

# WELLCOME SANGER INSTITUTE STANDARD OPERATING PROCEDURE PACKET

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# SANGER INSTITUTE STANDARD OPERATING PROCEDURE

SUBJECT: Neurological and Morphological Phenotypic Assessment (NaMPA) – V1

SOP Number: SOP0088	To be reviewed:	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

# **INTRODUCTION:**

The purpose of this procedure is to assess and record gross motor, neurological and morphological phenotypes in wild-type and genetically altered mice.

# **ABBREVIATIONS:**

**DCF** = Data Capture Form

IVC = Individually Ventilated Cage

**LAA** = Laboratory Animal Allergens

**NaMPA** = Neurological and Morphological Phenotypic Assessment

**PAF** = Project Authorisation Form

PIL = Procedure Individual License

**PPE** = Personal Protective Equipment

**PPL** = Procedure Project Licence

**QC** = Quality Control

**RA** = Risk Assessment

**RSF** = Research Support Facility

**SMP** = Sick Mouse Procedure

**SOP** = Standard Operating Procedure

**SLT** = Senior Leadership Team

# **QUALITY CONTROL (QC) DURING PROCEDURE:**

Refer to the table below for approved QC fail comments steps to be used during procedures.

If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

# **In-Life Procedures:**

Problem / Issue	QC fail reason
At any point during the procedure the	Fail whole DCF as 'Sick mouse' - for all
mouse is deemed sick and processed	tests that day
through Sick Mouse Procedure (SMP)	



Mouse incorrectly scheduled at wrong	Fail whole DCF as 'Scheduling Issue'
week Insufficient anaesthesia level affects	Fail whole DCF as 'Anaesthesia Issue'
the whole test DCF Insufficient anaesthesia level affects	Fail parameter(s) as 'Anaesthesia issue'
specific parameter(s)  A welfare issue makes it impossible to	Fail parameter(s) as 'Welfare issue'
collect specific parameters	. ,
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Glucose meter unable to record low blood values	Fail parameter(s) as 'Meter reading LO'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

# **HEALTH & SAFETY:**

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-3332

**Assessment Title:** Basic Mouse Procedures

Assessor: Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or down flow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.



All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual License (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

# **RESOURCES:**

# **Equipment:**

- 1. Tecniplast Interactive Cage Change Station
- 2. 70% Ethanol Hazardous substance: highly flammable
- 3. Hydrex Pink Hazardous substance: highly flammable
- 4. Hydrex Hard surface spray **Hazardous substance**: highly flammable
- 5. Paper hand towels
- 6. Tecniplast Mobile Individually Ventilated Cage (IVC) Rack
- 7. Clean cages (as defined by pipeline)
- 8. Diet (as defined by pipeline)
- 9. Nestlets
- 10. Perspex arena of 15cmx45cm
- 11. Clear 20 cm Perspex tubes with the following internal diameters: 20, 26, 30, 34mm
- 12. Click-box generating a 19.3 kHz tone at 90 dB
- 13. Fine cotton probe
- 14. Cirrus decibel meter (model: CR:821c)
- 15. Ruler
- 16. Post Procedure Check labels
- 17. Balance with weight boat
- 18. 'Phenotyping in progress' sign
- 19. 'Quiet please Behavioural work in progress' folding sign

#### **Associated SOPs/Documentation:**

- EQ18 Use of Techiplast Interactive Cage Change Stations
- SOP0062 Grip Strength
- **SOP0101** Taking and Returning Cages for Procedures
- 1) Equipment\_QC\_tool
- NaMPA Training Aid
- NaMPA Scoring and Health Record Guide
- Dysmorphology Catalogue
- WT Dysmorphology Images (all)
- Dysmorphology photos required

**Staff:** This test can be completed by 2 phenotypers.

# NOTE:

On some pipelines, this assay was performed in conjunction with Grip Strength.



Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

- 1. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 Taking and Returning Cages for Procedures).
- 2. Place 'Phenotyping in progress' sign on the outside of the door and the 'Quiet please Behavioural work in progress' folding sign before the door
- 3. Prepare change station for use (refer to EQ18 Use of Tecniplast Interactive Cage Change Stations).
- 4. Place all equipment and a clean cage in the change station. Clean equipment with 70% ethanol or Hydrex Hard surface spray and paper towels. Set equipment up according to work flow.
- 5. Open the Equipment QC tool to see if the click box meter QC is required. Perform if required.
- 6. Confirm the ID and sex of the mouse for testing.
- 7. Start the DCF and print the updated cage card with NaMPA procedure added.
- 8. Weigh mouse according to pipeline/DCF.

#### 9. NEUROLOGICAL OBSERVATIONS

# 9.1. Above the arena

- 9.1.1. Positional Passivity:
  - 9.1.1.1. If the mouse has a tail welfare issue do not lift the mouse, QC fail the parameters that cannot be measured and move onto step 9.2.
  - 9.1.1.2. Lift the mouse by the tail and suspend briefly, observing for typical struggling behaviour.
  - 9.1.1.3. If the mouse does not struggle when being held by the tail, hold the mouse in a loose scruff.
  - 9.1.1.4. If the mouse still does not struggle, lay the mouse supine without altering the scruff.

#### 9.2. In the arena

- 9.2.1. Transfer the mouse to one end of the arena from a height of ~25cm (drop the mouse into the arena following step 9.1) and score according to the current DCF, observing for an 'extended freeze'.
- 9.2.2. To perform the startle response test, hold the click box 30cm above the mouse and wait for it to stop moving before administering the stimulus.
- 9.2.3. Allow the mouse to explore the arena while observing for any abnormalities in locomotion or gait.

#### 9.3. On the cage grid

- 9.3.1. Transfer the mouse to the grid placed on top of the clean cage and score corneal and pinna reflexes using the cotton probe.
- 9.3.2. Contact Righting Reflex:



- 9.3.2.1. Allow the mouse to enter the Perspex tube of the appropriate size without using force. Ensure the mouse's whole body is inside the tube, including the tail.
- 9.3.2.2. Swiftly rotate the tube by 180° and observe whether or not the mouse attempts to orientate itself the right way up. Allow 10 seconds for the mouse to react.
- 9.3.2.3. If it is believed that the response has been affected due to the size of the tube, repeat with a tube of a different size.
- 9.4. Any abnormal behaviours or observations that are not included on the DCF should be noted in the comment section, indicating where they occurred.

#### 10. MORPHOLOGICAL OBSERVATIONS

- 10.1. Place the mouse on the cage grid to examine from the dorsal side. Keep in mind what is to be expected. Initially, observe for abnormalities in the following:
  - 10.1.1. Coat; check the presence, colour and appearance. Check the head, face, tail and dorsal coat. Check for guard hairs, hair behind ears and tail tip hairs.
  - 10.1.2. Skin condition and pigmentation; check the tail, pinna and skin on the dorsal side. To assess skin appearance and pigmentation, the coat is stroked from the back to the front.
  - 10.1.3. Fight wounds; check for presence and record location if any are observed.
  - 10.1.4. Tail morphological abnormalities; stroke the tail along its entire length with two fingers, both laterally and medially.
- 10.2. Holding the mouse in a scruff, observe for abnormalities in the following:
  - 10.2.1. Coat; look at the presence, colour and appearance of the ventral coat and limbs.
  - 10.2.2. Skin condition and pigmentation; check limbs, paws, digits and ventral coat.
  - 10.2.3. Fight wounds; check for presence and record location if any are observed.
  - 10.2.4. Ventral and lateral sides of head, body and ears.
  - 10.2.5. Eye abnormalities and eyelid opening (pipeline dependent).
  - 10.2.6. Whiskers; present, absent or abnormal.
  - 10.2.7. Shape, number and colour of the incisors. Shape of snout and mouth.
  - 10.2.8. Genitalia.
  - 10.2.9. Number and shape of digits, digit separation, nail number and length, paws and limbs morphology and size.
- 11. Record results according to the current DCF. If an abnormality is seen that requires an image or video, fill out and send a copy of the 'Dysmorphology photos required' email.
- 12. Repeat (steps 6-11) for all mice to be tested and perform a cage clean as defined by pipeline. If the orientation of the grid has been changed during the procedure, return it to its original position before replacing the lid.
- 13. Clean all equipment, surfaces and the floor. Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.
- 14. All cages must display the updated cage card. Place a 'POST PROCEDURE CHECK REQUIRED' label on all cages and register them to the correct rack



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whilst returning them to their destination/home rack in the animal room.
(Refer to SOP0101 – Taking and Returning Cages for Procedures).

# Neurological and Morphological Phenotypic Assessment (NaMPA) Training Aid

#### NOTE

This document provides description of the neurological parameters and recommendations on how to **record selected** morphological parameters in the DCF Neurological and Morphological Phenotypic Assessment.

- If observed morphological changes are not listed in the DCF, tick "Other abnormalities" under a corresponding parameter and describe them in the comment box. Contact a team photographer and they will organise the imaging.
- In the occurrence that a parameter cannot be recorded due to a body part being completely absent, QC fail all related descriptive parameters.
  - E.g. Tail is absent; QC fail parameters such as Tail Length, Morphology using the description 'Readout not possible'.
- In the occurrence that only one of a few body parts is absent select a relevant parameter to address this abnormality and score all related descriptive parameters for existing body parts.
  - E.g. If the majority of vibrissae are absent, score "**Sparse**", if all vibrissae are missing, score "**Absent**"
  - E.g. if some incisors are absent, score "Partly absent", if all incisors are missing, score "Absent"

#### **NEUROLOGICAL PARAMETERS:**

#### **Positional Passivity**

The response of the mouse to being held in position(s) of restraint. The next position is only attempted if the mouse does not struggle in the previous position. Struggling is defined by the mouse moving any part of its body during the period of restraint. This is most often seen in the movement of the paws and curling of the trunk from side to side.

**Struggles when held by the tail** – the mouse struggles when suspended by the tail for more than 5 seconds.

**Struggles when held by the neck** – the mouse fails to struggle when suspended by the tail, but struggles when held vertically in a loose scruff.

**Struggles when laid supine** – the mouse fails to struggle when suspended by the tail or in a loose scruff, but struggles when laid in supine (held horizontally in a loose scruff).

**No struggle** - the mouse fails to struggle when suspended by the tail, in a loose scruff or when laid in supine.

# **Trunk Curl**

**Present** – the mouse curls its torso, to bring its chin towards its stomach when held by the tail. Bending sideways does not count as a trunk curl.

**Absent** – the mouse shows no sign of a trunk curl for at least 5 seconds of being suspended by the tail.

# **Limb Grasping**

**Present** – the mouse brings its fore limbs together and grips with the paw or brings its hind limbs together and grips with the paw. If no grip is achieved or if the hind paw grasps the fore limb (or vice versa), this is not scored as limb grasping.

**Absent** – the mouse shows no signs of limb grasping for at least 5 seconds of being suspended by the tail.

# **Transfer Arousal**

The reaction of the mouse to being 'dropped' into the arena. Head movements are disregarded, but exploratory behaviours (where the fore paws are extended forwards, but the hind paws remain stationary, known as a 'stretch attend') count as locomotion.

**As Expected** – Any of the paws move within approximately 5 seconds of it landing in the arena. This includes stretch attends, grooming and locomotion.

**Extended freeze** – all the paws remains stationary for more than approximately 5 seconds after it lands in the arena.

# **Gait (inc. Ataxia)**

The way in which the mouse walks, including ataxia (coordination of the muscles in the limbs/body resulting in a 'wobbly walk', this can be identified by the base of the tail moving from side-to-side whilst the mouse is walking in a straight line), morphological abnormalities and voluntary/involuntary behaviours.

**Fluid movement** – there are no major defects in the way in which the mouse walks in the arena. Any 'slight limps', 'intermittent hops', 'mild ataxia' or any other minor defects should be noted in the 'gait related comment' section, but still be scored as fluid movement.

**Lack of fluidity in movement** – there are major defects (including severe limps, severe ataxia and retropulsion) in the way in which the mouse walks in the arena. All observations should be noted in the 'gait related comment' section.

#### **Tail Elevation**

The way in which the mouse holds its tail during locomotion in the arena. This should be scored by considering the angle of the middle third of the tail and its elevation above the floor of the arena. See appendix for diagrams.

