SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Wholemount Adult Mouse LacZ Staining

INTRODUCTION:

This document outlines the general procedures involved in the collection and staining of adult mouse tissues, for wholemount gene expression analysis of a LacZ reporter insert.

Two age-matched (8-12 weeks old) heterozygous mice per colony (1 male, 1 female), were collected and stained on the same day to reduce staining variation and facilitate comparison. Two wild-type mice (1 male, 1 female) were also routinely included in each collection to check for endogenous background staining.

ABBREVIATIONS:

DCF = Data Capture Form
DMF = Dimethylformamide
GM = Genetically Modified
LAA = Laboratory Animal Allergens
NVS = Named Veterinary Surgeon
PBS = Phosphate buffered Saline
PFA = Paraformaldehyde
PIL = Personal Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RSF = Research Support Facility
SOP = Standard Operating Procedure

QUALITY CONTROL (QC):

1. Mice collected from the same colony should be perfused and stained in the same session to minimise staining variation.
2. Tissues should be analysed and imaged for LacZ staining as soon as possible after collection before stain diffusion occurs.

Problem / Issue	QC action
A welfare issue makes it impossible to continue the procedure	Add a comment on the data capture form (DCF) with details. If no tissues can be used from a mouse: QC fail the DCF and request a replacement mouse. **Note:** Consider changing the severity level for the perfusion procedure on that mouse (consult with line manager).
A procedural error which affects the collection, e.g. a mix up of mice, genotyping samples or tissues.	Add a comment on the DCF with details. If no tissues can be used from a mouse: QC fail the DCF and request a replacement mouse.

Perfusion is interrupted, e.g. due to equipment failure, evacuation or power cut. **Note:** Perfusion pump should always be checked before injecting the first mouse	If perfusion not completed on a mouse add a comment on the DCF. In case of a power cut, perfusion may be completed manually by attaching a syringe to the perfusion needle. If no tissues can be used from a mouse: QC fail the DCF and request a replacement mouse.
Failed Genotype	QC fail the DCF and request a replacement mouse.
Inconsistent LacZ staining pattern found in male and the female tissues from the same colony.	Request additional 2 males and 2 females from the same colony to confirm staining pattern.
A tissue from the IMPC minimum annotation list (Appendix C) is missing from both mice collected for a colony (or a gender specific tissue is missing from one mouse).	Record No data for tissue. Request a replacement mouse.

HEALTH & SAFETY:

This procedure is covered by the following risk assessment WTSI-1204

- Appropriate personal protective equipment (PPE) is to be worn at all times when handling animals. This includes overshoes, gown and gloves.
- Entry procedure to the Research Support Facility (RSF) should be followed including the wearing of scrubs.
- **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.**
- Safety glasses must be worn when handling fixatives.
- Access to a functional down-flow table and fume hood is required.
- Users should familiarize themselves with the location of emergency equipment including: Eye wash stations, fire extinguishers, and spill kits.
- This procedure can only be performed during core working hours.
- All electrical equipment is to be inspected for damage before use.
- Lone worker alarms should be used when working alone in the RSF.
- New workers are to be supervised until deemed competent to perform this assay.
- Individual risk assessments for young persons and new or expectant mothers would be performed to define any exclusion for performing this assay.

RESPONSIBILITIES:

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).

RESOURCES:

Equipment and Reagents:

