Automating microseeding protein crystallography set-ups using mosquito®

Joby Jenkins, Rob Lewis, David Smith, Chloe Carter

TTP LABTECH

Introduction

Crystallising proteins, required for structure determination by X-ray diffraction, is a difficult and labour-intensive task. One of the many challenges facing the protein crystallographer is growing crystals of sufficient size and quality to successfully determine the protein's structure (this typically requires crystals of around 100-300 µm).

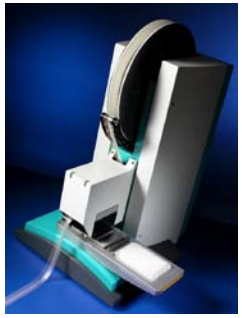
For structure based drug design a further challenge is being able to generate a sustainable crystal system able to produce liganded structures iteratively to support active chemistry.

Microseeding, where small crystals are crushed and suspended in a slurry of crystallisation buffer to produce new nucleation sites, is a recognised technique to improve crystal quality as well as promote the growth of larger, single crystals. However, it requires experimentation with varying concentrations of solutions to achieve successful results.

The mosquito® liquid handler (TTP LabTech) is ideally suited to automating the complex set-ups required for microseeding due to its precise handling of extremely low volumes of even viscous solutions, and its ability to perform multiple aspirations and dispenses with each pipette.

1. mosquito®

mosquito is a compact nanolitre pipetter that is ideally suited to automating all types of protein crystallography screening set-ups and has a number of advantages that facilitate the automation of microseeding.



2. Key Features of mosquito

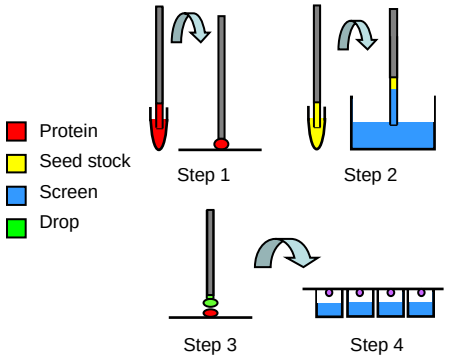
- instrument flexibility allows it to be used for both screening and optimisation without set-up changes
- nanolitre volume range: 25 nL–1,200 nL (or lower if 'multi-aspirate' functionality is used)
- aspirate, dispense and drop mixing capabilities enable easy set-ups for seeding and microseeding
- liquids of vastly varying viscosities can be handled accurately and without set-up changes or recalibration
- disposable positive displacement pipettes guarantee zero cross-contamination of even the stickiest samples
- excellent repeatability and accuracy: CVs of < 6% at 50 nL and < 4% at 100 nL
- intuitive operation and programming are ideal for a multi-user environment
- negligible dead volumes lead to reduced protein and seed stock wastage
- no need to prime so the instrument can be used minutes after switching on.

mosquito's multi-aspirate and dispense properties permit it to pick seed stock followed by the screen and then dispense a drop precisely on top of a protein drop (mixing if required) for subsequent vapour diffusion. Alternatively, all three drop components (protein sample, seed stock and reservoir solution) can all be aspirated sequentially and dispensed simultaneously, mixing if required.

mosquito can multi-aspirate and accurately dispense three different drops into each well of a 96-well hanging drop screen.

3. Automated Hanging Drop Microseeding Set-ups

mosquito can perform hanging drop, sitting drop and microbatch plate set-ups without instrument configuration changes. For automated microseeding hanging drop set-ups, the mosquito deck is loaded with an inverted hanging drop plate seal, a 96-well plate of screen buffers and reservoirs of protein sample and seed stock. The protocol is as follows:



Step 1: mosquito aspirates protein from the protein reservoir and multi-dispenses 100 nL drops across the substrate sheet.

Step 2: mosquito aspirates 10 nL of seed stock from the reservoir, then moves to the first column of the screen plate and muliti-aspirates 90 nL of screen.

Step 3: The combined drop is dispensed accurately on top of the first column of protein drops on the plate seal from step 1. Steps 2 & 3 are repeated to create a mirror image of the screen plate on the plate seal.

Step 4: the plate seal is then inverted over the screen plate (using a simple alignment jig), so that each protein + seed stock + screen droplet hangs over the corresponding screen well.

The entire set-up time is less than 3 minutes and no protein or seed stock is wasted.

4. Seeding and Growth

mosquito was used to automate a sitting drop plate set-up using the same conditions as the hanging drop experiment. Varying amounts of seed stock were added to the sitting drops, and the resultant crystal growth was monitored.

a) no seed stock was added, and this resulted in no nucleation

b) 10 nL of seed stock was added which promoted nucleation and crystal growth

c) over-seeding resulted in over nucleation and the growth of plate crystals

Conclusion

Seeding is a well established method that can improve the quality and reproducibly of crystals and can easily be accommodated in protocols for the mosquito robot. Automating seeding set-ups using mosquito offers the following benefits:

- improved nucleation and crystal growth
- aspirate, dispense and mix functions for automated drop set-up
- excellent repeatability and accuracy
- reduced protein and seed stock wastage.

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TTP LabTech Ltd, Melbourn Science Park, Melbourn, Royston, Herts, SG8 6EE, UK Phone +44 1763 262626 sales@ttplabtech.com