Screening Multiple PPI Targets in Parallel: Accelerating Portfolio-level Decisions

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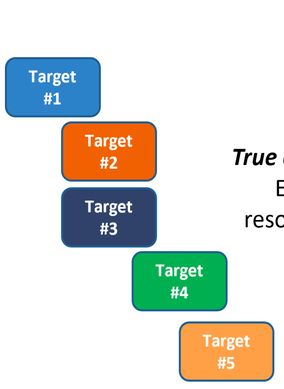
***Overview**: HTS projects for multiple targets were developed in collaboration with our client and performed in parallel in a cross-site collaboration. Up to 370,000 compounds from a variety of chemical libraries were screened in a 1536-well plate format using TR-FRET technology. During screening plate effects were observed and investigated, the cause was attributed to shipment of assay ready plates on dry-ice.*

1 INTRODUCTION

In a fast-paced evolving world, high quality data and trustworthy partnerships are the key components to success. At Charles River, we quickly developed multiple high throughput screening projects in collaboration with our client to detect small molecule inhibitors of protein-protein interaction (PPI) targets. The assays were performed in parallel in a unique cross-site collaboration between HTS groups in the UK and the Netherlands.

Assays were developed successively in 1536-well format using TR-FRET technology. With each successive target timelines were reduced by around 50% from assay-development to the final data packages, this was achieved by refining the assay development process and taking learnings from developed methods.

Compounds from both Charles River and Client libraries were screened simultaneously in a collaborative partnership. HTS scientists and medicinal chemists from both parties worked together to analyse the data and generate hit lists for progression through the HTS workflow and clients screening cascade.



True collaborative Partnership
Enabling the best use of resource planning to drive the wider portfolio

Library	Properties
Lead-like Library	• Collection of 153,000 sourced compounds with diverse chemical profiles • Structural alert-free and lead-like properties • > 95% of this library has calculated properties that favor blood-brain barrier penetration
SoftFocus Protein-Protein Interaction Library	• ≈ 3200 compounds designed towards major inhibiting protein-protein interaction
Client collection	• ≈ 210.000 compounds selected by the client
Prestwick Chemical library	• 1,264 compounds • Approved drugs with high chemical and pharmacological diversity • Assay validation set
SelleckChem FDA-approved Drug Library	• 966 compounds • Structurally diverse, medicinally active, and cell permeable • Assay validation set

2 METHODS

Assays for all targets were developed using the TR-FRET technology and the processes aligned for maximum efficiency ensuring robust assays were developed for HTS purposes. Optimisation experiments were performed for all detection reagents including testing a variety of detection pairings as well as Kd determinations for binding of the protein partners. Assays were validated using tool compounds when available and the tolerance to EDTA and DMSO were assessed. To assess the stability of each assay, plate uniformity studies were conducted followed by a pilot screen of 10,000 compounds tested in duplicate in the TR-FRET format before proceeding to the HTS. All assays produced a robust assay, acceptable plate statistics with Z' >0.7 and consistent signal to background following the workflow below.

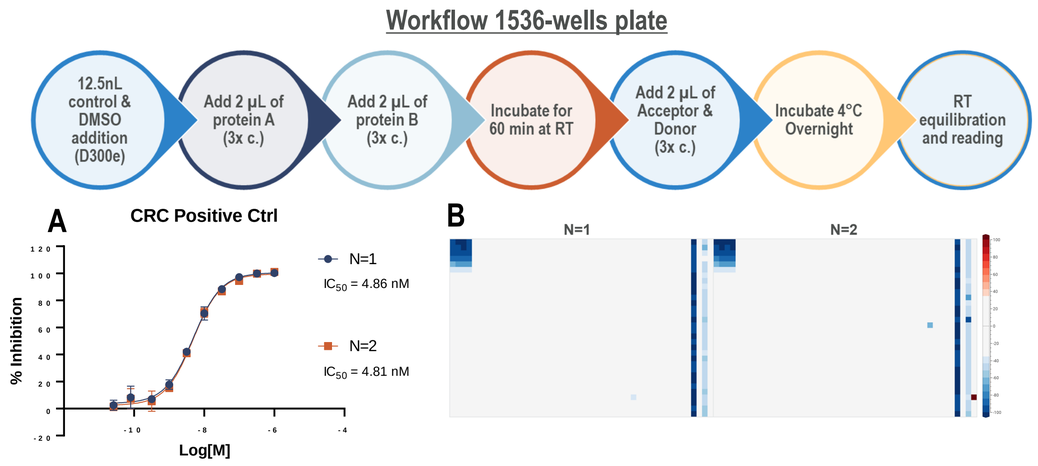


***Figure 1**: (A) Concentration response curves (CRC) for a tool compound added to each control plate in duplicate showing consistent calculated IC_{50} values (B) Heat maps of the 2 uniformity plates replicates showing low false positive rate.*

3 RESULTS

Once final conditions were identified and following successful pilot screens for each target, the TR-FRET assays were used to screen approximately 370,000 compounds comprising of the entire Discovery UK Lead-Like Library of approximately 155,000 compounds and a Client library screened using assay-ready plates containing 12.5nL of compounds and final assay compound concentration of 20µM.

