Improving Assay Optimisation and Pharmacological Sensitivity of a Nuclease by Employing a Computer-aided Biology Approach

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Poster
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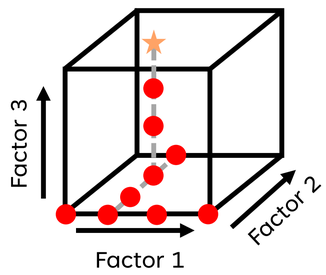
OVERVIEW

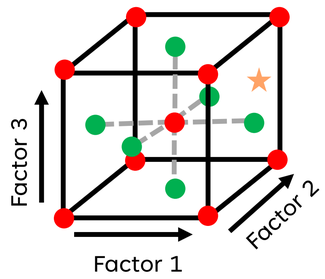
Successful implementation of computer-aided biology (CAB) to buffer optimisation. Resulting in increased assay signal, reduced reagent requirement, reduced assay development time.

INTRODUCTION

Traditional assay development methods employ labour-intensive and time-consuming iterative processes, which are limited by a lab scientist's experimental throughput. Design of experiment (DoE) is a statistical-based approach which designs multi-factorial experiments in smart combinations and uses the results of these experiments to statistically predict optimal conditions. CAB combines DoE with automation, enabling the execution of these complex DoE approaches. Here we demonstrate the application of CAB to optimising the buffer of a nuclease assay.



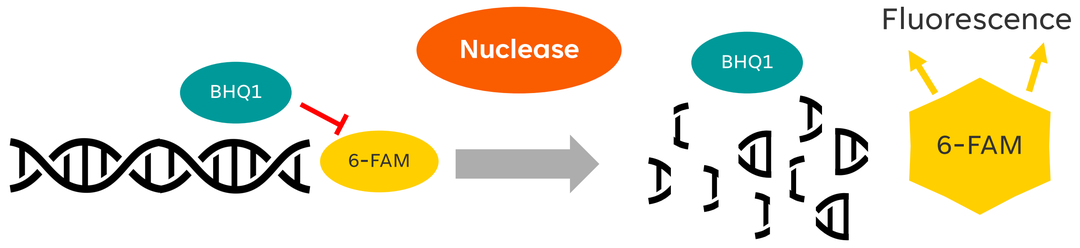
One-factor at a time (OFAT) buffer optimisation. Separate iterative experiments are used to determine optimal conditions.



DoE buffer optimisation. Statistical models are used to measure the interactions between factors to predict optimal conditions.

ASSAY METHODS

- DoE-designed buffers were dispensed directly into a 384-well assay plate from concentrated stock solutions using SPT Labtech's dragonfly® discovery low-volume liquid dispenser
- Nuclease and small molecule inhibitor were added and incubated for 30 minutes at room temperature
- Reaction was initiated via addition of fluorescently-labelled short-length DNA substrate
- Rate of fluorescent increase was measured on a PHERAstar® FSX at Ex 485 nm Em 520 nm over 40 minutes



Fluorescent nuclease assay schematic, using a 21-mer DNA substrate labelled with Black Hole Quencher 1 (BHQ1) and 6-carboxyfluorescein (6-FAM). Digestion of the DNA substrate by the nuclease target results in the release of 6-FAM from the BHQ1 and an increase in fluorescence.

Data Analysis

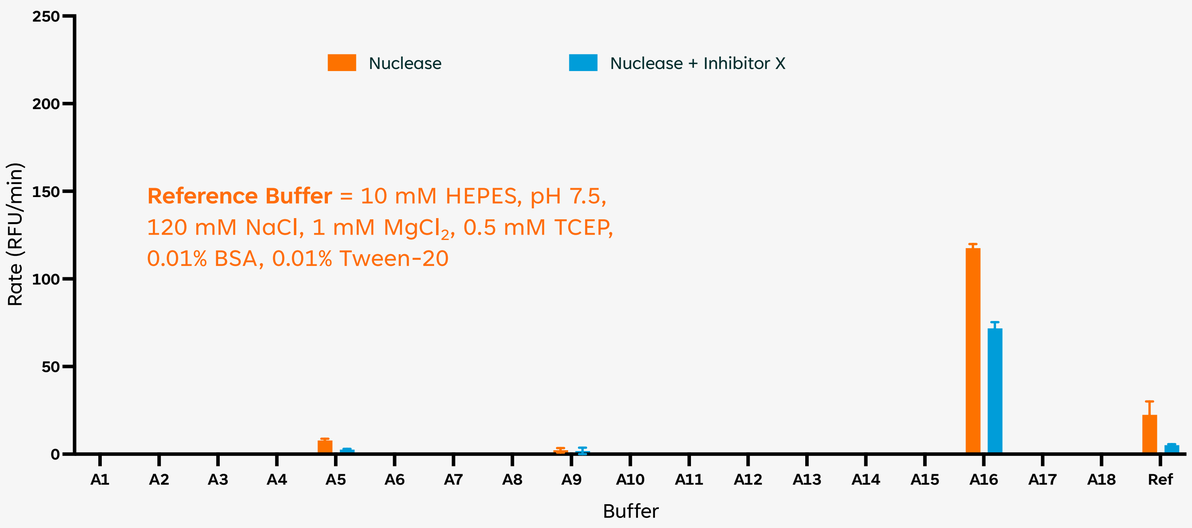
GraphPad Prism version 9.4.1 was used to fit all dose-response curves to a 4-parameter fit function.

