Supporting Information

Methods for Cryosectioning and Mass Spectrometry Imaging of Whole-Body Zebrafish

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Summary

S1. Standard Operating Procedure: Whole-body Zebrafish Cryosectioning for Mass Spectrometry Imaging

Figure S1. Workflow for preparation of whole-body zebrafish sections for mass spectrometry imaging and complementary histology.

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Scope/Applicability

Zebrafish are an important model vertebrate system for studying metabolism and disease. Whole-body mass spectrometry imaging (MSI) allows for direct visualization of lipid and metabolite distributions across various organs and tissues in a biological sample. The spatial resolution and chemical information afforded by this technology can be paired with complementary histological imaging to gain a deeper understanding of the biological mechanisms involved in a disease state or as a result of a particular treatment. Herein, the procedure for cryosectioning whole-body, adult male zebrafish at 6-months postfertilization for MSI is detailed. A histological staining method is also provided and can be used to obtain microscopy images for comparison with ion maps from mass spectrometry imaging data.

Reagents/Media

1. Carboxymethyl cellulose (Sigma, PN C4888-500G)
2. Gelatin from porcine skin type A (Sigma, PN G1890)
3. Room temperature Milli-Q water (e.g., ELGA Lab Water Purification System18 MΩ)
4. Refrigerated Milli-Q water
5. Ethanol for cyro-bath (Koptec, PN UN1170)
6. Optimal cutting temperature medium (Tissue-Tek, PN 4583)
7. 10% Neutral Buffered Formalin Fixative (Leica, PN3800602)
8. Deionized water
9. Ethanol for staining (Fisher BioReagents, PNBP28184)
10. Hematoxylin Solution, Harris Modified (Sigma-Aldrich, PNHHS16-500ML)
11. Eosin Y solution, alcoholic (Sigma-Aldrich, PNHT110116-500ML)
12. Permount (Fisher Chemical, PNX3P-1GAL)
13. Xylenes (Fisher Chemical, PNSP15-100)

Supplies/Materials

1. 500 mL beaker for water bath
2. Thermometer
3. 50 mL centrifuge tubes (Olympus, PN 28-106)
4. 100 mL beaker
5. Metal spatula
6. Cryostat specimen disks
7. Microtome blades (VWR, 95057-834)
8. Cooler with ice pellets

9. 250 mL beaker
10. Peel-A-Way mold (Thermo Scientific, PN 41744)
11. Forceps
12. Cooler with dry ice pellets
13. Disposable aluminum dishes (Fisherbrand, PN 08-732-106)
14. Precleaned microscope slides (Fisherbrand, PN 12-544-1)
15. Superfrost Plus microscope slides (Fisherbrand, PN12-550-15)

Equipment

1. Hot plate (VWR, PN 97042-740)
2. Vortex mixer (Scientific Industries, PN (G560) SI-0236)
3. Microwave oven (General Electric, PN JES738WJ02)
4. Cryostat (Leica CM 1950)

Safety Precautions

Personal Protective Equipment (PPE) must be worn while operating the cryostat; this includes safety glasses, lab coat, and disposable gloves. Never touch the cryostat blade with your fingers.

Procedure

I. Preparation of 5% Carboxymethyl Cellulose + 10% Gelatin Embedding Media:

1. Prepare a water bath by placing a 500 mL beaker of water on a hot plate and heating to 115 °C; use a thermometer to monitor the temperature. This will be used for step #6.
2. Add 1 g carboxymethyl cellulose (powder) and 2 g gelatin (powder) to a 50 mL conical tube. Shake the tube to mix the two powders.
3. Add 20 mL of room temperature Milli-Q water to the conical tube and vortex for 30 s.
4. Place the conical tube containing embedding media upright in a 100 mL beaker with the cap removed.
5. Heat the media in a microwave in 4 s intervals until the media is liquefied, stirring with a metal spatula periodically between heating intervals. The entire heating process is expected to take 30–40 s but may vary depending on the microwave oven used.
6. Remove the tube from the microwave and place in the 115 °C water bath to maintain liquid state. To prevent solidification, stir the media periodically.

II. Embedding and Sectioning Whole Zebrafish:

1. Cool the cryostat to −20 °C and verify that all components are thoroughly cleaned. Remove cryostat specimen disks from the cryostat and keep at room temperature.
2. Replace the cryostat blade and/or glass plate if either has an uneven surface that may cause tearing and poor sectioning.
3. During the cooling phase and throughout the sectioning process, the light inside of the cryostat should be kept OFF. (Having the light on was found to heat the sample and embedding media creating a tackier texture that was harder to section.)
4. Chill a 250 mL beaker of refrigerated Milli-Q water in a cooler filled with ice.

5. After the media has been in the water bath for approximately 15 min, euthanize the first fish in the chilled water.
6. Pour a small amount of media into a Peel-A-Way mold to form a uniform layer across the entire bottom of the mold. Pop any large bubbles with clean forceps.
7. Remove the fish from the euthanization water, dry with a paper towel and placed on top of the media layer with forceps.
8. Using the forceps, position the fish so that it lies completely flat along the bottom of the Peel-A-Way mold. Once the fish is correctly positioned, pour additional media on top of the fish to complete embedding and remove any large bubbles with forceps.
9. Place the mold containing the fish in an aluminum dish and float in a Styrofoam cooler containing a dry ice/ethanol bath (1:1 ratio of crushed dry ice and 95% ethanol).
10. Allow the embedding media to freeze completely (~15 min). Media should turn white.
11. Remove the embedded fish from the Peel-A-Way mold and place on a room temperature specimen disk using a small amount of optimal cutting temperature (OCT) mounting medium.
12. Place the disk inside the cooled cryostat to allow the OCT to freeze, adhering the embedded fish to the specimen disk.
13. Collect parasagittal sections by positioning the embedded zebrafish ventral side up.
14. Pare down the fish to the region of interest by setting the thickness to 30 µm.
15. Once the region of interest is reached, collect 16 µm serial sections by thaw-mounting onto a pre-cleaned glass microscope slide or HistoBond slide for IR-MALDESI MSI and histology, respectively.

