low-volume workflows improve the quality of drug discovery: two case studies

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ICAGEN
Advancing Early Drug Discovery

introduction

Assay miniaturisation benefits drug discovery workflows by decreasing the amount of samples and reagents that are required. At the same time, low-volume assays reduce costs.

The limiting factor in being able to miniaturise assays is typically the ability to pipette small volumes of various liquids, accurately and reproducibly.

Scientists at Icagen Tucson Innovation Center (Tucson, AZ, USA), a specialised pharmaceutical partnering organisation with a focus on early drug discovery, evaluated the use of SPT Labtech's mosquito® LV for low-volume qRTPCR and serial dilution of drug compounds. Here we demonstrate that automated liquid handling can successfully improve the quality of these applications in drug discovery.

miniaturising drug discovery applications with mosquito LV

Reducing assay volumes conserves compound used, minimises consumables required and because compounds are diluted initially in DMSO, ensures compound solubility.

These advantages can only be achieved with accurate and reproducible liquid handling.

SPT Labtech's mosquito® LV (25 nL - 1.2 µL) and mosquito® HV (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers (Fig1).

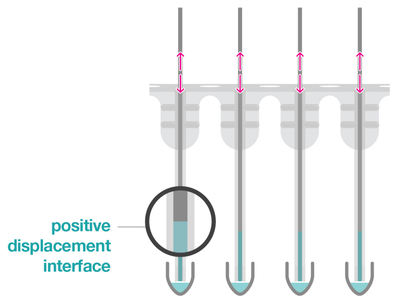
Fig 1. mosquito LV (8- or 16- channel) liquid handler

true positive-displacement technology:

- enables fast, accurate, gentle and contamination-free liquid transfer, essential for accurate and more efficient molecular biology applications and serial dilutions of drug compounds
- ensures low dead volumes of < 0.3 µL
- provides multiple-aspirate functionality for on tip mixing of sample

Each tip has its own individual piston that is in direct contact with the liquid being pipetted

- with no air gap the aspiration force remains constant enabling even the most viscous samples to be pipetted accurately and precisely
- stored in unique high-density spools of up to 36,000 tips with rapid tip exchange



case study 1: miniaturising qRTPCR reactions

Low-volume workflows for qRTPCR were tested, dispensing reagents and samples serially to ensure sufficient sensitivity and linearity of cDNA dilutions.

methods

qRTPCR standard curve reactions were set up in triplicate with either 1) dilutions of cDNA (target and housekeeping genes at 1, 0.5, 0.25 and 0,125x dilutions), or 2) 300 nL cell lysates from a rat s16 Schwann cell line treated with a selection of compounds. Master Mix was added to create final volumes of 2-3 µL respectively.

The required volumes of samples and reagents were dispensed into 384 well plates using mosquito LV.

qRTPCR gene analysis was performed with a Roche LightCyler (Fig 2).

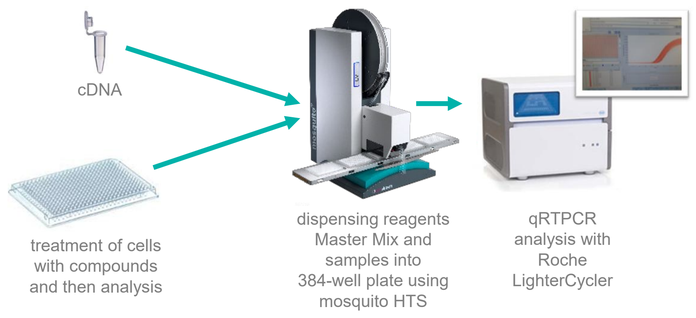


Fig 2. miniaturised qRTPCR workflows using mosquito HTS

results

In both experiments, the results demonstrated highly accurate and reproducible pipetting with CVs of < 3%, and a linear relationship between dilutions (Figs 3 and 4). The cell lysates were derived from drug treatment of cells. IC_{50} measurements calculated from the low-volume qRTPCR reaction of these cell lysates were comparable to established methods.

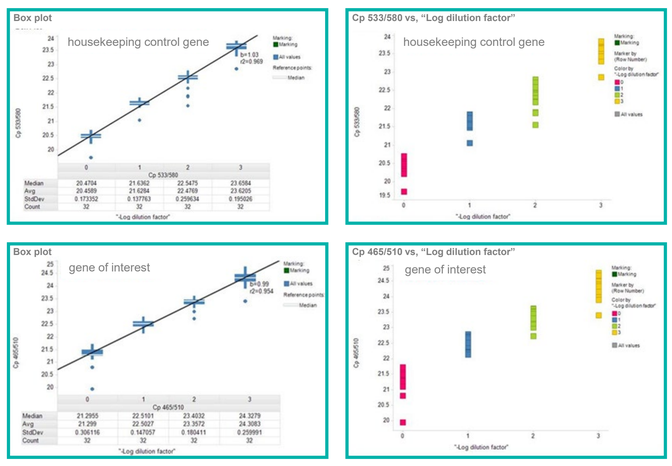


Fig 3. validation of low-volume cDNA standard curves prepared in 2 µL final volumes using mosquito LV. 0, 1, 2 and 3 represent dilutions of cDNA of 1x, 0.5x, 0.25x and 0.125x
observed CVs for technical repeats were < 3%. The graphs demonstrate cDNA maintained linearity of signal