1.	Fume hood and down flow bench	
2.	Polystyrene ice box & ice bucket	
3.	Butterfly needles	Harvard Apparatus #725966
4.	Injection needles	BD microlance 300635 0.4mm x 13mm (27G x ½")
5.	Syringes	BD Plastipak 3000013 1ml
6.	Disposable absorbent pad	Dynarex #1341
7.	Dissecting instruments	Fine Science Tools
8.	Peristaltic pump	Reglo Digital pump 4 channels
9.	Rocking platform shaker at 4°C	
10.	250 mL Amber HDPE Bottle	VWR; Catalogue No. 215-3446
11.	Sagittal brain matrix	Agar Scientific
12.	Single edge razor blades	
13.	Plastic histology cassette and lid	
14.	Dissecting board	
15.	Heat pad	
16.	Freshly made PBS (pH 8.0)	Invitrogen: Catalogue No. 70011051
17.	Cold PBS (pH8.0)	Sanger Media team
18.	Sodium hydroxide (4 mol/l (4 N) in aqueous solution) to pH PBS	BDH (VWR) 191373M
	Sodium hydroxide causes damage to organs and is corrosive. To be used only under an effective fume hood or on a functional downflow table wearing correct PPE.	
19.	32% Paraformaldehyde fixative Dilute to 4% PFA with PBS pH 8.0	Electron Microscopy Sciences; Catalogue No. 15714-S
	PFA is toxic, harmful, corrosive, an irritant, a skin sensitizer, possibly carcinogenic and can cause damage to organs. To be used under an effective fume hood and wearing correct PPE.	
20.	Anaesthetic: Euthatal® solution for injection. 200mg/mL pentobarbital sodium	Merial 08327/4112
	Pentobarbital sodium is toxic. Correct PPE must be worn.	
21.	50% Glycerol in PBS pH7.4	Sanger Media team
22.	70% Glycerol in PBS pH7.4	Sanger Media team
23.	Sodium Azide	Sigma: S8032 500g
	Sodium Azide is toxic and an irritant. To be used only under an effective fume hood wearing correct PPE.	
24.	LacZ Staining Solution: (Appendix B)	
	- 2 mM MgCl2.6H2O	Sigma: Catalogue No. M9272
	- 0.01% Deoxycholic Acid	Sigma: Catalogue No. D2510
	- 0.02% IGEPAL CA-630	Sigma; Catalogue No. I8896
	- 5mM potassium ferrocyanide (C6FeK4N6.3H2O)	Fisher: Catalogue No. P/4920/53
	- 5mM potassium ferricyanide (C6FeK3N6)	VWR: Catalogue No. 26807.297)
	- 0.1% (1mg/ml) X-Gal in DMF	Invitrogen: Catalogue No. 15520018 Sigma: Catalogue No. D4551

	Dimethylformamide (DMF) is harmful, flammable, causes damage to organs and an irritant. To be used under an effective fume hood and wearing correct PPE. Use glass cylinders as can melt plastic.	
	- Cold freshly made PBS pH 8.0	Invitrogen: Catalogue No. 70011051
26.	**Dissection and Imaging** Microscope Computer Light box	 Leica MZ16A stereomicroscope 0.5x Planapochromatic objective (10446157) Leica LAS v4.4 software
27.	Imaging plates: Deep 14cm diameter petri dish Silicon: Sylgard 184 Silicone elastomer kit Insect pins	 Sigma: Catalogue No. D9054-1CS VWR: 634165S Fine Science Tools

Staff: This procedure can be performed by one member of staff.

PROCEDURE A: Tissue collection set-up

1. **Heat mats** – Turn on heat mats

2. **On a down flow table**, **make 4% PFA and PBS pH8 and keep refrigerated**
   a. Sufficient PBS pH8 for washes and to dilute 32% PFA stock.
   b. ~50ml 4% PFA per mouse required for the perfusion.
   c. ~200ml 4% PFA per mouse in an amber HDPE bottle required for the collected tissues. Ensure the bottle cap is clearly labelled 'PFA'.

3. **Set-up perfusion equipment** – **On a down flow table,** set up pump and perfusion mats.
   a. **Disposable absorbent pad –** Tape 2 pads beneath perforated top of downflow table to receive perfusion waste liquid.
   b. **Perfusion Pump –** Plug the pump in and check it works. Stretch out and straighten all tubing and attach the needles. Place the prepared bottle of 4% PFA on ice and verify that the PFA can run through all the tubing correctly (flow rate 2.83mL/mn).
   c. **Cork Board –** Prop a cork board up on the downflow table to receive the perfusion mouse onto. The board should be angled to allow perfusion waste to flow down through the perforated table top.

