SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

Embryo collection

INTRODUCTION:

The main aim of this DCF was to cover the collection of any embryos before the DMDD project, the DCF therefore had to have a broad scale/generic abnormalities. The main two pipelines this DCF covered were:

1. The Recessive lethality screen which was set up to determine when homozygote mouse embryos were surviving until, embryos at a variety of timepoints throughout gestation were collected. Lines entered the pipeline if they were homozygous lethal or subviable at post-natal day 14 (P14).
    a. This is achieved by obtaining at least 28 genotyped embryos (per timepoint) from Het x Het mating's.
    b. During this process we identify potential embryonic abnormalities which could impair the survival of any homozygotes animals, if applicable abnormalities are recorded on data capture forms (DCFs) and images are taken; using a Leica MZ16A microscope and Leica LAS software.
    c. The embryos were fixed in Bouin's fixative, in some lines:
        i. 1 litter WM stained for LacZ reporter,
        ii. 1 litter was fixed in ethanol for a collaborator to study the skeletal structure
    d. Placentas were with fixed along with the embryos or separately fixed in PFA, depending on the line and timescale in the pipeline.
    e. If applicable, homozygotes and 3 wild types from each knockout line are sent for HREM and placental analysis.
2. If the lines were homozygous viable at P14 then one litter was taken at ED14.5 and wholemount stained LacZ to study the gene expression. The aim is to obtain 2 heterozygous E14.5 embryos stained. The embryos are scored for the presence or absence of lacZ staining. The term 'Ambiguous' is assigned when there is uncertainty as to the validity of the observed pattern e.g. staining may be very faint, background or an artefact due to trapping.
    a. This was achieved mainly through WT x Het matings but some Het x Het mating's were also performed depending on the line and timescale in the pipeline.

ABBREVIATIONS:

DCF – Data Capture Form
Hom – Homozygous
MIG – Mouse informatics group
NaN3 – Sodium Azide
PBS – Phosphate Buffered Saline solution
PFA – Paraformaldehyde
RT – Room temperature
WT– Wild type

HEALTH & SAFETY:

This procedure is covered by the following risk assessment WTSI_1380.

- Appropriate personal protective equipment (PPE) is to be worn at all times when handling the samples (white coasts and gloves).
- Access to a functional down-flow table and fume hood is required
- Safety glasses must be worn when handling fixatives
- Eye wash stations, fire extinguishers and spill kits are located in the area.
- New workers are to be supervised until deemed competent to perform this assay.
- Individual risk assessments for lone workers, 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) has 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).

STAFF: This procedure requires one member of staff per embryo litter to be performed optimally.

RESOURCES

Equipment and reagents

1. Fume hood and down flow bench
2. Polystyrene ice box & ice bucket
3. 50ml falcon tubes (No. PLTU0002.)
4. Ice cold PBS pH 7.4
5. Dissecting instruments (#5 forceps, scissors, perforated spoon; Fine Science Tools)
6. 10cm plastic petri dishes
7. 6cm plastic petri dishes
8. Bijoux 7ml tubes
9. Yellow and red boxes (Starlab)
10. Genotyping PCR plates (ThermoFisher UK Ltd: MPP-114-101R)
11. PCR plate cooler blocks (eppendorf)
12. Leica microscope (Leica (DCF495 – camera & M205c – Microscope)
13. Computer with Leica software (LAS) and sanger MIG database access
14. PBS pH7.4
15. Bouins Solution (Sigma: HT10132)
    Bouins is toxic, carcinogenic and an irritant.
    To be used under an effective fume hood and wearing correct PPE
16. 4% PFA (*EMS 15714-S)*
    Paraformaldehyde is toxic, carcinogenic and an irritant.

To be used under an effective fume hood and wearing correct PPE

17. PBS with 0.01% Sodium Azide (NaN3; Sigma: S8032)
    Sodium Azide is toxic and an irritant.
    To be used under an effective fume hood and wearing correct PPE
18. 50% Glycerol in PBS pH7.4
19. 70% Glycerol in PBS pH 7.4 and 0.01% Sodium Azide
20. LacZ staining solution (See Appendix A)

METHOD - Collection of Embryos in Animal house

Before performing the procedure, verify that this is the correct mouse for the procedure.

