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Sample Preparation for FLASH-PAINT

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Devin M. Fuller[1,2], Florian Scheuder[1], Thomas Melia[1,2]

[1]Department of Cell Biology, Yale University School of Medicine, New Haven, CT;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, 20 MD

Devin M. Fuller
Yale University

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Abstract

A protocol in how to prepare and image your samples using FLASH-PAINT

Materials

The
following reagents were purchased for FLASH-PAINT: Cy3B-modified DNA oligonucleotides were custom-ordered from IDT. Sodium chloride 5 M (cat: AM9759) were obtained from Ambion. Ultrapure water (cat: 10977-015) was purchased from Invitrogen. µ-Slide 8-well chambers (cat: 80807) were purchased from ibidi. Methanol (cat: 9070-05) was purchased from J.T. Baker. Glycerol (cat: G5516-500ml), protocatechuate 3,4-dioxygenase pseudomonas (PCD) (cat: P8279), 3,4-dihydroxybenzoic acid (PCA) (cat: 37580-25G-F) and (+−)-6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox) (cat: 238813-5 G) were ordered from Sigma. 1× Phosphate Buffered Saline (PBS) pH 7.2 (cat: 10010-023) was purchased from Gibco. Paraformaldehyde (cat: 15710) were obtained from Electron Microscopy Sciences. Bovine serum albumin (cat: 001-000-162) was ordered from Jackson ImmunoResearch. Triton X-100 (cat: T8787-50ML) was purchased from Sigma-Aldrich. DNA labeled Nanobodies were obtained from Massive Photonics.

Sample Preparation Protocol

1 Fix cells with fresh 3% PFA for 30 min at RT.

2 Immobilization and block with 3% BSA and 0.1% Triton-X.

3 Incubate sample with the desired antibodies with the lowest binding efficiency, one per organism in blocking solution (3% BSA + 0.1% Triton-X) overnight at 4°C (1:300 dilution as always). For example, we incubated our samples with the GFP Nanobody, ATG13 AB (rabbit) and WIPI2 AB (mouse).

4 For the remainder of the antibodies, prepare a preincubation: Mix 0.5 µL of primary AB with 5µL of 1xPBS and 2µL of nanobody in a PCR tube and let it incubate overnight at 4°C. The following substeps illustrate the antibodies that we used with their corresponding nanobody (NB), each of which was conjugated to a unique DNA barcode:

4.1 0.5 µL of LC3B-AB + 2 µL of 2nd NB-A20 + 5 µL 1xPBS

4.2 0.5 µL of ATG9A-AB + 2 µL of 2nd NB-A5 + 5 µL 1xPBS

4.3 0.5 µL of P62-AB + 2 µL of 2nd NB-A36 + 5 µL 1xPBS

4.4 0.5 µL of NBR1-AB + 2 µL of 2nd NB-A19 + 5 µL 1xPBS

4.5 0.5 µL of FIP200-AB + 2 µL of 2nd NB-A8 + 5 µL 1xPBS

4.6 0.5 µL of ATG13-AB + 2 µL of 2nd NB-A15 + 5 µL 1xPBS

4.7 0.5 µL of Syntaxin17 + 2 µL of 2nd NB-A38 + 5 µL 1xPBS

4.8 0.5 µL of ARFGAP1-AB + 2 µL of 2nd NB-A10 + 5 µL 1xPBS

4.9 0.5 µL of LAMP1-AB + 2 µL of 2nd NB-A39 + 5 µL 1xPBS

5 On the next day block incubate sample with the 2nd nanobodies corresponding to the antibodies that we incubated in the sample overnight for 1 hour at room temperature (e.g. NB-A25 26 2nd NB-A27 corresponding to ATG13 AB and WIPI2 AB).

6 Also on the next day block PCR tube mixes with 4 µL of unlabeled NB for 5 min.

7 Pool the content of all PCR tubes and put it on the sample for 2.5 h at room temperature.

8 Wash four times with 1x PBS for 30 seconds, 1 minute, and twice for 5 minutes each.

9 Post-fix the cells with 3% PFA and 0.1% GA for 10 minutes.

10 Wash four times with 1x PBS for 30 seconds, 1 minute, and twice for 5 minutes each.

Imaging on the microscope

11 Introducing the desired adapter (for example, A19-R2 (20 nM) to image NBR1) and the imager R2 (1 nM) in Buffer C.

12 After image acquisition, rinse 3 times with 1× PBS.

13 Erase: Add 200 µL of E19-R2 eraser (100 nM in Buffer C) to the sample and incubate for 5 min.

14 Next, introduce the adapter for the second target, e.g. FIP200: use A8-R2 (20 nM) and the R2 imager (1 nM).

15 After image acquisition, rinse 3 times with 1× PBS.

16 Erase: Add 200 µL of E8-R2 eraser (100 nM in Buffer C) to the sample and incubate for 5 min.

17 Iterate this process until all targets are imaged.

Protocol references

https://www.cell.com/cell/fulltext/S0092-8674(24)00236-8

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