WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Open Field Room Lights-V1

SOP Number: SOP0033	**To be reviewed:**
Author(s): Signed: Date:	
Editor: Signed: Date:	
Date modified:	

INTRODUCTION:

The purpose of this procedure is to measure the locomotion, habituation and fear/anxiety responses to a novel environment of wild-type and genetically altered mice.

HEALTH & SAFETY:

- **RA001** – Laboratory Animal Allergens; *Section RA001.3*
- **RA003** – Hazardous Substances; *Section RA003.2*
- **RA004** – Physical Hazards; *Sections RA004.1.4, RA004.2 & RA004.6*
- **RA007** – Musculoskeletal; *Section RA007.6 & RA007.10*

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment:

1. Weight scale
2. Techniplast Interactive Cage Change Station
3. 70% Ethanol and paper hand towels
4. Techniplast Mobile IVC Rack
5. Mobile cage transport rack
6. Clean cages (as required by pipeline)
7. Diet (as defined by pipeline)
8. Nestlets (as required by pipeline)
9. Open Field computer and processing unit with user account created and current version of Actimot
10. Open Field frames and arenas as required
11. Sensor height adjusting tool
12. Lux meter
13. Large tables x2
14. Cotton buds

Associated Documents & SOPs:

- **SOP0040** – Open Field Data Analysis
- **SOP0045** – Weigh Mice
- **SOP0065** – Use of Change Station
- TSE ActiMot V 7.01 Operating Manual

Staff Required: This test can be completed by one phenotyper.

NOTE:

This SOP can be used for both the 10 mins and 20 mins run time for the Open Field test carried out under room lights.

All phenotypers performing this will require a user profile on the Actimot system (see Appendix A).

PROCEDURE:

Before performing the procedure, verify that this is the correct procedure at this point in the pipeline by consulting the cage card(s) and confirming that the procedure has not already been performed on the mouse.

1. Adjust the light levels in the room to medium and allow a minimum of 5 mins for them to settle prior to any mice being brought into the room.

2. Prepare change station for use (see '**SOP0065** – Use of Change Station') leaving the light off.

3. Collect the scheduled mice from the animal room and transport to the holding equipment in the test area.

Note. Steps 4-6 must be performed in order. If the program is started before the processing unit is turned on, none of the rears will be recorded. You must close the program and then restart it once the processing unit is on.

4. Log into the Open Field computer.

5. Turn on the processing unit using the butterfly switch on the back of the apparatus.

6. Log into the ActiMot program using User Account details (to create a new User Account see appendix A).

7. Check the Pipeline 2 QC Tool to see if the QC of the height of the Z axes needs to be performed. If required ensure Z axes on all 4 frames are set to 6 'blocks' high using the measurement tool and record the date in the spreadsheet.

8. Set up the large tables and arenas ensuring that the arenas are correctly located within the frames, orientated correctly within the room and under each set of lights.
    - Position 'ARENA 1 MALES ONLY ROOM LIGHTS' in frame 1 under the light nearest the door.

- Position ‘ARENA 2 FEMALES ONLY ROOM LIGHTS’ in frame 2 under the light furthest away from the door, towards the back of the room.

9. Perform a sensor test (Systemtest -> Sensor Test) for each arena to be used ensuring an “X” is shown when the beam is broken. If the ports in the processing units have been altered, the appropriate port number should be selected for the frame.
   - If the system does not register a beam break or registers a beam break when there is no obstruction, remove the arena and clean the emitter and receiver on the frame with a blast of air. If the problem persists contact a Scientific Team Leader or Senior Phenotyper responsible for this test.

