workflow for hit-to-lead compound serial dilutions for multiplexed hepatotoxicity assays

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introduction

TTP Labtech has created a complete solution to automate and streamline hit-to-lead drug screening from hit-picking, serial dilution and cell-based phenotypic assays; addressing many common bottlenecks in the hit-to-lead process. Typical limitations include:

liquid handling

- cherry picking of hit compounds and subsequent reformatting to assay-ready plates is slow and laborious
- direct dilutions for concentration-response studies are difficult to perform, due to lack of instrument dynamic range
- assay and plate formats do not transition well between Assay Development, HTS and hit-to-lead laboratories, due to instrumentation and process differences

phenotypic assay

- multiplexed phenotypic assays are slow and expensive to run on automated microscopes
- biochemical assays are fast and simple, but can lack biological relevance

The integrated system described here enables rapid hit-picking and subsequent serial dilutions of compounds into 1,536 well plates followed by a phenotypic screen. In addition to reducing costs by low-volume liquid handling and miniaturisation of assays, TTP Labtech's novel automated and integrated hit-to-lead workflow ensures samples are treated consistently, improving sample integrity and data quality.

1. workflow for phenotypic HTS

Fig 1. workflow for a serial dilution apoptosis assay

2. cherry picking hit compounds from compound source plates

mosquito® makes assay miniaturisation simple, leading to significant savings on precious reagents and time. A novel, automated plate storage and processing system can be employed which combines a plate carousel with a robotic arm, and TTP Labtech's single- and multi-tip low-volume liquid handlers (mosquito® X1 and mosquito® HTS, respectively). The integrated system enables rapid hit-picking and subsequent serial dilutions of compounds into 1,536 well plates.

mosquito X1:

- single tip nanolitre hit picking system designed for hit confirmation and secondary profiling
- precision sampling of any individual well in any plate
- select small volumes of hits from a variety of primary screening plates and transfer them directly to the next screening stage
- disposable pipette tips prevent cross-contamination
- tips can directly pierce plate seals

Fig 2. mosquito X1 & mosquito HTS

Once hit compounds have been dispensed into a source plate, the next stage is to create a serial dilution of the compound.

mosquito HTS:

- nanolitre serial dilution of compound
- disposable pipette tips guarantee zero cross-contamination
- minimal dead volumes: reduced sample cost
- Mixing: good sample homogenaity

3. multi-reagent dispense

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

dragonfly discovery rapidly dispenses assay components (e.g. cells, media, dyes) to assay-ready plates providing:

- non contact, positive displacement: agnostic of liquid class, no clogging or blocking of nozzles
- disposable tips: zero cross-contamination
- high speed dispensing (fill 1,536 well plate in <3min)
- broad dynamic range 20,000:1
- low dead volume
- easy to set up and run, enabling DoE
- manual or auto feed reservoirs for hit-to-lead integration on screening systems of any size

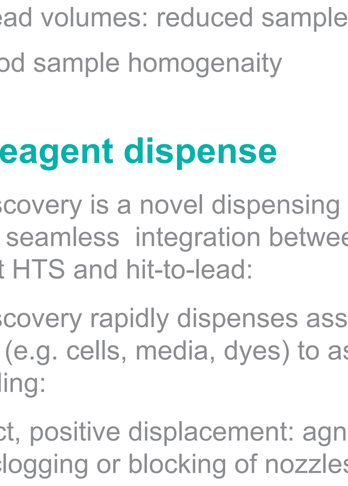

Fig 3. dragonfly discovery

Following serial dilution, the assay ready plate can then be moved onto dragonfly discovery and the rest of the assay components, cells/media/ dyes can be added to 384 or 1,536 well plates as required.

4. high-throughput phenotypic screening

Once the plates have been prepared and incubated, then the plate is moved onto the acumen® cellista microplate cytometer which is ideally suited for rapid phenotypic screening.