**As Expected** – the middle third of the tail is held in either a horizontal or an elevated position for a large proportion of the time that the mouse moves in the arena.

**Dragging tail** – the tail drags along the floor or the middle third of the tail is held at a low position or angle for a large proportion of the time that the mouse moves in the arena.

**Straub Tail** – the middle third of the tail is held in a straub position or angle for a large proportion of the time that the mouse moves in the arena.

#### Tremor

Involuntary shaking of the mouse's body.

**Present** – the mouse shudders for a large proportion of the trial. Tremors are sometimes seen for short periods of time in conjunction with urination or defecation, but should only be scored when the tremors are present throughout the duration of the trial.

**Absent** – the mouse does not show any of the above signs.

# **Startle Response**

The reaction of the mouse to a loud auditory stimulus made by a click box above the arena.

**None** – the sound from the click box does not elicit a reaction and the mouse appears to be deaf (does not show any of the behaviours below).

**Preyer reflex** – the sound from the click box elicits the preyer reflex (flicking of the ears), but with no other reaction or movement such as a whole body flinch, flick of the tail, jump or trying to flee. If a mouse shows signs of hearing the click, but does not show the preyer reflex, the reflex is late, performed slowly or only a slight twitch of the ears is seen, record this in the comments section.

**Reaction in addition to the Preyer reflex** – the sound from the click box elicits the preyer reflex (flicking of the ears) in conjunction with another reaction or movement such as a whole body flinch, flick of the tail, jump or trying to flee.

#### Pinna touch reflex

**Present** – the mouse reacts to the cotton thread placed in its ear by either flicking its ear or shaking its head.

**Absent** – the mouse fails to react to the cotton thread placed in its ear by flicking its ear or shaking its head.

#### **Corneal touch reflex**

**Present** – the mouse reacts to the cotton thread placed on the surface of its eye by blinking.

**Absent** – the mouse fails to react to the cotton thread placed on the surface of its eye by blinking.

# **Contact Righting Reflex**

**Present** – the mouse reacts to being inverted by turning its head or moving its limbs to attempt to right itself within 10 seconds of the tube being rotated. Normal head movements should not be counted.

**Absent** – the mouse fails to react to being inverted by turning its head or moving its limbs to attempt to right itself within 10 seconds of the tube being rotated.

#### Headbobbing/Circling

**Present** – the mouse exhibits head movements/orientation and/or circling behaviour which are indicative of balance deficiencies and vestibular defects. This can include repeated tilting the head to look upwards, repeated shaking of the head, running in circles, 'star-gazing', static head tilt etc.

**Absent** – the mouse does not exhibit any head movements or circling behaviour which are indicative of balance deficiencies.

#### Convulsions

**Present** – The mouse shows signs of an involuntary seizure at any point during the experiment. This can include a minor seizure where the mouse gasps and blinks repeatedly whilst remaining stationary, 'excited running' where the mouse seems to run in random directions and bumps into objects or a full body seizure where the body of the mouse contracts and relaxes rapidly and repeatedly.

**Absent** – no sign of involuntary seizures are seen at any point during the experiment.

#### **Comment section**

Any unusual behaviours (head tilt, stereotypic behaviours, etc) that are observed throughout the entire experiment should be noted in this section. This includes any comments on the status of the eyes (closed, partially open), any excessive rearing or grooming, anything that could be called as a behavioural phenotype or affect the behaviour of the mouse.

#### **MORPHOLOGICAL PARAMETERS:**

# COAT/HAIR

# Coat/Hair presence

**As expected –** coat/hair present;

Patchy - focal absence of hair growth in areas where hair is normally expected;

**Sparse** - body hair that is less dense;

Patchy/sparse- presence of less dense hair patches;

**Absent** – absence of hair due to loss of hair on that particular region;

**Other abnormalities** - absence or reduced amount of hair or abnormal onset of hair growth cycle or development, or abnormal hair pattern.

<u>Limb/Paw/digit Hair Presence</u> - If all hair is absent on a leg/paw/digits — record as hair "**Absent**" with a comment specifying number of leg/paw/digits being affected.