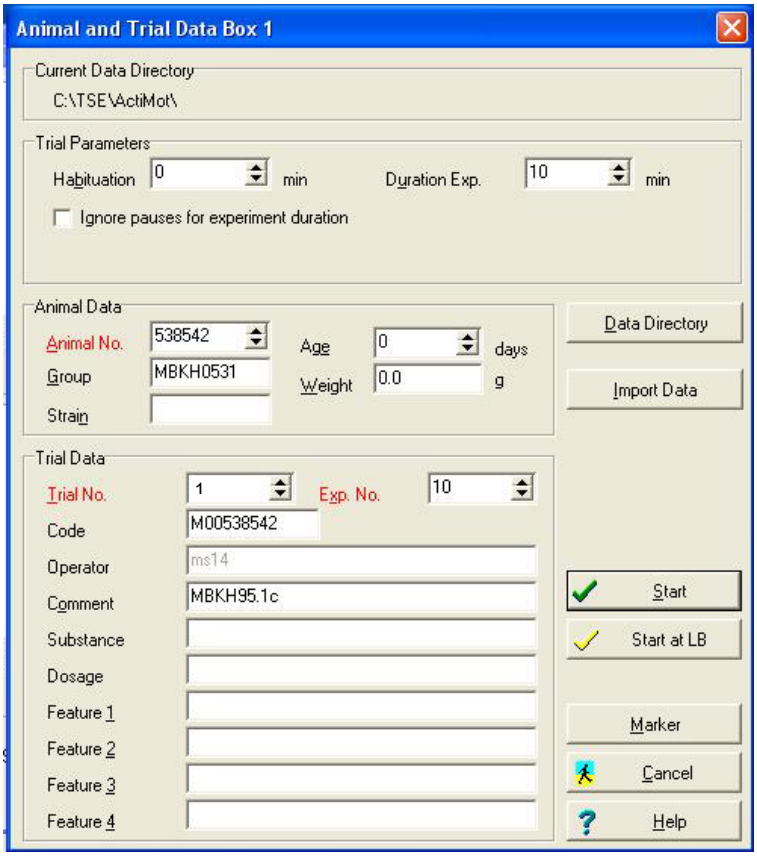
10. Position the arenas so that the lux meter reads within +/-5 lux in all 4 corners of each arena and the centre of Arena 1 is 163-168 lux and Arena 2 is 157-162 lux.

11. Clean each arena using 70% ethanol on a paper hand towel, paying special attention to the edges and the corners. A cotton bud soaked in 70% ethanol can be used to clean the edges and the corners if they require further cleaning.

12. Open the experiment screen on the program by selecting “experiment” or by clicking on the ActiMot icon.

13. Input the mouse details by clicking on the arena area to bring up the “Animal and Trial Data” screen (Image 1).
    The following fields should be entered:
    - Habituation (0 mins)
    - Duration Exp. (10 or 20 mins (depending on pipeline))
    - Animal No (xxxxxx of M00xxxxxx), e.g. 538542
    - Group (abbreviated cohort name with colony prefix and last 4 digits of the date), e.g. MBKH Mouse GP 20090531 would become MBKH0531
    - Trial No (1)
    - Exp No (10)
    - Code (M00xxxxxx), e.g. M00538542
    - Comment (Animal ID), e.g. MBKH95.1c

Image 1: Animal and Trial Data screen.



14. Select “Start at LB”.

15. Identify mouse to be tested by ear mark, causing minimal disturbance to the rest of the cage.

16. Transfer the mouse in the arena, half way along the Y axis from a height of 5 cm with its tail parallel to the wall. Leave the mouse undisturbed for duration of run.

17. Weigh mice (See ‘**SOP0045** – Weigh Mice’), after the experiment is complete and record on cage card.

18. Clean the arena using a moderate amount of 70% ethanol on a paper hand towel.

19. Repeat steps 11-18 for all mice to be tested and perform a cage clean as defined by pipeline.

20. Clean equipment and surfaces paying particular attention to remove all urine and faeces from the corners and surfaces. Transfer all other waste to a yellow offensive waste bag or clearly labelled waste container.

21. Transfer the weights recorded on the cage cards to the database.

22. Copy the raw data files from the ActiMot folder to the team drive and place in the folder for that week.

23. Shut down the program.

24. Turn off the control unit.

25. Log off/shut down the computer.

26. Ensure all cages display updated cage cards and return mice to animal room. Place ‘POST PROCEDURE CHECK REQUIRED’ labels on cages when returned.

APPENDIX A

Creating a new user profile in ActiMot software.

