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X-ray Micro Computed Tomography (MicroCT)

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

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Abstract

This protocol has been successfully used with colonial tunicates. It has been adapted to our needs based on the following publication: " MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues " by Brian D Metscher.

MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues.

Materials

4% PFA
PTA
IKI
Ethanol
low melting agarose
fixing paste (e.g. dental wax)
pipette tips 200uL or 1mL
10x PBS, pH 7.2 (store for up to 2 moths at RT)

	A	B	C
	Na2HPO4*2 H2O	0.1M	17.8g
	KH2PO4	18mM	2.4g
	NaCl	1.4M	80g
	KCl	27mM	2g
	water		1000mL

10x PBS (amounts calculated for 1L)

Fixation

1h 30m

1 Clean the slide with the colony of your interest. See **Cleaning colonial ascidians V.2**.

2 Anesthetize the animals following this protocol. See **Whole colony fixation V.1**

3 Fix the animals in 4% PFA Overnight at Room temperature . See **Whole colony fixation V.1**

4 Wash twice in 3.3x PBS for 00:30:00 . 30m

5 Rinse tree times with 1x PBS and leave in 1x PBS for 01:00:00 . 1h

Staining

4d

6 Detach the colony with a razor blade and put in a 2mL tube containing 1 mL PTA / 0.6 mL IKI/ 0.4 mL Ethanol.

7 Incubate for 96:00:00 at Room temperature on linear shaker (low speed), protect from light. 4d

8 Rinse quickly 3-4 times with 1x PBS.

Mounting

9 Prepare 10 mL of [M] 0.5 % volume low melting agarose.

9.1 When the agarose has cooled down a bit, pipette 125-150 µL if using a 200ul tip or 750-800 µL if using a 1mL tip (the tip size depends on the animal size).

9.2 Carefully place your colony vertically inside the tip (it should be as flat as possible, avoid folds).

9.3 Pipette some more agarose in order to cover the animal entirely.

10 Place the tip into a beaker filled with ice-cubes so that the agarose becomes solid.

11 Cover the tip with fixing paste.

12 Store at 4 °C or proceed to the next step.

13 Mount the tip on the tomograph support using fixing paste in such way that it is perfectly straight.

14 Place it inside the tomograph.

15 Follow the tomograph's manual and proceed to scan.

16 After scanning, the tip with the sample can be stored at 4 °C for several weeks and eventually can be reused.