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Systematic Evaluation of the Skin and Adnexa in Mutant Laboratory Mice

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Abstract

The skin and its adnexa, hair and nails, are one of the easiest organ systems to evaluate as it is the most accessible. However, mice are small, have lots of very fine hair of multiple types, and while major abnormalities are obvious, subtle ones, or the basis for these defects can be difficult to define. To assist in outlining basic approaches to evaluating mice clinically as well as microscopically with the help of a pathologist, methods are provided here that are used routinely in many laboratories. The mouse is a very useful mammalian model system for studying normal and abnormal (disease) development and there is a high degree of correlation with not only human biology and medicine but that of most other mammalian species. Utilizing basic approaches standardizes analysis and provides quality samples for analysis.

Keywords

skin; hair; nails; tissue collection

INTRODUCTION

The skin has been jokingly called the largest of the intermediate sized organs based on calculated mass (Goldsmith, 1990). As a barrier to the outside world, it is also a large immunological and somatosensory organ and is important in physical attraction (reproduction), thermoregulation, and has many other functions (Chuong et al., 2002; Sundberg et al., 2012). Because it is so easy to visualize, the skin is the most obvious organ system for clinical evaluation to identify abnormalities and is generally recognized as the first and most obvious system to determine overall health and wellbeing of any mammalian species, especially humans. However, for the mouse, being a relatively small mammal, the skin (hard to see because of the high density of hairs) and nails (due to their small size) are easily overlooked, although abnormalities of the hair (length, waviness vs. straight, or partial or *in toto* absence) may be observed and often provides a justification for maintaining colonies of mice with such changes by mouse fanciers. While observing that the animal, in this case the mouse, is abnormal, figuring out what is actually wrong, the underlying

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pathophysiology and genetics, can be a daunting feat. Systematic analysis of the clinical features, taking advantage of experts in dermatology and dermatopathology, can quickly find comparable human and domestic animal diseases from which much information is usually available to direct investigators to more specific studies and tests to confirm the preliminary diagnosis. Table 1 lists broad categories of types of skin diseases which provides a starting point for specific diagnostic approaches (Sundberg and King, 2000a; Sundberg and King, 2000b). We provide here an overview on how to start to collect samples for these types of diagnostic assays.

Physical examination

Mice are completely covered with hair. While the ears and tail superficially appear to not have hair, in fact they do, albeit short and more widely spaced than body (truncal/pelage) hair. Vibrissae, the long straight hairs on the muzzle and around the eyes (and less obvious, but around the lower legs above the feet as well), stand out and are used for sensing the environment. The lay term for these specialized hairs is whiskers. “Whisking is a behaviour in which the facial whiskers (vibrissae) of an animal are repetitively and rapidly swept back and forth” (http://en.wikipedia.org/wiki/Whisking_in_animals). There is no structural homolog to vibrissae in humans.

A cursory view of the mouse may reveal areas of hair loss, missing hair types in special regions (the anhydrotic ectodermal dysplasia mutants, downless, tabby, crinkled, sleek, and other allelic mutations in this series lack pilosebaceous units on their tail and zigzag hairs behind their ears) (Sundberg, 1994a; Sundberg, 1994b; Sundberg, 1994c), diffuse hair loss, or changes in color or structure of the hair shafts (curly, misdirected, etc.). Inbred strains should have uniform hair texture and color. Variations, especially in color, often indicate the mice are not inbred. This is a common misunderstanding with genetically engineered mice where a mutation is maintained on a segregating genetic background.

The skin may be abnormal but this may not be easily visualized when hair is present. Shaving with mechanical clippers, depilating with chemicals (Nair®, etc.), mechanical removal of hair (plucking or wax stripping), or simply pushing the hair back may help with a quick evaluation. Nails are very small on mice and can be easily overlooked. All of these structures can be evaluated in mice under general anesthesia or following euthanasia using a dissection microscope and more details can be visualized using scanning electron microscopy (Fleckman et al., 2013).

Necropsy and Tissue Collection

Abnormalities (lesions) are phenotypic deviations from known traits (normal anatomy). Any abnormalities should be recorded in as much detail as possible. Anatomic and pathologic terms should be used if known, but careful descriptions using lay terms can often be translated into anatomic nomenclature by a medically trained collaborator.

Simple and specific descriptive terms commonly used by pathologists are summarized in Table 2. Combined with detailed anatomic location, these descriptions provide a great deal of information on lesions observed. Normal anatomy of the mouse is detailed in several books (Feldman and Seely, 1988; Popesko et al., 1992; Treuting et al., 2012).

