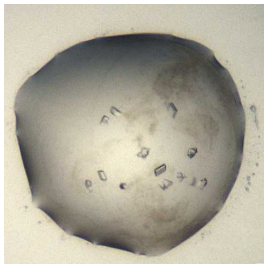
Jun 05, 2025 Version 2

Crystallisation of Enterovirus D68 3C protease V.2



Forked from a private protocol

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzjp45lx1/v2

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Ryan Lithgo: The principle crystallographer on the Enterovirus 3C protease;

ASAP Discovery

Peter Marples
Diamond Light Source

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Oxford Lab Technologies crystal shifter https://doi.org/10.1107/S2059798320014114

Abstract

The development of effective broad-spectrum antivirals forms an important part of preparing for future pandemics. A cause for concern is the currently emerging pathogen Enterovirus D68 (EV-D68) which primarily spreads through respiratory routes causing mostly mild to severe respiratory illness but, in severe cases, acute flaccid myelitis. The 3C protease of EV-D68 is a potential target for the development of antiviral drugs due to its essential role in the viral life cycle and high sequence conservation. This protocol was used to grow D68 3C ProB crystals that were applied high-throughput crystallographic follow up compound screening on D68 3C. In this version we correct the protein expression and purification protocol, and also added the Addgene id. Also, this protocol should be deprecated and we will add modifications to the following protocol.
https://www.protocols.io/view/asap-protocol-crystallization-of-enterovirus-d68-3-n92ldm6jnl5b/v1?version_warning=no

Materials

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

[M] 1 Molarity (M) Tris adjusted to pH 7.8 with NaOH, Molecular Dimensions, Catalog # MD2-027-PH 7.8
[M] 1 Molarity (M) Ammonium acetate, Molecular Dimensions, Catalog # MD2-002-PH
50% w/v PEG 3350, Molecular Dimensions, Catalog # MD2-250-9

Purified D683C protein ([M] 35 mg/mL) in [M] 10 millimolar (mM) HEPES, pH 7.5 , [M] 0.5 Molarity (M) NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

Protocol materials

Enterovirus D68 (EV-D68) strain EV-D68 STL 2014 12 3C protease. **addgene Catalog #**228644

Safety warnings

Follow all handling warning for the chemicals used in the crystalllisation screen composition.

Enterovirus D68 3C protease expression and purification

1 The protein used for crystallisation was expressed and purified using the following protocol.

Enterovirus D68 (EV-D68) strain EV-D68 STL 2014 12 3C protease. **addgene Catalog** #228644

Protocol

NAME

Small-scale Expression and Purification Protocol for His-SUMO Tagged Enterovirus D68 Strain STL 2014 12 3C Protease in E. coli

CREATED BY

korvus.wang

Preview

Equipment needed

2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito

Equipment

Mosquito HV	NAME
High Volume 16-Channel Robotic Liquid Handler	TYPE
SPT LabTech	BRAND
3097-01057	SKU
https://www.sptlabtech.com/products/liquid-handling/mosquito-hv/	LINK

P100 8 multi-channel pipette
SwissCI 3 lens plate

Crystallisation experiment

1d

3 **Prepare seed stock:**

Protocol

NAME
Diamond XChem Seeding Protocol

CREATED BY
Peter Marples

Preview

1: 1 000 000 dilution Sample seeds

4 **Protein and buffer requirements:**

14.4 µL [M] 35 mg/mL Sample
2.88 mL Crystallisation screen
7.2 µL seeds, dilution 1:1 000 000

5 **ccCrystallisation screen composition:**

[M] 0.1 Molarity (M) Tris pH 7.8

[M] 0.2 Molarity (M) Ammonium acetate
26% w/v PEG 3350

Stock solutions used:
[M] 1 Molarity (M) Tris adjusted to pH 7.8 with NaOH
[M] 1 Molarity (M) Ammonium acetate
50% w/v PEG 3350

Note

The crystallisation screen can be stored in a duran bottle or aliquoted into 96 deep well block for easy dispensing into SwissCI 3 lens plates.

For long term storage keep the crystallisation screen in the fridge at 4°C.

6 Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 50 µL [M] 35 mg/mL Sample to each lens using the SPT mosquito.
Dispense 100 µL Crystallisation screen to each lens using the SPT mosquito.
Dispense 25 µL Seeds to each lens using the SPT mosquito.

