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Brain Clarification for Neuromelanin visualisation by OPT

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for the clarification of whole mouse brains to allow neuromelanin visualisation by Optical Projection Tomography

Brain processing

- 1 Mice are transcardially perfused in 4% PFA and postfixed in the same fixative overnight
- 2 After rinsing several times in PBS, brains are embedded in 1% low melting point agarose in water
- 3 Brains are dehydrated in 100% methanol covered in aluminum foil to protect the specimen from light; change the methanol three times during the next 24 h or until the specimen is completely dehydrated
- 4 Once dehydrated the brains are incubated during 4 hours shaking in 66% dichloromethane (DCM) /33% methanol at room temperature
- 5 The brains are incubated with shaking in 100% DCM 30 minutes twice to complete the delipidation process
- 6 The brains are chemically cleared with BABB (a combination of 1 part of benzyl alcohol and 2 parts of benzyl benzoate)
- 7 The brains are subsequently immersed in a BABB-filled chamber for Optical projection tomography (OPT) imaging

Brain imaging

- 8 The brains are scanned with transmission light with three different filters (a cyan fluorescent protein -CFP-filter: emission 460-500nm, a green fluorescent protein -GFP- filter: emission 500-550nm and a DSR filter: emission >610nm) using an OPT imaging system mounted on a Leica MZ 16 FA microscope
- 9 The brain images are visualised using the open source software Fiji