Mouse embryo preparation for microCT imaging

Materials

- 4% paraformaldehyde, pH 7.2 (4% PFA).
- 16% paraformaldehyde, pH 7.2 (for preparing the stabilization buffer).
- STABILITY buffer (SB): [4% w/vol PFA, 4% w/vol acrylamide (BIO-RAD, Cat# 161-0140), 0.05% w/vol bis-arcylamide (BIO-RAD, Cat# 161-0142), 0.25% VA044 initiator (Wako Chemical, Cat# 017-19362), 0.05% w/vol Saponin (Sigma, Cat# 84510) in 1x PBS].
- 0.1N Iodine solution (Sigma, Cat# 38060).
- 1% agarose (AMRESCO).
- Transport tubes with screw caps (VWR, Cat# 89497-738, 16 mm x 56 mm).

Sample Preparation (E9.5 to E12.5)

1. Dissect embryos in warm 1X PBS and fix in 4% PFA immediately and leave at 4 °C overnight on a nutator.

2. Wash with 1X PBS three times, 10 min each at RT.

3. Immerse the sample in 0.1N (vol/vol) iodine solution (Sigma) overnight at room temperature.

Note: Use glass sample tubes instead of plastic vials for staining. We noticed that plastic tubes absorb iodine, which will reduce the iodine concentration in the solution and reduce the contrast in the sample.

4. Mount embryos in 1% agarose (AMRESCO) made in sterile MilliQ water in either capped 2 ml tubes (E9.5) or 16 mm x 56 mm sample tubes (E12.5, VWR) right before imaging.

5. Acquire the images at 3 um/pixel (E9.5) and 5 um/pixel (E12.5) on SKYSCAN 1272 with the X-ray source at 70 kV and 142 uA with no filter selected.

Sample Preparation (E15.5 to E18.5)

1. After dissection, fix samples directly in 4% PFA at 4 °C (E15.5: overnight; E18.5: 3 days) on a nutator.

2. Transfer each sample to a 50 ml conical tube and immerse in 20 ml of STABILITY buffer [4% w/vol PFA (Sigma), 4% w/vol acrylamide (Bio-Rad), 0.05% w/vol bis-arcylamide (Bio-Rad), 0.25% VA044 initiator (Wako Chemicals), 0.05% w/vol Saponin (Sigma) in 1x PBS] and incubated at 4 °C for 3 days for the polymer to diffuse through the samples.

3. Place the sample tubes in a desiccator to replace the air with nitrogen gas.

4. Incubate the samples at 37 °C to initiate the crosslinking reaction for 3 hours for the acrylamide-PFA to crosslink with the tissue and create a hydrogel-tissue mixed structure.

5. After crosslinking, carefully remove the external hydrogels from the specimens in a fume hood and store the samples in 1X PBS with 0.1% sodium azide at 4 °C until ready for imaging.

6. The staining time for E15.5 in 0.1N iodine is overnight; it takes at least 3 days for E18.5. For better contrast, replace the iodine solution every 3 days.

7. Mount embryos in 1% agarose (AMRESCO) within capped 16 mm x 56 mm sample tubes (E12.5, VWR) right before imaging.

8. Acquire the images on SKYSCAN 1272 at 11um/pixel with the X-ray source at 70 kV and 142 uA with 0.5mm aluminum filter selected.

Sample Preparation (P0 to P4)

1. Euthanize neonates via CO_2 inhalation with a SmartBox Auto CO_2 system for 90 mins followed by immersion in ice cold 4% paraformaldehyde at 4 °C for 3 days on a nutator.

2. Transfer each sample to a 50 ml conical tube and immerse in 20 ml of STABILITY buffer [4% w/vol PFA (Sigma), 4% w/vol acrylamide (Bio-Rad), 0.05% w/vol bis-arcylamide (Bio-Rad), 0.25% VA044 initiator (Wako Chemicals), 0.05% w/vol Saponin (Sigma) in 1x PBS] and incubated at 4 °C for 3 days for the polymer to diffuse through the samples.

3. Place the sample tubes in a desiccator to replace the air with nitrogen gas.

4. Incubate the samples at 37 °C to initiate the reaction for 3 hours for the acrylamide-PFA to crosslink with the tissue and create a hydrogel-tissue mixed structure.

5. After crosslinking, carefully remove the external hydrogels from the specimens in a fume hood and store the samples in 1X PBS with 0.1% sodium azide at 4 °C until ready for imaging.

6. The staining time for neonates take at least a week for the iodine to perfuse thoroughly. For better contrast, replace the iodine solution every 3 days.

7. Mount embryos in 1% agarose (AMRESCO) within capped16 mm x 56 mm sample tubes (E12.5, VWR) right before imaging.

8. Acquire the images on SKYSCAN 1272 at 11um/pixel with the X-ray source at 70 kV and 142 uA with 0.5mm aluminum filter selected.