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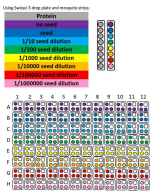
Diamond XChem Seeding Protocol



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ASAP Discovery | OpenBind Consortium

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We use this protocol and it's working

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Disclaimer

N/A

Abstract

The use of seeding in protein crystallisation is a highly effective method for increasing the quantity of usable crystals from a single plate and can be crucial for obtaining crystals in a highly reproducible manner. Even crystallisation experiments which did not previously require seeding can benefit from the use of seeds when transferring protocols between labs.

Image Attribution

N/A

Guidelines

N/A

Materials

Formulatrix Protein Crystallization Imager
SPT mosquito
P10 pipette
SwissCI 3 lens plate
Seed beads
Crystalline material
Vortex

Safety warnings

Follow safety guidlines in regards to the chemicals present in the protein buffer and crystallisation condition.

Ethics statement

N/A

Before start

Prepare crystalline material needed to produce crystals.

Equiptment needed

1 Formulatrix Protein Crystallization Imager
SPT mosquito
P10 pipette
SwissCI 3 lens plate [UVXPO-3LENS3W96T-PS3W96T-UVP]
Seed beads [Hampton HR4-782]
Crystalline material
Vortex
0.5 mL Eppendorf tubes [Eppendorf Catalog No. 0030121023]

Seeding experiment

17m 40s

2 Produce crystals to use for seeding. Micro crystalline protein material can be used to produce a seed stock as well.

3 Set up the serial dilution for step 10 using the reservoir solution used to obtain the starting crystalline material On ice . Make up an appropriate volume; most seeding uses 20-50 nL of seed stock, meaning a SwissCI 3 lens 96 well plate could require ~ 14.5 µL of seed stock per plate.

5m

4 Add seed beads to the undiluted tube in the serial dilution (Check manufacture instructions).
For Hampton glass beads, use 2 beads in a 0.5 mL Eppendorf tube

1m

5 Under a microscope, open the well containing the crystal material being used and crush the crystals. **From this step onwards proceed rapidly as the seed stocks are metastable and need to be frozen as soon as possible.**

1m

6 Pipette 2 µL from the reservoir well into the drop well

20s

7 Mix the solution by aspiration a minimun of three times

10s

8 Transfer to the first tube set up for the serial dilution that will remain undiluted and is marked "undiluted seed stock"

10s

9 Repeat steps 5-7 until there is no more crystal in the drop well. To ensure all available crystal material is recovered, a minimum of around 30 µL of reservoir solution

2m

should be used in this process

10 Vortex the undiluted tube containing the seed beads – 30 seconds on vortex then 30 seconds on ice, repeating three times. Take 2 µL of the seed stock and check under microscope whether crystals have been crushed. 3m

11 Carry out the set up serial dilution using the undiluted seed stock and freeze stocks at -80 °C 5m

Interpreting results

12 Based on what your project needs, decide which condition made from the serial dilution makes the best results with good reproducability. If needed, further optimise around the dilution e.g. if 1/100 works best, try using 1/20, 1/50 and 1/200; repeat until crystals are acceptable for your project and its timeframe.

Protocol references

Further reading:

Procedure for making the microseed stock
Hampton Research Seed bead user guide
A method to produce microseed stock for use in the crystallization of biological macromolecules