

Implementation of the HP D300 digital dispenser improves accuracy and reproducibility of concentration response curves

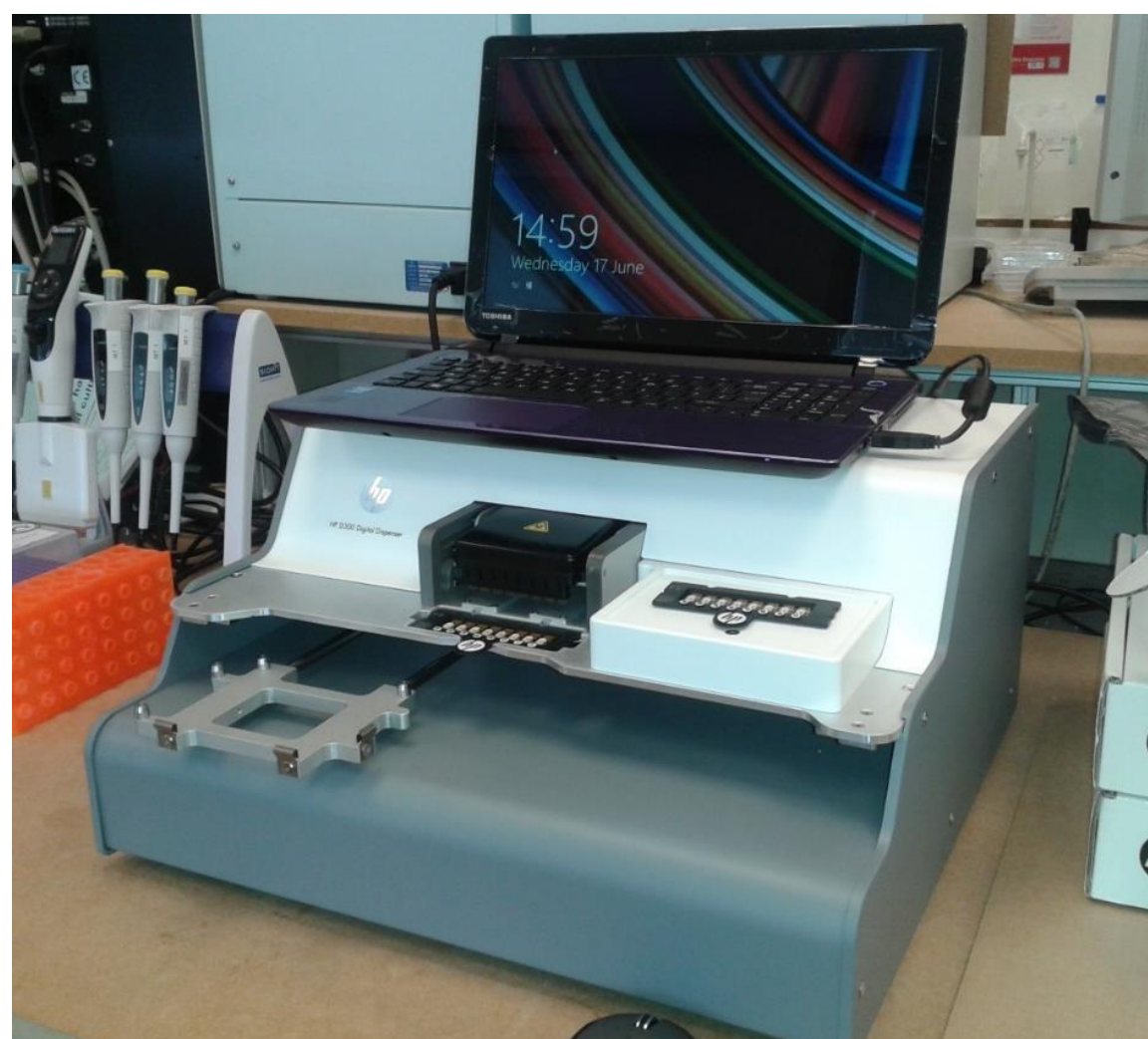
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Introduction

The HP D300 digital dispenser is designed to dispense picolitre to microliter volumes of compound (DMSO) stocks directly into multi-well plate formats. It is rapid and highly accurate yet gentle enough to not disturb a cell monolayer. The ability to add low volumes of stock compound directly to wells has a number of advantages including; eliminating the need for intermediate aqueous buffer dilutions, maintaining low final DMSO concentrations and maximising the solubility of compounds across the concentration range.

