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morphoFISH Protocols

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

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Abstract

morphoFISH combines tissue clearing, submicron whole-brain imaging, and Expansion-Assisted Iterative Fluorescence In Situ Hybridization (EASI-FISH) to assign molecular identities to fully reconstructed neurons in the mouse brain. This protocol aggregates the morphoFISH procedures, including perfusion, tissue-clearing, and probing of transcripts.

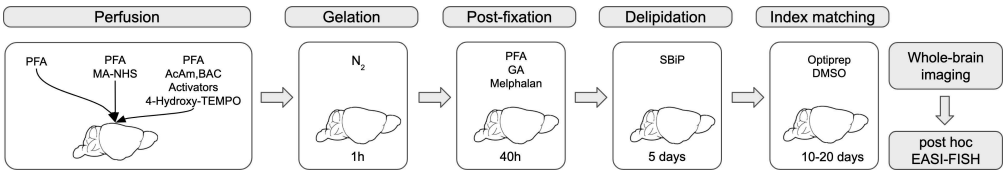


Image Attribution

Tiago Ferreira

Materials

- Nuclease-free water or water filtered using a Milli-Q filtering system **Ambion Catalog #**AM9932
- Paraformaldehyde 32% (methanol free) **Electron Microscopy Sciences Catalog #**15714
- 4-Hydroxy-TEMPO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**176141
- N,N,N′,N′-Tetramethylethylenediamine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T7024
- Ammonium persulfate **Catalog #**A3678
- DMSO, Anhydrous **Thermo Fisher Catalog #**D12345
- Methacrylic acid N-hydroxysuccinimide ester **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**730300-1G
- 40% Acrylamide Solution **Bio-Rad Laboratories Catalog #**1610140
- OptiPrep - Density Gradient Media (Iodixanol) **Cosmo Bio Catalog #**AXS-1114542
- Acrylamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9099
- N-N-Bis(acryloyl)Cystamine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A4929-5G
- 10x Phosphate Buffered Saline **Gibco - Thermo Fisher Scientific Catalog #**70011044
- Agarose-Ultra Pure **Invitrogen - Thermo Fisher Catalog #**16500-500
- RNAse AWAY **Molecular BioProducts Catalog #**7000
- MOPS pH 7 **Thermo Fisher Scientific Catalog #**AAJ61821AK
- DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**472301
- Peel Away Disposable Embedding Molds **Electron Microscopy Sciences Catalog #**70183

Recipes:
Reagents:

	A	B	C	D	E
	4HT	0.5 wt%	50mg	9.95mL water	store -20°C, several months
	TEMED	10 vol%	50uL	450uL water	store single use aliquotes at -20°C, several months
	APS	10 wt%	50mg	450uL water	store single use aliquotes at -20°C, several months

	A	B	C	D	E	F
		A	B	C	D	E

	A	B	C	D	E	F
	1	1M MA-NHS	1M	186.3mg	1mL anhydrous DMSO	Make fresh day of perfusion
	2	BAC	20%	120mg	600ul anhydrous DMSO	Make fresh day of perfusion

Melphalan

	A	B	C	D	E
	Melphalan	2mg/mL	250mg	125mL DMSO	store -20°C for several months

**Make solution on a hot plate with a stir bar. Mix for 02:00:00 at 37 °C until completely dissolved. Aliquot and store at -20 °C for two months. Discard if turns yellow in color.

Perfusion buffers:

	A	B	C	D	E
	Perfusion fixative				
				tot vol:	**50 mL**
		Stock	final		volume (mL)
	32% PFA (%)	32	4		6.25
	Nuclease free water				43.75

	A	B	C	D	E
	Protein anchoring solution				
				tot vol:	**50**
		Stock	Final		Volume
	PFA	32	4		6.25
	Nuclease free water				42.7

	A	B	C	D	E
	MA-NHS (mM)	1000	20		1

Add MA-NHS immediately prior to perfusion

	A	B	C	D	E
				tot vol:	**50**
	Gel solution				
		stock	final		volume
	AcAm (%)	40	20		25
	BAC (%, in DMSO)	20	0.1		0.25
	PB pH7.5 (mM)	1000	100		5
	PFA (%)	32	4		6.25
	TEMED pH7.5 (%)	10	.2		1
	4HT (5)	.5	0.01		1
	APS (ppt)	10	.2		1
	water				10.5

***Add 4HT, TEMED, and APS immediately prior to perfusion

	A	B	C	D	E
	A	B	C		D
	Post-fixation solution				
				Tot vol:	**50**
		Stock	Final		Volume (mL)
	PFA (%)	32	4		6.25
	Glutaraldehyde (%)	10	.05		.250

	A	B	C	D	E
	MOPS pH 7 (mM)	1000	10mM		1
	Melphalan (mg/mL)	2	1		25
	Nuclease free water				17.5

Brain Perfusion and Fixation

1

Protocol

NAME

morphoFISH Perfusion

CREATED BY

Monique Copeland

Preview

Aqueous Delipidation

2

Protocol

NAME

Aqueous (SBiP) Delipidation for Whole Mouse Brain After morphoFISH Perfusion

CREATED BY

Monique Copeland

Preview

Agarose Embedding (5%Agarose)

1m 30s

3 Rinse 500ml glass bottle with RNAse AWAY.

4 Add 100 mL 1x PBS to beaker and warm in microwave for 00:01:30 1m 30s

Note

Use caution glass bottle will be hot. Use appropriate protection.