1. Prepare 50 mL falcon tubes of cold PBS pH pH7.4 (one per litter collected) and place on ice.

   Note: Once removed from the mother the uterus should spend **no more than 30 minutes on ice.**

   A maximum of one litter per dissector should be collected at any one time.

2. **On a down-flow table,** sacrifice the female by cervical dislocation. Check if the female is unresponsive. Damp the fur with 70% Ethanol. Open up the body cavity and confirm death my removal of the heart.

3. Remove the uterus. Count the embryos (and resorptions if necessary) and transfer them to the cold PBS. Store on ice. Label the 50ml tube with identification of sample.

4. Record the number of embryos on the mating card for database entry later.

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

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

5. Transfer embryos, on ice, to lab.

METHOD – Embryo Dissection in the Lab

6. Cut open uterus. Remove the embryo & placenta from uterus by holding onto the uterus with one set of forceps and using the other forceps to guide the embryo & placenta away from the uterus. Be careful not to damage the placenta or embryo.

7. Recount the embryos (and resorptions if necessary). Transfer embryos into a dish of clean PBS (to avoid contamination) and place on ice.

8. Detach embryos from the placenta using fine dissection tools. Remove the yolk sac and the embryonic membrane.

9. Collect two small pieces of yolk sac (at earlier points in the pipeline history embryo tail tips ± yolks sac were taken for genotyping) into two 96 well genotyping plate (on cooler block). One for genotyping and one for back-up. Label plates accordingly.

10. Check the gross morphology of the embryos. If the embryo appears abnormal, take a photo to show the dsymorphology (see appendix) and make notes on the cage card.

11. Transfer embryo, using a perforate spoon, into 7ml Bijoux tubes containing ice cold PBS. If the placenta was to fixed the same as the embryo then the placentas was transferred together with the embryo. Mark the lid of the tube with the colony prefix and embryo name (i.e.MGPY65.1a) for first embryo, and embryo name (65.1a).

    a) If the placentas were to be fixed separately the placenta were transferred, using a perforate spoon, into 7ml Bijoux tubes containing ice cold PBS. Mark the lid of the tube with a red pen with the colony prefix and embryo name (i.e.MXXX65.1a) for first placenta, and embryo name (65.1a).

12. Repeat until all samples for litter are dissected.

    PBS waste containing biological contaminants must be treated with Virkon for 10 minutes, then diluted 5 times with water and disposed of down a laboratory sink.

    Solid biological waste must be frozen in the -20C in a biohazard bag and given to the Sanger Waste Team for disposal.

    Transfer all other waste to a yellow clinical waste bag or clearly labelled waste container.

13. Add litter to mating:
    a) Enter the number of embryos collected or check the box marked Not pregnant.
    b) Enter any abnormalities observed in the mother (e.g. inflamed uterus). Cull the female and under Licensing Procedure enter the relevant Procedure (Embryo Harvest (post E10.5)), PIL and PPL and Start Date.

14. Print tube labels for embryo and placenta collections and stick onto tube(s) – this can be done either at the start or end of dissecting. Place Embryo tubes in yellow box and label box with date of death. Place Placenta tubes in red box and label box with date of death (for other coloured boxes please see white board area for descriptions).

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

METHOD – Fixation of Tissues

Important Note: Embryos should never be left dry. When changing solutions, always keep embryos in enough residual solution to cover them as they are extremely fragile at this stage and can easily dry out and become damaged.

1. **In a fume hood**, remove the PBS from the embryos and placenta using a Pasteur pipette or perforated spoon. Take care not to remove or damage the embryos.

2. Replace the PBS with fixative

    a) Bouin's Solution. **Store at RT in a safety cabinet.**
    b) 100% ethanol. **Store at RT in a safety cabinet.**
    c) 4% PFA Solution. **Store at 4C**
    d) Lacz staining solution (see appendix A)

3. Mark litters on the white board under the relevant section and leave to fix for 24 hours, or 48 hours over the weekend. Earlier in the pipeline history samples were permanently stored in Bouins/ethanol.

    PBS waste containing biological contaminants must be treated with Virkon for 10 minutes, then diluted 5 times with water and disposed of down a laboratory sink.

METHOD – Database entry

4. **Create cohort:** Enter a description of the cohort (e.g. gene names) and select the MGP Recessive Lethality pipeline. Then add the appropriate litters to the cohort and save it.

5. **Create and register genotyping list:**

    a) If tissues were not collected for any reason, ensure that this is recorded on the genotyping grid.
    b) **Yolk sac genotyping samples:** Print off a copy of the genotyping grid for the samples and spin the plates in the lab centrifuge. Submit both paperwork and genotyping plates to the genotyping team freezer.
    c) **Back-up Yolk sac genotyping samples:** Place the yolk sac genotyping plate in freezer. No paperwork is required, but the plate MUST be submitted on the database. The yolk sac samples are a temporary resource and will be transferred to the genotyping freezer once a box is

full. We rotate the embryo boxes and old samples are thrown out after 6-8 months.

6. **Data Capture:** Add the 'Embryo collection' DCF to the appropriate litters.
    a) For the first embryo of the litter only (i.e. for "xxxx.1a" only), enter the number of reabsorptions which were observed (enter 0 if there were none).
    b) Fill in the DCF as appropriate with fixatives and any relevant abnormal dysmorphology, add any comment if needed.

7. **Annotate Embryo dsymorphology images:**
   Check that any images taken have been uploaded onto the database and ensure the minimum annotations are applied (see appendix).

METHOD – Removal of Fixative

Important Note: Embryos should never be left dry. When changing solutions, always keep embryos in enough residual solution to cover them as tare extremely fragile at this stage and can easily dry out and become damaged.

Depending on the pipeline and workflow some samples were removed from the fixative and placed in to PBS containing 0.01% NaN3, or another solution depending on the workflow. Below is the description of the workflow towards the end of the RL screen history when samples stored long term in PBS containing 0.01% NaN3.

1. After 24 hours fixation, **in a fume hood,** remove the Bouins from the embryos, using a Pasteur pipette, and wash 3 times with PBS pH7.4.

   Bouins waste must be disposed into the Bouins waste bucket and given to the Sanger Waste Team for disposal.

2. **In a fume hood** also remove the PFA from the placenta, using a Pasteur pipette, and wash 3 times with PBS pH7.4

   PFA waste must be disposed into the PFA waste bucket and given to the Sanger Waste Team for disposal.

3. Fill tubes with PBS containing 0.01% NaN3. Store the embryos at RT in a safety cabinet, and store placentas at 4C.
    - Consolidate boxes to maximise storage use.
    - Once the safety cabinet is full, embryo boxes are stored in the extra storage space at RT
    - Once the fridge is full placenta boxes are transferred to fridge for collaborator collection or long term storage in the extra storage space at RT.

Appendix A: WM LacZ

LacZ staining solution

1. 2 mM MgCl2.6H2O	Sigma: Catalogue No. M9272
2. 0.01% Deoxycholic Acid	Sigma: Catalogue No. D2510

Deoxycholic acid is harmful. To be used wearing correct PPE

3. 0.02% IGEPAL CA-630	Sigma; Catalogue No. I8896
4. 5mM potassium ferrocyanide (C6FeK4N6.3H2O)	Fisher: Catalogue No. P/4920/53

Potassium ferrocyanide is harmful to acquatic life. Care should be taken when disposing of waste

5. 5mM potassium ferricyanide (C6FeK3N6)	VWR: Catalogue No. 26807.297)
6. 0.1% (1mg/ml) X-Gal	Invitrogen: Catalogue No. 15520018
a. in DMF	Sigma: Catalogue No. D4551

Dimethylformamide (DMF) is harmful, flammable, cause's 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.

7. Cold freshly made PBS pH 8.0	Invitrogen: Catalogue No. 70011051

Initial Dissection:

1. Cut open uterus. Recount the embryos (and resorptions if necessary).
   - A fresh dish should be used for each embryo to avoid contamination. Place each dish on ice until ready for dissection.
   - Remove the embryos with yolk sac and placenta intact.
2. Extract embryos from yolk sac.
3. Place each embryo in a clean vial with PBS pH 8 on ice.
4. Let embryo bleed out for a while in PBS (Hb inhibits the staining reaction) keeping the embryos on ice.
5. For genotyping, collect a piece of tail in a 96 well genotyping plate (on cooler block). When finished, seal the plate and freeze it at -20°C.
6. When all the embryos are dissected, replace PBS by 4% Paraformaldehyde pH8.
7. Fix in 4% paraformaldehyde with mixing at 4°C for 30 minutes
8. After fixation ensure 2 x 30 min washes in large volume of PBS at 4°C (Dispose of fixative and washes into liquid aldehyde fixative waste bottle, stored in the ventilated cabinet under the fume hood).

Dissection

9. Pin down embryo, belly up, through mouth, above tongue and at base of tail.
10. Cut skin and underlying muscles straight up from tail base to chin, along either sides of body.
11. Turn embryo over and pierce the nape of the neck.
12. Place the dissected embryo in fresh PBS for the third and final 30 minute wash before staining.

LacZ staining

13. Incubate embryos in LacZ staining solution for up to 48h in the dark at 4°C. (Note: if embryos are collected on a Thursday: ED 14.5 should be incubated in LacZ staining solution for up to 24h – until Friday pm)
14. Postfix with 4% paraformaldehyde overnight at 4°C with gentle agitation. (After this stage the staining can be checked and the staining result for each embryo recorded on the database)
15. Clear with 50% glycerol overnight at 4°C with gentle agitation
16. Final clearing and storage in 70% glycerol in the dark at room temperature. (Note: Ensure that the staining result for each embryo is recorded on the database before moving them to storage).

Take pictures for the database of embryos with a heterozygous genotype:

17. Picture the placenta if staining is present.
18. Take a side, front and back view picture of the whole embryo lying horizontally with the head to the left, facing upwards.

Quality control:

19. The staining score and genotype should match.
20. If this is not the case, a confirmation of the genotype and of the staining check is asked.
21. If this is not the case and the genotype is confirmed, the embryo is QC failed in the database.

Troubleshooting

If crystals on embryos, wash them in PBS + 0.1% tween during one or 2 days after the post-fixation

Appendix A-i: Solutions for LacZ staining

For Whole-mount embryos (ED14.5): 6mL each

PBS

PBS from 10x stock solution

- For 1L 1xPBS; mix 100mL 10xPBS with 900mL water
- Adjust to pH 8.0 with sodium hydroxide
- Adjust to pH 7.4 with hydrochloric acid (for glycerol)

4% Paraformaldehyde (solution stock 32%)

(PFA is toxic, mutagenic and teratogenic. It must only be used in an effective fume hood and appropriate PPE must be worn.)

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

✓ *With powder*, if needed, Add 4g of PF to 100mL PBS, shake under fume hood, put on hotplate and warm up gently with stirring. The solution is being cloudy to clear when it is ready. Allow to cool. When cooled, transfer to 4C with correct label and date

4% X-Gal stock solution in DMF

(Dimethylformamide is toxic, mutagenic and teratogenic. It must only be used in an effective fume hood and appropriate PPE must be worn.)

- Dissolve 40 mg of X-Gal in 1 ml of DMF (or 1g in 25mL) protected from light
- Aliquot 1.5mL/eppendorf and 750μL (enough for 600mL and 300mL staining solution) protected light sensitive and aliquots should be stored in the dark at – 20°C

Deoxycholic acid – Sol. Stock 10% in water

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

- For 100mL 10% deoxycholic acid, in a 250mL beaker put 10g deoxycholic acid and make the volume up to 100mL with water. Mix thoroughly on a stirrer.
- Note: it is best to get a little of the water stirring in the beaker before adding the deoxycholic acid. Use the remaining water to rinse the sides of the beaker as you add it.
- Continue stirring while aliquotting 1mL into eppendorf tubes. Store at -20C

10% Sodium Azide – stock solution

10% sodium azide in water

- For 100mL 10% sodium azide stock solution; in a 100mL bottle put 10g sodium azide make up the volume to 100mL with water. Mix thoroughly on a stirrer.

50% Glycerol

50% Glycerol in PBS pH 7·4

- For 1L 50% Glycerol; Mix 500mL Glycerol and 500mL PBS pH7.4. Mix overnight on a stirrer.
- Note: Add the PBS first, start the stirrer and gradually add the glycerol.

