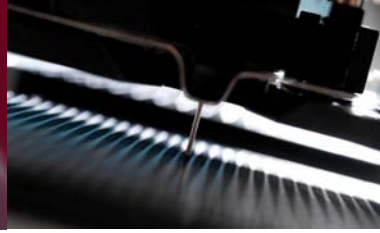
Automated Nanolitre Hit-Picking Using A mosquito® X1 Low Volume Pipettor

Joby Jenkins, Rob Lewis, Tristan Cope, Wayne Bowen
TTP LabTech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, SG8 6EE, UK



Abstract

A prerequisite for efficient primary screening is the automated selection of "hits" for confirmation and secondary profiling. Solutions exist for low density microplates, however, the 1536 well format presents significant challenges for many liquid handling systems. The mosquito® X1 offers precision sampling of any individual well in 48-, 96-, 384- or 1536-well plates. This enables researchers to quickly select small volumes of "hits" from primary screening plates and transfer them directly to the next screening stage without further dilution. mosquito® X1's disposable pipette tips guarantee zero cross-contamination. The system's unique positive displacement pipetting technology allows it to pipette accurately and reproducibly throughout the 25 nL - 1.2 µL range, producing CVs of <10% at 25 nL with a cycle time of around 7 seconds. The mosquito X1 can be integrated with common plate handlers for vastly increased walk-away time.

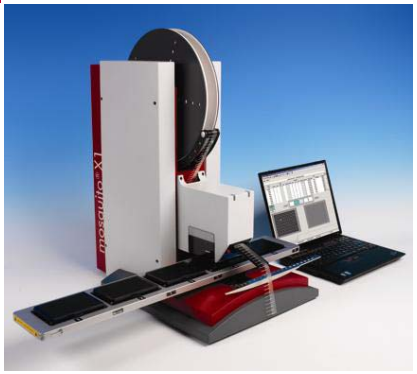
Introduction

Following their identification in primary screens, compounds deemed "hits" must be retrieved for secondary confirmation and profiling. Where compounds are stored in microplates, hit-picking solutions must be able to address wells on an individual basis whist avoiding cross-contamination of stocks. Solutions exist for low density microplates, however, the 1536 well format presents significant challenges for many liquid handling systems.

Based on a unique positive displacement pipetting technology, the mosquito® X1 offers nanolitre hit-picking from any individual well in 48-, 96-, 384- or 1536-well plates. The instrument is capable of pipetting volumes from 1.2 µL down to 25 nL with no washing required and extremely rapid tip changing (~ 2 seconds). The mosquito® X1 also offers a multi-dispense option which can dispense into multiple wells - or wells within multiple plates - from a single aspiration, thus increasing speed and saving on consumables for sample replicates.

Here, we demonstrate mosquito® X1's capability in compound profiling using a variety of microplate formats.

1 mosquito® X1 Instrument

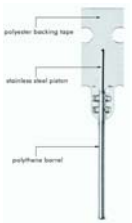


mosquito X1 is a low volume liquid handling instrument combining a disposable tip system with a positive displacement pipette. mosquito is capable of pipetting volumes from 1.2 µL down to 25 nL with no washing required and extremely rapid tip changing (~ 2 seconds).

2 Applications

- **Hit-Picking** – selection of hit compounds from primary screening plates for confirmation and secondary screening.
- **Clone Selection** – retrieval of positive cells from cell culture plates for clonal expansion.
- **Assay Analysis** – aspiration of assay constituents for secondary evolution in a second assay.
- **Protein Crystallography** – preparation of cover slides for hanging drop experiments.

Single mosquito tip addressing a 1,536 well microplate

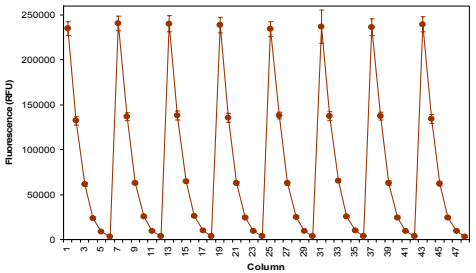


Disposable Positive Displacement mosquito Pipette Tip

3 Performance – Pipetting Precision

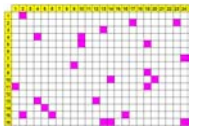
- **Cycle time** - 7 seconds average for aspirate dispense and tip change.
- **Pipetting modes** – multi-aspirate, and multi-dispense modes available, giving much reduced cycle times.
- **Compatible lab-ware** - 48, 96, 384 and 1536 well plates can used as source or destination, glass slides and cover slips can also be pipetted on to.
- **Volume range** – 25 nL -1.2 µL aspirate and dispense range.
- **Repeatability & accuracy** - CVs of <10% at 25 nL across a 384 well plate, accuracy within ±10% at all volumes.
- **Dead volume** - less than 500 nL in 'V' bottomed source plates.
- **Zero Cross-contamination** - guaranteed due to use of disposable tips.

5 Low Volume Hit-Picking and Serial Dilution in 1536 Well Plates



256 hits were randomly selected from four 1536 well 'source' plates, and a volume of 775 nL transferred into a single well in a 1536 destination plate using a mosquito X1. Each hit was subsequently serial diluted (half-log) using a standard 16-tip mosquito and the resultant fluorescence quantified using an Acumen Explorer microplate cytometer. The maximum CV for any series of terminal dilutions was 8% (n = 32) indicating the high precision and accuracy of pipetting, and consistency of mixing, using nanolitre volumes in 1536 well plates.

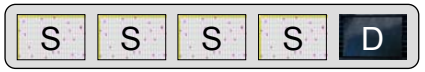
4 Hit-Picking in 384 and 1536 Well Plates



384 well hit layout

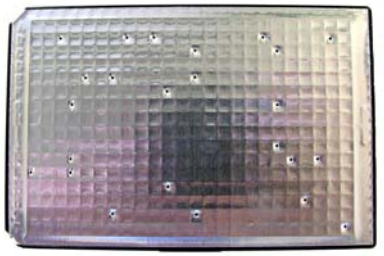
Task

- Plates: 4 Source and 1 Destination
- Each source plate contained 24 hits
- Hits were randomly distributed
- 100 nL sample volume



	384	1536
Time to complete task	10 min	10 min
Average time per pick	6.5 sec	6.5 sec
CV for picked samples	5%	6%

6 Hit-Picking From Foil-Sealed Microplates



Test compounds (50 µL) were placed in a 384 well microplate and foil sealed. 24 hits were randomly selected, and a volume 100 nL retrieved using a mosquito X1. This was achieved by piecing the foil seal directly with the mosquito disposable pipette tip and subsequent aspiration of the solution. The method can also be applied to 1536 well plates and 'V'-bottomed 384 well plates containing as little as 500 nL total volume.

Conclusion

- The mosquito X1 can address wells in microplates of varying well density, including 1536 microplates with high positional accuracy.
- This capability permits the use of source and destination plates with different well densities during hit-picking.
- Sample volumes as low as 25 nL can be applied at high precision resulting in conservation of stock compound solutions.
- The integration of mosquito X1 with a standard 16-tip mosquito fully enables hit-picking and serial dilution of compounds in 1536 well plates.

TTP LABTECH

TTP LabTech Ltd., Melbourn Science Park, Melbourn, Hertfordshire, SG8 6EE, UK. Phone +44.1763 262626, Visit us at www.ttplabtech.com/mosquito