4. **IP injection –** Obtain key to drug safety cabinet, and retrieve Euthatal® solution (200mg/mL pentobarbital sodium).

5. **Genotyping –** Set-up genotyping plates in cold blocks and print genotyping tissue layout.

6. **Labels** – Print a pot label for each mouse.

7. **Mice** – Collect mice from animal room and record the number taken in the day book.

PROCEDURE B: Mouse Perfusion (Regulated procedure)

Before performing the procedure, verify that this is the correct mouse for the procedure. For genetically altered (GM) mice check the severity level that the mice are being held at on the database. For any mice listed as moderate severity, verify whether special handling measures are required before proceeding.

Injection and genotyping

1. On a down flow table, anaesthetize the mouse: IP injection of 0.1mL Lethobarb® /Euthatal using a 0.4mm x 13mm (27G) needle and 1ml syringe.

2. Place the mouse back in its cage and place the cage on the heat mat.

3. When the mouse is clearly anaesthetised (loss of the pedal reflex), collect an ear clip and tail sample in an appropriate tail/ear clip plate for genotyping. The sample plate should be kept either on a cold plate or on ice.

Perfusion

4. Transfer anaesthetised mouse to angled cork board in perfusion set-up.

5. Start the perfusion pump and ensure the fixative is running before proceeding.

6. Dampen down fur, then cut the skin overlaying the rib cage. Keep the rib cage intact by cutting up each side, then cut through the diaphragm to open the chest cavity.

   Take care not to pierce any organs, particularly the liver, lungs and heart, which could compromise the perfusion.

7. Insert the perfusion needle through the left ventricle of the heart and cut the right atrium.

8. Perfuse around 50mL of fixative. Check the mouse for rigidity and colour change in the liver (red to brown yellow) (average time 10-15min).

Perfusion and injection needles should be disposed of in a sharps bin which once full should be sealed and left for support staff to collect.

Spillages of Lethobarb®/Euthatal should be absorbed using Uni-Safe gel and disposed of in a yellow clinical waste bag. Any expired Lethobarb®/Euthatal should be returned to the NVS.

PROCEDURE C: Dissection (see Appendix A for dissection list summary)
Dissect out the following tissues and drop fix in amber HDPE bottle with 4% PFA

1. Take a piece of **dorsal skin from the back** and open it to spread it flat.

2. Remove the **brown fat** from between the shoulders.

3. Collect **the rib cage** (sternum and ribs) and remove the skin from the surface of the rib cage before fixing.

4. Cut through the **skin** on the abdomen and take a piece from one side of the lower abdomen, ensuring the **abdominal mammary glands** are collected.

5. Dissect all tissues **from the tongue to end of the colon and all the associated tissues and viscera.** Avoid any damage to any tissues, especially the intestine. Gently lift the **genital area** and pull up all the organs in one piece.

6. Collect both **pinnae**.

7. Remove **brain** from skull. Drop fix whole.

8. Collect the **head,** including the **eyes** and **pituitary**.

9. Cut a piece of spinal cord from the lumbar region, approximately 2cm.

10. Collect the right **hind limb** as close to the spine as possible, and remove the skin before fixing.

11. Cut ~3cm piece of **tail**.

The residual animal carcass must be disposed of in a cadaver waste bag, sealed, and put in the appropriate freezer.

Clean equipment and surfaces. All dissection instruments should be scrubbed clean of blood in the sink with antibacterial solution.

The absorbent pad used to collect PFA and blood must be placed in an offensive waste bag, sealed and left out for the support staff to collect.

All waste should be transferred to a yellow offensive waste bag, sealed and left out for the support staff to collect.

The top and inside of the down-flow tables should be scrubbed clean of blood, hair and animal tissue.

