SOP Test 2 - Measuring Locomotion in Adult Zebrafish

1.0 Purpose:

1.1 The purpose of this standard operating procedure (SOP) is to measure the locomotion of mature adult zebrafish.

2.0 Scope:

2.1 This protocol is suitable for individuals who have been trained in zebrafish handling and care.

2.2. Any queries, comments or suggestions, either relating to this SOP in general, or to a specific problem encountered during the procedure should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.3. Any deviation from this protocol should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.4. All zebrafish should be kept, propagated and handled in accordance with the institutional guidelines on animal safety. Please also keep in mind the principle of replacement, refinement and reduction.

3.0. Safety Requirements

3.1. General laboratory safety procedures should be followed, which include: no eating, no drinking and no applying of cosmetics in the work area. Laboratory gloves must be worn at all times in the work area, unless the protocol specifically notes otherwise.

4.0. Associated Documents:

5.0 Notes:

5.1. This protocol is designed to compare animals that have been raised under similar conditions. Fish density, feeding regimes and age will play a significant role in modifying the level of adult locomotion.

5.2. Adult zebrafish do not show sex-specific difference in locomotion levels when measured using this protocol. Fish of both sexes can thus be combined in the experiment.

5.3. Environmental factors can play a significant role in changing locomotion levels. Behaviour should be recorded in a silent behavioural room with minimal experimenter disturbance. Lighting, temperature and time of day should be kept constant during testing.

6.0 Quality Control:

6.1. The measuring tanks with both 70% ethanol and then fresh system water before starting the experiment. A similar amount of water should be placed in the tank for each recording session.

6.3. Fish should be raised in groups of a defined number (20-25 fish in a group) from larval stages onwards.

6.4. Fish need do not need to be habituated before analysis in the behavioural setup. Fish can be brought to the testing room in their home tanks immediately before analysis begins.

6.5. Care must be taken that the tank is illuminated evenly without shadows or reduced light at the corners.

7.0 Equipment:

7.1. The boldness setup contains two parts:

a) A computer recording system that contains VideoTrack software from ViewPoint S.A.

b) 15 standard large plastic fish tanks (30 x 30 x 50 cm) filled with 20L of water. The tanks are made of transparent plastic.

c) A mounting apparatus, made of a floor that allows infra-red illumination (ViewPoint S.A.), with a video camera and a uniform white light source arranged above it.

8.0. Supplies:

Zebrafish for analysis, 12 – 15 for each genotype or treatment group.

(Optional) drugs or chemicals to modify adult behaviour,

System water to fill setup.

9.0. Procedure:

9.1. Adult fish are raised to adulthood in groups of 15 or more. Immediately before testing, fish are transported to the behaviour room (ideally in their home tank, or if not possible then in a 10L plastic mouse cage).

9.2. The observation tank is filled with system water up to a depth of 10cm. The tank is lit from both beneath (providing Infrared light for camera) and above by a circular white-light light bulb.

9.3. The VideoTrack programme needs to be started before behaviour is recorded. Switch on the computer and double-click on the ViewPoint space rocket icon. Launch the “Videotracking” option within the VideoTrack menu. In the detection threshold menu, set animal colour to black and detection threshold to 12 (this value may need to be calibrated for each new experimental setup).

Make sure that the programme is able to track the fish smoothly, without either miss-tracking or losing the animal.

9.4. In order to measure time on the non-preferred side several areas need to be delineated within the videotrack programme. Draw one area ("area 1") that encompasses the entire tank.

Measurement Process:

9.5. Fish are placed singly into the locomotion setup using a standard fish net. Their position in the tank is then videotracked for the next 10 minutes. Fish are then placed in a holding tank, or back in their home tank following testing.

9.6. The VideoTrack software should be started next. Choose "Execute" from the Experiment menu and input an name for the experiment – e.g. WT 1. Press the background and then start buttons.

9.7. The behaviour of many fish can be measured in parallel. Our current setup allows 15 tanks to be arranged on the infra-red illumination floor.

Results Analysis:

9.8. The results should be exported into Microsoft Excel and the data points analysed. For locomotion the total distance swam in a ten-minute time period can be compared.

9.9. Plot the data as a histogram, and use appropriate statistical tests to compare the different groups (either a Student's *t*-test, or ANOVA followed by an appropriate post hoc test).

10.0 Supporting Information:

11.0 History Review:

12.0 Emergency Procedures: