For more information

Request an online demonstration or webinar for your lab

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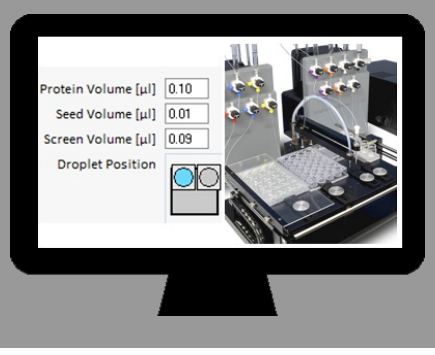


Douglas Instruments
Success in protein crystallization

rMMS Microseeding

Dear Crystallographer

Get the most out of your rMMS experiments

Random Microseed Matrix Screening 'rMMS', where a seed stock is added along with protein to a 'random' screen (D'arcy et al., 2014) should be used routinely as part of the screening stage and can be used whenever candidate seed crystals are available (see below).

Which crystallization screen should I use?

Use a screen that you have already used with your target protein without seeding. This will allow you to identify conditions that rely on seeding - i.e. crystals that only grow with seeds. Focus on these conditions - you will have much more control. Any commercial or homemade screening kit can be used - the more 'random' the better.

How should I make the seed stock?

Use a crystal crusher and seed bead to prepare the seed stock (available from Hampton Research). Consider combining diverse crystals from multiple wells to make a more powerful seed stock in the early stages of a project. A video showing how to produce the seed stock is below.

How can I avoid salt crystals?

If your crystallization 'hit solution' contains less-soluble salts such as phosphate, calcium or magnesium, it is likely that salt crystals will form when the seed stock is dispensed to a random screen. To avoid salt crystals, try to choose a crystal hit that does not contain less-soluble salts. If your crystal hit is in a condition that includes less soluble salts, check the stability of your crystals in a solution without these components (e.g. suspend the seeds in PEG only). For more information see Shaw Stewart et al., 2011.

Can I use 'cross-seeding'?

If you have crystals of a related or homologous protein, you can certainly use these to cross-seed to a similar protein. If you have multiple candidate crystals, it may be worth preparing multiple seed-stocks. In some cases, cross-seeding works better than self-seeding (Obmolova *et al.,* 2014).

References

[1] D'Arcy, A., Bergfors, T., Cowan-Jacob, S.W. and Marsh, M., 2014
Microseed matrix screening for optimization in protein crystallization: what have we learned?
Acta Crystallographica Section F: Structural Biology Communications , *70*(9), pp.1117-1126.

[2] Shaw Stewart, P.D., Kolek, S.A., Briggs, R.A., Chayen, N.E. and Baldock, P.F., 2011
Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization
Crystal Growth & Design, *11*(8), pp.3432-3441.

[3] Obmolova, G., Malia, T.J., Teplyakov, A., Sweet, R.W. and Gilliland, G.L., 2014

Protein crystallization with microseed matrix screening: application to human germline antibody Fabs

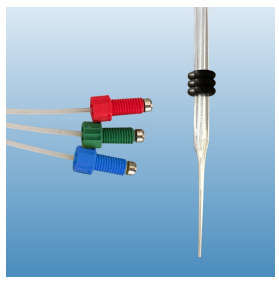
Acta Crystallographica Section F: Structural Biology Communications, 70(8), pp.1107-1115

Request more information

Douglas Instruments Products

Oryx Robots

Crystallization Plates



Oryx Microtips

Conferences:

Douglas Instruments is attending the following meetings:

ACA 2021
30 July - 5 August 2021

IUCr 2021, Prague, Czech Republic
14 August - 22 August 2021

Recently published research using Oryx protein crystallization robots:

Porous assembly of an antifungal protein mediated by zinc and sulfonato-calix [8] arene.

Guagnini, F., Huber, A., Alex, J.M., Marx, F. and Crowley, P.B., 2021.

Antiviral research, *185*, p.104971.

Structural characterization of the interaction between the C-terminal domain of the influenza polymerase PA subunit and an optimized small peptide inhibitor

Hejdánek, J., Radilová, K., Pachl, P., Hodek, J., Machara, A., Weber, J., Řezáčová, P., Konvalinka, J. and Kožíšek, M., 2021.

Antiviral research, *185*, p.104971.