IV. Histological Staining with Hematoxylin and Eosin (H&E):

1. Fix sections in 10% natural buffered formalin for at least 1 h.
2. Rinse slide in deionized water.
3. Rinse with 95% ethanol for 30 s.
4. Rinse with 70% ethanol for 30 s.
5. Rinse with deionized water for 30 s.
6. Stain 16 µm thick sections in hematoxylin for 45 s (Harris).
7. Rinse with deionized water for 30 s.
8. Rinse with 70% ethanol for 30 s.
9. Rinse with 95% ethanol for 30 s.
10. Stain with eosin for 1 min.
11. Rinse with 95% ethanol for 30 s.
12. Rinse with 100% ethanol for 30 s.
13. Soak in xylenes for 2 min.
14. Drop Permount on slide, preventing bubbles, and cover with cover slip.

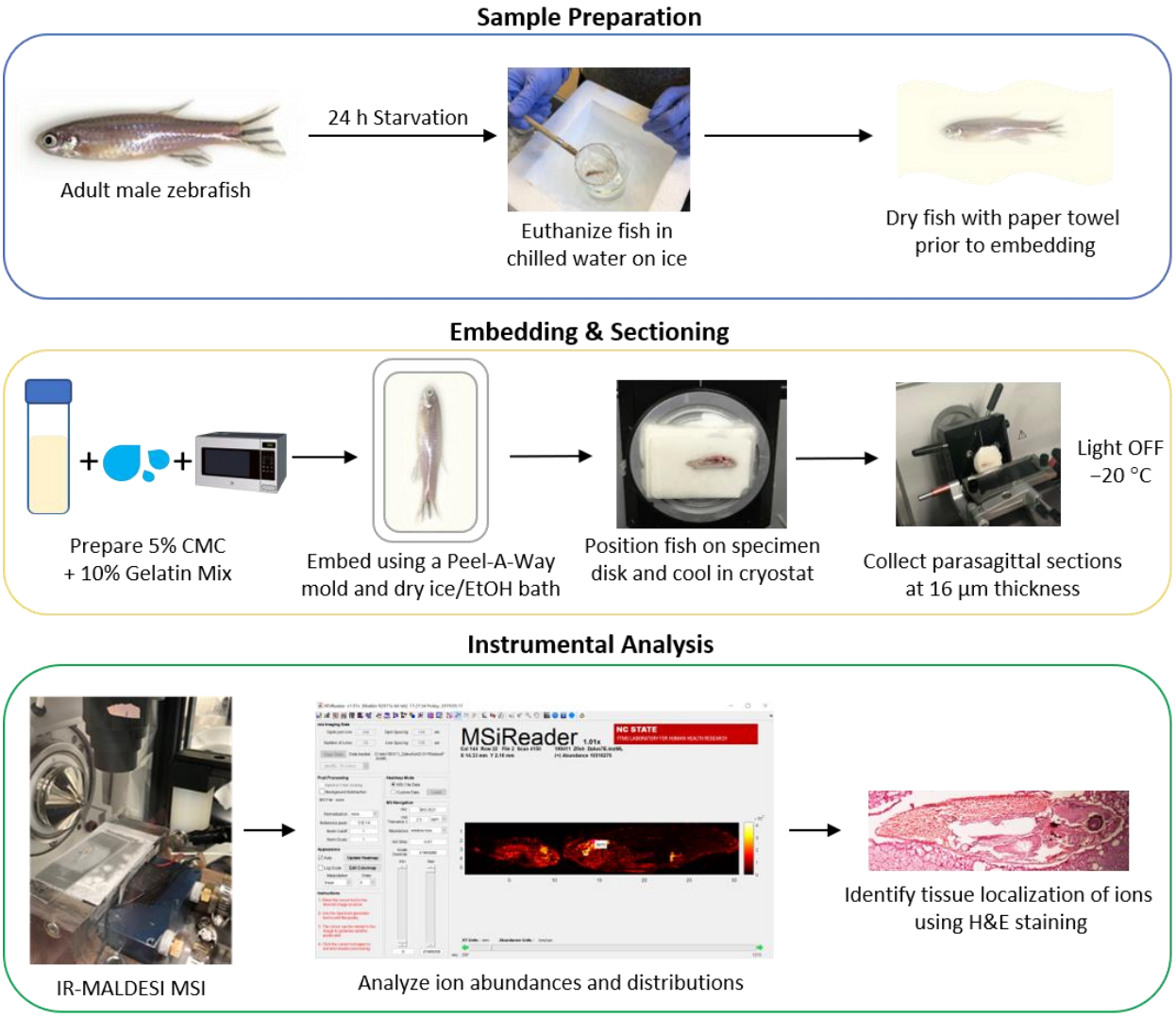

Figure S1. Workflow for preparation of whole-body zebrafish sections for mass spectrometry imaging and complementary histology.