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# Pathological analysis of mosue embryos

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## Abstract

A protocol for the pathological analysis of mouse embryos.

1 Pregnant dams were euthanized at estimated embryonic day 12.5 using a carbon monoxide chamber (filled gradually to 70%) followed by creation of pneumothorax.

2 The uterus was extracted in its entirety, after which fetuses (n=6 per litter) and their membranes were dissected intact from the uterus. The tail of each fetus was harvested for genotyping.

3 Fetuses were fixed in Bouin's solution for 48 hours, followed by bisection along the sagittal plane. Cassetted tissues were submitted for standard paraffin embedding, processing, and generation of 20-30 5µm sections at 20µm intervals.

4 Sections were stained with hematoxylin and eosin.

5 Immunoperoxidase stains were performed using unstained 5µm paraffin sections using primary antibodies at a 1:100 dilution.

6 After deparaffinization and rehydration, antigen retrieval was performed using sodium citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0) at 95-100$^{O}$C for 10 minutes.

7 Immunostaining was performed using a Dako autostainer.

8 Label was visualized by 0.05% 3',3'-diaminobenzidine (DAB) as a chromogen, precipitated by 0.01%
hydrogen peroxide.

9 Light microscopic images were taken using a Zeiss Axioskop and with Axiocam MrC camera.