Euthanasia

Laboratory mice are usually provided alive for necropsy. Mice have a very high metabolic rate and decompose rapidly after death making histology useless in most other circumstances. A variety of euthanasia methods are approved by the American Veterinary Medical Association (Leary et al., 2013). Care should be taken to ensure that humane treatment is provided. However, as approval for use of all these methods vary between institutions and change over time, methods approved by the Institutional Animal Care and Use Committee must take priority in determining which method is used and your attending veterinarian must be consulted for the appropriate method that can be used at your institution.

Fixatives

A variety of chemical solutions are available to preserve tissues for histologic, immunohistochemical, and ultrastructural studies (Table 3, 4). Each has advantages and disadvantages. The choice depends upon the goals of the study, tissue processing available in your histology laboratory, experience, and preference of the investigators, especially the preference of the pathologist who will interpret the results (Mikaelian et al., 2004). Fixatives should be prepared in advance and be available in adequate volume in appropriately sized containers before the necropsy is started. There are two general rules for histology. (1) Fixatives penetrate cut tissue surfaces to various degrees and should be individually tested before use. Penetration is usually between 1–2 mm on any cut surface, so skin should generally be trimmed. (2) Approximately 20 times the volume of fixative should be used to the volume of tissues to obtain optimal preservation.

Necropsy Procedure

There are numerous published methods on complete necropsies in laboratory mice (Relyea et al., 2000; Seymour et al., 2004; Silva and Sundberg, 2012; Treuting et al., 2012) which should be consulted and the methods most appropriate for your studies adopted. We will limit our discussions here to specialized methods for collecting skin and adnexa for analysis.

Analysis of Samples

The methods described provide an approach to standardize tissue collection for a wide variety of analyses. Most of these require the expertise of a pathologist to interpret the results accurately. This is best done by a veterinary anatomic pathologist as they are most familiar with species variations and specific disease processes in mice. However, to accurately correlate findings with homologous human diseases, the veterinary pathologist should work with an M.D. pathologist, preferably a dermatopathologist. This approach has been very successful in generating the critical data necessary for defining mouse models of human diseases. These same general approaches can be applied to any species when analyzing skin abnormalities.

Basic Protocol 1: Skin Collection for Cryosectioning

Cryosectioning is one of the diagnostic tools used to identify skin abnormalities, cell structure, morphology and diseases within the skin using immunohistochemistry, *in situ*

hybridization, etc. Tissue freezes quickly and can be used immediately whereas fixation with Fekete's acid-alcohol-formalin, paraformaldehyde, glutaraldehyde, and other forms of fixation can take days.

Materials

Mouse

Electric clippers (e.g., Oster Finisher Trimmer, Cat. # 76059-030, Oster Professional Products, McMinnville, TN)

Gloves

Cork dissection board. The cork board should be approximately 14 × 21.5 cm in size and 1.0 cm thick.

95% ethanol

Micro-Dissecting scissors 4.5" curved sharp/sharp (Cat # RS-5913 Roboz Surgical Instruments, Gaithersburg, MD)

Micro-Dissecting forceps serrated slight curve 4" (Cat # RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

Nitex fabric (Sefar, Buffalo NY or small quantities from [Amazon.com](https://www.amazon.com))

Embedding molds (22 × 22 × 20 mm deep; VWR International, Bridgeport, NJ)

Cryo-marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

O.C.T. Compound (Cat # 62550-01, Electron Microscopy Sciences, Hatfield, PA)

Dry ice

Bench top dry ice chest (Cat # C3378-1 Cardinal Health, Dublin, OH)

Aluminum foil

Thin metal platform

Procedure

1. Following euthanasia, the dorsal skin (and ventral if needed) is shaved using electric clippers to remove the hair as hair is very hard and can interfere with sectioning.
2. According to the size and age of the mouse surgically remove a rectangular piece of skin from the dorsal using micro-dissecting scissors and forceps while maintaining orientation of the tissue, anterior to posterior (A–P). This is important as hair follicles grow at an angle and one objective of the skin sections is to obtain complete or nearly complete longitudinal sections of hair follicles.