The dispenser also enables a wide range of titration series to be programmed, not limited to the manual serial titrations typical in drug discovery e.g. half-log or 2-fold dilution. This has significant benefits and allows for unique curve profiles to be developed for compounds with poorly defined activities, whereby a cluster of compound concentrations can be targeted to any region of the concentration curve.

Here, we demonstrate the increased accuracy of the digital dispenser compared to manual titration series in a both cell-based and biochemical formats. Further to this we demonstrate a number of potential targeted titration curves designed for compounds with poorly defined activities or IC50/EC50 values.



Materials and Methods

- HEK 293 CRE-*luc2P* luciferase cell line
- NIH 3T3 luciferase reporter cell line
- Steady-Glo luciferase assay system, Promega
- Nuclear hormone LanthaScreen Assay (Life Technologies)

Reporter Assays

HEK 293 cells were plated at 6000 cells/well in 384 well plates. NIH 3T3 cells were plated at 4000 cell/well in 384 well plates.

For manual pipetting, compounds were serially diluted in to a 384 well plate and transferred to the cells using a 16-channel pipette or the CyBi-well 384 pipettor.

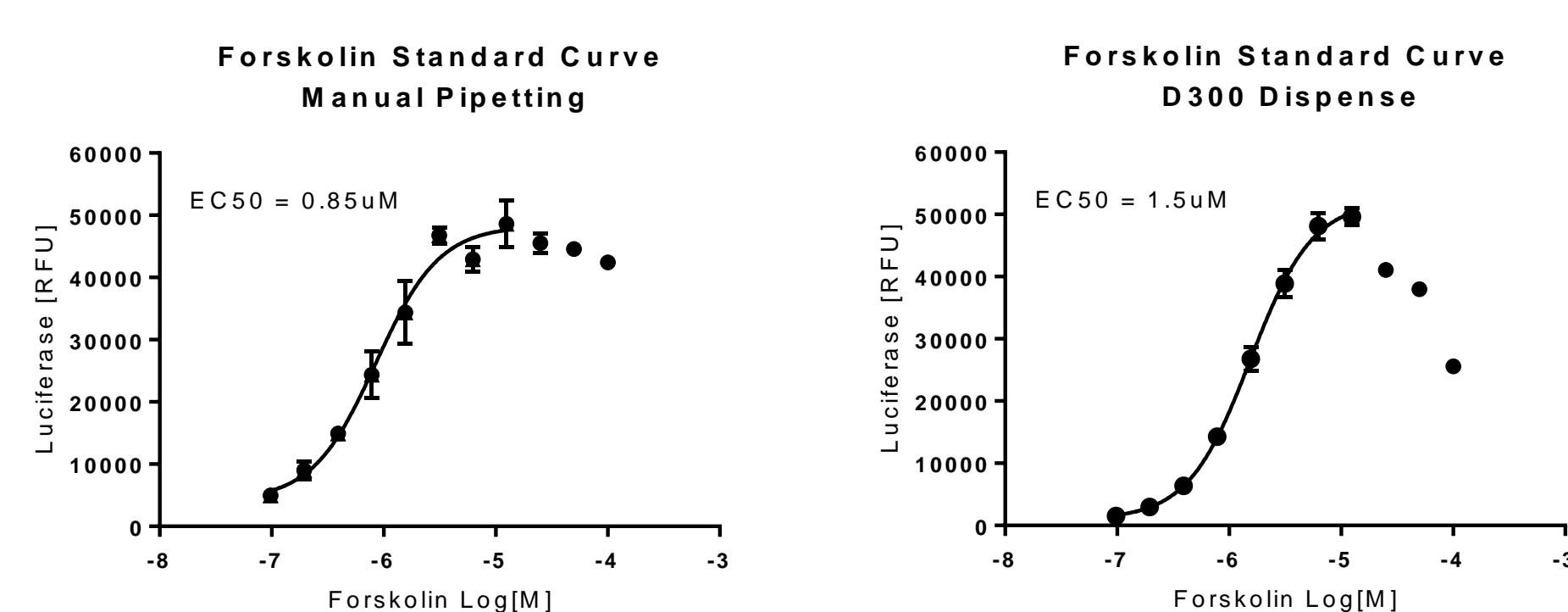
For compound addition using the D300, compounds were loaded onto the instrument at 10mM in DMSO. Compounds were added 24hr post seeding.