Fig 4. acumen Cellista

The acumen Cellista microplate cytometer enables multiplexed phenotypic assays on high-density microplates. Combine the value of a high content approach with the convenience of a biochemical assay

acumen Cellista combines:

- rapid whole well scanning - plate analysis in under 5 minutes
- multiplex readouts with up to 3 lasers and 4 fluorescence channels
- high-throughput capability and rapid analysis means it can analyse large numbers of compound

5. case study: hepatoxicity assay

The concentration-dependent cytotoxicity was investigated in a high throughput, high content screening assay using 3 markers:

- TMRM was used to determine mitochondrial health
- TOTO-3 identifies nuclei of dead or dying cells
- Hoechst staining facilitates two readouts of total cell number to normalise cytotoxic responses.

6. assay method

HepG2 cells were treated with compound (in culture medium) to give desired working concentration. Cells were then incubated at 37ºC, 5% CO_2 until appropriate timepoint when 10 µL per well of detection mix was added (Life technologies products TOTO-3 [4 µM, cat# T-3604], TMRM [2 µM, cat# T-668] and Hoechst 34580 [4 µM, cat# H21486]) in culture medium. Cells were incubated with detection mix in the dark for 1 hour at 37ºC, 5% CO_2, prior to scanning on the acumen Cellista.

results

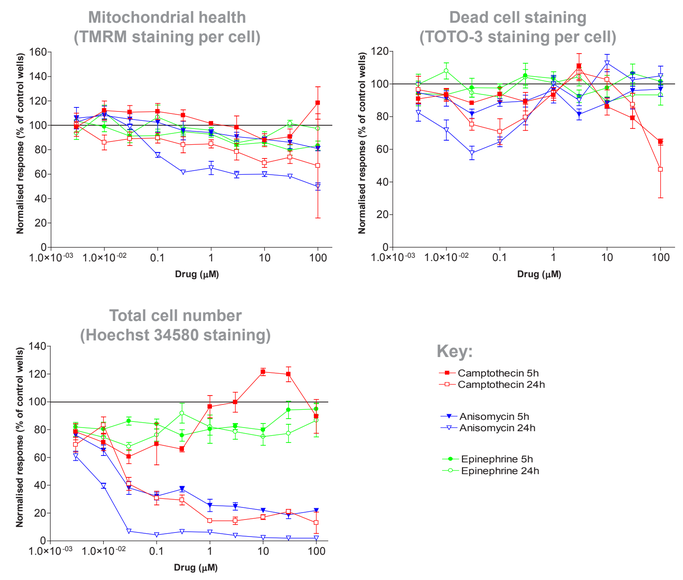

Fig 5. Concentration-dependent changes in cell health in HepG2 cells. Dotted line indicates no change relative to control wells. Data sets are from quadruplicate wells. Error bars indicate S.E.M.

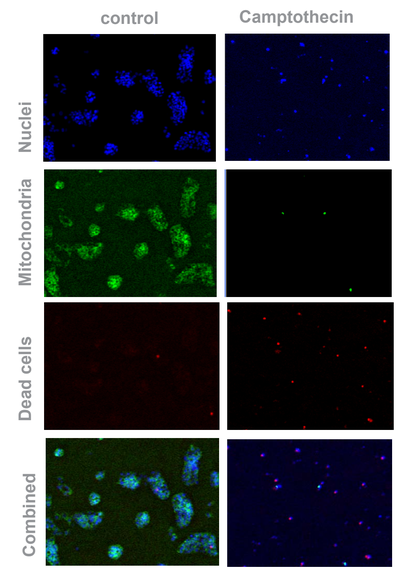

Fig 6. Treatment of HepG2 cells with Camptothecin (2µM) for 72 hours significantly reduces cell proliferation, decreases mitochondrial health and increases the proportion of dead cells. Open source TIFF files generated by the acumen Cellista were false-coloured using ImageJ

conclusions

Data presented here demonstrates a simple, accurate workflow to pick hit compounds, create serial dilution plates, add all assay reagents and screen a complex phenotypic assay:

- accurate, no contamination, compound hit picking with or without plate seals
- assay ready serial dilutions, minimizing compound dead volumes
- rapid addition of multiple assay reagents, including cells
- rapid high content multi-parametric analysis of hepatocyte cell health