Assay Miniaturisation using Hummingbird and synQUAD Systems

Malcolm Willson

Genomic Solutions Ltd., 8 Blackstone Rd, Cambridgeshire, UK

Introduction:

Research and development organisations are under constant pressure to effectively manage their compound assets, which are the result of many years of chemical development and can also represent a large financial investment. The value of these company assets will not be realised without effective management and as compound collections grow in size, there is increasing pressure on HTS departments to become more efficient. Advances in assay technologies, data handling and improvement in plate readers along with increased assay sensitivity are enabling higher density formats (*e.g.* 1536 well plates) and lower volume assays to be performed without sacrificing data quality.

Assay miniaturisation is not only applicable to HTS, it can also be used to streamline secondary screens. The advantages of reduced reagent costs, more efficient use of compounds and higher density assay plates enable more data to be acquired without increasing plate storage or waste. For mode of action studies (*e.g.* K_i determination) with multiple copies of compound dilution, plates can significantly improve screening efficiency.

DMSO tolerance often limits the volume used in assays. Historically, dispensing technologies for compound addition have been limited by the assay volume size. Reducing the amount of DMSO by pre-diluting the compound is not ideal and can cause compound solubility issues.

Typical reagent additions:

	96 well assay	miniaturised 1536 well assay
Compound/100% DMSO	1 μl	50 nl
Enzyme	25 μl	2.5 μl
Assay reagents	25 μl	2.5 μl

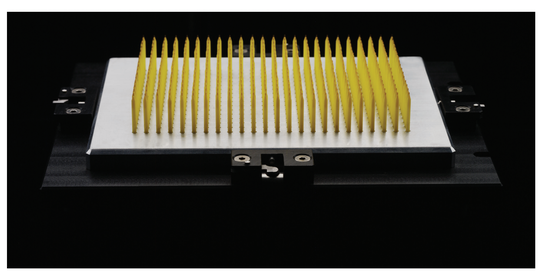
Consequently nanolitre dispensing capabilities are required for assay miniaturisation both at the compound and "bulk reagent" assay component stages.

The assay assembly stage of the overall compound screening process can be divided into two processes:

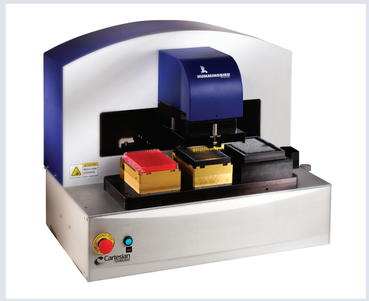
- Plating compounds into assay plates
- Assay reagent addition

Hummingbird for Compound Reformatting / Plate Replication:

Compound plates can be replicated (96 to 96 or 384 to 384 wells) or reformatted (4 x 96 to 384 or 4 x 384 to 1536) with Hummingbird capillary sizes ranging from 25 to 1000 nL. In many facilities, such as Schering AG in Germany, the Hummingbird is interfaced to a robotic plate handler and plates are prepared and stored for future use.

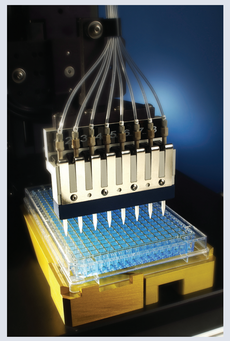


Hummingbird Capillary Cassette



Hummingbird

- Accurate and precise non contact dispensing - CV < 10%
- Wide dispense range - 25 nL to 1000 nL
- No need to pre dilute compounds
- 96 and 384 capillary format available - replication and reformatting

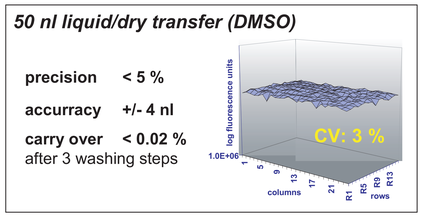


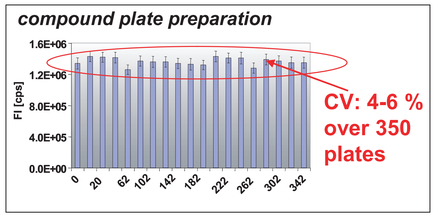
synQUAD

- Fast - non contact 'on the fly' dispensing for assay assembly
- Wide dispense range - 20 nL to 20 μL
- Dispense multiple reagents simultaneously
- Variety of reagents dispensed with CV< 10%, including: SDS/protein solutions, bead solutions, mammalian cells