Luciferase expression was measure at various time points depending upon the cell line and stimulus (4hr – 48hr post compound addition).

Nuclear Hormone TR-FRET Assay

The kit contains assay buffer, ligand binding domain (LBD), antibody and labelled coactivator peptide. The LBD was dispensed into a low volume 384 well plate followed by compound either manually diluted in buffer with 10% DMSO and then transferred to the well using a 16-channel pipette or added directly to the well from a 10mM stock with the D300. After 10 minutes the antibody and coactivator peptide were added. The assay was incubated for 2hrs prior to measuring the TR_FRET signal.

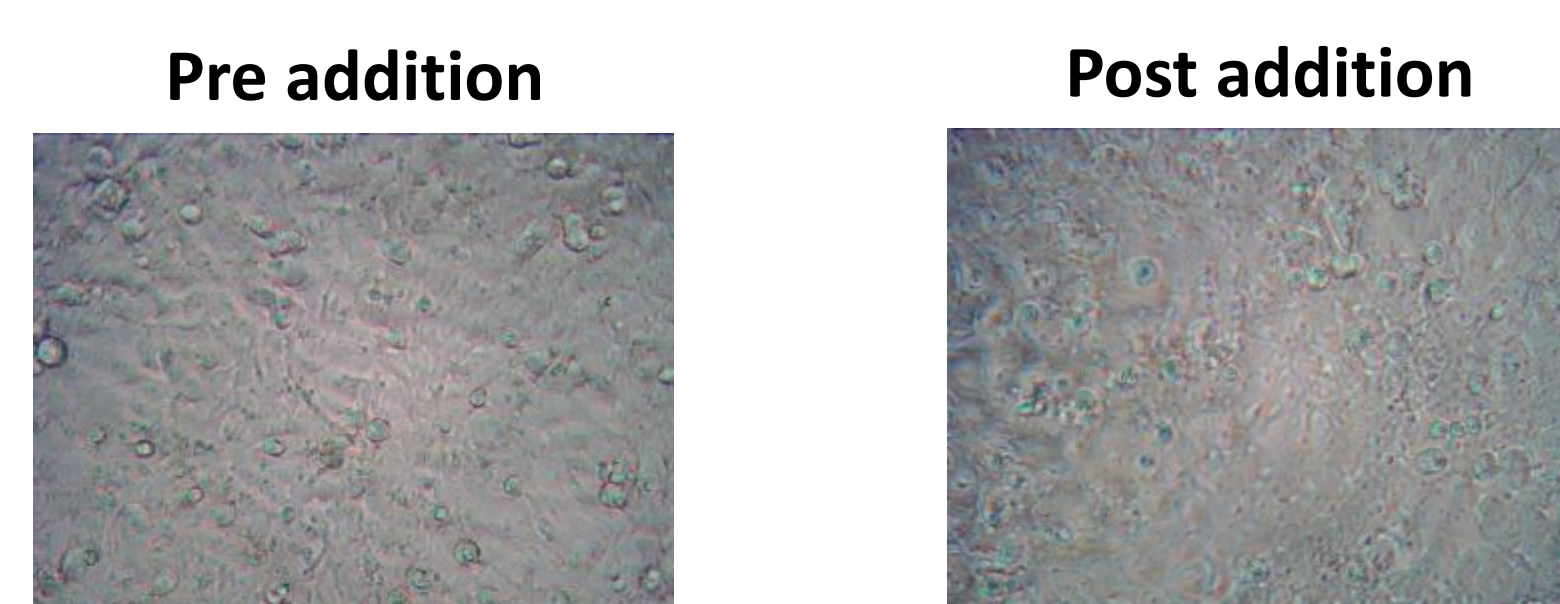
