Buccopharyngeal morphology of tadpoles in Scanning Electron Micrography Version 2

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Abstract

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Protocol

Step 1.

Wash quickly the specimens in tap water and dissect tadpoles following Wassersug (1976: p. 4-5).

NOTES
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Usually tadpoles are fixed and conserved in 10% formalin, buffered or not. I'm here assuming that your samples are already in formalin.

Step 2.

Fix the dissections in 4% Glutaraldehyde.

REAGENTS
Glutaraldehyde EM Grade 25% G5882-50ML by Sigma Aldrich

DURATION
02:30:00

NOTES
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To make this fixing solution, you'd use 200 mL of Millonig's phosphate buffer to 0.5 g of 1% Tannic Acid, and 6 mL of 25% Glutaraldehyde

Step 3.

Post fix the dissections in 1% Osmium tetroxide solution.

AMOUNT
20 ml Additional info:

REAGENTS

Osmium tetroxide solution 2% 75633 by Sigma Aldrich

DURATION
02:30:00

EXPECTED RESULTS

At the end of this step all your specimens would have to be entirely black.

NOTES
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Usually you'd have a 2% solution and you'd have to dilute it in Millonig's phosphate buffer solution until your dissections are fully covered.

Safety check: this solution is highlytoxic and volatile, so this procedure is better done in exhaust hoods.

Step 4.

Dehydrate in a ascending series of acetones, starting at 30%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 5.

Put in acetone 50%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 6.

Put in acetone 70%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 7.

Put in acetone 90%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 8.

Put in acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 9.

Put in another solution of acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 10.

Put in another solution of acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 11.

Put in acetone 100%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 12.

Put in another solution of acetone 100%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 13.

Now take your specimens to a Critical Point Dryer

LINK:
http://www.leica-microsystems.com/products/em-sample-prep/biological-specimens/room-temperature-techniques/drying/details/product/leica-em-cpd300/

EXPECTED RESULTS

Specimens are completely dried, rigid, and somewhat whitish

Step 14.

Mount the specimens in stubs and attach them with a double-sided tape

LINK: https://us.vwr.com/store/catalog/product.jsp?catalog_number=100492-314

Step 15.

Take the specimens to the High Vaccum Coater and you're ready to analyze them.

LINK: http://www.leica-microsystems.com/pt/produtos/preparacao-de-amostras-para-microscopia-eletronica/especimes-biologicos/tecnicas-em-temperatura-ambiente/revestimento/detalhes/product/leica-em-ace600/