3. From the rectangular portion of full thickness skin, approximately 1.0×1.5 cm is trimmed.
4. Maintaining A–P orientation of the skin, lay the skin flat on the Nitex fabric.
5. Place two plastic embedding molds in the work area. Fill one mold with O.C.T. Compound and the other mold 1/3 of the way full.
6. Label the mold to be frozen with a cryo-marker for identification.
7. In the first mold “wash” the sample for about a minute to saturate it with O.C.T. Compound and to remove any attached bubbles with forceps. The objective is to obtain a consistent texture of the O.C.T. Compound between the block and tissue.
8. Remove the skin from the mold and remove the Nitex fabric from the skin. Place the skin on a small piece of aluminum foil with the cut section (A–P orientation) at the edge of the foil. Lightly cover the specimen with O.C.T. Compound on one side of the cut section and using a bench top dry ice chest filled with block or crushed dry ice and place the specimen on a thin metal platform. Dry ice is preferred over liquid nitrogen because when tissues are frozen in O.C.T. Compound in liquid nitrogen the O.C.T. Compound will crack and freeze unevenly. By freezing the skin on dry ice the skin will have a more uniform consistency for cryosectioning. We found a galvanized cage card holder worked better than blocks of steel.
9. If there is a point of interest, lightly cover the specimen with O.C.T. Compound opposite the point of interest.
10. At this point place the second mold into the dry ice container and watch until the O.C.T. Compound begins to turn white. Remove the frozen sample from the aluminum foil and quickly place it with the cut side into the semi-frozen O.C.T. Compound. Either cut side is fine unless there is a point of interest. Hold the skin upright with chilled forceps until it is frozen in place. Holding skin longer than a few seconds will cause the forceps to stick to the skin and skin will come out of the mold.
11. Fill the mold with O.C.T. Compound, close the top of the container and allow the O.C.T. Compound to freeze *in toto*. Remove bubbles with forceps as the sample freezes. The bubbles create artifacts and can be problematic when sectioning the skin.
12. Remove the mold and wrap the sample in labeled aluminum foil to protect the tissues from drying out.
13. Transfer tissues on dry ice and store in a -80°C freezer until needed. Thawing and freezing the skin may create ice crystals that can cause artifacts when cutting the skin.

Basic Protocol 2: Snap Freezing Skin Samples

Snap freezing the skin is used for protein and a variety of other assays including RNA extraction for q-PCR (quantitative real time RT-PCR) to be used in genetic studies. Snap

freezing the tissues reduces the chance of water which creates crystals and will cause distortion in the tissues. Snap freezing also reduces the chance of contamination and will yield cleaner proteins/RNA.

Materials

Mouse

Gloves

Electric clippers

95% ethanol

Micro-Dissecting scissors 4.5" curved sharp/sharp (Cat # RS -5913 Roboz Surgical Instruments, Gaithersburg, MD)

Micro-Dissecting forceps serrated slight curve 4" (Cat # RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

Cork dissection board. The cork board should be approximately 14 × 21.5 cm in size and 1.0 cm thick.

Cryo-marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

RNase-free cryo-vials (Cat # 89094-810, VWR, Radnor, PA)

Liquid nitrogen

Long forceps (Fisher, Pittsburgh, PA)

Liquid nitrogen flask (Dewer flask, Fisher, Pittsburgh, PA)

Cryo-boxes (Thermo-Scientific, Pittsburgh, PA)

Procedure

1. After the mouse has been euthanized, the dorsal skin (and ventral if needed) is shaved using electric clippers to remove the hair and cleansed with 95% ethanol to remove any remaining hair thus decreasing contamination.
2. Using micro-dissecting scissors and forceps remove a rectangular piece of skin from the dorsal (size depends on age and size of the mouse) and cut into 0.5 cm² pieces.
3. Place tissue into a sterile cryo-vial and using a long forceps submerge the vial approximately halfway into the flask of liquid nitrogen for 20–30 seconds and release into the liquid nitrogen. The liquid nitrogen should be filled halfway as immersing the vial(s) will cause the liquid nitrogen to boil. Liquid nitrogen should only be used with the proper container such as a high density polyethylene base and cover with a polyethylene coated handle for strong, shatterproof performance. The

flask has double-walled sides with chlorofluorocarbon-free (CFC-free) foam urethane insulation minimizes heat leakage so samples stay cold longer, per recommendations of the manufacturer.

4. Transfer vials in the liquid nitrogen and store in cryo-boxes at -80°C until needed.

Basic Protocol 3: Freezing skin for *in situ* hybridization

In situ hybridization (ISH) is an important tool for identifying specific mRNA and detecting mRNA within preserved tissues fixed in 4% paraformaldehyde needed to study cell regulation and function within the skin along with protein analysis studies.

Materials

Mouse

Gloves

Electric clippers

Embedding molds ($22 \times 22 \times 20$ mm deep; VWR International, Bridgeport, NJ)

O.C.T. Compound (Cat # 62550-01, Electron Microscopy Sciences, Hatfield, PA)

95% ethanol

Cork dissection board. The cork board should be approximately 14×21.5 cm in size and 1.0 cm thick.