Manual vs D300 compound addition



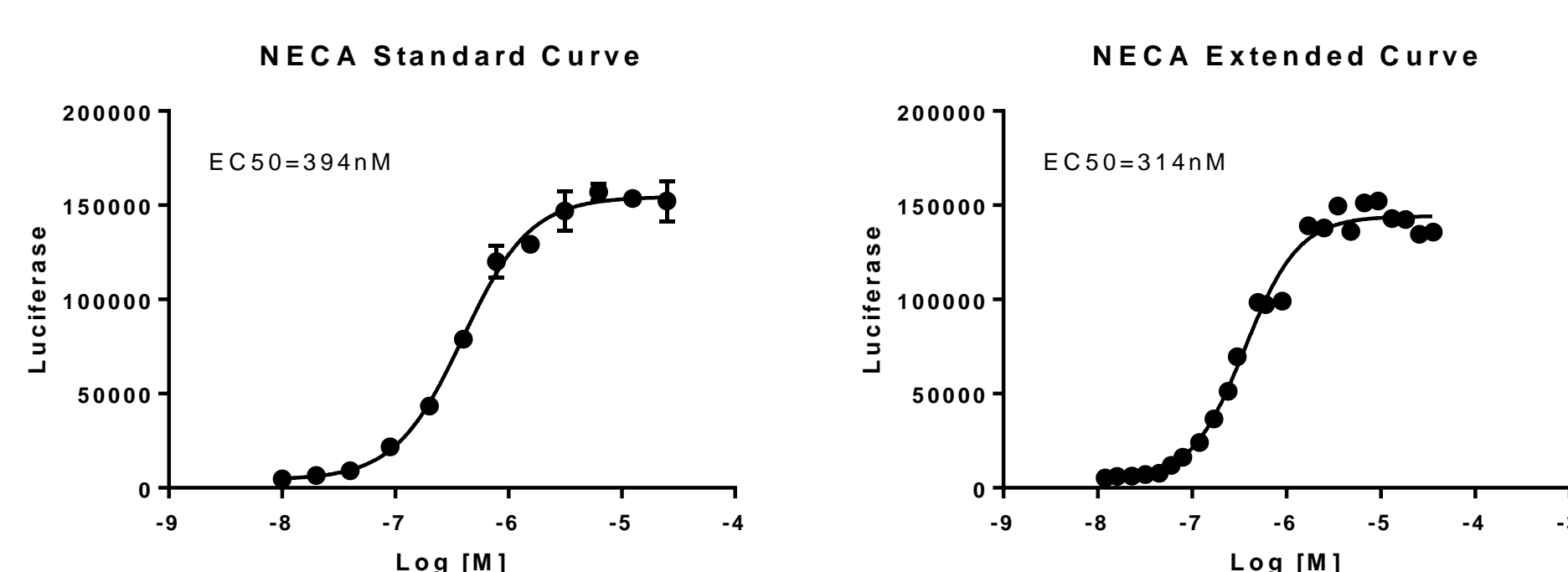
Addition of the compounds using the D300 reduces variability and improves accuracy.

A series of Forskolin dilutions was added to HEK 293 CRE cells either by manual pipetting or using the D300. Data point variability is reduced when the compound is added using the D300 (error bars represent the standard deviation of triplicate values). Furthermore, addition with the D300 produces a bell shaped curve suggesting Forskolin insolubility at high concentrations with manual pipetting.

Direct addition of the compound to the plate using the D300 does not disrupt the cell monolayer.