1. Log in to the program.
2. Open the “User Administration” screen (Parameters -> User Administration).
3. Enter the new user name and press “Save”.
4. Exit the program.
5. Enter the new user name and “User” (case sensitive) as the password.
6. Click “Change password”.
7. Enter the new password.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Open Field Data Analysis – V1

SOP Number: SOP0040	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Date modified:		

INTRODUCTION:

This procedure is undertaken to extract open field data from the ActiMot software and prepare it for upload to the MCMS database.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment:

1. TSE Open Field computer

Associated Documents & SOPs:

- **SOP0033** – Open Field Room Lights
- **SOP0034** – Open Field Light Lids

Staff Required: This test can be completed by one phenotyper.

PROCEDURE:

Data Extraction

1. Log on to the ActiMot program using your user name and password.

2. Open the Data Selection screen (Analysis -> Data Selection).

3. Clear all of the previously selected records by clicking the “None” button.

4. Filter the available data records by using the data filter. Use the start date for the days that you want to upload. Once the filter is complete, check that you have found the correct number of data records and select them by clicking “All”. The “All” button will add any data records to the list currently selected, so you can filter for each day individually and add them to the selection.

NB: Records for 10 and 20 minute experiments cannot be analysed together as they need different interval durations. The durations for each record can be seen in the experiment column. If there are both 10 and 20 minute experiments run during the same week then they should be analysed separately.

5. Check that the selected data records contain all of the data records that you need to select and save the selection by clicking "OK".

6. Open the "Interval Analysis" screen (Analysis -> Interval Analysis).

7. Select the following parameters by using the right and left arrows on the screen to move the selections from "Available Parameters" to "Selected Parameters". It is vital that they appear in the following order.

Parameter	Description
5	Total Distance (cm)
8	No. of Rearings in Z1
13	Dist. Center/Total Dist. (%)
11	Time Center/Total Dist. (%)

8. Select an interval of 5 for 20 min procedures and 2 for 10 min procedures.

9. Click on "Export" and save the file as *int*. The "int" stands for interval.

10. Clear the "Selected Parameters" and enter the following parameters in this order.

Parameter	Description
5	Total Distance (cm)
1	Time Resting
84	Lokomotion Speed (cm/s)
16	Distance Periphery (cm)
108	Resting Time Periphery
14	Time Periphery
106	Speed Periphery (cm/s)
12	Distance Center (cm)
110	Resting Time Center
10	Time Center
109	Speed Center (cm/s)
101	Latency 1. entry Center
83	Visits in Center

11. Select an interval of 20 for 20 min procedures and 10 for 10 min procedures.

12. Export this to the same folder, but save it as *tot*. The "tot" stands for total.

Data Analysis

13. Open the files using excel.

14. In the "tot" file, delete the rows that are not needed (all rows that do not contain data specific to an individual mouse).

15. Order the data by animal number using the custom sort function.

16. Remove all of the columns that do not contain the data to be uploaded, apart from the mouse number.

17. On the “int” file, delete the rows that are not needed (all rows that do not contain data specific to an individual mouse) and sort the data by both “RunNo” and then “Anim. No.” using the custom sort function. This should order the data so that all of the first time interval (run no 1) should be grouped together in animal number order followed by the second interval (run no 2) and the third (run no 3), etc.

18. Copy and paste the four headings (Dist (cm), Rear Z1, Dis Ce % and Tim Ce %) the appropriate number of times for the duration of the experiment (4 times for the 20 min experiment and 5 times for the 10 min experiment) in the heading row and label them with the appropriate bin number.

19. Move the data for each bin to the same level as the first under the corresponding headings. This should mean that all of the data for each mouse is on the same row. Delete all of the extra rows that contain the animal numbers and run numbers etc for all of the data that has been moved up.

20. Move all of the columns so that all of the parameters are grouped together and ordered by run number. (Dist (cm) 1,2,3,4,5 followed by Rear Z1 1,2,3,4,5 followed by Dis Ce % 1,2,3,4,5 followed by Tim Ce % 1,2,3,4,5).

21. Copy and paste the data from the “tot” sheet after the data from the “int” making sure that all of the data for each mouse is on the same row. You can make sure that this is the case by copying the animal number with the data from the “tot” sheet and subtracting it from the one from the “int” sheet. If you are also unsure about whether the correct binned data went into the correct row then you can calculate the difference between the total of the binned distances and the whole arena distance.

22. Save this file.