to advance his project.



The RecBCD assay principle.

(A) RecBCD activity is modulated by the ATP:Mg^{2+} ratio.

(B) QuantiFluor dsDNA dye (F) fluoresces when bound to dsDNA; RecBCD (orange, blue and green complex) exonuclease processing of dsDNA is detected as a decrease in fluorescence.

Adam brings together his research toolbox

Experiment methodology

Adam recognised that optimising this complex assay required him to evaluate interactions among ten variables.

Design of Experiments (DOE)

Learn more

- Systematic and simultaneous evaluation of variables (multivariate), enabling quantitative identification of interactions
- Efficient use of resources
- Improved reproducibility and robustness of experimental outcomes

Experiment planning

A multivariate design, in which all variable setpoints change for each observation, adds complexity to experiment planning.

Synthace

Learn more

- Integration of DOE designs and experimental methods
- In silico experiment simulation with automated stock calculation and labware planning
- Instruction generation for automated liquid handling systems
- Automatic structuring of experiment design, metadata, and results

Experiment execution

Using the generated experiment instructions; Adam required an automated liquid handler to perform accurate, reproducible, and rapid low-volume transfers.

sptlabtech dragonfly® discovery

Learn more

- 10 independent non-contact dispensing channels for ultimate flexibility
- Accurate and repeatable low volume dispensing
- Supports all liquid viscosities
- Rapid programming and high speed plate setup

Data analysis

Instead of curve descriptors, Adam wanted to model full reaction time courses to link experimental variables to changes in curve shape.

JMP STATISTICAL DISCOVERY Functional Data Explorer

Learn more

- Models entire data structure, keeping the context and nuance that summarization loses
- Includes smoothing and functional principal component analysis (FPCA) making key features stand out
- Model assay reaction curves as a function of experimental variables

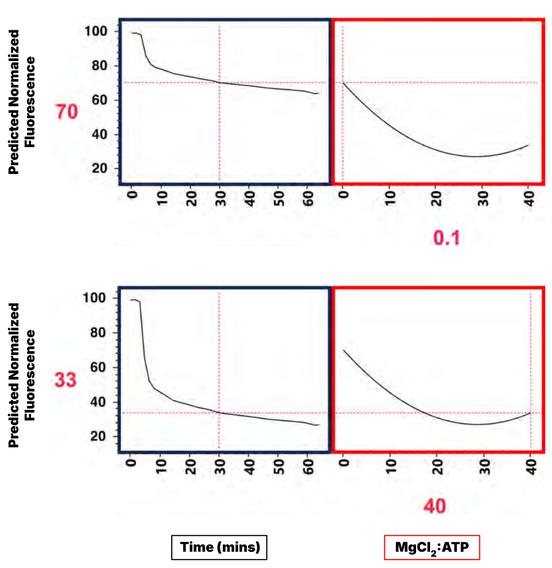
Scan to read the research paper ➡

Results of Adam's research using the toolbox

⬅ Scan to watch Adam discuss his experience

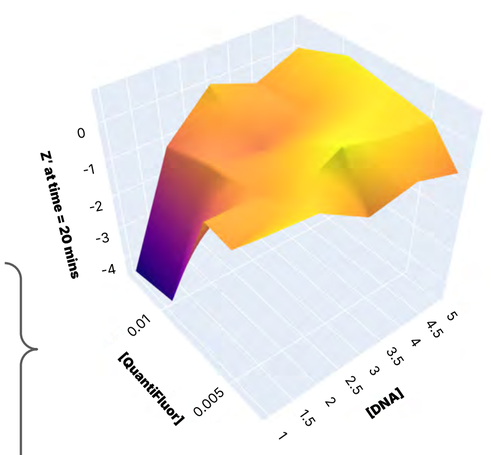
✔ **10 variables** investigated ✔ **3 weeks** (2 experiment iterations and assay validation) ✔ **0.59** Z′ value of assay

Experiment Iteration 1



Consistent with literature, a 4-factor DOE identified optimal $MgCl_2$:ATP ratios that enhance nuclease activity.

Experiment Iteration 2

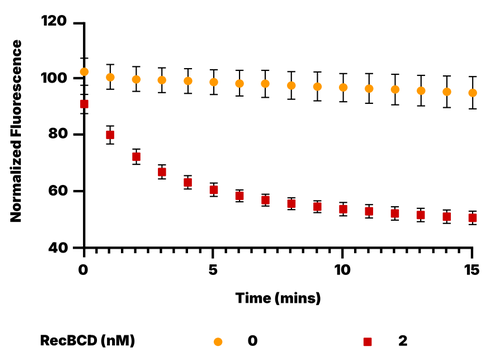


A 10-factor space-filling DOE highlighted key and non-impactful factors using Z′.

A significant three-way interaction between pH, [BSA], and [RecBCD] was elucidated.

Assay Validation

To check the robustness of the assay, the best assay conditions were repeated 128 times, yielding a Z′ of 0.59 (measured after 10 mins).



DOE, Synthace, the dragonfly® discovery, and JMP enabled rapid development of a robust RecBCD assay, offering a versatile toolbox for novel assay optimisation.

Stop by Booth 2628 for Synthace and Booth 616 for dragonfly® discovery insights!