Micro-Dissecting scissors 4.5" curved sharp/sharp (Cat #, RS-5913 Roboz Surgical Instruments, Gaithersburg, MD)

Micro-Dissecting forceps serrated slight curve 4" (Cat #, RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

Unlined index type cards

4% paraformaldehyde

Cryo marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

Cryo-vials (Thermo-Scientific™ Nunc™ Cryotube vials, Thermo-Scientific, Pittsburgh, PA)

Rolling wave rotator (Eberbach, Ann Arbor, MN)

30% sucrose

Dry ice

Bench top dry ice chest (Cat # C3378-1 Cardinal Health, Dublin, OH)

Aluminum foil

Thin metal platform

Procedure

1. Label both the vials and embedding molds with a cryo-maker.
2. After the mouse has been euthanized the dorsal skin (and ventral if needed) is shaved using electric clippers and washed with 95% ethanol to remove any remaining hair.
3. Using micro-dissecting scissors and forceps remove a rectangular portion in an A–P orientation (size depends on age and size of the mouse) of full thickness skin, and trim to approximately 0.3×1.0 cm.
4. Maintaining A–P orientation, place the skin on a piece of unlined index type card, transfer to a cryo-vial and fix in 4% paraformaldehyde overnight at 4°C.
5. After overnight fixation the skin is transferred to 30% sucrose at 4°C and placed on the rolling wave rotator overnight.
6. The following day O.C.T. Compound is added to the sucrose for a half and half mixture and placed on the rolling wave rotator for 4 hours at 4°C.
7. After 4 hours the skin is embedded in O.C.T. Compound A–P orientation on dry ice as described in protocol #1.
8. Only one section of skin per mold and make certain to mark the orientation on the mold. Care must be taken to remove all bubbles so artifacts are not created that will cause problems when sectioning the skin.
9. Once the sample has been frozen wrap the mold in labeled aluminum foil and transfer tissues on dry ice and store at –80°C until needed.

Basic Protocol 4: Direct mounting and evaluation of hair shafts

Abnormalities of hair shafts are often easy to observe during a physical examination. Once mice are euthanized or anesthetized, hair shafts on the dorsal (back) skin can be plucked. In adults the hair follicles are usually in the resting growth phase, telogen, for long periods of time (unlike humans that have hair in anagen, the growing phase, for long periods making removal difficult and painful). Because adult mouse hair follicles are often in telogen, the hair shafts can be easily plucked using slight traction with one's fingers with no pain to the mouse.

Materials

Mouse

Gloves

Cryo-marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

Cryo-vials (Thermo-Scientific™ Nunc™ Cryotube vials, Thermo-Scientific, Pittsburgh, PA)

Micro-Dissecting forceps serrated slight curve 4" (Cat # RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

Glass slides (Fisher, Pittsburgh, PA)

Acrymount (Stat Lab Medical Products, Inc. McKinney, TX)

Cover slips (Thermo-Scientific, Pittsburgh, PA)

Slides boxes (Fisher, Pittsburgh, PA)

Procedure

1. Euthanized or anesthetized mouse.
2. With the fingers pluck hair using slight traction.
3. Place plucked hair in a cryo-vial until processed. When collecting multiple mice for a study it is crucial to pluck hair from the same area on all the mice. It is important to change gloves and forceps between mice to avoid cross contamination. Hair shafts are visualized using a dissection microscope or standard compound research microscope if laid flat and immobilized.
4. To view the hair shafts remove 20+ hairs from the vial.
5. Lay hair on a labeled clean glass slide and gently separate with forceps.
6. Once hairs have been separated apply a drop of Acrymount cover slip mounting media. Drop a cover slip at an angle to allow for mounting media to spread across the slide. This forces the hair to lie flat in one plane and, once the mounting media is dry, allows for easy visualization with a compound microscope for photography.
7. After evaluation, slides can be stored in 100 count slide boxes or metal slide filing cabinets indefinitely. Slides can be viewed with standard white light microscopy for structural defects. However, without changing the position of the sample, changing to polarized or dark field microscopy can reveal interesting changes not easily seen with white light.

Basic Protocol 5: Scanning (SEM) and Transmission (TEM) Electron Microscopy

Hair shafts and nail units can be better evaluated by scanning electron microscopy. The differences between SEM and TEM are the fixatives used, methods for sample collection, the dehydration process and how the samples are to be viewed. SEM offers a view of the surface area while TEM examines what is inside of the sample. It is important to collect tissues from the same area of every mouse for the consistency in the study.

Materials

Mouse

Gloves

Electric clippers

20 ml scintillation vial (Sigma-Aldrich, St. Louis, MO)

Cryo-marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

SEM fixative, 15% glutaraldehyde in phosphate buffer

TEM fixative, with 4% paraformaldehyde, 5% glutaraldehyde in cacodylate buffer

Nitex fabric (Sefar, Buffalo NY or small quantities from [Amazon.com](https://www.amazon.com))

95% ethanol

Micro-Dissecting scissors 4.5" curved sharp/sharp (Cat # RS-5913 Roboz Surgical Instruments, Gaithersburg, MD)

Micro-Dissecting forceps serrated slight curve 4" (Cat # RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

Cork dissection board. The cork board should be approximately 14 × 21.5 cm in size and 1.0 cm thick.