70% Glycerol

70% Glycerol in PBS pH 7·4 with 0·01% Sodium azide (Sodium azide is toxic – appropriate PPE must be worn).

- 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.

Appendix A-ii: Processing LacZ stained embryos to FFPE sections

Tissue-processing programs utilised are all “ethanol-start” so it is necessary to bring the embryos through increasing alcohol gradients to 95% EtOH

If embryos are currently in 70% glycerol

a) Decant 70% glycerol, embryos incubated in 50% glycerol for 24hrs
b) Decant 50% glycerol, embryos Incubated in PBS for 24hrs
c) Decant PBS, embryos incubated in 50% EtOH for 6hrs
d) Decant 50% EtOH, embryos incubated in 70% EtoH overnight
e) Decant 70% EtOH, embryos incubated in 95% EtOH 6hrs or overnight

If embryos are currently in PBS/Formalin

1. Decant PBS/Formalin, embryos incubated in 50% EtOH for 6hrs
2. Decant 50% EtOH, embryos incubated in 70% EtoH overnight
3. Decant 70% EtOH, embryos incubated in 95% EtOH 6hrs or overnight

Processing Embryos to paraffin

ED10.5 and ED14.5 embryos are processed to paraffin using the Thermo-Shandon tissue processor located in the Histopathology Lab. For the general use SOP for this apparatus refer to ThermoShandon Excelsior Tissue Processor guide

Embryos should be apportioned into labelled histology cassettes

The program process is as follows:

1. Ethanol (30 mins)
2. Ethanol 30 mins
3. Ethanol 9hrs 30mins (basically just a holding step so that we can run the program overnight and take the embryos off in the morning)
4. Xylene (30 mins)
5. Xylene (30 mins)
6. Xylene (30 mins)
7. Wax (1hr)
8. Wax (1hr)
9. Wax (1hr)

making it an overnight program. The ED10.5 program is ~6hrs so it can be run in the morning (load before 10.00 am) and removed in the afternoon if required

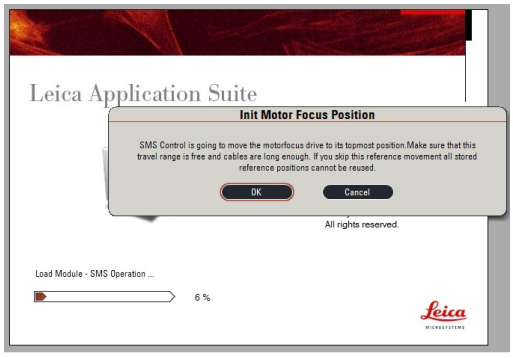
Embryos can then be embedded and sectioned as needed

Appendix B: Use of LeicaLAS for microscope imaging

1. ***Turn on microscope***

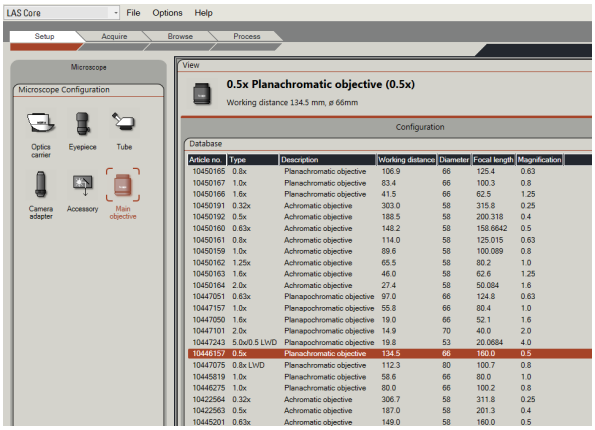
2. ***Start Leica LAS v4.4 software***

3. **Motor Focus Position** - Upon start-up, the software will request to move the microscope so it can calibrate the zoom/focus and scale the images correctly. Ensure the microscope is uncovered and the cables do not restrict its movement, and then click "OK".



