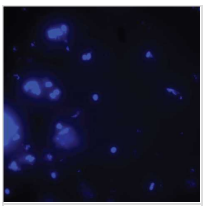

Apr 02, 2022

Single-Nuclei Isolation From Snap Frozen Axolotl Brain with Injected EdU

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protocol .

QuadBio

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This protocol enables isolation of single nuclei with EdU incorporation from frozen pallium dissections (from axolotl) for the purpose of generating single-nuclei gene-expression libraries following a modified protocol from 10x (Demonstrated protocol CG000365, Rev B), Div-Seq (DOI: 10.1126/science.aad7038), and EdU FACs protocol. In brief, we prepared and precooled wash and lysis buffers (see Materials). Lysis buffer was added to the sample and dissociated via short pulses with an electric grinder. The pestle of the grinder was washed with a wash buffer before centrifugation. Supernatant was removed and the pellet gently washed. After a final centrifugation the supernatant was removed and the pellet was resuspended in PBS + BSA. Resulting nuclei were then assessed (count and viability) using Trypan Blue assay, counted using the automated cell counter Countess (Thermo Fisher).

EdU staining was performed immediately using Click-iT EdU Flow Cytometry assay Kit (Thermo Fisher Scientific, #C10424), 500 µl reaction buffer was added directly to the resuspension buffer (mix is made following the manufacturer's protocol), mixed well and left in RT for 30min. 3ml of wash buffer was added to the resuspended nuclei and mixed well, then nuclei were spun down for 5 min at 500xg (4°C), supernatant was removed and nuclei were resuspended in 500 µl PBS + 0.5% BSA with DAPI and FACS sorted immediately (sorted for DAPI+/EdU+). FACS nuclei are then ready for downstream analysis, including but not limited to 10x Genomics gene expression (single-nuclei RNA sequencing).

Ashley Maynard, Fides Zenk 2022. Single-Nuclei Isolation From Snap Frozen Axolotl Brain with Injected EdU. **protocols.io** https://protocols.io/view/single-nuclei-isolation-from-snap-frozen-axolotl-b-b6yqrfvw

protocol

Single-cell analyses of axolotl forebrain organization, neurogenesis, and regeneration Katharina Lust, Ashley Maynard, Tomás Gomes, Jonas Simon Fleck, J. Gray Camp, Elly M. Tanaka, Barbara Treutlein bioRxiv 2022.03.21.485045; doi: https://doi.org/10.1101/2022.03.21.485045

Nuclei isolation, Axolotl, Pallium, EdU, Div-seq

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For this protocol you will need an electric grinder with pestle, we recommend:

- Kimble 749521-1500 Polypropylene Pellet Pestle Only, 1.5mL Capacity (Case of 100)
- Kimble Pellet Pestles 749540-0000 Drive Unit Cordless Motor with Two AA Batteries

Wash/Resuspension Buffer:

A	B	C	D
Reagent	**[Stock]**	**[Final]**	**Volume**
Tri-HCL (pH 7.4)	1 M	10mM	60ul
NaCl	5M	10 mM	12ul
MgCl2	1M	3mM	18ul
BSA	10%	1%	600ul
RNase Inhibitor	M0314S 40000U/ul (dilute 1:100 first = 400U/ul [working])	1U/ul	15ul
Nuclease-free Water			5.295ml

Volume will make 6mL

Div-Seq Lysis Buffer:

A	B	C	D
Reagent	**[Stock]**	**[Final]**	**Volume**
Tri-HCL (pH 7.4)	1 M	10mM	100ul
NaCl	5M	10 mM	20ul
MgCl2	1M	3mM	30ul
Tween-20	10%	0.01%	10ul
NP-40	10%	0.01%	20ul
Digitonin (dissolve at 65C)	5%	0.001%	2ul
BSA	10%	1%	1000ul
DTT	1M	1mM	10ul
RNase Inhibitor	M0314S 40000U/ul	1U/ul	2.5ul
Roche Protease Inhibitor	100x (x1 tablet in 500ul of water) cOmplete, EDTA-free Protease Inhibiotr Cocktail 11873580001 Roche	1x	100ul
Nuclease-free Water			8.8ml

Volume will make 10mL

Prepare Click-iT® EdU reagents

1. To make a 10X stock solution of the Click-iT® EdU buffer additive (Component G), add 2 mL of deionized water to the vial and mix until the Click-iT® EdU buffer additive is fully dissolved. After use, store any remaining stock solution at ≤–20˚C. When stored as directed, the stock solution is stable for up to 1 year.
2. Prepare a working solution of Alexa Fluor® 647 azide (Cat. no. C10424 by adding 130 µl of DMSO to Component B and mix well. After use, store any remaining working solution at ≤–20°C. When stored as directed, this working solution is stable for up to 1 year
3. Prepare 1X Click-iT® EdU buffer additive by diluting the 10X stock solution 1:10 in deionized water (2 reactions = 10ul + 90ul)

Prepare the **Click-iT® reaction cocktail** (according to number of reactions needed):

A	B	C	D
Reaction component	**Number of reactions**		
	x1	**x2**	**x3**
PBS	438 µl	875 µl	1.314 mL
CuSO4 (Component F)	10 µl	20 µl	30 µl
Fluorescent dye azide	2.5 µl	5 µl	7.5 µl
Reaction Buffer Additive	50 µl	100 µl	150 µl
Total reaction volume	500 µl	1 mL	1.5 mL

Prepare Buffers

1 Prepare the buffers as described in the Materials section. Store buffers at **4 °C** or **On ice** .

Nuclei isolation 10m 10s

2 Use pre-cooled buffers and store **On ice** , perform isolation steps **On ice** , use pre-cooled micro-centrifuge at **4 °C** .

3 Put tissue in cold **1.5 mL** tube

4 Add **50 µL** of lysis buffer

5 Using an electric grinder. Grind the tissue for **00:00:10** (or 2-5 pulses depending on if the tissue persists) in the tube. Rinse the pestle with **150 µL** wash buffer. 10s

6

Optional: Check an aliquot of the nuclei on the Evos or at the Nikon

7 Spin down **00:05:00** at **500 x g** (at **4 °C**) 5m

8

Optional: Keep the supernatant and check an aliquot on the Evos or at the Nikon

9

Wash the pellet with **200 µL** of wash buffer **(do not disturb the pellet for optimal recovery)**

10 Spin down at **500 x g** for **00:05:00** (at **4 °C**) 5m

11 Resuspend the pellet in **55 µL** of wash/resuspension buffer

12 Count with typan blue (**5 µL** sample + **5 µL** trypan)

Stain for EdU 35m

13 Add **500 µL** **Click-iT® reaction cocktail** to nuclei, mix well, and incubate at **Room temperature** for **00:30:00** protected from light 30m

14 Wash the nuclei once with **3 mL** of 1% BSA in PBS.

15 Spin down at **500 x g** for **00:05:00** (at **4 °C**) 5m

16 Remove the supernatant. Dislodge the pellet and resuspend the nuclei in **500 µL** of Wash/Resuspension Buffer, add **5 µL** DAPI and FACS immediately.

FACS of cells

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To obtain our EdU+ cells, FACS a negative control first (no EdU injection in sample but also stained for EdU (steps #13-16)). From this you can gate for DAPI+ and EdU+ cells.