Phosphate buffer

Cacodylate buffer

Procedure

1. Following euthanasia, the dorsal skin (and ventral if needed) is shaved using electric clippers to remove the hair and cleansed with 95% ethanol to remove any remaining hair.
2. Using micro-dissecting scissors and forceps remove a rectangular portion in an A–P orientation (size depends on age and size of the mouse) of full thickness skin from the dorsal for both SEM and TEM.
3. Maintaining A–P orientation, lay skin flat on the Nitex fabric.
4. Skin for SEM is trimmed to 1.0 × 1.0 cm and placed in a labeled 20 ml scintillation vial with 15% glutaraldehyde in phosphate buffer fixative overnight at 4°C.
5. Skin for TEM trimmed to 1.0 × 1.0 cm, feathered and placed in a 20 ml scintillation vial with 4% paraformaldehyde, 5% glutaraldehyde in cacodylate buffer overnight at 4°C.
6. Following overnight fixation tissues for both SEM and TEM are placed in their appropriate buffers until they are processed.

Basic Protocol 6: Skin collection for RNA Extraction

RNA is used for a variety of methods to detect cellular development and function as well as determining abnormalities and diseases in the skin. These methods include microarray analysis, gene expression *in situ* hybridization and protein studies.

Materials

Mouse

Sterile gloves

Electric clippers

Sterile Micro-Dissecting scissors 4.5" curved sharp/sharp (Cat # RS-5913 Roboz Surgical Instruments, Gaithersburg, MD)

Sterile Micro-Dissecting forceps serrated slight curve 4" (Cat # RS-5135, Roboz Surgical Instruments, Gaithersburg, MD)

DEPC treated water (Fisher, Pittsburgh, PA)

Sterile paper towels

Cork dissection board. The cork board should be approximately 14 × 21.5 cm in size and 1.0 cm thick.

Sterile Nitex fabric

Disposable plastic bags

Cryo-marker (Cat # 14-905-30 Fisher, Pittsburgh, PA)

RNase-free cryo-vials (Cat # 89094-810, VWR, Radnor, PA))

RNase Zap Decontamination Solution® (Ambion)

RNase Zap Decontamination Wipes® (Ambion, Austin, TX).

RNAlater® Stabilization Solution (Ambion, Austin, TX)

Cryo-boxes (Thermo-Scientific, Pittsburgh, PA)

Procedure

1. The work area is to be thoroughly clean and wiped down with RNaseZap Wipes followed by wiping down with DEPC treated water. The work area, sterile instruments, and Nitex fabric are sprayed with RNase Zap followed by wiping down with DEPC treated water to guarantee an RNase-free environment.
2. Wrap the cutting board in a disposable plastic bag.

3. Wipe the plastic covered cutting board with RNaseZap Wipes.
4. Lay clean sterile paper towels on the cutting board.
5. Following euthanasia, the dorsal skin (and ventral if needed) is shaved using electric clippers to remove the hair and cleansed with 95% ethanol. When collecting tissues for RNA extraction it is important to know that it is done in a very timely manner. The tissues begin to degrade as soon as the animal has been euthanatized.
6. Wearing sterile gloves the mouse is wiped with RNaseZap Wipes to remove any remaining hair to reduce contamination and yield high quality RNA. After the mouse has been wiped, gloves are changed, sprayed with RNaseZap and the skin is collected. It is essential instruments, gloves, disposable plastic bags and paper towels be changed between mice to ensure there is no cross contamination since RNases are secreted through the skin which can result in RNA contamination.
7. Using sterile micro dissecting scissors and forceps remove a rectangular piece of skin from the dorsal (size depends on age and size of the mouse).
8. Lay the skin flat on the Nitex fabric and cut into 0.5 cm² pieces allowing the *RNAlater* to be absorbed in the skin.
9. After the tissue has been cut, the Nitex fabric is removed before placing the tissue into the cryogenic RNAase-free vials containing *RNAlater* to permeate the cells and stabilize the RNA for later use. The amount of *RNAlater* to be added is a minimal 5 times the amount of tissue collected. It is important to have the correct tissue and *RNAlater* volume ratios as the consequence of too little *RNAlater* will result in degraded RNA. However make sure not to overfill the vial with *RNAlater* as it will freeze and possibly break the vial.
10. Store the tissue overnight at room temperature.
11. Place vials in a cryo-box and transfer to a –20°C freezer and allow samples to freeze before transferring samples to a –80°C freezer until needed for analysis.