PFA Fixation and 3x PBS washes:

12. Fix tissues in 4% paraformaldehyde mixing at 4°C for 1 hour maximum.

13. **On a downflow table or in a fumehood**, remove the PFA and wash with PBS pH8.0, for 30 minutes at 4°C on a rocker.

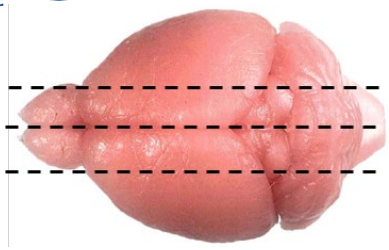
14. **On a downflow table or in a fumehood**, wash twice more with PBS pH8.0 at 4°C for 30 minutes on a rocker (PFA inhibits LacZ staining).

PFA waste and washes must be disposed of in an appropriate waste container and given to the Sanger Waste Team for disposal

PROCEDURE D: Sub-dissection – optional (required for tissues collected for BaSH consortium)

Sub-Dissect tissues in PBS:

1 **Brain:** Lay the brain ventral side down in a sagittal brain matrix. Using a fresh single edge razor blade for each brain, cut the brain down the middle and cut two thick sections to expose the striatum and hippocampus.



2 **Kidney**: Use a disposable scalpel to cut transverse sections (1mm) across the middle of one kidney to obtain sections across the renal pelvis. Bisect the second kidney longitudinally.

3 **Spleen:** Use a disposable scalpel to cut the spleen transversely into thick sections ~0.5 mm.

15. Once sub-dissected, place all tissues in fresh PBS pH8, 4°C.

Razor blades and scalpels should be disposed of in a sharps bin which once full should be sealed and given to the Sanger Waste Team for disposal.

PROCEDURE E: LacZ Staining (see Appendix B for stock reagents)

1. **In a fume hood**, make up LacZ solution (excluding x-gal) 200ml per mouse in a glass bottle.
   Add the X-gal just before use. Ensure the LacZ solution is thoroughly stirred using a magnetic stirrer.

Final Concentration	Volume of Stock reagent	
2mM MgCl2.H2O	2	mL
0.02% IGEPAL	2	mL
5mM potassium ferrocyanide	10	mL
5mM potassium ferricyanide	10	mL
Cold PBS pH 8.0	170.8	mL
0.01% deoxycholic acid	200	uL
0.1% (1mg/ml) X-Gal in DMF	5	mL
Total	200 mL	

2. **In a fume hood**, pour off the PBS from tissue samples and replace with LacZ staining solution. Incubate adult tissues in LacZ staining solution for 48hrs in the dark, on a rocker at 4°C.

3. **In a fume hood**, remove the LacZ stain solution and replace with 4% paraformaldehyde. Leave overnight on a rocker at 4°C.

4. **In a fume hood**, remove the PFA and replace with 50% glycerol. Leave to clear overnight on a rocker at 4°C.

5. Remove the 50% glycerol and replace with 70% glycerol. Leave to clear overnight on a rocker at 4°C. Cleared tissues in amber HPDE bottles can be left at room temperature until imaged.

6. Image stained tissues within 3 months (LacZ stain has been found to diffuse away over time). For long term storage, transfer tissues to 70% glycerol with 0.01% sodium azide.

LacZ stain waste, paraformaldehyde waste and glycerol waste must be disposed of into appropriately labelled waste containers and given to the Sanger Waste Team for disposal.

PROCEDURE F: Staining Analysis and Imaging (see appendix C for standardised annotation list and representative tissue imaging)

1. Check that the genotype of the mouse has been confirmed before proceeding.

2. Dissect and examine the tissues of each mouse under a microscope for the presence or absence of LacZ staining. Record the staining result for each tissue on the 'Wholemount expression' DCF as "Present" or "Not detected".

   If the staining pattern is typical of the endogenous staining seen in wild-type tissues, record it as "Not detected". Record an "Ambiguous" staining result where the staining result is uncertain e.g. very faint, non-discrete staining but atypical for endogenous staining.

