An integrated solution for automated nanolitre hit-picking at BioFocus

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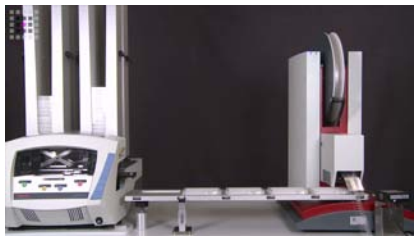
introduction

A pre-requisite for efficient primary screening is rapid, automated selection of "hits" for confirmation and secondary profiling. The mosquito® X1 (TTP Labtech) can be easily integrated into larger screening systems to offer precision sampling of any individual well in any plate. This enables researchers to quickly select small volumes of hits from a variety of 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, and ensure accurate and reproducible pipetting throughout the 25 nL -1.2 µL range.

This poster describes a section of BioFocus's screening workflow where mosquito X1 is integrated with a RapidStak plate stacker (Thermo Scientific) using TTP Labtech's CherryPicker software. This allows the mosquito X1 to work unattended for extended periods. The CherryPicker software drives the system automatically by converting pick lists provided by BioFocus' LIMS system into mosquito protocols, and feeding plates via the RapidStak.

1. integration hardware

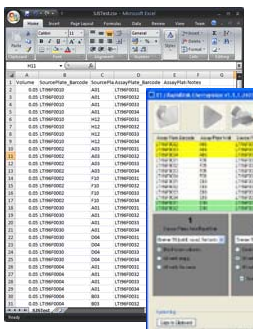
mosquito® X1 is a single tip 'hit-picking' instrument that can access any well in any microplate and transfer nanolitre quantities of hit compounds. mosquito X1 is capable of pipetting volumes from 1.2 µL down to 25 nL with no washing required and extremely rapid tip changing (~1 second).



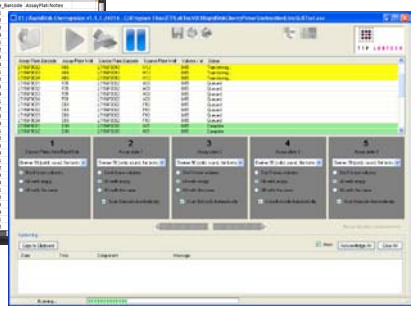
RapidStak is a fast, automated microplate loader and stacker, available in a variety of bench-top formats offering a capacity of 30-150 plates and load/unload times of only 6 seconds/plate.

2. CherryPicker software

BioFocus' LIMS system identifies hit compounds for verification and follow up. The CherryPicker software converts a CSV file of transfers from the LIMS system into an optimised protocol for the mosquito X1 and microplate loader, then manages the run.



CSV file to CherryPicker infterface



Barcoded plates can be stacked in any order in a microplate loader (here a RapidStak). As a plate is loaded, the barcode is read and the correct transfers for that plate are carried out. Additional plates are easy to load and process.

The CherryPicker software automatically optimises all mosquito protocols for efficient tip usage and optimal dispense heights, and, if required, could also track the source plate volumes for subsequent reporting

3. workflow at BioFocus

HTS Phase

Plate-based compound selection (up to 880,000 unique compounds on 2,944 plates): Plate preparation with PlateMate Plus (Matrix Technologies)

Well-based compound selection (generally up to 2,000 compounds): Automated cherry-picking with mosquito X1 (TTP Labtech)

Verification Phase

Determination of actives based on compound activities and chemoinformatic tools (typically 1-2% of all screened compounds): Automated cherry-picking in duplicates with mosquito X1 (TTP Labtech)

Concentration Response Phase

IC50 and purity determination for verified actives (up to 0.1% of screened compounds)

Small volumes: Cherry-picking with mosquito X1 and serial dilutions with mosquito X1 or mosquito ® HTS (TTP Labtech)

Large volumes: Cherrypicking with Janus (PerkinElmer) and serial dilutions with PlateMate Plus (Matrix Technologies)

Hit Expansion Phase

Well-based compound selection based on HTS outcome (approximately 2,000 compounds):
Automated cherry-picking in duplicates with mosquito X1 (TTP Labtech)

Concentration Response For Hit Expansion Phase

IC50 and purity determination for actives

Small volumes: Cherry-picking with mosquito X1 and serial dilutions with mosquito X1 or mosquito HTS (TTP Labtech)

Large volumes: Cherry-picking with Janus (PerkinElmer) and serial dilutions with PlateMate Plus (Matrix Technologies)

4. mosquito accuracy and performance

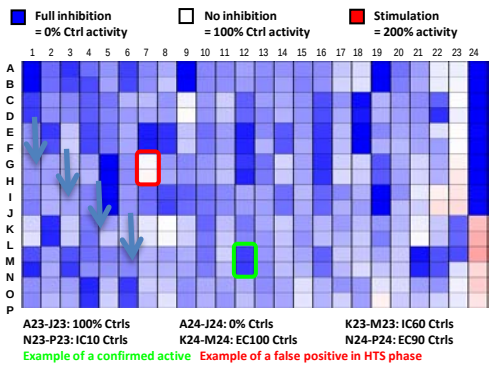
For BioFocus' CV determination, mosquito X1 pipetted volumes of 0.1% Tartrazine solution in 100% DMSO into a 384-well Greiner flat bottomed plate. The wells were then filled with 50 µL of water before OD determination.

Vol (µL)	1.00	0.50	0.25	0.10	Mean
CV (%)	2.2	1.1	1.0	3.1	2.2

Other mosquito X1 benefits:

- Rapid cycle time: averages only 6 seconds for aspirate, dispense and tip change.
- Multi-aspirate and multi-dispense pipetting ensures speed and efficient consumable usage.
- Dead volumes are under 100 nL in 'V' bottomed source plates
- Zero cross-contamination is guaranteed using disposable tips
- Rapidly accesses any well in any type of plate
- Directly pierces plate seals with no additional equipment.

5. verification phase plate



This figure illustrates a representative heatmap of a verification plate tested in a biological assay system. In columns 1-22 the plate contains actives identified in a HTS campaign and cherry-picked in duplicates with the mosquito X1 system. Columns 23 and 24 contain different controls, which are used for data normalization or for an estimation of the assay sensitivity.

conclusion

- BioFocus chose the mosquito® X1 system because it is an accurate, true walk-away cherry-picking system in the 25 nL to 1.2 µL range with an attractive throughput.
- mosquito X1, RapidStak and CherryPicker software offer a very easy to use system which rapidly converts pick lists provided by a LIMS system to optimised mosquito protocols, which can be run unattended.
- Nanolitre sample volumes can be applied with high precision resulting in conservation of stock compound solutions.
- Direct piercing of plate seals means that compounds dissolved in DMSO (or other solvents) are not exposed to the atmosphere prior to hit-picking.
- mosquito X1 enables the use of source and destination plates with different well densities during hit-picking.
- mosquito X1's multi-drop feature allows simple and fast creation of replicated assay wells or plates.

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