<u>Hair behind the ears</u> - If missing this should be recorded as "**Head Coat/Hair** 

Presence: Other Abnormalities" with the comment "Hair behind ears absent".

<u>Guard hairs</u> - If missing this should be recorded as "**Dorsal Coat/Hair: Other Abnormalities**" with the comment "Guard hairs absent".

<u>Tail tip hairs</u> - If missing this should be recorded as "**Tail Coat/Hair Presence**:

Other Abnormalities" with the comment "Tail tip hairs absent".

<u>Note:</u> If there is a finger-nail sized patch of mid-dorsal fur missing this could be due to Hair Follicle Cycle testing. If this is seen **please check previous tests and only score as abnormal (patchy) if Hair Follicle Cycling has not been performed** on the mouse.

#### Coat /Hair Colour

**As expected** – coat/hair colour commonly observed in the WT population of the corresponding genetic background;

**Abnormal** - irregular or unusual pigmentation of the hair.

<u>Digit Coat/Hair Colour</u> - presence of white hair on digits as well as absence of white hair on digits should be scored "**Digit Coat/Hair Colour**: **as Expected**".

# **Coat/Hair Colour pattern**

**As expected** – coat/hair colour pattern commonly observed in the WT population of the corresponding genetic background

**Abnormal** – different patterns of irregular colouration of the hair

"Two white belly patches".

White patches of fur on ventral coat - When 1 or more white patches are present on the abdomen area of the ventral coat this should be recorded as "Ventral Coat/Hair Colour: Abnormal" and "Ventral Coat/Hair Colour Pattern:

Abnormal". This should be followed with a comment "White belly patch" or

# **Coat/Hair appearance**

**As expected** - coat/hair appearance commonly observed in the WT population for the corresponding genetic background;

**Rough** - coat does not have the usual smooth appearance. Rough coat is often due to fighting, if mice show signs of fighting score as "As expected".

Matted - coat hairs sticks together to form clumps and does not lie flat;

Waved - hair or fur having undulations or a sinusoidal shape.

**Other Abnormal Coat Appearances**- any other irregular or unusual appearances of the structure of the hair.

<u>Note:</u> If the appearance of the fur (on any part of the body) is greasy please check the pipeline and diet that the mouse is assigned to:

- If the diet assigned is normal chow then this is not expected and should be scored as: Other Abnormal Coat Appearances for each coat/hair relevant parameter with the comment 'greasy coat'.
  - If the whole coat is found to be greasy then selection of "other abnormal Coat Appearance" would apply to head, dorsal, ventral, limb, paw, digit and tail appearance parameters with a comment of "whole coat greasy".
- If the diet assigned is high fat diet, the greasy appearance can be caused by the diet and should be scored as 'As Expected'

#### **SKIN**

#### **Skin Appearance**

**As Expected** – skin appearance commonly observed in the WT population of the corresponding genetic background;

**Dry** - skin characterized by the lack of natural or normal moisture;

**Wet** - skin characterized by the excess of natural or normal moisture;

Scaly - skin covered with shedding scales or flakes;

Loose - condition in which the skin hangs in folds;

Other abnormal skin appearances – any other anomalies in the visual aspect of the skin.

#### **Skin Colour**

**As Expected** - skin colour commonly observed in the WT population of the corresponding genetic background;

**Abnormal** – any variation of skin colour or complexion.

<u>Dorsal/Ventral non-synchronous skin colour -</u> It is common to see non-synchronous patterns of hair growth cycles between different hair follicle populations in adult mice. Therefore the presence of patchy regions of black, shades of grey and pink colours on both ventral and dorsal sides of the body should be recorded "as Expected".

<u>Tail Skin Colour</u> - lack of pigmentation of the skin of any part of the tail should be scored "as Expected". Variation of skin color or complexion of other parts of the body should be scored as "Abnormal".

<u>Paw Footpad Skin Colour</u> - When recording dark footpads record this as "**Abnormal**" and please add the comment "Dark footpads on one/both fore/hind/all paws".