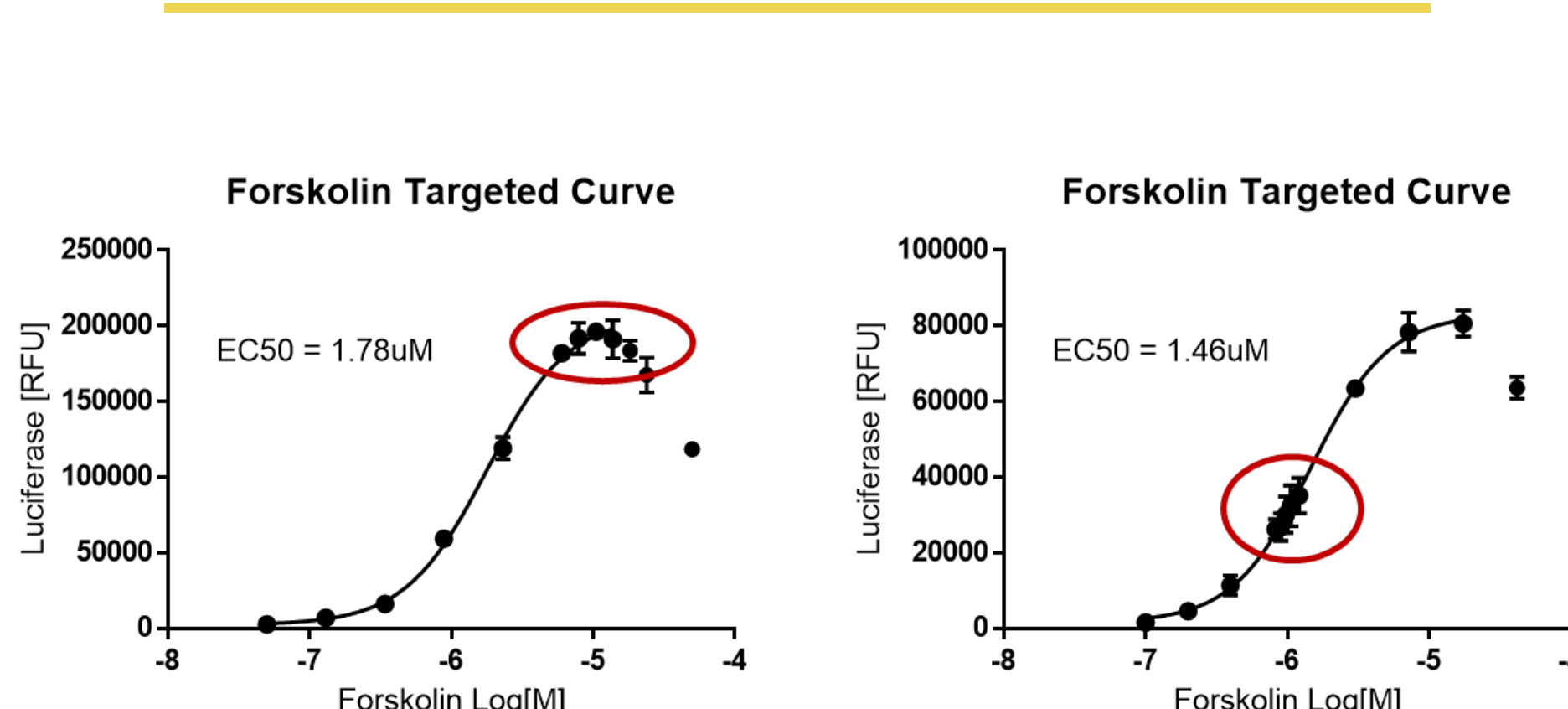


Targeted and multiple point curves



The accuracy and dosing flexibility of the D300 means you can use fewer wells of a multi-well plate for standard curves and controls.

A series of NECA dilutions was added to HEK 293 CRE cells using the D300. The titration curves consisted of either a 12 point, triplicate curve or a 24 single point curve. A 12 point dose response curve in triplicate uses 36 wells from a 384-well plate. However, due to the accuracy of the D300 a 24 point, single dose curve gives equivalent accuracy with fewer wells used.