COMMENTARY

Background Information

While it is easy to collect samples, interpretation takes expertise. Working with a pathologist or dermatopathologist familiar with mouse tissues is critical for an accurate interpretation. Experience is needed to learn to consistently orient samples and collect them quickly so as to optimize results. Once this becomes routine, consistent, high quality samples can be obtained which will enable comparisons between mutant and control tissues as well as with samples collected in other investigations. Basic histology textbooks (Treuting et al., 2012) provide illustrations of normal anatomic structures in the skin and adnexa which can be used for comparison to reference the quality of the work generated.

Critical Parameters

Performing a careful physical examination will help with initial evaluation of the mutant or diseased mouse. However, systematic evaluation of the skin and adnexa will avoid missing lesions. Working with a pathologist who is trained to scan the slides to find abnormalities will further optimize interpretation. Once a general diagnosis or better yet, a specific diagnosis is made, more focused assays can be performed for confirmation of the diagnosis and then to work out the detailed pathogenesis and genetics of the disease.

Trouble Shooting

Mice have a very high metabolic rate and decompose quickly. Therefore it is imperative to practice these methods in order to do them quickly and efficiently so as to get tissues fixed as quickly as possible. This avoids autolysis, the process of decomposition, which dramatically reduces the quality of the samples. Having the results screened by a pathologist will provide quality control. Reading the slides with the pathologist will help you to understand the anatomy, abnormalities, and artifacts present. In so doing, you can learn to separate the artifacts you induced by the tissue collection from those of the other services, such as the histology laboratory, so that further modifications can be made to optimize the protocols.

Anticipated Results

By using systematic approaches you will develop a standard approach regardless of gross clinical features which will minimize missing major lesions. One's skills will improve with practice and experience. Working with clinical investigators, especially working with pathologists, will help you gain an understanding of the lesions present, how to interpret them, and to compare these lesions with those seen in human patients so as to develop mouse models for specific human diseases.

Time Considerations

Initially these protocols are slow and labor intensive. The old surgeon's adage, "Time is trauma", is valid here as well. The longer it takes to collect and fix tissue samples the greater the loss of integrity of the samples due to autolysis. However, speed should not supersede care. As one gains experience the speed will come eventually making this an efficient procedure.

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Table 1

General types of cutaneous mutant mouse phenotypes (Sundberg and King, 2000b).

<p>Hair color (pigmentation): Any variation from normal of the inbred strain.</p>
<p>Eccrine gland disorder: Histologic diagnosis based on anatomy of gland (change in anatomy or aplasia, total loss). Neoplasia may be seen by masses on the footpads where these glands are located.</p>
<p>Sebaceous gland defects: Histologic diagnosis on body skin hair follicles; may present as alopecia (hair loss).</p> <p><i>Meibomian glands</i> are specialized sebaceous glands in eyelids. Abnormalities present as closed lids, opacity of corneas, or exudation from eyes.</p> <p><i>Preputial/clitoral glands</i> are specialized glands in the male/female genital areas. These normally undergo atrophy with duct ectasia as mice age. Aplasia, failure to develop, may be seen at the time of necropsy.</p> <p><i>Zymbal's gland</i> is a modified sebaceous gland located by the ear canal. Atrophy, failure to develop is seen in some mutant mice.</p>
<p>Primary scarring disorders (cicatricial alopecia) present as hair loss (alopecia).</p>
<p>Hair growth and structural defects can present as mice with long hair (extended hair cycle) or most commonly, as alopecia, hair loss. Hair shaft breakage may be evident with a magnifying lens or visualized using a dissection microscope.</p>
<p>Noninflammatory proliferative skin diseases (ichthyosiform dermatoses and keratodermas) are observed as mice with thick scaly skin grossly that lack a major inflammatory cell component histologically.</p>
<p>Inflammatory proliferative skin diseases (psoriasiform dermatoses) are observed as mice with thick scaly skin that have any of a number of different types of inflammatory cell infiltrates in the dermis at the histological level.</p>
<p>Autoimmune diseases may have lymphocytes in the dermis and around hair follicles (cell mediated) or be seen as separation of layers of the epidermis and hair follicles (humoral) at the histological level. Alopecia (hair loss) or ulceration may be the presenting signs.</p>
<p>Bullous and acantholytic defects are blisters due to any of a number of causes. Histologic evaluation of the blister can yield a definitive diagnosis. However, once the blister ruptures, an ulcer develops which is not diagnostic.</p>
<p>Nail growth and structural defects may be seen at the gross level as long, curved nails, broken nails, lost nails, or ulcers at the end of the digits.</p>
<p>Papillomatous diseases are wart-like proliferative lesions. As a laboratory mouse papillomavirus has now been discovered (Ingle et al., 2011; Joh et al., 2011; Joh et al., 2012) this may be one cause. Some transgenes or chemical carcinogens can also cause these lesions which may or may not be neoplastic.</p>
<p>Neoplasia can be seen as unpigmented (squamous cell carcinoma, trichoepithelioma, amelanotic melanoma) or pigmented (melanoma) proliferative lesions anywhere on the skin.</p>

