Protocol

Necropsy Guide for the Collection of Tissues from Mice with or without Tumors

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Mice that die at any stage of a mutational analysis, whether during early life or during ageing or longitudinal studies such as tumor survival studies, can yield important information. This protocol provides a necropsy guide for the collection and processing of tissue samples to provide material for complete histological or immunostaining analysis.

INTRODUCTION

During a longitudinal tumor study to determine a tumor-free survival curve, regular monitoring will identify sick or dead animals or mice with visible signs of tumors. Mice with tumors and moribund animals should be sacrificed for necropsy and collection of tumor and tissue samples for histopathology. Necropsy on dead animals can also yield useful data provided the animals have not degraded significantly. For each animal that is sacrificed or found dead, a complete histopathological workup, including normal tissue as well as any tumors, will provide valuable information as part of the tumor study.

MATERIALS

It is essential that you consult the appropriate Material Data Safety sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Appropriate fixative for histology or immunostaining (e.g., Bouin's (Bouin's fixative <R>; 4% paraformaldehyde [PFA] <R>) or other special stains) (see Protocol: **Fixation of Mouse Embryos and Tissues** [Nagy et al. 2007]).

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From the Mouse Phenotypes collection by Virginia E. Papaioannou and Richard R. Behringer.

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Equipment

Dissection instruments

METHOD

1. Generate a list of tissues that will be collected from every mouse in addition to samples of any tumors detected. This may depend on the nature and expression of the gene under study or on information such as tissue susceptibility to tumor formation.

   You can never take too many samples because you cannot predict whether you will have to go back to a certain tissue for more analysis.

2. Perform necropsy as soon as the mouse is euthanized or is found dead. If the necropsy cannot be performed immediately, refrigerate the body. Never freeze the body because this will cause tissue distortion.

   However, specific tissues may be frozen for RNA (transcriptome) and DNA (loss of heterozygosity or copy number analysis) studies. In addition, specific tissues can be processed for frozen sections.

3. Fix tissues minimally at a 1:10 ratio of tissue to fixative at room temperature (Bouin's) or 4°C (PFA).

   Ideally, tissue samples should be no thicker than 5 mm for good penetration of the fixative. The length and width can be greater. Sometimes you may have no choice but to fix a large sample (e.g., the entire head).

4. Keep detailed notes as part of your permanent record during necropsy. Devise a numbering system for each animal and tissue to be used consistently during the entire duration of the tumor study, which may last for several years and involve multiple investigators. Describe the location, size, shape, pattern, amount, consistency, involvement, and color of each tumor. Take tissue samples from the tumor and nearby control tissues.

5. Acquire images of the tumor in situ and dissected away from the body. Establish an image numbering system to link images to a specific mouse and tissue sample (see Step 4).

RECIPES

Bouin's Fixative

2 g picric acid powder *or* saturated aqueous picric acid (see below)

Picric acid is explosive in crystal form!

20 g paraformaldehyde
Formalin (40% w/v formaldehyde), as an alternative to paraformaldehyde (see below)
NaOH (1 N)
2× Phosphate-buffered saline (PBS)
Glacial acetic acid (to be used with saturated picric acid and formalin; see below)

To prepare 1 L of Bouin's fixative, dissolve 2 g of picric acid in 500 mL of H_2O. Filter through a Whatman No. 1, or equivalent. Add 20 g of paraformaldehyde, and heat to 60°C in a fume hood. Add a few drops of 1 N NaOH to dissolve. Cool and add 500 mL of 2× PBS.

Alternatively, combine 75 mL of saturated picric acid, 25 mL of formalin, and 5 mL of glacial acetic acid. Store at 4°C.

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Paraformaldehyde (PFA; 4%)

Reagent	Quantity (for 100 mL)	Final concentration
Paraformaldehyde (PFA)	4 g	4%
Phosphate-buffered saline for immunohistochemistry (10×), diluted to 1× <R>	100 mL	

Slowly dissolve PFA in 1× phosphate-buffered saline over low heat until solution clears. Cool. Use immediately or aliquot and store for up to 1 yr at −20°C.

REFERENCES

Nagy A, Gertsenstein M, Vintersten K, Behringer R. 2007. Fixation of mouse embryos and tissues. *Cold Spring Harb Protoc* doi:10.1011/pdb.prot4702

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