   For examples of endogenous LacZ staining in wild-type tissue, refer to lab WT image example catalogue and any wild-type tissues included in the collection.

3. For any tissues with staining, take a representative image for the colony. Where possible, take all representative images from the same heterozygous mouse per colony.

   Image tissues in 70% glycerol using a Leica MZ16A stereomicroscope (0.5x objective).

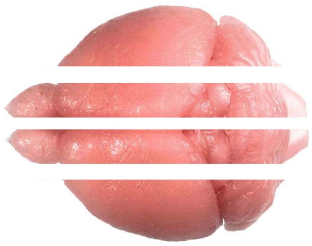
- Use petri-dish with transparent silicon base and insect pins to immobilise tissues as required.
- Adjust the lighting, microscope diaphragm and exposure time to achieve a well illuminated sample with a uniform white background.
- Images acquired using Leica LAS v4.4 software
  - In the form "Image Data" scan in the correct annotations for the image. The annotations will carry over to the next image so be careful to check the annotations are correct before acquiring the image:
    i. **Image Name:** Enter the mouse name e.g. XXXX12.3a_
    ii. **Description:** Wholemount Expression
    iii. **Notes:** Adult

4. Annotate the pictures on the database using MA ontology OLS.

APPENDIX A – Dissection list:

1	Abdominal Mammary gland	Leave on skin, open up flat.
2	Rib cage (sternum and ribs)	Needs to be a large piece; cut along the sides of the rib cage. Remove skin.
3	Skull	Ensure trigeminal nerves, pituitary gland and eyes are in place on the skull.
4	The tongue to end of the colon and all the associated tissues and viscera	One block – be careful to avoid damaging any tissues and ensure the reproductive organs are collected.
5	Skin from the back	Open up flat.
6	Brown fat	
7	Lumbar region spinal cord/vertebrae	2 cm piece.
8	Brain	Remove from the skull and drop fix whole. Ensure olfactory bulbs are collected intact.
9	Pinna	Both pinnae.
10	Hind limb	Whole hind limb from hemi-pelvis.
11	Tail	3 cm piece.

Sub-Dissection list (optional):
Occurs after the initial PFA tissue fixation, in PBS (before the samples are LacZ stained)

1	Brain	3 Sagittal thick section cuts using brain matrix: • Midline Cut • Cuts on either sides of the olfactory bulbs to expose the striatum and hippocampus 
2	Kidney	TS cuts through pelvic region to produce thick sections of one kidney, second kidney cut LS.
3	Spleen	TS cuts to produce thick sections.

APPENDIX B – Reagents:

Fixation and washes

PBS from 10x stock solution

For 1L 1xPBS; mix 100mL 10xPBS with 900mL water

- Adjust to pH 8.0 with sodium hydroxide

Sodium hydroxide causes damage to organs and is corrosive.
To be used only under an effective fume hood or on a functional downflow table wearing correct PPE.

4% Paraformaldehyde (solution stock 32%)

PFA is toxic, harmful, corrosive, an irritant, a skin sensitizer, possibly carcinogenic and can cause damage to organs. To be used under an effective fume hood and wearing correct PPE.

For 1L 4% PFA; mix 125mL 32% PFA stock with 875mL cold PBS pH8.0

Glycerol for clearing and storage

50% Glycerol in PBS pH7.4

For 1L 50% Glycerol; Mix 500mL Glycerol and 500mL PBS pH7.4 overnight on a stirrer.

- Note: Add the PBS first, start the stirrer and gradually add the glycerol.

70% Glycerol in PBS pH7.4

For 1L 70% Glycerol; Mix 700mL Glycerol and 300mL PBS pH7.4 overnight on a stirrer.

- Note: Add the PBS first, start the stirrer and gradually add the glycerol.

70% Glycerol in PBS pH 7·4 with 0·01% Sodium azide

For 1L 70% Glycerol; Mix 700mL Glycerol and 300mL PBS pH7.4. Add 1mL 10% sodium azide. Mix overnight on a stirrer.

