

Dear Stefan

New data-collection methods sometimes require greater control or reproducibility of crystallization conditions. Here are some useful ways this can be achieved with an Oryx:

Microseeding

Microseeding is recommended as part of the screening stage if you have crystals of a related protein to make seed stock (rMMS microseeding). Oryx robots can dispense volumes of seed stock down to 5 nL to a drop. Microseeding can be used to:

- Increase the number of crystallization conditions
- Cross-seed using seeds of a related protein
- Identify new and sometimes completely different crystallization buffers
- Improve the quality of crystals

Microseeding can also be used to control the number of crystals in a drop. The metastable zone (see below) can be identified by comparing experiments with and without seeds. Crystals only grow in conditions in the metastable zone when seeds are added. Moreover, the number of crystals can be controlled by diluting the seed stock. Different dilutions of seed stock can be systematically tested using the **Cross-Matrix optimization** script.



Microbatch-Under-Oil

Crystals usually grow much quicker with modern microseeding methods – typically within 24 hours. By sealing the drop with oil, you can be sure of the exact crystallization conditions, and so increase control. Oil is added to cover the drop automatically with an Oryx robot. For **microbatch crystallization** experiments, typically light paraffin oil is used to cover the drop,

which almost completely prevents equilibration. Oil can reduce the amount of skin forming on the drop interface. Also, as the drop concentration remains constant, conditions can be scaled with greater reproducibility. If you do want to allow evaporation from the drop, Al's oil (50:50 paraffin and silicon oil) and other mixes of oils can be used to vary the rate of evaporation.

Additive screening

Additive screening (including fragment screening) can combined with microseeding to increase control and improve reproducibility. Oryx8 systems can be used to dispense 4 ingredients simultaneously to the drop (protein, precipitant, additive screen and seed stock). Vapor diffusion drops can also be covered with oil to control the rate of equilibration.

Video on YouTube: How to make seed stock for rMMS



Coming in 2024 - a new plate for Microbatch:

- We are excited to announce that a new plate for microbatch-under-oil is currently under development
- Details will be announced soon

Conferences:

Douglas Instruments is attending the following meetings:

BCA Spring Meeting, Leeds

25 March - 28 March 2024

ACA Annual Meeting, Denver

7 July - 12 July 2024

ECM 34, Padova

Visit our booth for:

Live demonstrations rMMS microseeding toolkits Updates about our latest projects including crystallization methods for serial crystallography.

Recently published research using Oryx protein crystallization robots:

Ethoxylate Polymer-Based 96-Well Screen for Protein Crystallization

Demmer, U., Lemaire, O.N., Belhamri, M. and Ermler, U., 2023.

Crystals, 13(10), p.1519.

Arginine cluster introduction on framework region in anti-lysozyme antibody improved association rate constant by changing conformational diversity of CDR loops

Maeta, S., Nakakido, M., Matsuura, H., Sakai, N., Hirata, K., Kuroda, D., Fukunaga, A. and Tsumoto, K., 2023.

Protein Science, 32(9), p.e4745.



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