

mosquito[®] Crystal and mosquito[®] LCP: fast, reliable automation of protein crystallisation drop set-up

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introduction

The automation of protein crystallography screening has contributed significantly to the rapid progress of crystallography-based structural biology. Automation offers increased throughput and repeatability. It also offers the ability to accurately dispense smaller volumes of both protein and screen solutions, which saves valuable protein and reduces reagent costs.

Automation of protein crystallisation screening trials requires accurate placement of nanoliter volumes of protein and screen drops, in addition to the reproducible and accurate dispensing of solutions of varying viscosities. This is particularly important for the set-up of the highly viscous lipid mesophases in the Lipidic Cubic Phase (LCP) crystallisation technique for membrane protein crystallisation trials.

This poster describes the features of TTP LabTech's mosquito[®] Crystal and mosquito[®] LCP, showing their ability to address the issues inherent in the automated set-up of protein crystallisation screen trials. An instrument capable of automating both microbatch and vapour diffusion methods of protein crystallography (sitting drop, hanging drop) as well as crystallisation of membrane proteins using the bicelle and LCP methods, without instrument configuration changes, offers significant flexibility for the crystallography laboratory

mosquito Crystal and mosquito LCP offer fast throughput, high precision, unrivalled reproducibility and low volume accuracy.

1. mosquito[®] Crystal

mosquito[®] Crystal is a compact low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination.

mosquito is capable of pipetting volumes from 1.2 µL -25 nL with no washing required. It is ideal for all stages of crystal formation from primary screening set-up, optimisation, microseeding and final crystallisation set-up



2. mosquito[®] LCP

mosquito[®] LCP is a low volume liquid handling instrument that has been specifically designed to overcome the challenges presented by the LCP method and provide an automated solution to LCP screening techniques.

LCP is highly viscous, thus difficult to manipulate. This in turn makes the automation and the miniaturisation of the LCP crystallisation set-up very challenging.

With its unique features, mosquito[®] LCP solves these problems.



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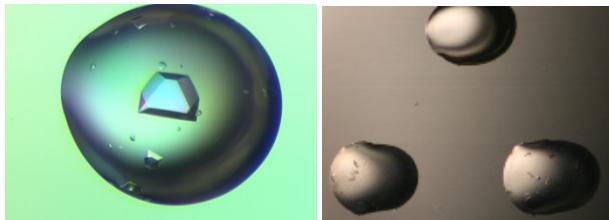
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3. automated hanging drop set-up

For automated hanging drop set-ups, the mosquito deck is loaded with an inverted, self-adhesive, hanging drop plate seal, a 96-well plate of screen buffers and reservoir(s) of protein sample, seed stocks or additive screens could also be added.

mosquito pipettes from, and into, plates one column at a time allowing protein solution to be aliquoted from a single source column to all 96 'windows' on the hanging drop plate seal. Droplets of the solutions in the screen plate are placed on top of the protein drops in a mirror image. The plate seal is then inverted using a simple alignment jig – placing droplets over their correct wells.



mosquito can set up single drops or multiple drops in one well.

mosquito's accuracy, repeatability and speed allows users to create several multi-component drops per well – even in hanging drop set-ups. Such drops allow different constructs, volume ratios or protein concentrations to be assessed at the same time.

This can yield 288 conditions in a single plate which can be set-up in less than 4 minutes.

4. automated sitting drop set-up

mosquito's X, Y and Z axes are accurately driven by a stepper motor with a resolution of <0.05 mm. This, along with the tightly tolerated and relatively short pipette tips, means that drops can be placed with a high degree of accuracy in the centre of the sub-wells of any standard crystallization plate.



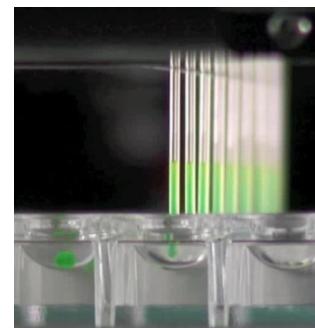
Triple well sitting drop iQ plate set up with protein, seeds and buffer in 200 nL total drop volume

mosquito Crystal advantages for hanging and sitting drop set-up include:

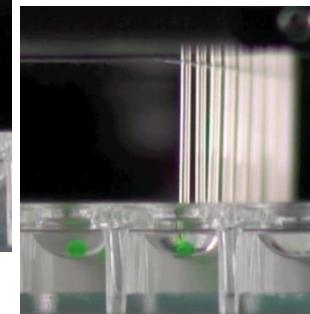
- Drop set-up completed in <2 minutes.
- Uses normal (inexpensive) flat bottomed 96-well plates and 96-well plate seal for hanging drops.
- Disposable pipettes for screen addition avoids slow washing steps and eliminates cross-contamination.
- Unrivalled low volume accuracy and repeatability.
- Highly accurate drop placement.
- Easy set-up of microseeding or additive screening in either sitting or hanging drops.
- Ability to rapidly set up multi-component drops for more expansive initial screening.

4. automated microbatch set-up

mosquito's multi-aspirate, dispense and drop mixing capabilities enables both protein and screen solutions to be aspirated consecutively within the same tip and then to be pipetted directly through oil for microbatch set-up. This ensures that screen and protein drops always combine.



mosquito dispensing a combined drop through oil for microbatch set-up

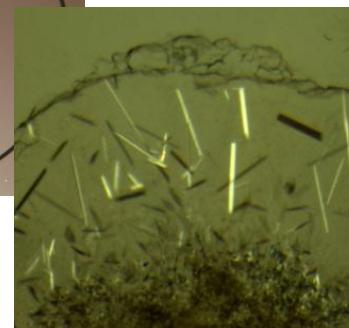


5. mosquito LCP: all the functionality of mosquito Crystal – and more

- mosquito LCP has all the functionality of the standard mosquito Crystal and enables zero loss dispensing of protein solutions or expensive/highly viscous additives.
- mosquito LCP enables both LCP and traditional crystallisation experiments to be set up in commercially available plates using the same instrument.
- Sample volumes as low as 25 nL can be applied at high precision resulting in conservation of valuable protein samples and additives.
- Accurate, auto-calibrated LCP syringe and pipette positioning permits highly precise drop-on-drop placement of the LCP solution which facilitates automated imaging.



LCP crystal image supplied by Janet Newman, C3, CSIRO, Australia



LCP crystal image supplied by Jim Fairman/Susan Buchanan, NIDDK, NIH, USA

conclusion

mosquito's positive displacement technology enables consistent and accurate nanoliter pipetting for repeatable drop setting irrespective of liquid viscosity and surface tension.

Disposable micropipettes handle a range of viscosities, even at very low volumes, eliminating carry over and cross-contamination risks to valuable protein or reagent stocks.

No instrument set-up changes are required for either mosquito Crystal or mosquito LCP to perform automated vapour phase, microbatch, LCP and bicelle set-ups, or for microseeding and additive screening. This can save researchers valuable time as well as offering significant flexibility.

Reliable and robust hardware, together with simple, user friendly software, make mosquito Crystal and LCP the ultimate tool for every crystallography laboratory.