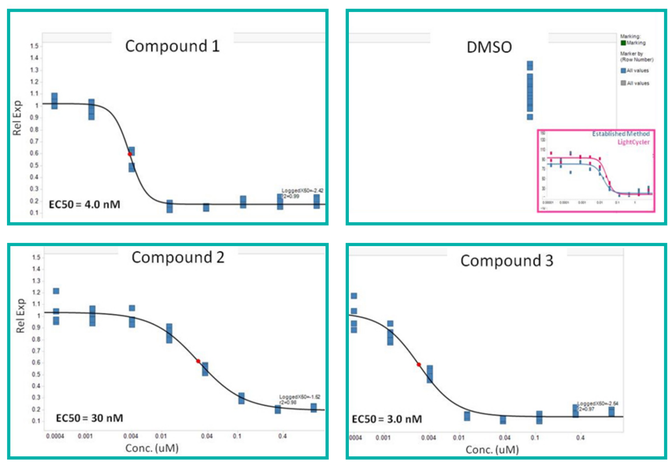


Fig 4. evaluation of qRTPCR gene analysis of 300 nL cell lysates prepared in 3 µL final volumes using mosquito LV
observed CVs for technical repeats were < 3%. The calculated IC_{50s} were comparable to established methods (inset).

case study 2: low-volume dilution curves for drug compounds

Scientists at Icagen Tucson Innovation Center routinely analyse serial dilutions of lead optimisation compounds in simultaneous biochemical and cellular assays for a rare disease project.

The assays are designed to identify compound activation in both assays in parallel on replicate plates using the same compound dilution plate.

Further, errors in reproducibility or manipulation are minimised since assays are run on the same day with the same dilution plate.

method

A 7-point 2x dilution curve of compounds plus DMSO (final volume 500 nL) was prepared in a 384 well plate using mosquito HTS.

These prepared plates were further diluted in reaction buffer with a Biomek FX and dispensed to replicate daughter plates for analysis of compounds in molecular and cellular assays.

Detection of a fluorescent product produced from the reaction of compounds with substrate was performed with a BMG Labtech Clariostar (Fig 5).

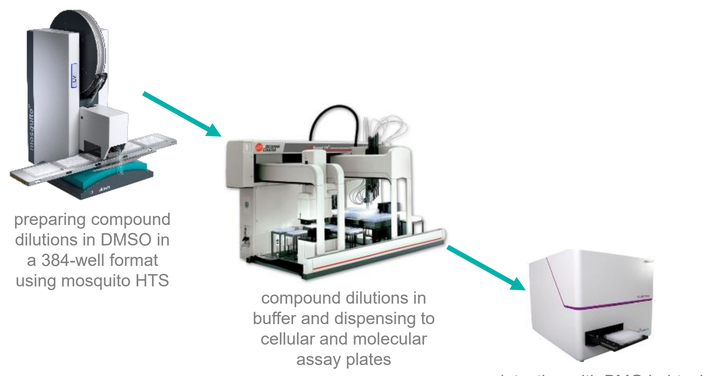


Fig 5. low-volume drug dilution workflow using mosquito HTS

results

The results demonstrated it was possible to decrease compound consumption to as little as 0.5 µL per assay.

Reduced consumables used for replicate plates and replicate assays reduced costs and minimised errors.

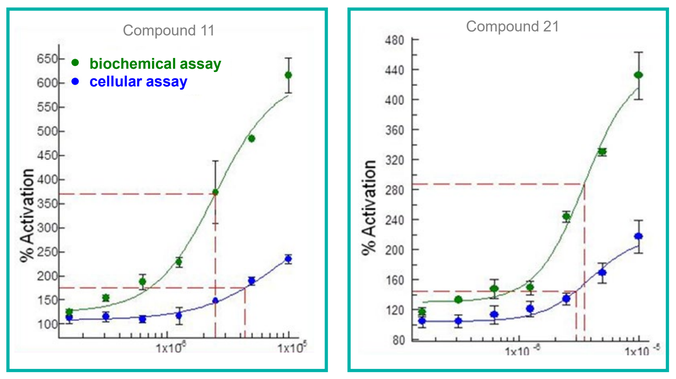


Fig 6. concentration curves for compound activity in both biochemical and cellular assays

conclusions

SPT Labtech's mosquito LV was validated for two different drug discovery applications:

qRTPCR low-volume workflow

- low CVs for technical replicates of <3% in 2 µL working volume
- cDNA titration maintained linearity of signal
- suitable gene expression was detected using as little as 300 nL of cell lysates
- IC_{50s} were calculated in line with established methods

Low-volume drug dilution for use in multiple assays

- reduced compound consumption
- ensured compound solubility
- facilitated assay-ready plate preparation
- reduced consumables used
- reduced errors in reproducibility by running biochemical and cellular assays from the same drug dilution plate

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