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Expansion microscopy with R1441C LRRK2 MEF cells: visualization of Myc-RILPL1 and TMEM55B

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We use this protocol and it's working

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Abstract

Expansion microscopy is a super-resolution imaging technique that uses expandable hydrogels to increase the physical distance between fluorophores from fixed cells on coverslips. By isotropically expanding samples, the traditional wavelength-limited confocal microscopy imaging techniques can be improved upon ~4X in resolution. Here we use expansion techniques developed previously by others to confocal image TMEM55B and RILPL1. See below for more details and prior methods.

Citation

Laporte MH, Klena N, Hamel V, Guichard P (2022)
. Visualizing the native cellular organization by coupling cryofixation with expansion microscopy (Cryo-ExM).. Nature methods.

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Citation

Truckenbrodt S, Sommer C, Rizzoli SO, Danzl JG (2019)
. A practical guide to optimization in X10 expansion microscopy.. Nature protocols.

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Materials

Materials

Paraformaldehyde 16% (Electron Microscopy Sciences 50-980-487)
Sodium Acrylate (Pfaltz & Bauer 50-750-9773)
Bis-acrylamide (VWR 97061-138)
Acrylamide (Alfa Aesar A17157)
PBS (Cold Spring Harbor protocol)
6 Well Glass Bottom Plate (Cellvis P06-1.5H-N)
Microscope cover glass (Fisher 12-545-81)
FuGENE 6 transfection reagent (Promega E2691)
myc-RILPL1 plasmid
TMEM55b polyclonal antibody (Proteintech 23992-1-AP)
Myc antibody (Biolegend 626802)
Donkey anti-Rabbit 568 Alexa Fluor (Thermo Fisher A10042)
Donkey anti-Mouse 488 Alexa Fluor (Thermo Fisher A-21202)
NaCl (Fisher S271-500)
Omnipure Tris (Milipore 9210-500gm)
Sodium Dodecyl Sulfate (Sigma L3771)
DMEM high glucose (Cytiva SH30243.01)
Fetal bovine serum (Sigma #F0926)
MLi-2
Spinning disk confocal microscope

Solutions

4% PFA
Make from 16% stock of PFA in PBS

AA-FA solution
1% Acrylamide and 0.7% PFA in PBS
Prepare fresh.

Monomer solution (MS) (19% sodium acrylate* 10% acrylamide 0.1% N,N′-methylenebisacrylamide in x1 PBS)
19% sodium acrylate*
10% acrylamide
0.1% N,N′-methylenebisacrylamide
In PBS
*Proper gel formation is sensitive to the quality of sodium acrylate. Sodium acrylate must be fresh and high quality.

Stocks to prepare fresh:
38 % (w/v) sodium acrylate in DI water
50 % (w/v) acrylamide in DI water

2% (w/v) Bis-acrylamide in DI water
* It will take time to dissolve each component in water. Putting tubes on a rotator at room temperature for the duration of dissolution can be helpful.

Can store aliquots of monomer solution in -20ºC.

Gelation solution
Monomer solution
Add 10% TEMED in DI water (final 0.5%)
Add 10% APS in DI water (final 0.5%)

Denaturation Buffer
200mM SDS
200mM NaCl
50 mM Tris pH 9.0
In DI water

Troubleshooting

Transfection of Myc-RILPL1 in LRRK2 R1441C MEF cells

1 Seed LRRK2 R1441C MEF cells at 50-60% confluency on 12 mm glass coverslips in a 24 well plate in 500 µL of complete DMEM (DMEM containing 10% FBS and 1% penicillin-streptomycin) 24 hours before transfection.

2 Transfect cells with Myc-RILPL1 plasmid using FuGENE 6 transfection reagent (E2691) at a 3:1 (3 µl FuGENE:1µg plasmid) ratio according to manufacturer's guidelines.