4. Software Settings

- Check the correct objective is selected
  - Go to the Set up tab
  - Click on Main objective

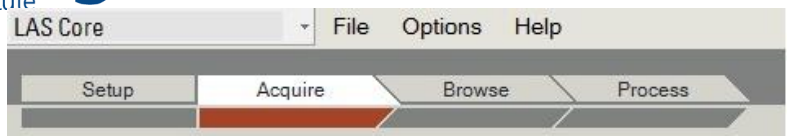


  - **The objective displayed** (at the top of the screen in section 'view') **must match the lens on the microscope.**
  - If not select the correct objective The standard/default objective is 10446157 - 0.5x Planapochromatic objective - working distance 123.5 – Diameter 66 – Focal length 160.0 - Magnification 0.5
  - Turn off software and microscope and wait 5 -10 seconds
  - Turn on microscope and then the LASV4 software
  - Check that the right objective is selected.

- This is the default settings for all the microscopes, as they are all fitted with the same lens. Once you have followed these steps, it should also be the default on your LASV4 software program.
- **If changing the objective** from 0.5x to 1x, or vice versa, make sure you **change *the software settings before changing the lens.*** Avoidance of doing so will result in a wrong scale bar after the change of lens. If you wish to change the objective, our x1 lens is 10447157.
- Change the lens on the main objective tab, then physically change the lens, then turn off the software and the microscope, wait 5-10 seconds and turn back on. **Always check the scale bar using a ruler each time you change the lens** to ensure it has been set up properly. If you change the lens, **always return the 0.5x objective and the software to its default settings at the end of your session**.

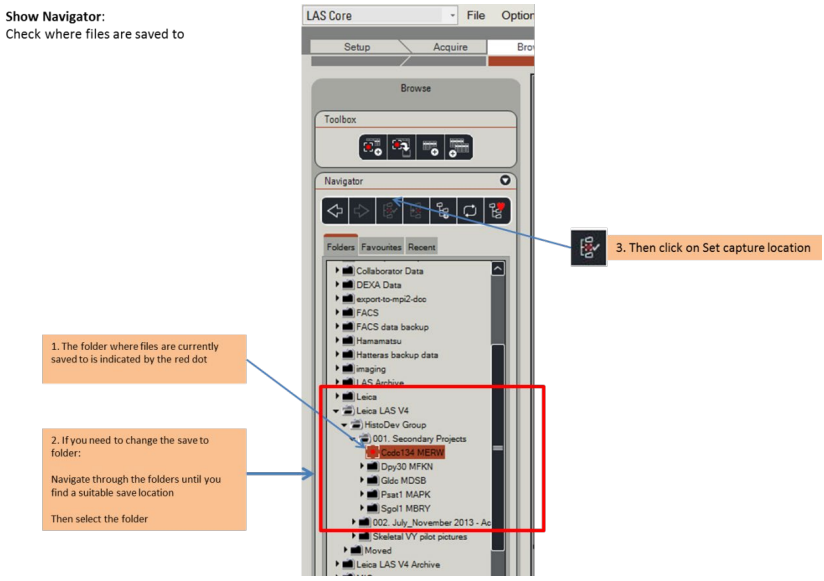
***Important note*:** LASV4 software will save your lens choice, but it does this in a profile specific manner. Therefore you need to check the software settings on your own profile on all 3 microscopes.

5. Use the ***tabs at the top to navigate*** through the software:



Setup: Shows the hardware which is connected – this should be setup already
Acquire: Where images are captured
Browse: Shows where the files are saved to, can be used to view images once they are taken
Process: Used for post-processing images (e.g. cropping, adding arrows etc) – we don't need this

6. ***Saving images to MIG drive*** (see figure below):
   - Click on the "browse" tab
   - Path to save images to:
     Mig_Data\Leica LAS V4\HistoDev Group
     - Secondary projects – Create one folder per project named "Gene_Colony Prefix"
     - "Month – ACTIVE" – for all other images. Periodically the active folder will change as a new folder is created(i.e. 03.March – ACTIVE)
   - When set to the correct file location, click on the  icon