Drop ratio: 2:4:1
Final drop volume: 175 nl

7 Incubate at 20 °C for 24:00:00 h in Formulatrix Rock Imager. 1d

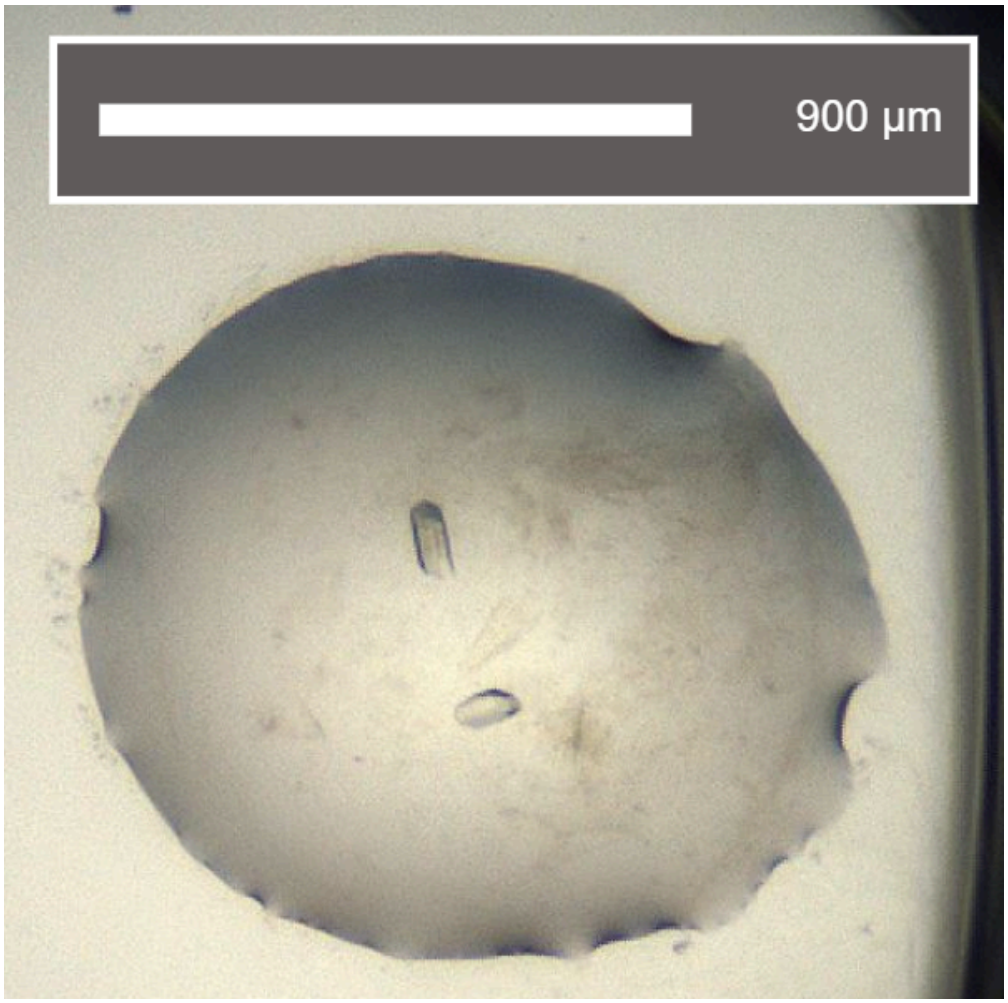
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

8

Expected result

Crystals typically appear after 24 hours and reach their maximum size after ~24 h with some precipitation often remaining.

Morphology: small shards.
Size: ~40 µm in length and ~40 µm in width, depth of the crystals is ~20 µm, giving a glass shard appearance
Average resolution: 1.5 Å
Space group: $P2_1$
Unit cell: 39.7 Å, 105 Å, 43.5 Å
90.00°, 110.00°, 90.00°



An example of a drop containing D68 3C protease crystals.

Data collection at Synchrotron

9 Diamond Light Source
Unattended Data Collection (UDC)
Data Collection Temperature: 100K
Detector: DECTRIS EIGER2 X 9M
Beamline: I04-1
Wavelength: 0.9212 Å
Resolution (Å): 1.62
Beam Size (µm): 60 X 50
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0020
Transmission (%): 100
Flux (ph/s): 9.50e+11

Protocol references

Crystallographic fragment screen of Enterovirus D68 3C protease and iterative design of lead-like compounds using structure-guided expansions, https://doi.org/10.1101/2024.04.29.591650