Hummingbird Performance

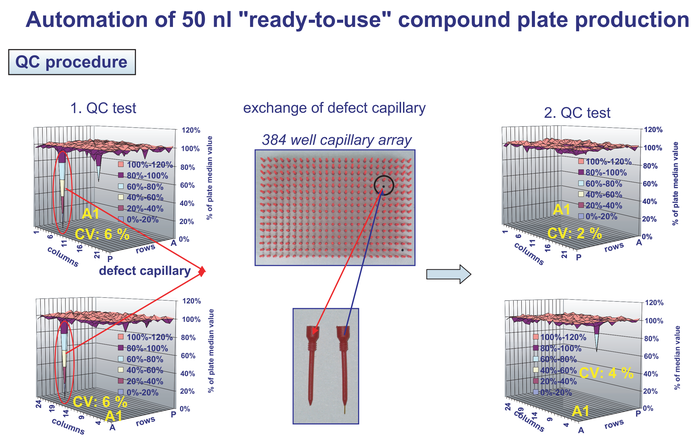
It is important to not only maintain precision between wells in the microplate, but to also maintain precision from plate to plate. Accuracy and precision data for 50 nL transfers over 350 plates is shown below (data courtesy of Dr. Christian Bergsdorf, Schering AG).





Hummingbird QC

When processing large numbers of plates, QC checks are performed to ensure dispensing across the entire plate. If a capillary needs to be replaced this can be done easily as capillary tips are individually screwed into place.

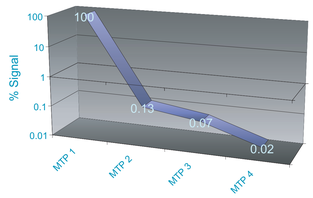


Tip washing

Efficient tip washing is important and it is possible to use solvent wash solutions, such as DMSO, on the Hummingbird to avoid moisture uptake into compound solutions. The data below demonstrates effective washing using high concentration dyes or fluorescent solutions to show carry over reduced significantly between washes (data courtesy of Dr. Johannes Ottl, Novartis Pharma AG).

Effective Tip Washing on Hummingbird

- 100nl reformatting of TAMRA* with Hummingbird™ in first Quadrant
- 100nl reformatting of 90% DMSO in Quadrant 2 - 4
- addition of 4µl Buffer** with FlexDrop™
- Fluorescence Intensity (FI) measurement on LjL Analyst GT™



* TAMRA 4µM, 20µl / well, 4 wash cycles (DMSO 10%)

** 50mM TRIS, 150mM NaCl, 0.1% CHAPS, pH 7.4

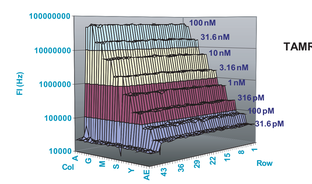
synQUAD systems for Assay reagent addition

At Novartis compound reformatting is performed on an integrated system utilising the Hummingbird, followed by addition of assay reagents using Genomic Solutions synQUAD dispensing system. The data below demonstrates the high degree of accuracy and precision that is maintained by the Hummingbird and synQUAD dispensing systems at low volumes (data courtesy of Dr. Johannes Ottl, Novartis Pharma AG).

It is important to demonstrate that data quality remains intact when assay volumes are reduced. Data from Novartis shows no significant loss in data quality on assay miniaturisation using Hummingbird and synQUAD systems

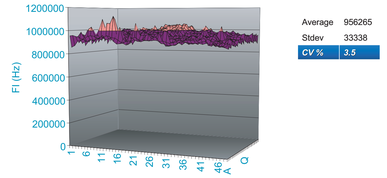
Performance of Nanopipetting with Hummingbird

- 100nl reformatting of TAMRA on Hummingbird™ and addition of 4µl Buffer on Synquad™ in *1536w* NTP with high accuracy



Performance of Nanodispensing with synQUAD

- Dispensing of 4µl TAMRA 2nM in Nunc *1536w* with synQUAD™
- Fluorescence Intensity (FI) readout



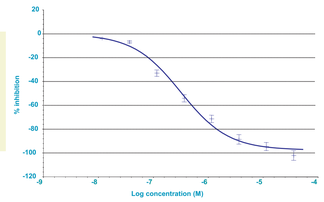
Summary

The data shown demonstrates that assay integrity is maintained when dispensing nanoliter volumes to 1536 well plates. Significant savings in reagent and assay costs can be realised from assay miniaturisation with the additional advantage of getting more assays and data from compound stocks before depletion, a very important consideration since many of these compound stocks are in limited supply.

Screening Validation Data

- IC50 data determined with reformatting 50nl on Hummingbird and screening on Mark III in *1536w* NTP (3µl/well) compatible with data derived from 96w screening (100µl/well)

IC50 measured in miniaturized 1536w format:
0.317 µM
IC50 measured in 96w format:
0.256 µM



Acknowledgements

My thanks to Christian Bergsdorf from Schering Berlin, Johannes Ottl and Oliver Bruttger from Novartis Pharma AG, Basle for the use of their data.

GENOMIC SOLUTIONS®
A Harvard Bioscience Company