high-throughput phenotypic screening workflow for cytotoxicity determination

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introduction

Running targeted small molecule library screens for phenotypic assays has a number of hurdles to overcome to be successful.

- firstly, selecting the compounds for a specific targeted library to be screened can be time consuming to pick and assemble
- secondly, transferring a protocol from assay development into HTS can be troublesome, mainly caused by using different liquid handlers and/or, plate densities in the two processes
- finally, running phenotypic assays in high number compound screens can cause difficulties when using traditional imaging techniques due to; imaging time, data analysis and also storage of large quantities of data

Here we present a workflow using comPOUND® to rapidly cherry pick individual compounds, dragonfly® discovery for rapid reagent dispensing into 1,536 well plates and acumen® Cellista for reporting medium content data from multiplexed phenotypic screening assays.

We highlight the benefits using cell viability and proliferation assays as a case study. Cell health is a fundamental tool in the drug discovery process used to evaluate both the potency of compounds, as well as their toxicity profiles for drug safety assessments.

1. Phenotypic HTS workflow

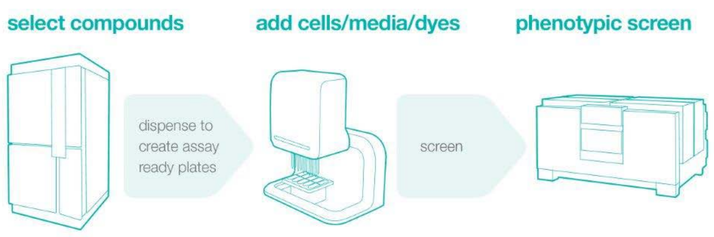

Fig 1. HTS workflow using comPOUND, dragonfly discovery and acumen Cellista

2. modular sample tube storage

TTP Labtech's comPOUND modular sample store comprises a high-density storage unit and an additional suite of specialised delivery and processing modules to enable easy integration into any compound management or screening system.

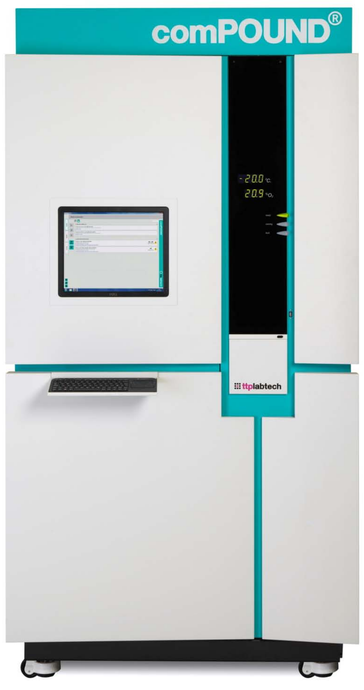

Fig 2. comPOUND automated high speed modular sample storage down to -20°C

With comPOUND:

- select any library subset quickly to meet the needs of your screening department
- reduce potential damage to library stocks by only cherry-picking the samples you need
- ensure sample integrity with temperature controlled, dry, inert, hermetically sealed environment
- ensure throughput grows with your library by processing samples in parallel
- easily expand sample storage capability with additional modules to respond to growing library size
- can be located remotely from screening laboratories

Finally, comPILER, represents a high throughput processing system creating assay plates directly from stored compound libraries. comPILER can cherry-pick microtubes from up to 12 comPOUND stores simultaneously, allowing the system to retrieve, process and re-store over 30,000 microtubes a day.

Once compounds have been selected, they are dispensed into assay ready plates.

3. reagent dispense & assay plate creation

Assay ready plates are transferred onto dragonfly discovery where the rest of the assay components, cells/media/ dyes are added to 384 or 1,536 well plates as required.

dragonfly discovery is a novel dispensing technology designed for seamless integration between assay development, HTS and hit-to-lead; providing:

- accurate and repeatable non contact dispensing, agnostic of liquid class
- due to positive displacement pipetting by disposable tips eliminates cross contamination
- rapid plate filling (1,536 plate fill < 3 minutes)
- easy to set up and run on automated screening systems
- enhanced system reliability no routine maintenance required
- auto feed reservoirs for HTS

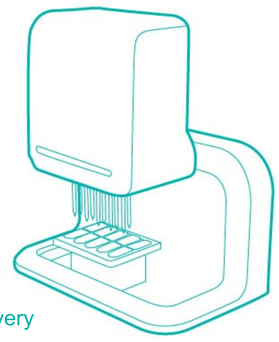

Fig 3. dragonfly discovery

4. phenotypic HTS imaging

Post-incubation, plates are transferred to the acumen Cellista microplate cytometer for rapid phenotypic screening.

