

HISTOPATHOLOGY AND EMBRYOLOGY

PRESENTATION

Anatomy / histopathology evaluation is a key step in characterizing and evaluating genetically engineered mice produced by transgenesis or targeted mutagenesis and related technologies.

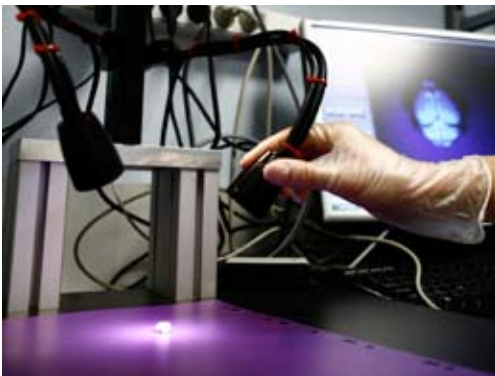
The mutant mice entering the histopathology screen are often seen as 'black boxes':

- They are either viable or die at variable age in the postnatal period, for various causes
- The expression domain of the mutated gene is ubiquitous and potentially associated with pleiotropic effects
- The outcome of the mutation in living mice is often unknown, having not been assessed yet or remaining cryptic

Alternatively, the mutant mice may display known defects which are, however, only manifested in a restricted subset of the tissues where the gene of interest is expressed.

Outcomes of the standard histopathology screen of mutant mice can be summarized as follows :

- No morphological defects are identified. In such case, no further histopathology screening is required.
- Morphological defects are identified; however they are not correlated with the mutation as they occur at the same penetrance and with the same expressivity in wild type mice. In such case, no further histopathology screening is required.
- Morphological defects are identified which are also identified in wild type mice at a lower frequency. In such case it is recommended to repeat the standard screen on a larger sample to obtain statistically significant data.
- Morphological defects are identified. In such a case mice can be screened further with organ / pathology specific assays.



Macroscopical examination

1. DISSECTION

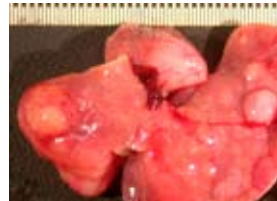
Examples of pathologies



Normal male genital tract



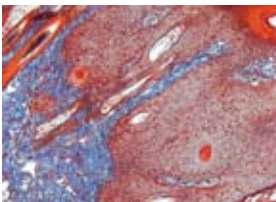
Unilateral hydronephrosis



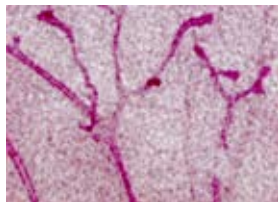
Hepatoma

2. EXAMINED ORGANS

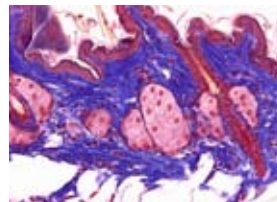
Integument (Skin, Mammary gland)



Basal cell carcinoma
Mallory's trichrome and hematoxylin stain

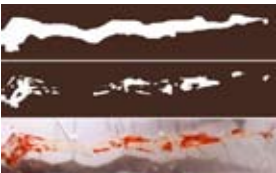


Mammary gland
Carrine stain



Normal skin
Mallory's trichrome and hematoxylin stain

Cardiovascular (Heart, Aorta)



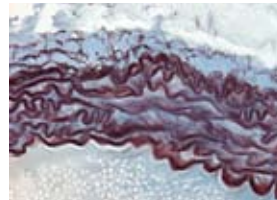
a) En face Sudan-IV-stained aorta



b) lesion areas



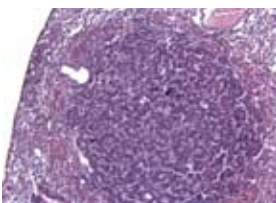
c) total aortic area



Transverse section of aorta.
Orcein stain for elastic fibers

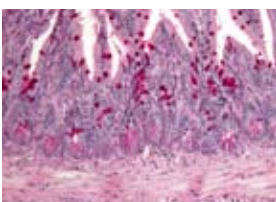
Respiratory (Trachea, Lung, Pleura)

Immune/Hematopoietic (Bone marrow, Thymus, Spleen, Lymph node)



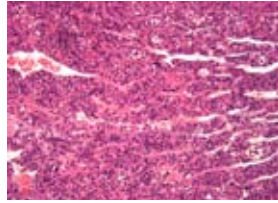
Lung adenoma
Hematoxylin and eosin stain