Table 2

Basic components of a pathological description

Organ or tissue name (Skin, ear, tail, or nail)
Specific site (dorsal, interscapular or lumbar skin; left ear/pinna, proximal or distal tail, digit 1, left front foot, etc.)
Location
Medial or lateral - toward the middle or periphery
Dorsal or ventral - back or abdominal region
Cranial or caudal - toward the head or the tail
Promximal or distal - close to the body or far end of a limb, away from the body
Site specified by anatomic proximity (lumbar vs. thoracic, over the lower back or chest region)
Pattern and or number:
Focal - Localized circumscribed process
Patchy - Alterations that are multiple and poorly delineated
Multifocal - Indication of the specific number of foci contributes to the visual picture
Diffuse - Total involvement of the structure
Specific alteration and or morphologic diagnosis (i.e. hemorrhages, abscesses, edema)
Color
Shape
Size - Measure with a ruler, preferably in metric units; enlargement or decrement if that change is uniform in size
Severity - Degree – mild, moderate, severe, extreme
Etiology
Gross examination - Identify parasites if present
Impression smears - Identify infectious agents by appearance
Special tests - Bacterial or fungal culture, tissue culture for viruses, PCR or serology for detecting a known infectious agent's DNA or antibodies produced against it.

Table 3

Commonly used fixatives for mouse histopathology

Fekete's Acid Alcohol Formalin (Tellyesniczky's/Fekete's Solution)	This is a commonly used fixative in mouse laboratories (Mikaelian et al., 2004; Sundberg and Boggess, 2000; Sundberg et al., 1998). The Fekete (10% formalin) modification of Tellyesniczky's Solution (5% formalin) (Fekete, 1938) provides rapid and surprisingly deep tissue fixation. Specimens are transferred to 70% ethanol following overnight fixation after which they are trimmed and processed. This fixative yields high quality specimens in histologic sections and is one of the best for looking at details in the skin. Long term storage can be a problem as ethanol is flammable and evaporates easily. Once dry the specimens are useless. Artifacts associated with this fixative include erythrocytes (red blood cells) becoming leached so that they appear only as pink ghosts within vessels and a variety of vacuoles and crystalline structures forming in neurons and neuropile, respectively. Of the commonly used fixatives, Fekete's acid alcohol formalin is the best general purpose fixative for maintaining epitopes for immunohistochemistry (Mikaelian et al., 2004).
Bouin's Solution	This fixative uses formalin, acetic acid, and picric acid, which stains everything permanently yellow. Delicate detail is not often well preserved, especially for the skin, but this fixative is preferred by some pathologists and researchers, especially for neurological tissues. Penetration in tissue is moderate. Bouin's fixed specimens must be washed in running tap water for 2–4 hours after initial overnight fixation and stored in 70% ethanol or they become very brittle. Bouin's solution can be used for bone decalcification since the acids will demineralize specimens that are left in the fixative for several days or weeks. If used for decalcification, the Bouin's solution must be changed weekly to optimize demineralization.
Neutral Buffered 10% Formalin Solution	This is the most commonly used fixative in most pathology laboratories and is the fixative of choice for participants in the Human Mouse Models Cancer Consortium (Cardiff et al., 2000). Specimens can be fixed overnight and left indefinitely in the fixative. Specimens can be processed at any time as long as they remain wet, sometimes many years after initial collection. This fixative is particularly useful for retrospective evaluation of lipids or other substances that are soluble in ethanol and would be lost during tissue processing. For example, the presence of fat in adipocytes can be demonstrated if you take wet tissue fixed in neutral buffered 10% formalin, trim the tissue, cut frozen sections, and stain the sections with oil-red O or other lipidophilic histochemical stains. Most of the other fixatives are alcohol based and remove lipid. Long term storage in neutral buffered 10% formalin causes continued cross-linking of amino groups resulting in changes in the tertiary structure of proteins. As a result, many antigenic epitopes are lost or changed, making immunohistochemistry problematic. Transfer of tissues into 70% ethanol after overnight (12 hour) fixation can reduce but not eliminate this effect (Mikaelian et al., 2004). Neutral buffered 10% formalin is noxious and should be used in a fume hood. Buffering is needed to reduce acid hematin formation, an artifact of fixation.
B5 Fixative	This fixative is sometime recommended for immunohistochemistry. B5 fixative contains mercury salts making it difficult to dispose of and fine precipitates can form with some tissue stains. Tissues fixed in B5 may be difficult to cut.
Carnoy's Fixative	This treatment fixes tissues rapidly. It preserves glycogen and enhances the staining of mast cell granules. Nissle granules are also well preserved. Because it is an alcohol/acid based fixative, it lyses red blood cells and acid soluble granules.