Fig 4. acumen Cellista

The acumen Cellista HTS solution provides:

- rapid whole well imaging (~5 minutes/plate)
- total cell counts/well for data normalisation, cell proliferation, or toxicity indication
- multiplexed assays screening up to 400K wells/24hr day
- rapid data driven decisions by removing data analysis/validation bottlenecks
- small data output files (Mb) to prevent data mountains
- integration into Genedata Screener

5. case study: cell health assay

Here we demonstrate the implementation of an 1,536 compatible phenotypic assay using inexpensive commercially available dyes (Calcein-AM and propidium iodide) to profile the drug-responses of several well-characterised anti-cancer agents on HeLa cells following overnight compound treatment.

The results are compared to a more expensive classical biochemical assay readout ATP-luminescence (ref. ASSAY and Drug Development Technologies. Vol.14, No.7, September 2016.).

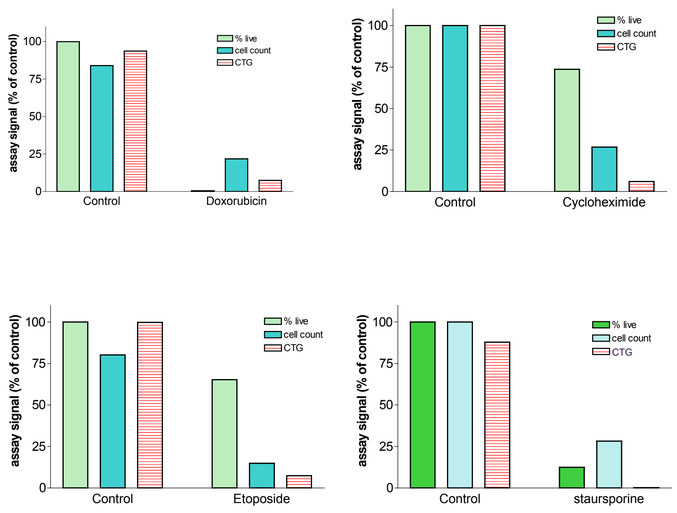

Fig 5. drug response curves comparing acumen phenotypic readouts (% live cell) to an ATP-luminescence measurement (CTG)

Hit wells were selected based on cytotoxic parameters. Well views of hit wells are shown in Figure 6.

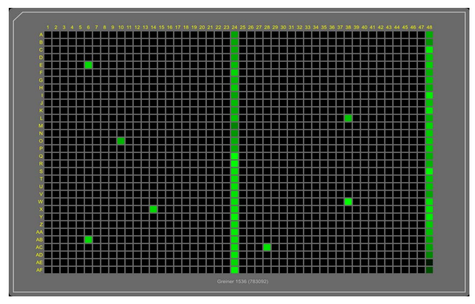

Fig 6. Typical screening plate. Negative control wells, columns 1 & 25. Positive control wells, columns 24 & 48. Seven hit wells also shown

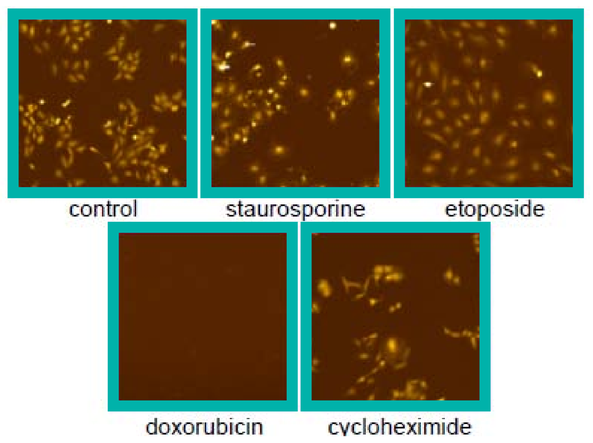

Fig 7. representative TIFF images of control and four hit wells

conclusions

The workflow presented here demonstrates the utility of TTP Labtech's products in solving many of the problems associated with running HTS:

- comPOUND can rapidly cherry pick individual compounds to assemble targeted libraries for screening
- comPILER can process tubes from up to 12 comPOUNDs to retrieve 30,000 tubes per day in preparation for assay ready plate generation
- dragonfly discovery enables a smooth transition from assay development into HTS by rapidly dispensing many liquid types, including cells, into 1,536 well plates accurately, reliably and with no cross contamination
- acumen Cellista offers the data quality of phenotypic analysis, with the speed and simplicity of a plate reader at a 7 fold savings in cost