#### **HEAD**

#### **Snout morphology**

**As Expected** - snout morphology is commonly observed in the WT population of the corresponding genetic background.

**Abnormal** - any structural anomaly of the anterior facial part of the face or muzzle containing the oral and nasal regions

Several snout morphological abnormalities have been so far recorded in populations of mutant mice. MP terms describing these abnormalities are following:

<u>Asymmetrical snout</u> - presence of a snout that lacks mirrored identity on either side of the median plane;

<u>Upturned snout</u> - muzzle has a curve or tilt such that the tip points upwards;

<u>Downturned snout</u> - muzzle has a curve or tilt such that the tip points downwards;

Short snout - reduced length of the anterior facial part of the muzzle;

If you observe all or some of these abnormalities, please record as "Snout morphology: Abnormal" and enter corresponding standardised term/terms (i.e. asymmetrical, upturned, short) into the comment box to describe these anomalies.

# **Eyelid Opening (DCF dependent)**

**As Expected** – eyelids on both sides are fully open with the eye clearly visible.

**Abnormal** – one or both eyes are partially or fully closed. Score each eye accordingly.

E.g.

"Left/Right/Both eye closed"

"Left/Right/Both eye partially closed"

"Left/Right eye closed. Left/Right eye partially closed"

#### **Eye Morphology (DCF dependent)**

As Expected – both eyes are black, smooth and reflective of light.

**Abnormal** – any morphological abnormalities to one or both eyes. Score each eye accordingly.

E.g.

"Left/Right/Both corneal opacity"

"Left/Right/Both corneal ulcer"

"Left/Right/Both abnormal eye colour, eye (list colour)"

"Left/Right/Both eye bulging"

"Left/Right/Both white/clear eye discharge"

#### Vibrissae

**As Expected** – Whiskers present.

Sparse – More than 50% if vibrissae are missing.

**Absent** – No vibrissae present.

#### Vibrissae size

**As expected** – Whiskers are regular in length.

**Increased size** – At least 50% of whiskers are abnormally long (If all whiskers are long comment "whiskers long"; otherwise comment "majority of whiskers long").

**Decreased size** – At least 50% of whiskers are abnormally short (If all whiskers are short comment "whiskers short"; otherwise comment "majority of whiskers short").

e.g. A mouse has only a few whiskers remaining and they are all short. Score Vibrissae as "Sparse" and Vibrissae Size as "Decreased Size" and comment "Majority of vibrissae missing. Vibrissae short".

# Vibrissae Shape

**Curly** – Whiskers curl.

**Disorientated** – Whiskers point in random directions.

**Other abnormal shape** – Any other abnormality in whiskers shape.

#### **Vibrissae Colour**

**As Expected** – Vibrissae are white/grey.

**Abnormal** – Whiskers are a colour other that white or grey.

#### Incisors

**As Expected** – two sets of anterior prominent long teeth present;

**Partly Present** – absence of one or more but not all of the pairs of long teeth that are the most anterior and prominent in the jaw;

**Absent** - absence of the pairs of long teeth that are the most anterior and prominent in the jaw;

# **Incisors Morphology**

As Expected – incisors have the same morphology (length, shape and orientation) as commonly observed in the WT population of the corresponding genetic background; Malocclusion – incisors are orientated in such a way that the do not align Other Abnormal Incisor Morphology – any other abnormal incisor length, shape or

orientation.

#### **Incisor Colour**

**As Expected** – bottom set of incisors "off white" while top set of incisors "yellowish" (can be less apparent in younger mice).