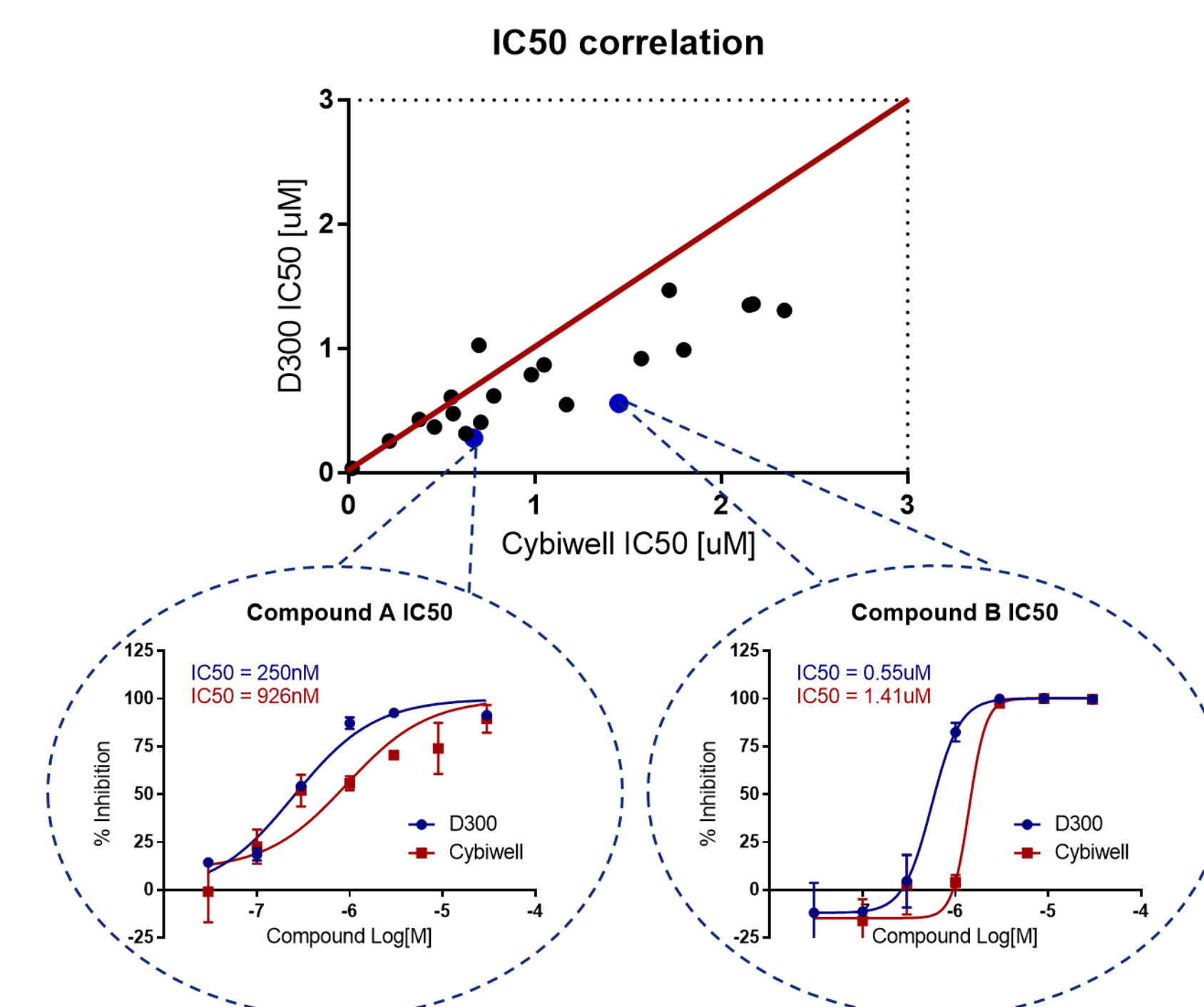


The D300 targeted titration function allows for unique curve profiles.

A series of Forskolin dilutions was added to HEK 293 CRE cells using the D300 with a focus of compound dilutions at either the predicted EC50 or top of the curve.