5 Add 5 g agarose powder to warm 1x PBS, mix well by rotating the bottle.

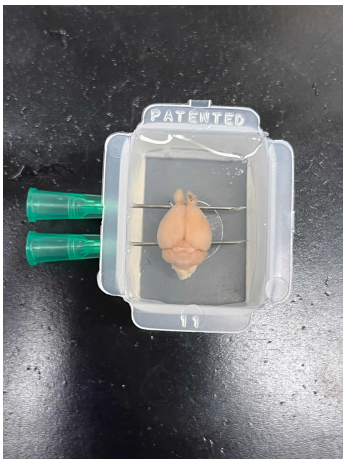
6 Place glass bottle in 57 °C incubator for 02:00:00 or until agarose is dissolved 2h

7 Submerge the brain in 25 mL agarose in a 50mL conical tube for 00:15:00 15m
37 °C

Note

Equilibrating the brain to agarose helps create a better seal around the brain when embedding in the mold. Store remaining agarose at 37 °C for later use.

8 Prepare 20×20 embedding mold by inserting two needles parallel to each other through the side of the mold about 2-3mm from the bottom. This creates a platform for the brain to rest while agarose is setting, ensuring brain remains in the middle of the mold.



8.1 Fill embedding mold with 15mL of 5% agarose

9 Quickly place the brain in the center of the mold resting on the needles and allow agarose to set 00:10:00 4 °C 10m

Note

The agarose will turn slightly white in color and harden to the touch when it is completely set.

9.1 Remove the agarose block from the mold by gently pulling the edges of the mold apart. Trim the block as desired and proceed immediately to index matching.

Index Matching 6d

10 Equilibrate brain in 100 mL 30% DMSO for 72:00:00 a 40 rpm, Room temperature protected from light. 3d

Note

The agarose block will initially float in each index matching solution, but will gradually sink as it equilibrates to the solution.

11 Equilibrate brain in 100 mL 50:50 DMSO:Optiprep for 72:00:00 at 40 rpm, Room temperature protected from light 3d

12 Equilibrate brain in 100 mL 60:40 DMSO:Optiprep for 72:00:00 at 40 rpm, Room temperature protected from light. 3d

13 Replace with 100 mL 60:40 DMSO:Optiprep for 168:00:00 (7-10 days or until brain sinks to bottom) at 40 rpm, Room temperature protected from light. 1w

Tissue Sectioning and imaging 10m

14 Trim agarose block as needed

15 Glue block to specimen disc and fill imaging tray with 60:40 DMSO:Optiprep

16 Proceed to imaging as per **Economo et al., 2016**; **Winnubst et al., 2018**

Neuronal Reconstruction

17

Protocol

NAME

Reconstructing Complete Neurons in Whole Brains Using HortaCloud

CREATED BY

Tiago A Ferreira

Preview

Slice collection

10m

18 Collect slice from imaging tray with sterile rod or brush into a 24-well plate. Briefly shining light into the imaging bath may assist in locating the brain slices for recovery

19 Rinse with 1 mL RNAse-free water for 00:05:00 at Room temperature to rinse off the DMSO. Trace amounts of DMSO left in the tissue may impact RNA transcript detection by hybridization chain reaction (HCR). Slices become opaque in aqueous solutions

5m

20 Rinse 2x with 2 mL 70% EtOH for 00:05:00 at Room temperature

5m

Note

XFP labeling (GFP, tdTomato, etc.) will become dim in 70% EtOH. Signal strength should recover once the brain slice is rehydrated in 1x PBS.

21 Store in 70% EtOH at 4 °C until further processing. Seal well plate with parafilm (or sealing film) to minimize evaporation. Brain slices are stable for at least 3 months, although it is recommended to proceed to RNA detection as quickly as possible

Note

Check ethanol level in the wells frequently to monitor evaporation. Replace 70% EtOH as needed.

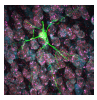
RNA detection via EASI-FISH

20m

22

6d

Protocol



NAME

Expansion-assisted iterative fluorescence *in situ* hybridization (EASI-FISH) for morphoFISH

CREATED BY
Mark Eddison

Preview