Design of Experiments

SAS JMP® 16 software was used for all DoE studies.

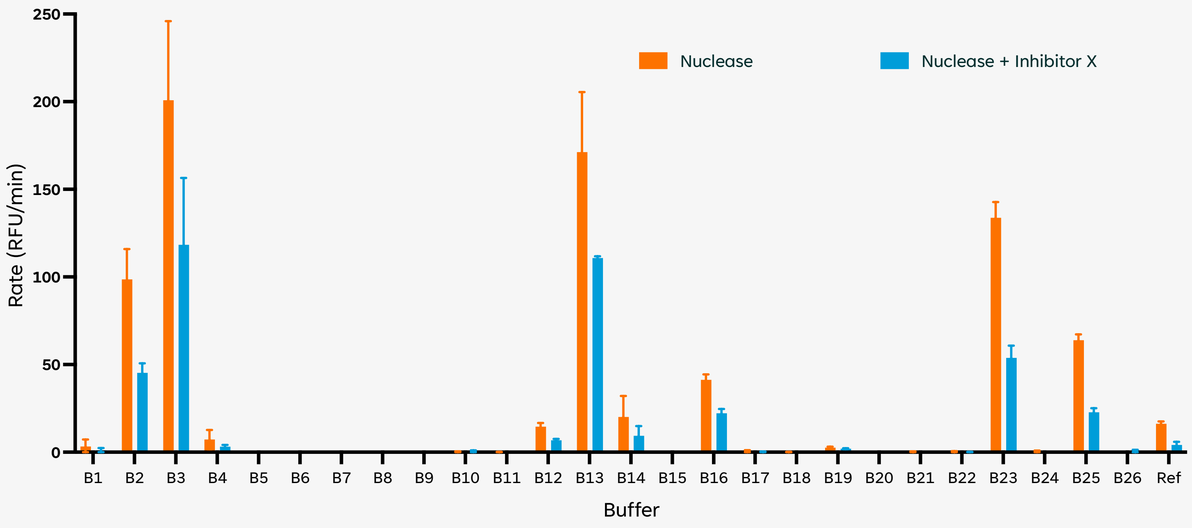
RESULTS

Figure 1: Round 1 DoE general buffer composition optimisation enhanced the assay signal by 5-fold



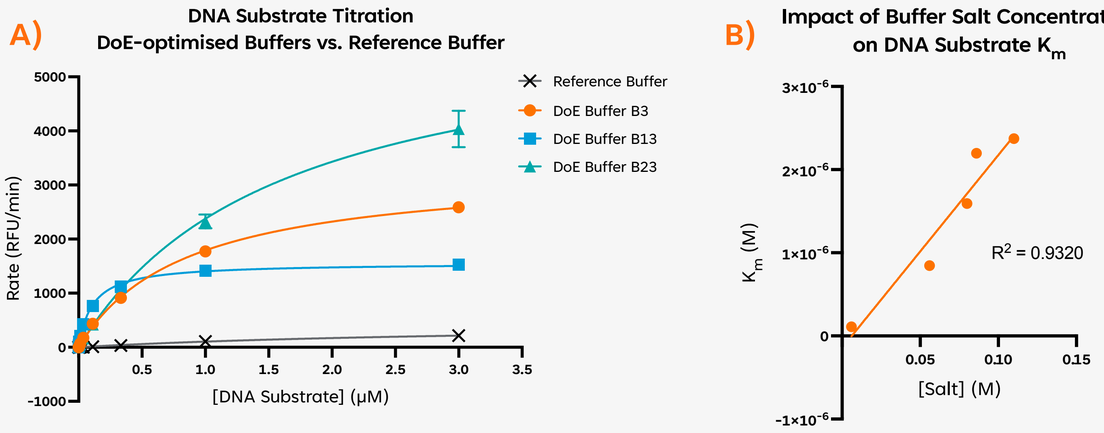
18 different buffer compositions with various concentrations of KCitrate, KOAc, KCl, $Mg(OAc)_2$, $MgCl_2$, DTT, Triton X-100, glycerol and buffering agents (Tris-HCl, HEPES) were evaluated. A standard buffer imported from an external source was used as reference. A potent and specific inhibitor, X, was used as additional experimental validation. Buffer A16, which contained no KCitrate, KOAc or KCl, enhanced the assay signal by 5-fold in comparison to the reference buffer.