- Note: Add the PBS first, start the stirrer and gradually add the glycerol.

10% Sodium Azide – stock solution

Sodium Azide is toxic and an irritant. To be used only under an effective fume hood wearing correct PPE

For 100mL 10% sodium azide stock solution; 10g sodium azide in 100ml RO water. Mix thoroughly on a stirrer.

Stock reagents for LacZ staining

4% X-Gal stock solution in DMF (40 mg/ml) – stock solution

Dimethylformamide (DMF) is harmful, flammable, causes damage to organs and an irritant. To be used under an effective fume hood and wearing correct PPE. Use glass cylinders as can melt plastic.

Dissolve 1g X-gal in 25mL DMF.

- Store in a foil covered bottle in the dark at -20°C.

10% Deoxycholic acid in water – stock solution

Deoxycholic acid is difficult to dissolve and requires stirring for several hours before aliquoting and storage at -20C

1. For 100mL 10% deoxycholic acid; Measure 10g deoxycholic acid in 100ml RO water. Mix thoroughly on a stirrer.
2. Aliquot into 1-2 ml eppendorf tubes. Store at -20C.

- Note: use a 250ml beaker and get some of the water stirring before adding the deoxycholic acid.

The following LacZ staining stock solutions should be changed either monthly, or when the potassium ferrocyanide solution becomes yellowish instead of colourless – whichever occurs soonest:
$MgCl_2.6H_2O$, IGEPAL, Potassium Ferrocyanide, Potassium Ferrocyanide.

200mM $MgCl_2.6H_2O$ in water – stock solution

FW: 203.31g/mol for 100mL of sol. stock 200mM: 4.07g - store at 4C
for 50mL of sol. stock 200mM: 2.04g - store at 4C

For 100mL 200mM $MgCl_2.6H_2O$ stock solution; in a 100mL bottle put 4.07g $MgCl_2.6H_2O$ and make up the volume to 100mL with water. Mix thoroughly on a stirrer.

2% IGEPAL (CA-630) in water – stock solution

For 100mL 2% IGEPAL stock solution; in a 100mL bottle put 2mL IGEPAL and make up the volume to 100mL with water. Mix thoroughly on a stirrer.

100mM Potassium ferrocyanide II ($K_4Fe(CN)_6 \cdot 3H_20$) in PBS pH8 – stock solution

FW: 422.41g/mol 100mM stock: 42.24g/L
Light sensitive - store at 4C.

For 500mL 100mM Potassium Ferrocyanide stock solution; in an amber 500mL bottle put 21.12g Potassium Ferrocyanide and make up the volume to 500mL with PBS pH8.0. Mix thoroughly on a stirrer.

100mM Potassium ferricyanide III ($K_3Fe(CN)_6$) in PBS pH8 – stock solution

FW: 329.24 g/mol 100mM stock: 32.92g/L
Light sensitive - store at 4C.

For 500mL 100mM Potassium Ferricyanide stock solution; in an amber 500mL bottle put 16.46g Potassium Ferricyanide and make up the volume to 500mL with PBS pH8.0. Mix thoroughly on a stirrer.

LacZ Staining solution for wholemount adult tissue: 200mL per mouse

		1 Adult	2 Adults	3 Adults	4 Adults	5 Adults	6 Adults	7 Adults	8 Adults	9 Adults	10 Adults	11 Adults	12 Adults
2mM MgCl2.H2O	mL	2	4	6	8	10	12	14	16	18	20	22	24
0.02% IGEPAL	mL	2	4	6	8	10	12	14	16	18	20	22	24
5mM potassium ferrocyanide	mL	10	20	30	40	50	60	70	80	90	100	110	120
5mM potassium ferricyanide	mL	10	20	30	40	50	60	70	80	90	100	110	120
Cold PBS pH 8.0	mL	170.8	341.6	512.4	683.2	854	1024.8	1195.6	1366.4	1537.2	1708	1878.8	2049.6
0.01% deoxycholic acid	mL	200uL	400uL	600uL	800uL	1	1.2	1.4	1.6	1.8	2	2.2	2.4
0.1% (1mg/ml) X-Gal in DMF	mL	5	10	15	20	25	30	35	40	45	50	55	60