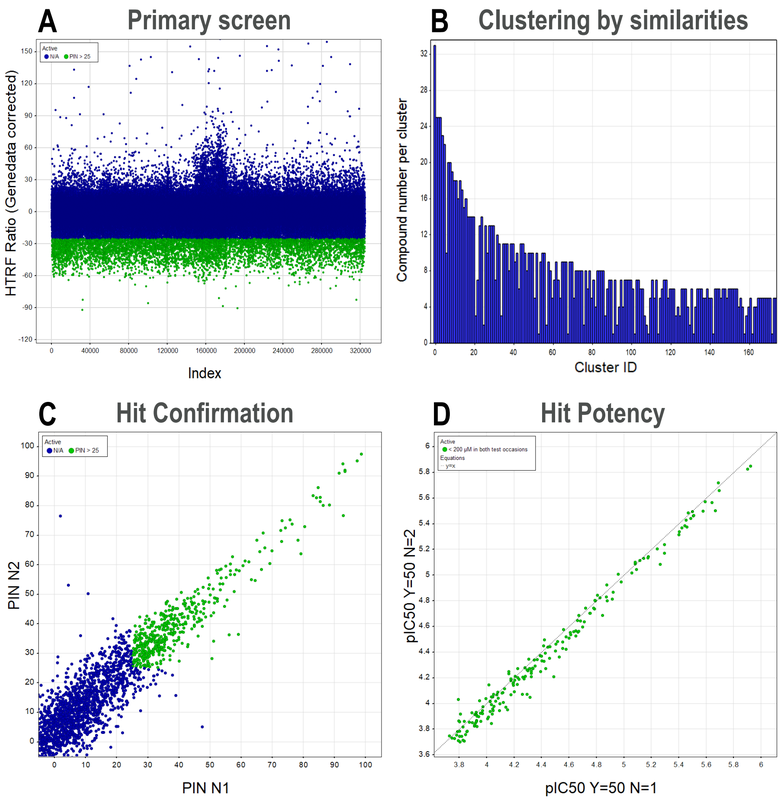
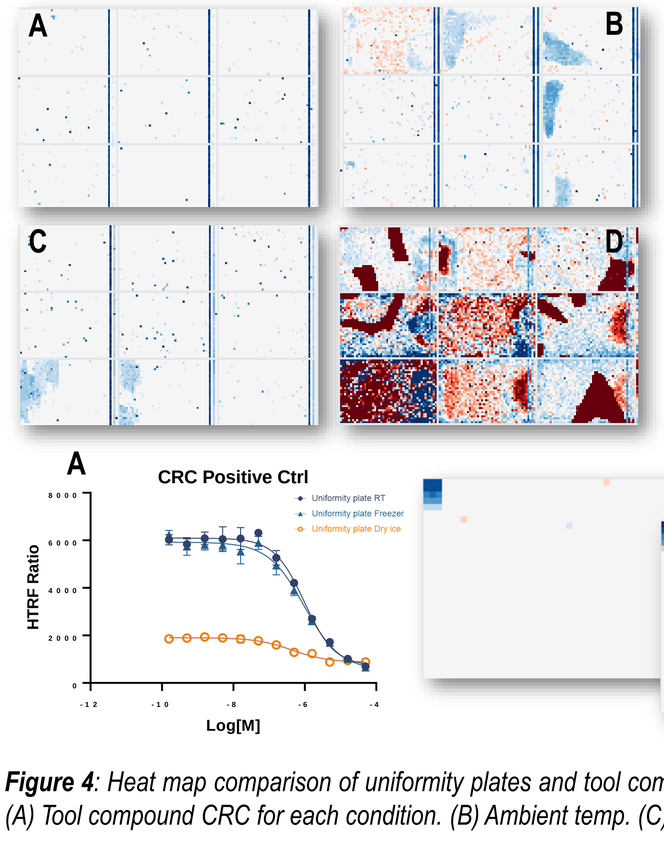


***Figure 2**: (A) 370,000 compounds were tested at single concentration (20µM) for each target. (B) After filtering out compounds interfering with the read-out, frequent hitters or high Lilly demerit score* a cluster analysis was performed showing a Percentage inhibition of ≥ 25 using Tanimoto similarity of 0,6. (C) compounds selected for hit confirmation were subjected to re-test in duplicate showing a good correlation. (D) pIC50 correlation of potency determination of confirmed hits.*

During the collaboration and screening activities an unusual plate effect which affected assay quality of all the targets was observed on assay-ready plates. An investigation to the cause of this was subsequently attributed to the dry ice used in the shipment with the particular plate type utilised in the assays.



The investigation to determine whether the plate effects were due to the shipping conditions was conducted as follows. Assay-ready plates were subjected to either exposure to ambient temperature, -80 ° C or dry ice. The results below clearly demonstrate that exposure to dry ice is responsible for the plate effects observed.

***Figure 3**: (A) A typical heat map observed during plate uniformity studies and pilot screens, showing no plate effects. (B), (C) and (D) show varying plate effects observed affecting plate statistics and assay quality to varying degrees.*

***Figure 4**: Heat map comparison of uniformity plates and tool compound results taken from the same batch of plates. (A) Tool compound CRC for each condition. (B) Ambient temp. (C) -80 ° C freezer exposure (D) dry ice exposure.*

4 CONCLUSION

Through the collaboration with our client, we have successfully developed high-throughput TR-FRET assays for a range of targets. The example given here highlights the HTS capabilities at Charles River across multiple sites not only utilising Charles River's Discovery UK compound libraries but also our clients or 3rd party compound collections to aid our partners hit finding campaigns and increasing chances of success. Our highly experienced HTS scientists and medicinal chemists work in close collaboration with our client's counterparts to deliver high quality assays and hits to progress the client's drug discovery process. We also highlight the importance of the shipping conditions used when transferring assay ready plates between facilities, we demonstrated that dry ice shipments can have a detrimental effect on the assay quality. Following the hit identification by the medicinal chemists, the client is now progressing compounds through the screening cascade and is currently confirming activity and mechanism of action through SPR studies at Charles River and in-house. Collaborating with a knowledgeable partner like Charles River can help our partners develop a good hit-finding approach, avoid pitfalls and ensure that the hits they identify are of high quality. Charles River's drug discovery experts support the hit identification process and the subsequent steps in the drug discovery workflow.

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