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XChem crystallographic fragment screening

Forked from XChem solvent test

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We use this protocol and it's working

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Disclaimer

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Abstract

The XChem Facility at Diamond Light Source provides routine, large scale crystal graphic fragment screening, supporting the entire crystal to deposition process. X-ray crystallography is an essential part to the FBDD toolkit. It is sensitive enough to identify weak binders and directly yields structural information about the interactions on a molecular level. Here we summarise a generic layout of a crystallographic fragment screening protocol.

Guidelines

N/A

Materials

SwissCI 3 lens crystallization plates **https://swissci.com/product/3-lens-crystallisation-plate/** Codes: Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

Uni Pucks https://moleculardimensions.com/en/product/MD7-601 Codes: Catalog # MD7-601
Uni Wand https://moleculardimensions.com/en/product/MD7-411 Codes: Catalog # MD7-411
Loops https://moleculardimensions.com/en/category/mounted-litholoops Codes: Catalog # MD7-131, Catalog # MD7-133, Catalog # MD7-135, Catalog # MD7-138

DMSO https://www.sigmaaldrich.com/GB/en/substance/781367685 Codes: **D5879**
Glycerol https://www.sigmaaldrich.com/GB/en/substance/920956815 Codes: **G5516**
Ethylene Glycol https://www.sigmaaldrich.com/GB/en/substance/6207107211 Codes: **324558**

Safety warnings

Follow all handling warning for the chemicals use.

Use of cryogen liquid nitrogen; cold burns can occur with exposure of skin to very low temperatures, contact with eyes can cause permenant damage, evaporatin liquid nitrogen can cause oxygen depletion, asphixia and death. Wear appropriate footware and PPE when handling including cryo gloves and safety glasses. Use apportiate volumes of liquid nitrogen for the ventialtion of the laboratory while in use.

Ensure centifuge is properly balanced when used.

Before start

Grow any necassary crystal plates in sitting drop plates.

Imaging

1 Image all sitting drop crystal plates required for the experiment in a crystal plate imagers.

Selecting crystals

2 Based on the images, select drops with suitable crystals for the experiment. Fragment dispensing is done in singletons. i.e. one compound per drop of crystals. Ensure enough drops for the total number of compounds required for the experiment.

3 If you have a targeting system, target the drops for dispensing compound, not directly targeting the crystals directly to avoid damaging crystals.

Fragment dispensing

3h 2m

4 Based on the results of your solvent test, calculate the % v/v required of compound to dispense into crystal drops. This percentage chosen will now include compound to dispense into crystal drops.

Note

When changing from solvent to compound, the tolerance of crystals often is reduced, so the optimal soaking time may need to be lowered by 5% v/v compared to the solvent test results.

Protocol

NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

5 Take the compound plate containing the fragments and spin the plate in a benchtop centrifuge for 00:02:00 at 200 x g . 2m

6 Dispense the required volume of compound into the corresponding crystal drop.

7 Store the plate in the incubator for 01:00:00 to 03:00:00 depending on the results of your solvent test at either 4 °C or 20 °C depending on the crystal growth temperature. 3h

Harvesting crystals

8 In preparation for crystal harvesting, if cryoprotectant is required add the necessary volume of cryo onto crystal drops just before full soaking time has ended.

9 Select a tray of the appropriately sized loops for the crystals (35 µm , 75 µm , 150 µm or 300 µm). Importantly, choose a loop size that matches the size of the crystal as closely as possible to improve the data quality.

10 Place the pucks in a foam dewar and cool them down with liquid nitrogen.

Safety information

Wear appropriate PPE when handling liquid nitrogen.

11 If the crystal has survived, mount the crystal in the loop and plunge into the liquid nitrogen, placing the sample into a puck.

12 If the crystal has been successfully mounted, record the success and continue to the next sample. Otherwise record the failure and continue to the next sample until all samples that can be harvested have been mounted.

13 Place lids on the pucks and store.

14 Send the pucks to be collected.

Protocol references

Douangamath, A., Powell, A., Fearon, D., Collins, P. M., Talon, R., Krojer, T., Skyner, R., Brandao-Neto, J., Dunnett, L., Dias, A., Aimon, A., Pearce, N. M., Wild, C., Gorrie-Stone, T., von Delft, F. Achieving Efficient Fragment Screening at XChem Facility at Diamond Light Source. *J. Vis. Exp.* (171), e62414, doi:10.3791/62414 (2021)

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