Total Volume	mL	200	400	600	800	1000	1200	1400	1600	1800	2000	2200	2400

APPENDIX C – Minimum annotation list for IMPC:

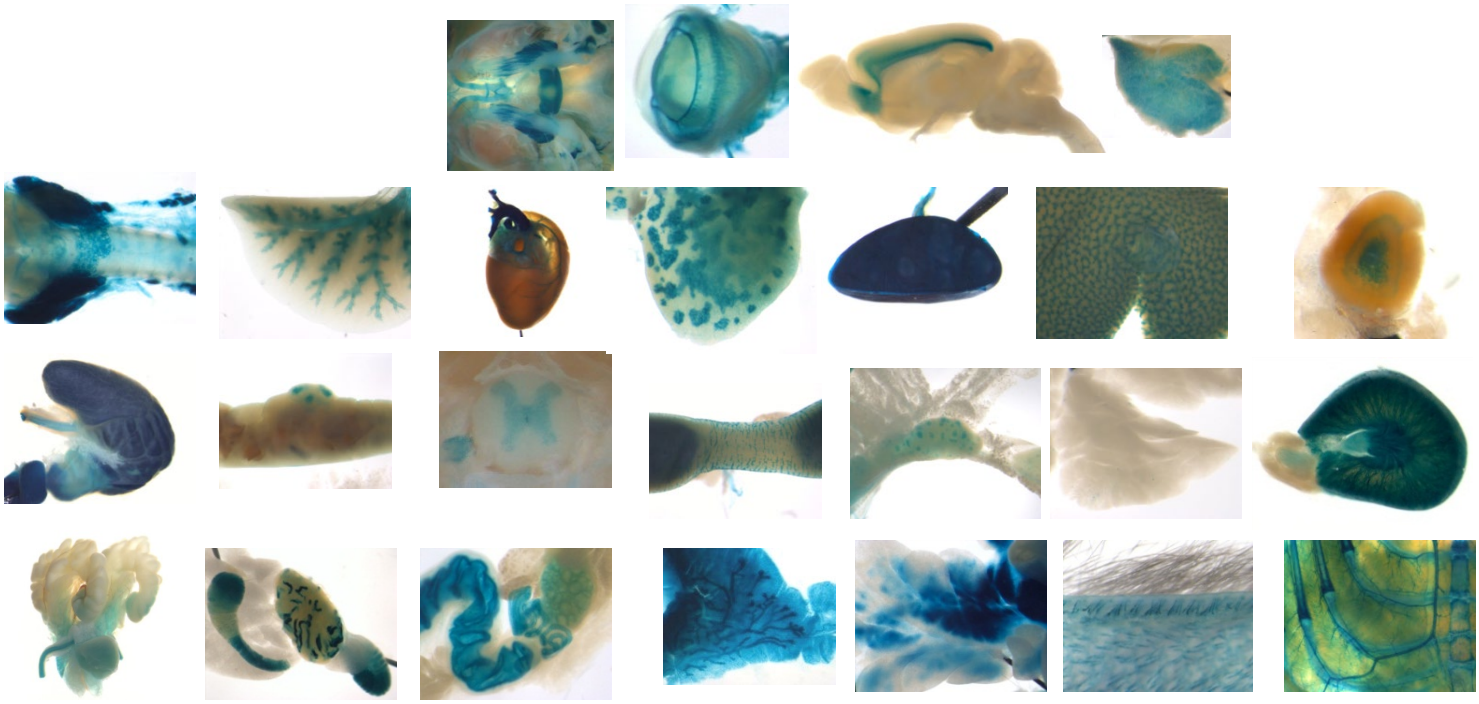
Tissue	MA term	Adult MA id
Adrenal Gland	adrenal gland	MA:0000116
Aorta	aorta	MA:0000062
Blood Vessel	Blood vessel	MA:0000060
Brain	brain	MA:0000168
Brainstem	brainstem	MA:0000169
Cartilage Tissue	cartilage tissue	MA:0000104
Cerebral Cortex	cerebral cortex	MA:0000185
Cerebellum	cerebellum	MA:0000198
Eye	eye	MA:0000261
Heart	heart	MA:0000072
Hippocampus	hippocampus	MA:0000191
Hypothalamus	hypothalamus	MA:0000173
Kidney	kidney	MA:0000368
Large Intestine	Large Intestine	MA:0000333
Liver	liver	MA:0000358
Lower Urinary Tract	lower urinary tract	MA:0002636
Lung	lung	MA:0000415
Lymph Node	lymph node	MA:0000139
Mammary Gland	mammary gland	MA:0000145
Oesophagus	oesophagus	MA:0000352
Olfactory Lobe	olfactory lobe	MA:0002413
Ovaries	ovary	MA:0000384
Oviduct	oviduct	MA:0000385
Pancreas	pancreas	MA:0000120
Peripheral Nervous System	peripheral nervous system	MA:0000218
Peyers Patch	peyer's patch	MA:0000137
Pituitary Gland	pituitary gland	MA:0000176
Prostate	prostate gland	MA:0000404
Skeletal Muscle Tissue	skeletal muscle tissue	MA:0002439
Skin	skin	MA:0000151
Small Intestine	small intestine	MA:0000337
Spinal Cord	spinal cord	MA:0000216
Spleen	spleen	MA:0000141
Stomach	stomach	MA:0000353
Striatum	striatum	MA:0000891
Testis	testis	MA:0000411
Thymus	thymus	MA:0000142
Thyroid Gland	thyroid gland	MA:0000129
Trachea	trachea	MA:0000441
Uterus	uterus	MA:0000389
White Adipose Tissue	white adipose tissue	MA:0000058