Figure 2: Round 2 DoE buffer optimisation of salt concentration enhanced the assay signal by 15-fold



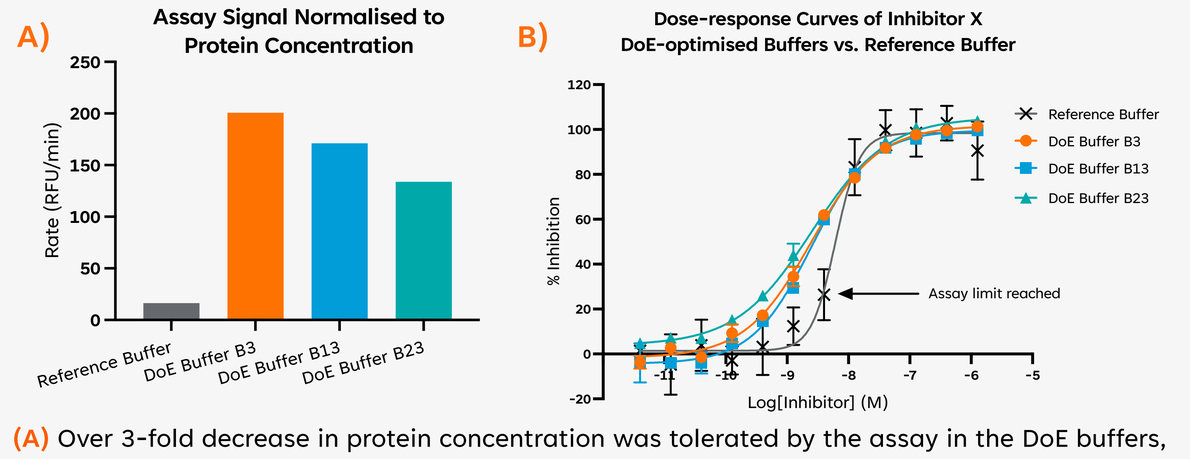
Buffer conditions were optimised to provide the highest nuclease activity based on the results from Round 1 (**Figure 1**), testing 26 different buffer compositions with various concentrations of salt components (KOAc, KCl, $Mg(OAc)_2$, $MgCl_2$) and fixed concentrations of Tris-HCl, DTT and Triton X-100. Buffers B3, B13 and B23 contained little to no K^+ salt and low Mg^{2+} salt conditions and enhanced the assay signal by between 9-15-fold in comparison to the reference buffer.

Figure 3: Low salt buffer conditions are optimal for DNA substrate affinity



(A) DoE-optimised buffers resulted in over 5-fold increase in maximal velocity (V_{max}) in comparison to the reference buffer. **(B)** K_m decrease is observed as the total buffer salt concentration decreases.

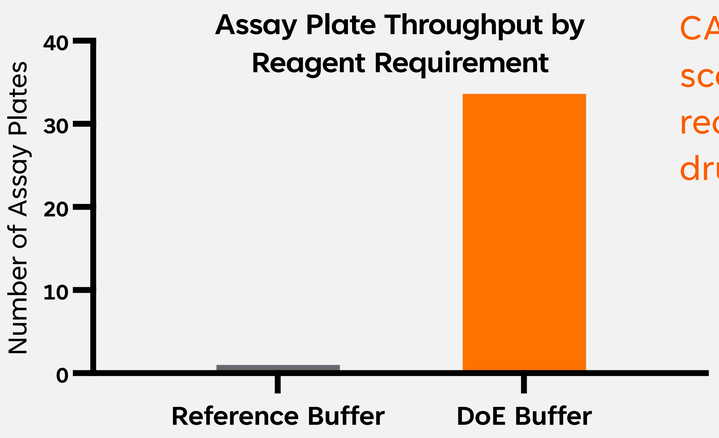
Figure 4: Optimised DoE buffers increased the pharmacological sensitivity of the nuclease assay towards potent inhibitors



(A) Over 3-fold decrease in protein concentration was tolerated by the assay in the DoE buffers, due to improved, higher signal. **(B)** Increased lower assay limit was achieved with the DoE buffers due to the reduced protein concentration. An increased hill slope of 2.5-fold in the reference buffer indicates that the potent inhibitor X is nearing the assay limit under these conditions.

CONCLUSIONS

- A CAB approach (combining design of experiments and automation) to buffer optimisation increased the signal of a fluorescent nuclease assay by 15-fold, alongside over a 3-fold decrease in protein requirement
- Unexpectedly low salt conditions were observed to be optimal for this nuclease target, which would otherwise not have been predicted from literature



CAB has the potential to radically improve the scope and efficiency of assay development; reducing the cost, labour and timelines for drug discovery programmes.

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