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We use this protocol and it's working

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ABSTRACT

Expansion microscopy is a technique to visualize biological structures with higher spatial resolution than traditional microscopy methods.

ATTACHMENTS

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MATERIALS

Materials

Stock X solution

	A	B
	Sodium acrylate 33% (w/v)	8.6% (w/v)
	Acrylamide 50% (w/v)	2.5% (w/v)
	N,N´-methylenebisacrylamide 2% (w/v)	0.15% (w/v)
	5 M NaCl	11.7% (w/v)
	PBS	1X

Digestion buffer

	A	B
	Triton X-100	0.5% (w/v)
	EDTA 0.5 M, pH 8	0.2% (v/v)
	Tris-Cl 1 M, pH 8	5% (v/v)
	NaCl	4.67% (w/v)
	proteinase K	8 U/ml

- 10% (v/v) normal goat serum
- 0.1% (v/v) Triton X-100
- PBS
- secondary antibody (Alexa Fluor, Invitrogen)
- Acryloyl-X SE solution (Thermo Scientific)
- 10% (w/v) TEMED
- 10% (w/v) APS stock solution
- poly-L-ornithine-coated coverslips
- acryloyl-X SE solution
- 0.5% (w/v) 4-hydroxy-TEMPO stock solutions

- Leica TCS SP8 confocal microscope (Leica, Germany)
- GraphPad Prism version 9.0.0 (RRID:SCR_002798)

Expansion microscopy

6h

1

Note

This protocol refers to the expansion microscopy (ExM) protocol described in Asano et al., 2018 with some modifications.

Block cells with 10% (v/v) normal goat serum (NGS) in 0.1% (v/v) Triton X-100 in PBS and incubate it with primary antibodies in blocking solution Overnight.

2

3h

After a 3-h incubation with the corresponding secondary antibody (Alexa Fluor, Invitrogen), wash the samples and treat with 0.1 mg/ml Acryloyl-X SE solution (Thermo Scientific) in PBS for 03:00:00 at Room temperature.

3

The freshly prepared gelling solution consisted of Stock X solution (8.6% (w/v) sodium acrylate 33% (w/v), 2.5% (w/v) acrylamide 50% (w/v), 0.15% (w/v) N,N´-methylenebisacrylamide 2% (w/v), 11.7% (w/v) NaCl 5 M, and PBS 1X), water, 10% (w/v) TEMED and 10% (w/v) APS stock solution in a 47:1:1:1 ratio.

4

3h

Perform gel digestion Overnight in digestion buffer (0.5% (w/v) Triton X-100, 0.2% (v/v) EDTA 0.5 M, pH 8, 5% (v/v) Tris-Cl 1 M, pH 8, 4.67% (w/v) NaCl and 8 U/ml proteinase K).

5

Add the gelling solution to each well and covered by a 15-mm coverslip to ensure the formation of a smooth, flat and thin gel.

6

1h

Incubate coverslips for 01:00:00 at 37 °C for complete polymerization.

7

1h

Expand the gel in water for 01:00:00 and mount in 10 undetermined poly-L-ornithine-coated coverslips to immobilize the gel for picture acquisition.

8 Acquire images using a Leica TCS SP8 confocal microscope (Leica, Germany) equipped with a 100× /1.4 numerical aperture oil-immersion objective. For each condition, acquire 5 images from at least three independent experiments.

9 Analyze Images using Diffraction PSF 3D, DeconvolutionLab2, and EzColocalization plugins in Fiji-ImageJ.

10 Use GraphPad Prism version 9.0.0 (RRID:SCR_002798) to calculate Spearman's rank correlation value (ρ) to identify colocalization of fluorescence signals.

11 The following is a variant of the protocol in case of using midbrain organoid sections:

Fix midbrain organoids and perform immunofluorescence staining as described above.

12 Treat sections with 0.1 undetermined acryloyl-X SE solution in PBS at Room temperature Overnight.

13 Perform gelation in a 47:1:1:1 ratio of Stock X, 10% (w/v) TEMED, 10% (w/v) APS, and 0.5% (w/v) 4-hydroxy-TEMPO stock solutions.

14 Perform gel digestion and expansion as described above.

15 Acquire images using a Leica TCS SP8 confocal microscope (Leica, Germany) equipped with a 100× /1.4 numerical aperture oil-immersion objective. For each condition, acquire 5 images from at least three independent experiments.

16 Analyze Images using Diffraction PSF 3D, DeconvolutionLab2, and EzColocalization plugins in Fiji-ImageJ.

17 Use GraphPad Prism version 9.0.0 (RRID:SCR_002798) to calculate Spearman's rank correlation value (ρ) to identify colocalization of fluorescence signals.