3 Allow cells to attach on coverslips in a 37 °C incubator with 5% CO_2 for ~ 32:00:00 . 1d 8h

4 For MLi-2 conditions, add [M] 200 nanomolar (nM) MLi-2 and leave cells in the incubator for 01:00:00 before continuing. 1h

Expanding cells in gels

5 Aspirate media and wash cells on coverslips by performing three quick 1 mL washes with PBS.

6 Fix cells by incubating them in PBS containing 4% PFA for 00:10:00 at Room temperature . 10m

7 Incubate cells in AA-FA solution Overnight at 37 °C . 16h
During this incubation, thaw monomer solution (MS) and keep it on ice. Pre-cool gelation chamber at 4ºC (we use an old 1 mL pipette-tip box with added water for humidity or a 10 cm Petri dish with moistened Kimwipes and Parafilm), and prepare a 10% TEMED and a 10% APS solution diluted in DI water. Keep all solutions On ice .

8 To make Gelation solution (80 µl per coverslip; recipe below makes enough for ~5 coverslips):

i. Monomer solution	378 µL
ii. Add 10% TEMED (final 0.5%)	21 µL
iii. ADD 10% APS (final 0.5%)	21 µL

Note

*Do not add APS to the final gelation solution until you are in the cold room, because polymerization will occur quickly

9 In the cold room, add 80 µL droplets of gelation mixture onto parafilm in a pre-cooled gelation chamber.

Note

Keep solution on ice and work quickly as the solution will polymerize quickly.

10 Place coverslip (cell-side facing down) onto droplet making sure the coverslip is parallel to the parafilm. Wait 5 minutes in the cold and then transfer the chamber containing the coverslips to a 37 °C room for 01:00:00 Be careful not to disturb the coverslip and gel.

1h

11 Gently remove coverslips and gel from the parafilm and place each coverslip in a 6cm dish containing 5 mL denaturation buffer.

Note

The gel should be firmly attached to the coverslip at this step and it should be possible to cleanly lift the coverslip and gel off the Parafilm.

12 Incubate gels and coverslips fully submerged in denaturation buffer for 00:15:00 at room temperature with gentle rotation. This step is necessary to cleanly remove gels from the coverslips.

15m

13 Place each gel into a 1.5 mL tube also filled with 1mL fresh denaturation buffer. Incubate at 95 °C using a heat block for 01:30:00 .

1h 30m

14 Remove gels from the 1.5 mL tube and place them each into a 10 cm petri dish with enough DI water to completely cover the gel (~10mL). Incubate the gels twice for 00:30:00 per incubation on a gentle rotator at Room temperature, replacing the water each time. Perform a final incubation with fresh DI water Overnight at Room temperature with gentle rotation. 1h

15 The next day, gels should appear expanded. Incubate gels with enough PBS to completely cover the gel (~10mL) three times for 00:30:00 each time at Room temperature with gentle rotation. Gels should shrink significantly, but will not shrink back to their original size. 30m

Antibody Staining to Visualize Myc-RILPL1 and TMEM55B 2h 15m

16 Incubate gels with primary antibody (anti-TMEM55b (1:500; 2µl); Anti-Myc (1:250; 4µl) diluted in 1 mL PBS containing 2% BSA) using a 1.5 mL tube Overnight at Room temperature on a rotator. 30m

17 Wash gels three times with enough PBS-T (PBS + 0.1% Tween20) to completely cover the gel (~10mL) for 00:15:00 each time at Room temperature using a 10 cm petri dish with gentle rotation. 15m

18 Incubate with secondary antibodies (Donkey anti rabbit 568 (1:500; 2µl), donkey anti-mouse 488 (1:500, 2µl) diluted in 1 mL PBS containing 2% BSA) with gentle rotation Overnight at Room temperature in a 1.5 mL tube. 15m

19 Wash gels three times with enough PBS-T (0.1% Tween20) to completely cover the gel (~10 mL) for 00:15:00 each at Room temperature using a 10 cm petri dish with gentle rotation. 15m

20 Wash gels twice with enough DI water to completely cover gel (~10mL) for 00:30:00 each at Room temperature, rotating gently during each wash. 30m

21 Incubate gels with enough fresh DI water to completely cover gel (~10mL) Overnight at Room temperature for a final expansion rotating gently. 30m

22 Place gels (cell side down) in an 6-well chamber for confocal imaging.

Note

Gels must remain immersed in a small amount DI water to prevent drying out during imaging. Note that the gels may also drift during imaging. Placing a coverslip on top of the gel can help avoid this issue.

23 Image gels!

Citations

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