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We use this protocol and it's working

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Cryo-EM Grid preparation

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ABSTRACT

This protocol details Cryo-EM grid preparation.

ATTACHMENTS

852-2198.pdf

MATERIALS

Materials

- PI3KC3-C1~RAB1A sample with n-Octyl-β-D-Glucopyranoside (OG) at a final concentration of 0.05%
- QUANTIFOIL R 2/1 mesh Cu 300 holey carbon grids
- Vitrobot cryo-plunger (Thermo Fisher Scientific)
- Cryo-EM microscope (300 kV Titan Krios with X-FEG and energy filter)
- K3 Summit direct electron detector (Gatan)
- SerialEM software
- Liquid nitrogen
- Humidity control system

Grid Preparation 1m

1 Freshly glow-discharge the QUANTIFOIL R 2/1 mesh Cu 300 holey carbon grids using a glow-discharge system (PELCO easiGlow) at 25 mA current for 00:01:00. 1m

Sample Application

2 Mix the PI3KC3-C1~RAB1A sample with 0.05% n-Octyl-β-D-Glucopyranoside (OG) to achieve the desired final concentration.

3 Apply a small volume (typically 3-4 µL) of the sample onto the glow-discharged side of the grids.

Vitrification 3s

4 Load the grids with the sample into the Vitrobot cryo-plunger.

5 Set the Vitrobot to the following conditions:

5.1 Maintain 100% humidity inside the chamber.

5.2 Set the temperature to 4 °C.

5.3 Wait for 00:00:03. 3s

5.4 Blot using a blotting force of -15 (may need calibration depending on your particular instrument) .

Data Acquisition Setup

6 Prepare the Cryo-EM microscope (300 kV Titan Krios) for data acquisition.

7 Used X-FEG and an energy filter set to 20 eV.

Data Collection Parameters

8 Use SerialEM software for automated data collection.

9 Set the following parameters for data collection:

- Magnification: 81,000x.
- Super-resolution pixel size: 0.525 Å.
- Defocus range: -0.8 to -2.2 micrometers.
- Collect 50-frame image stacks.

Data Collection

10 Initiate the data collection process using the specified parameters.

11 Monitor the data acquisition progress and ensure that the images are being recorded.

Cumulative Dose Control

12 Each movie has a cumulative electron dose of 50 e/Å².