4% Paraformaldehyde.	This is used as a fixative for electron microscopy and <i>in situ</i> hybridization and has become popular as a general histologic fixative in laboratories that use these techniques. The paraformaldehyde should be prepared in a buffered solution at pH 7 and refrigerated until use. It will keep for several weeks this way. It is one of the least useful fixatives for maintaining epitopes for immunohistochemistry (Mikaelian et al., 2004).
Zinc based fixatives	A variety of proprietary fixatives, based on variations of the classic fixatives, were developed and marketed, not so much as fixatives for routine, high quality histopathology, but rather to optimize antigenic epitopes for immunohistochemistry (Tome et al., 1990). Such fixatives are the basis for large-scale tissue arrays that provide large numbers of small tissues on individual slides. A variety of proprietary zinc based fixatives (such as IHC Zinc, BD Biosciences PharMingen, San Diego, CA) yield good immunohistochemical results while providing adequate fixation for histopathology.
Glutaraldehyde.	This fixative is commonly used for ultrastructural studies (transmission and scanning electron microscopy). Tissue penetration is minimal, approximately 1 mm on any cut surface, so specimens have to be finely minced with a sharp razor blade to achieve adequate fixation. Several different buffers can be used. The most common are phosphate and cacodylate based buffers. Phosphate buffers are safe and yield good results when used fresh. Fine electron dense precipitates may form that will render a specimen useless if the buffer used is old. Cacodylate buffer is arsenic based, which is toxic and can be difficult to dispose of properly (Bechtold, 2000).
Karnovsky's Fixative	This fixative is used for plastic embedding for transmission electron microscopy. Karnovsky's is a general term for any fixative combining glutaraldehyde and paraformaldehyde in a phosphate buffer. Glutaraldehyde has minimal penetration ability. Paraformaldehyde penetrates deeper, but fixation is unstable. Karnovsky's fixative combines the positive points of both these chemicals.
JB4 fixative	This fixative is used for plastic embedding for 1 µm sections used in light microscopy. It combines glutaraldehyde and paraformaldehyde in a cacodylate buffer (Bechtold, 2000).
O.C.T. compound	O.C.T. Compound (Tissue-Tek®, Sakura Finetek U.S.A., Inc., Torrance, CA) is not a fixative but rather an embedding medium for frozen tissue sections. It is a thick, clear fluid used in conjunction with plastic base molds such as CMS Tissue Path-Disposable Plastic Base Molds (Curtis Matheson Scientific, Inc. Houston, TX) to bind fresh tissues for freezing and sectioning. Tissues are floated in the mold and placed on dry ice. The O.C.T. solidifies and turns white as it freezes, after which the block has to be stored frozen at -80°C until sectioning.

Table 4

Formulations of commonly used histological fixatives

<i>Tellyesniczky/Fekete Solution</i>	70% Ethanol (100 ml) Glacial acetic acid (5 ml) 37–40% Formaldehyde solution (10 ml)
<i>Bouin's Solution</i>	Saturated aq. picric acid (85 ml) Glacial acetic acid (5 ml) 37–40% Formaldehyde solution (10 ml)
<i>10% Neutral Buffered Formalin Solution</i>	37–40% Formaldehyde solution (100 ml) Distilled water (900 ml) Sodium phosphate-monobasic (4 g) Sodium phosphate-dibasic (6.5 g)
<i>B-5 Fixative</i>	Mercuric chloride (6 g) Sodium acetate-anhydrous (1.25 g) Distilled water (hot; 90 ml) Add 37–40% Formaldehyde solution (10 ml) use before use
<i>Carnoy's Solution</i>	Absolute ethanol (60 ml) Chloroform (30 ml) Glacial acetic acid (10 ml)
<i>4% Paraformaldehyde Solution</i>	16% Paraformaldehyde (10 ml) Phosphate buffered saline (pH 7.2, 30 ml)
<i>Glutaraldehyde Solution</i>	15% Glutaraldehyde (10 ml) 0.2 M Phosphate buffer (pH 7.2, 50 ml) Distilled water (40 ml)
<i>Karnovsky's Solution</i>	25% Glutaraldehyde (8 ml) 16% Paraformaldehyde (12.5 ml) 0.2 M Phosphate buffer (pH 7.4, 50 ml) Distilled water (29.5 ml)
<i>JB4 Solution</i>	25% Glutaraldehyde (16 ml) 16% Paraformaldehyde (12.5%) 1 M Cacodylate buffer (20 ml) Distilled water (151.5 ml)