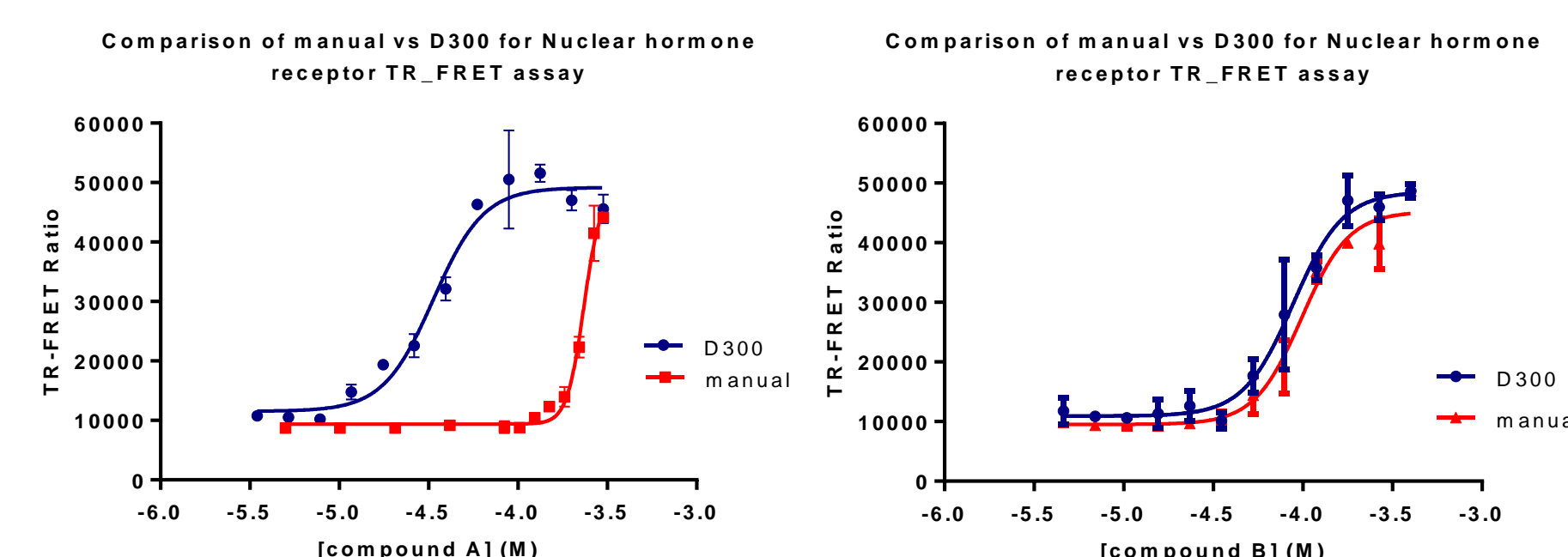
This clustering of concentrations more accurately defines critical regions of the curve and has significant benefits for compounds with poorly defined activities. Here we use the compound clustering to define the top of the agonist curve and the predicted EC50 region.

Insoluble compounds



Addition of poorly soluble compounds using the D300 increases compound potency and dosing accuracy.

Compound A was added to 3T3 reporter cells either via the D300 or from a manually prepared titration curve and the CyBi-well 384 pipettor. The manually prepared curve involved an intermediate aqueous dilution. The IC50 was significantly lower for the compound when it was administered using the D300. By dispensing the compound directly from DMSO stocks it removes the need for aqueous buffer dilutions and therefore reduces compounds loss due to insolubility.



Use of the D300 in a biochemical assay increases compound potency and dosing accuracy.

Two sterol compounds were tested in a TR-FRET nuclear hormone assay. The manually prepared curve involved an intermediate aqueous dilution in 10% DMSO, whilst the D300 added compound directly to the assay well. In the case of compound A the solubility in 10% DMSO clearly affected the subsequent titration series. The IC50 was significantly lower for compound A when it was dispensed with the D300. Compound B was more soluble in 10% DMSO hence the manual and D300 curves were similar. For compound types like sterols which are poorly soluble in aqueous the D300 ensures that the desired compound concentration is achieved in the well and reduces compound loss due to insolubility.

Summary and Conclusion

The D300 digital dispenser dispenses picolitre to microliter volumes of compound (DMSO) stocks directly into multi-well plates.

We have demonstrated the following benefits over manual pipetting of compounds including:

- Improved accuracy
- Speed of addition
- Elimination of user induced variability
- Targeted titration curves
- Increased flexibility of titration curve dilutions (1:N)
- Maximising solubility of compounds across the concentration range
- Efficiency of compound usage (maximum volume of the dispenser chip is 10ul)