Digestive tract (Oesophagus, Stomach (fore stomach, corpus and antrum), Duodenum, Ileum, Colon)

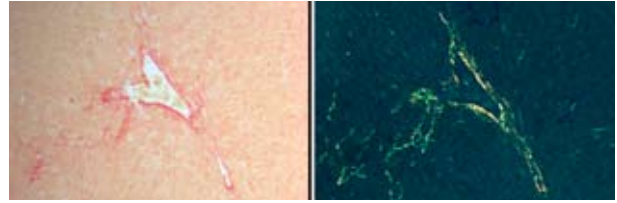


Ileum.
Periodic-acid Schiff (PAS) stain

Digestive organs (Salivary glands: parotid, submandibular and sublingual, Liver; Pancreas (exocrine))

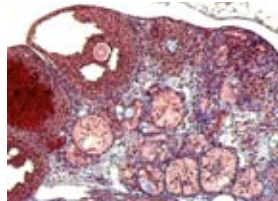


Hepatoma
Hematoxylin and eosin stain

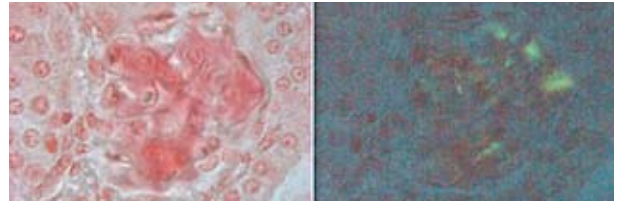


Fibrosis in a liver treated with CCL4.
Sirius red stain (bright field (left) and polarized (right))

Urogenital female (Kidney, Urinary bladder, Ovary, Oviducts, Uterus, Vagina)

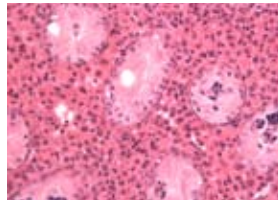


Ovary with Sertoli cell metaplasia.
Mallory's trichrome
and hematoxylin stain

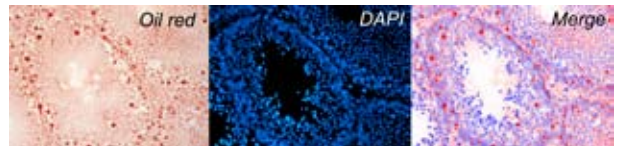


Amyloid staining with Congo red in the glomeruli from a mutant mouse.
Right: same section viewed under polarizing light to demonstrate the characteristic «apple green» birefringence of amyloid.

Urogenital male (Kidney, Urinary bladder, Testis, Epididymis / Ductus deferens, Prostate, Seminal glands, Accessory glands)



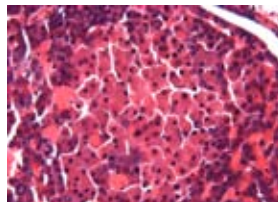
Testis with Sertoli cell only syndrome.
Hematoxylin and eosin stain



Adult testis in a mutant mouse showing large lipid inclusions in the seminiferous epithelium. Oil red O stain for neutral lipids

Musculoskeletal (Striated muscle, Knee joint)

Endocrine system (Pituitary gland, Thyroid gland, Adrenal gland, Pancreas (endocrine), Ovary, Testis)



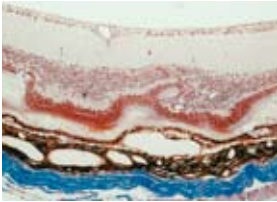
Pituitary adenoma.
Hematoxylin and eosin stain

Central Nervous System (Cerebral cortex, Hippocampus, Basal ganglia, Cerebellum, Brain stem)



Cerebral cortex and Hippocampus:
Luxol fast blue and cresyl violet stain

Sensory organs (Eye and adnexa, Tongue)



Retinal dysplasia. Mallory's trichrome and hematoxylin stain

2. FIXATION TECHNIQUES

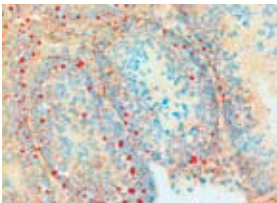
- Tissue fixation with 10% buffered neutral formalin
- Tissue fixation with glutaraldehyde
- Tissue fixation with 4% buffered paraformaldehyde
- Tissue fixation by perfusion
- Tissue fixation with periodate-lysine-2% paraformaldehyde (plp)
- Tissue fixation with Bouin's solution