Optional Annotation List:

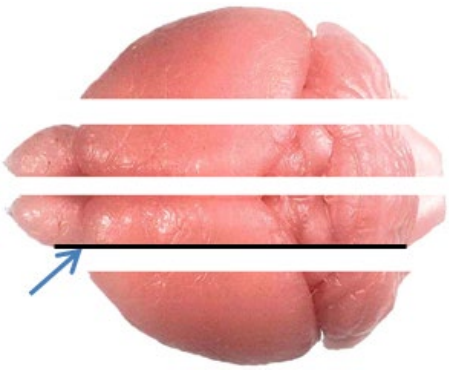
Tissue	MA term	Adult MA Id
Ascending colon	ascending colon	MA: 0001541
Bone	bone	MA: 0001459
Brown adipose tissue	brown adipose tissue	MA: 0000057
Descending colon	descending colon	MA: 0001542
Duodenum	duodenum	MA: 0000338
Gall Bladder	gall bladder	MA: 0000356
Inguinal Lymph node	inguinal lymph node	MA: 0002829
Ileum	ileum	MA: 0000339
Jejunum	jejunum	MA: 0000340
Mesenteric Lymph node	mesenteric lymph node	MA: 0002829
Parathyroid gland	parathyroid gland	MA: 0000128
Seminal vesicles	seminal vesicles	MA: 0000410
Stomach: Forstomach	forstomach	MA: 0001606
Stomach: Glandular	glandular	MA: 0001613
Thalamus	thalamus	MA: 0000179
Tongue	tongue	MA: 0000347

Adult LacZ expression – Minimum standardised list of 25 images:

Only tissues containing lacZ staining are imaged.
Additional images may be taken of 'optional' tissues, or to better show the expression pattern



Brain Imaging: Section indicated by the arrow on the diagram below is the surface which should be imaged.



Brain Annotation – Gensat brain atlas: www.gensat.org