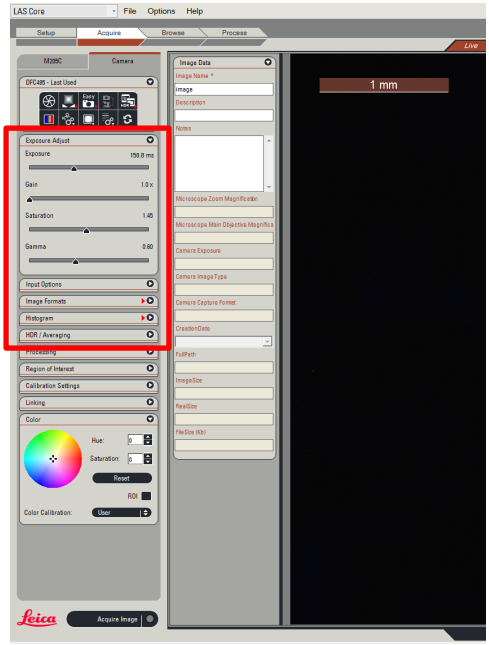


7. ***Capturing an image*** : the "Acquire" Tab

- **Use the guide** 'Leica LAS –Guide to Annotations and Processing' located X:\Team109\Recessive Lethality Pipeline\Equipment and Orders\Microscopes (Leica)

- Setting up the image

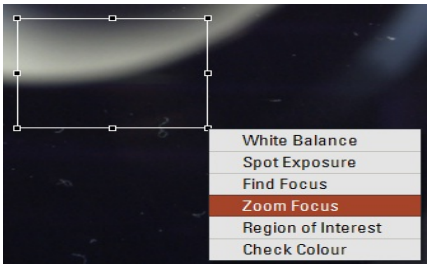
  - Adjust the lighting, microscope diaphragm and exposure time to achieve a well illuminated sample with a uniform black or white background.
    - Using the functions in the camera settings in the acquire tab



- Ensure the image is focussed on the screen (use the zoom focus if necessary)
- If needed adjust the white balance
    i. Draw a square around the relevant section and click white balance

Draw box on image holding down left mouse button:

- White balance – small box over entirely white/black region



8. Image Data

- Depending on your experiment, locate the appropriate 2D tags sheet by the microscope (‘Embryo Dysmorphology’ or ‘Wholemount Expression’)
- 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:

    - **Image Name:** Enter the mouse name e.g. PMCZ31.1a_ (make sure to place an underscore at the end)
    - **Description:** Scan in the appropriate experiment e.g. Embryo Dysmorphology/Wholemount Expression

- **Notes:** Scan in the appropriate annotation tags e.g. Time point= E9.5/E14.5, etc.; Tissue = Adult/Embryo/Placenta; Orientation = dorsal/lateral, etc.
- **Comments:** to add comments manually add '^' at the start and '$' at the end e.g. Comment^ccdc134$ (do not leave gaps)

- **To guarantee all the image annotations are maintained** for the picture you will acquire, press 'Tab' to move the cursor away from the last annotation field you filled in. (i.e. press tab until the cursor moves into the "microscope zoom magnification" box).

- When the image is ready click acquire image button at the bottom left-hand corner of the screen or press 'F3' on the keyboard.

Appendix C: Annotating Embryo Dysmorphology Images

1. Verify images are uploaded on WTSI Mouse Database

2. Check that the number of uploaded images matches the number of images that were taken that day.

3. Verify that the below minimum annotations are present for LacZ stained, RL and DMDD specimens pictures

   a. *Experiment:* Embryo Dysmorphology or Wholemount Expression
   b. *Tissue:* Embryo or Placenta
   c. *Age:* As appropriate (e.g. Embryo E14.5)
   d. *Orientation:* As appropriate (Note: use this tag with discretion; it is supposed to be informative so if the orientation of the tissue is not obvious e.g. due to a strange angle, then do not apply this tag)
   e. *Comment*: use the comment box for extra information, i.e. if photographed with another embryo, state embryo name

4. QC Images:

a. QC Fail images that are:

- Out of focus (cannot see phenotype/other images available)
- Too dark (cannot see phenotype/other better images available)
- Duplicated
- Wrong embryo name (if possible correct embryo name on LAS software so the image can be reloaded with the correct name)

Note: QC of images is performed in batch on a weekly basis. However, everyone taking pictures while dissecting is responsible for their QC failing that same day if any of the above criteria is true.