**Abnormal** – incisors white or any other colour other than the one described above.

#### LIMBS

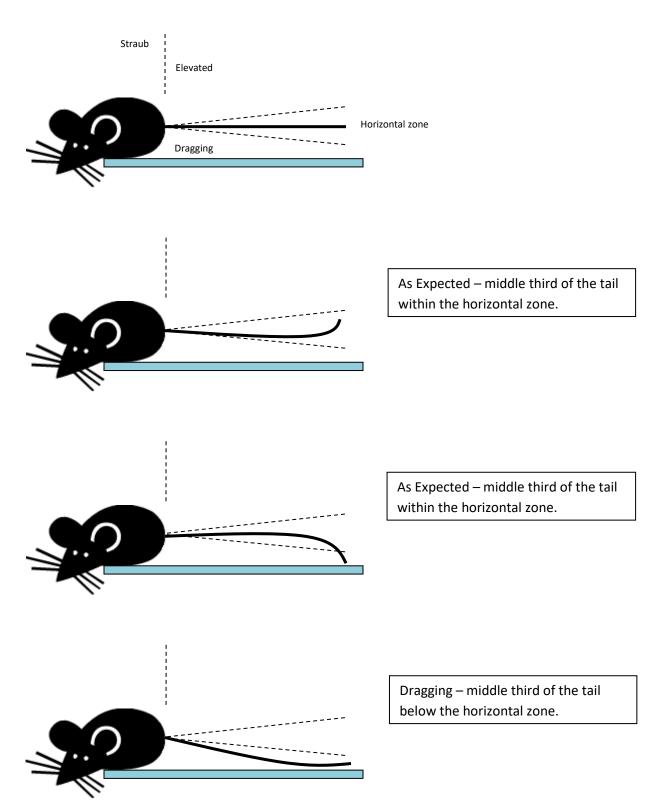
#### **Fore/Hindlimb Nail Count**

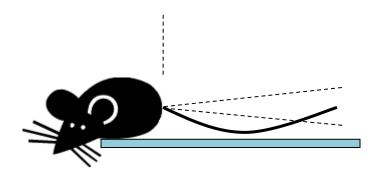
**As expected** - All nails are present or no more than one nail is missing on any paw. **Abnormal** - Absence of more than 1 nail should be recorded as "ABNORMAL" with a comment saying how many nails are missing.

<u>Fore/Hindlimb interdigit webbing</u> - a fold of skin, or web, between the digits that is not normally present.

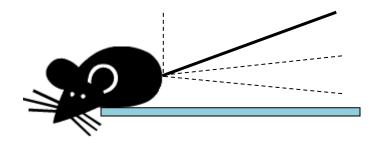
# **Appendix: Tail Elevation**

The elevation of the tail is scored by observing the position and angle of the middle 3<sup>rd</sup> of the tail. The dashed lines indicate the limits of the different zones (if the majority of the middle third of the tail is in a given zone the tail is scored as indicated below). Horizontal and elevated tails are combined to be scored as 'As Expected'.

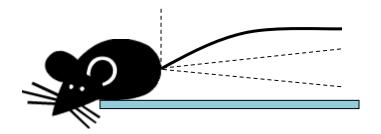




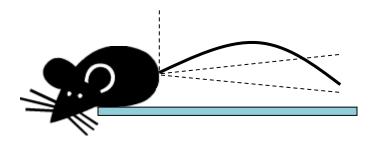
Dragging – middle third of the tail below the horizontal zone.



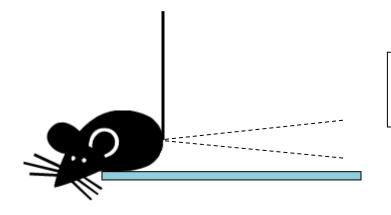
As Expected – middle third of the tail above the horizontal zone.



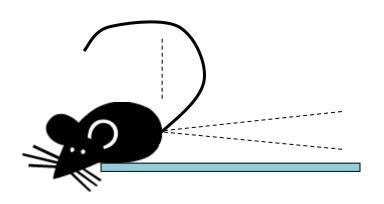
As Expected – middle third of the tail above the horizontal zone.



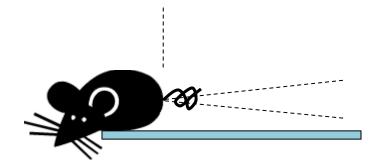
As Expected – middle third of the tail above the horizontal zone.



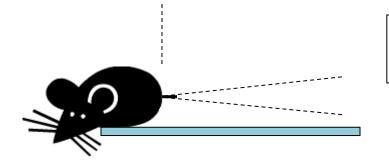
Straub tail – middle third of the tail is raised at an angle greater than 90 degrees from horizontal.



Straub tail – middle third of the tail is raised at an angle greater than 90 degrees from horizontal.



QC Fail – middle third of the tail is not identifiable and the parameter is not applicable.



QC Fail – middle third of the tail is not identifiable and the parameter is not applicable.