dragonfly® discovery to enable and accelerate early stage drug discovery processes

M. Pemberton[1], S. Desai[1], P. Craggs1, J. Evans[1] , C. Ashby[1], M. New[2], C.Schofield[2], R. Lewis[3], E. Schwan[3], G. Cochrane[3], R. Pardoe[3], J. Jenkins[3]
[1]GlaxoSmithKline, Screening Profiling & Mechanistic Biology, Stevenage, UK. [2]GlaxoSmithKline, Functional Genomics, Stevenage, UK. [3] SPT Labtech Ltd, Melbourn Science Park, Herts, UK

Introduction

The complexity of experimental design in early stage drug discovery is rapidly increasing with reagent requirements for a growing number of assays becoming progressively more complex and difficult to dispense. In order to address these challenges, we have utilised the dragonfly® discovery to enable a broad range of applications within functional genomics, phenotypic and biochemical assay workflows.

dragonfly® discovery

- Positive displacement liquid Dispenser
- Dispense volumes 200nl-400ul
- 96, 384 and 1536 well plates
- Low dead volume
- Up to 10 different reagents at a time
- Any syringe to any well at any time
- Covered plate position for incubations
- Optional plate Stacker
- Automatic reagent refill options
- Chilled reagent reservoirs

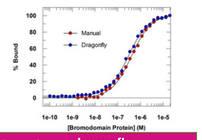
Assay development and Routine screening

FP Assaydevelopment

- ALL assay development and validation experiments by dragonfly discovery.
- All experiments in 1536, HTS ready.

Reduced
-Assay dev time
-Labour
-Reagent

Bromodomain FP Binding curves



Binding curve feature	Manual	dragonfly
Microplate density	384-well	1536-well
No. ofreplicates	3	8
Experimental preparation	Serial dilution	Discrete gradient
Experiment time	~1 hour	~20 mins
Reagent dead volume	~1mL	280uL

Protease Assay

- Compound profiling for small molecule inhibitors
- Weekly, 2-14 plates (384 well)
- Walk away

12.5ul Substrate
↓
12.5ul Enzyme
↓
Incubate 60min
↓
40ul Quench
↓
Remove plates from dragonfly discovery
↓
Analyse on Rapid Fire Mass Spec system

Autofill reservoir for reagent delivery

autofill reservoir valve

Plate stacker

CRISPR-Cas9 Gene Editing

High-efficiency, arrayed genome-editing screen.

- In primary CD4+ T cells.

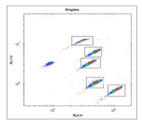
Workflow

Day 1 — Gene Editing

Media changes

Day 14 — Low level stimulation

Day 17 — Multiple end point assays



Gene editing– dragonfly® discovery

- Sterile dispensing
  - T-cells, recombinant Cas9 protein, media and dilution of RNP components.
- Low dead volume
  - Low volume reservoir ~150ul
  - Significant savings on use of costly recombinant Cas9 protein.
  - Temperature control of Cas9 protein.

Low volume reservoir and cooling block

- Accurate dispensing

Figure 1. T-cell dispense, 6 syringes, %CV 4.3 (whole plate excluding col 18), Z prime 0.88, readout using CellTitre-Glo® reagents and BMG PHERAstar plate reader. Note col18 media only.

Design of Experiment (DoE)

The use of statistical methods to design experiments and analyze data.

- Less experiments, more data, reduced cycle times and costs.
- Complex plate patterns required.

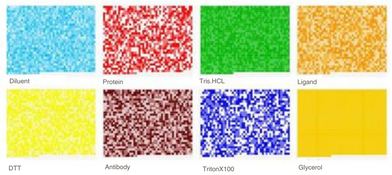
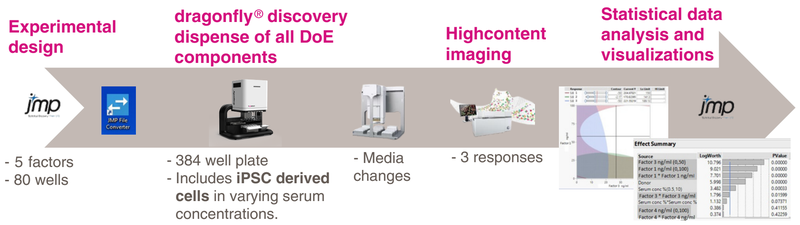


Figure 2. dragonfly discovery dispense layouts for a DoE to optimize assay conditions for a TR-FRET assay. 1536 well plate, randomized dispense, multiple concentrations per reagent.

DoE workflow for optimization of an iPSC derived cell assay.



3D Organoid Cultures

- The use of organoid cultures asrobust pre-clinical in vitro models is becoming more established.
- The dragonfly® discovery is in use to develop methodology to grow 3D organoid cultures at a scale suitable for screening.

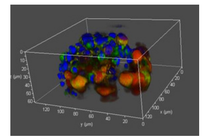
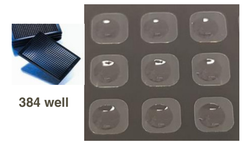


Figure 3.3D confocal image of fluorescently labelled cells in an organoid, dispensed by dragonfly® discovery.

Printing organoids in 3D Matrigel droplets



- Controlled dispense speeds
- Sterile
- Uniformed droplets
- Chilled reservoir for optimal handling of Matrigel

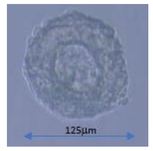


Figure 4. Phase contract image of an organoid, dispensed by dragonfly® discovery.

Enzyme Mechanistic Study

- Utilizing the time-map feature for scheduled dispensing to a 384 well microplate.
- Precision multi-reagent dispensing.
- Discontinuous time course over 6 hours.
- Plate covered between time points.
- Low dead volume.
- High quality data.
- Minimal user interaction.

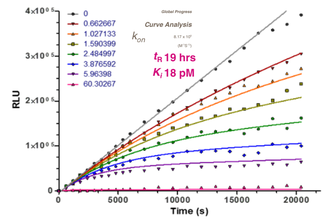


Figure 5. The onset of inhibition progress curves were Globally fit to a one-step model to determine binding rate constants kon, and an estimate of koff (residence time (tR) = 1/koff and Ki = koff/kon). Quoted tR value determined using a jump dilution experiment.

Conclusion

Key features of the dragonfly discovery

- Any syringe to any well at any time.
- Low dead volume.
- Temperature control.
- Dispense speed control.
- Accurate dispensing across a range of liquid classes.

These features have resulted in rapid development of complex phenotypic assay workflows in primary cells, iPSC derived cells and organoids, which have then been employed in screening workflows for both compound and arrayed CRISPR screens.