3. EMBEDDING

- Demineralization of long bones using EDTA.
- Freezing tissues for histopathological purposes
- Fixation, decalcification, processing and paraffin embedding of whole adult mouse head
- Tissue processing and embedding in paraffin

4. SECTIONING

- Sectioning from paraffin-embedded tissues
- Cryosectioning



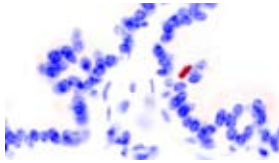
Mutant testis. Oil red O and DAPI counterstain

5. STAINING

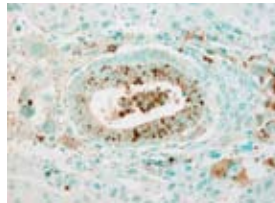
- Hematoxylin and eosin staining of histological sections standard
- Oil red o staining of histological sections
- Detection of cholesterol esters on histological sections
- Congo red stain for amyloid on histological sections
- Luxol fast blue and cresyl violet staining of brain and spinal cord on histological sections
- Von kossa's silver nitrate method for detection of calcified tissue deposits
- Periodic acid Schiff (PAS) staining of histological sections (glycoproteins, mucins)
- Orcein stain for elastic fibers on histological sections
- Modified Mallory's trichrome staining of histological sections
- Sirius red stain for collagen on histological sections
- Alizarin red and alcian blue method for staining bones and cartilages in the adult mouse, in toto
- Mammary gland whole mount preparation carmine-alum staining
- Staining for β -galactosidase activity in transgenic mice
- Succinic dehydrogenase (SDH) staining for mitochondria
- Alcian blue method for staining of cartilage on histological sections

6. PROLIFERATION AND APOPTOSIS

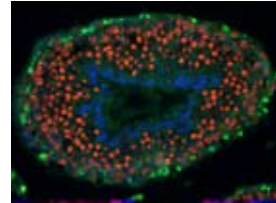
- Detection of BrdU incorporation
- Immunodetection of the cell proliferation (anti-ki-67 immunostaining)
- Immunodetection of cells in mitosis (anti-phosphorylated H3 immunostaining)
- In situ detection of cell death (TUNEL assay)



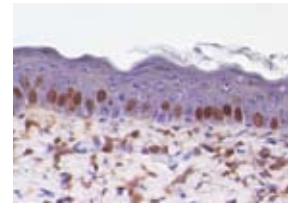
Prostate
Detection of mitotic cells (red signal)
with an anti-phosphorylated H3
antibody



Mutant embryo at E7.5
Tunel analysis



Adult testis
Double immunostaining for detection
of TIF1 beta and BrdU



Skin
Detection of BrdU incorporation
Peroxidase activity was revealed
using DAB
Hematoxylin counterstain

7. IMMUNODETECTION OF ADIPOPHILIN

Immunodetection of adipophilin

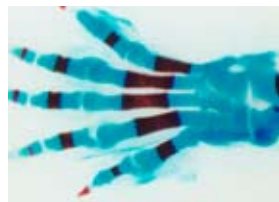
8. IMMUNOHISTOCHEMICAL ASSAYS

Experimental assays can be run on commercially available antibodies (references and protocols required) or with antibodies provided by the customer.

9. EMBRYOLOGY

The objective of this activity is to detect developmental defects in prenatal specimens. Embryos and fetuses are analyzed macroscopically, then fixed, eventually decalcified, embedded in paraffin, serially sectioned and stained. Whole skeletal analyses are performed on fetuses.

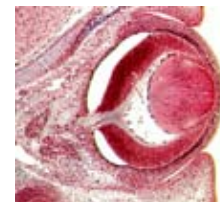
- H&E stained slides of serial histological sections of embryos and fetuses.



Foot plate
Alcian blue and alizarin red stain



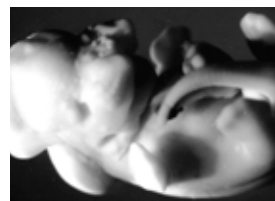
Fetus at E18.5
Mallory's trichrome
and hematoxylin stain



Fetal eye
Mallory's trichrome
and hematoxylin stain



External view of a E9.5 embryo



Exencephaly and facial cleft



Vertebrae of E18.5 mouse fetus.
Von Kossa stain