Challenging Cellular HTS to Identify Small Molecule Upregulators of a Critical Biological Process with the Target Driven Biosensor Readout

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ABSTRACT
High Throughput Screening (HTS) using inventively designed cellular models can provide a high volume of data on the activity of a large number chemical compounds, their potential mechanisms of action and can differentiate biological activity from unwanted toxicity. The continued advancement of technology in this field allows the development of sensitive, reliable and robust phenotypic cellular assays to triage active compounds from large screening libraries. We outline a case study for a challenging cellular HTS campaign that was successfully performed and resulted in the identification of diverse chemical matter to support the early drug discovery process. We present the approach for transforming an existing complex cellular assay to allow successful execution of a ~500,000 compound screen. We also present the strategies we used to overcome the scientific and technical challenges faced during the HTS including data handling and the hit calling approach.

WORkFLOW OPTIMISATION FOR HTS

The cellular assay was used to screen approximately 500,000 compounds (384 well plates) with a consistently acceptable performance based on robust Z’-factor >0.5. Multiple factors affected the robustness and consistency of the assay and the workflow required significant optimization. These factors included:
- Initial expression levels of the target protein which was driven by concentration and time of doxycycline exposure
- Random variation in protein expression between plates
- Cell growth rates during above two phases
- Compound expression levels depending on the induction threshold and loss of expression after multiple cell passages.

ASSAY SENSITIVITY AND TECHNICAL CHALLENGES DURING SCALE UP FOR HTS

Additional technical challenges were encountered and overcome during the scale up and batch testing ahead of HTS, including:
- Relative instability of the expression system
- Reduced cell health due to reduced incubator airflow and critical temperatures
- Post potency determination, ~250 potent compounds identified with structural diversity and minimal toxicity

RESULTS AND DISCUSSION CONTINUED...

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The distribution of active compounds was found to be uniform across all the HTS runs based on the hit rate. Data was analyzed with Genedatas software and a hit calling approach based on the use of robust Z-score (RZ-score) was used to take account of the variability seen between HTS runs. The normalized percent control data relative to DMSO and positive control wells on each plate was used as a criterion to understand biological activity of compounds in the assay. Genedatas correction function was applied to the normalized data to mitigate any positional effects on the distribution of hits.

SUMMARY AND CONCLUSIONS
- A new throughput assay protocol developed by our Partner was successfully converted into a format amenable to HTS
- Overcame significant scientific and technical challenges to successfully screen 0.5M compounds with robust assay performance
- Post potency determination – 250 potent compounds identified with structural diversity and minimal toxicity
- These results highlight Charles River’s capabilities to execute challenging cellular HTS campaigns.

MATERIALS AND INSTRUMENTS

Human embryonic kidney cells (HEK-293) were supplied to Charles River by our Partner, expanded and frozen stocks prepared for HTS purposes. HEK-293 cells were CRISP/HiP knock out for the wild type copy of the target protein and replated with a Doxycycline inducible mouse uromit to avoid cell lethality. Cells were further modified to express the Nano-BiT® based biosensor (Promega) for live cell monitoring of the biological process under investigation. The assay was multiplexed with CellTiter-Glo® (CTG) to measure cell viability. The assays were performed in 384-well white, cell culture treated plates (Perkin Elmer) and luminescence was measured on Envision plate reader. For HTS purposes, cells were added directly to acoustically treated 384-well white plates (Perkin Elmer) and luminescence was measured on Envision plate reader. For HTS purposes, cells were added directly to acoustically treated 384-well white plates (Perkin Elmer) and luminescence was measured on Envision plate reader.