# SOP Test 10 - Measuring Aggression in Adult Zebrafish

## 1.0 Purpose:

1.1 The purpose of this standard operating procedure (SOP) is to measure the aggression level of mature adult zebrafish by using mirror-induced stimulation.

## 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 expression of aggression.

5.2. Adult zebrafish do not show sex-specific difference in aggression 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 the expression of aggression. Aggression should be recorded in a silent behavioural room with minimal experimenter disturbance. Lighting, temperature and time of day should be kept constant during testing.

5.4. Manual analysis of aggression films should be carried out by two independent observers who do not know the genotype of the fish being quantified. The two data sets can then be compared in order to remove observer bias in the quantification of behaviour.

**6.0 Quality Control:**

6.1. The aggression setup should be thoroughly cleaned with fresh water before starting the experiment. The mirror placed outside of the setup should be cleaned using a 70% ethanol solution. The white opaque material (EVA foam rubber) placed on three internal sides of the tank absorbs water. This material should be thoroughly wetted before measurements starts, and any trapped air bubbles removed using a plastic ruler.

6.2. The recording computer should be checked in order to make sure that enough hard-disk space is available for recording 5 minute AVI films (file sizes can become large).

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

6.4. Fish need to be handled for one week before analysis in the behavioural setup. The night before, fish need to be habituated to the presence of white material on the tank walls (see below).

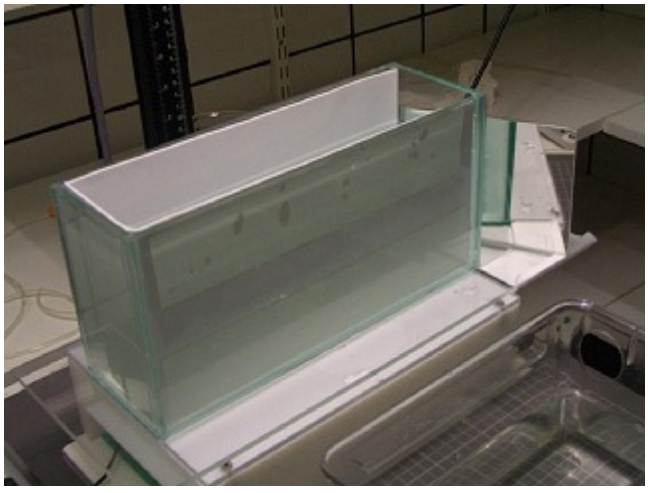
**7.0 Equipment:**

7.1. The aggression setup contains three parts:

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

b) A mount for the aggression tank, including an infra-red light box, a video camera attached to a vertical arm (all from ViewPoint S.A.) and a standard desktop strip lamp to provide white light.

c) A glass tank (home-made), covered with white foam rubber on three sides and with a glass mirror placed outside the fourth side of the tank, offset at an angle of $22.5^0$.



**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 aggression setup.

**9.0. Procedure:**

9.1. Adult fish are raised to adulthood in groups of 15 or more. One week before the experiment the fish are caught and released from a net ("handled") to reduce the stress of being placed in the behaviour setup.

9.2. On the night before recording, all fish are placed in a new holding tank which has white paper on the outside of the walls. This habituates the fish to the novel white walls.

9.3. The aggression tank is filled with system water up to a depth of 10cm, and the mirror is positioned outside of the tank at an angle of $22.5^0$. The aggression tank is lit from both beneath (providing Infrared light for camera) and above by using a desktop strip lamp that provides white light.

9.4. 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 "Tracking with fast camera" option within the VideoTrack menu. In the detection threshold menu, set animal colour to black and detection threshold to 0 (the animals will not be tracked in this setup). Make sure that the "record an AVI" button is activated in the preferences menu.

9.7. The brightness and size of field can be changed by adjusting the rings on the camera lens. The image size can also be modified by increasing- or decreasing the height of the camera over the aggression tank.

**Measurement Process:**

9.6. Fish are placed singly into the aggression setup using a standard fish net. Their reaction to the mirror is recorded for 5 or 10 minutes. Fish are then placed in a holding tank, or back in their home tank following testing.

9.8. 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.

9.9. Record the behaviour of fish from all treatment groups or genotypes separately.

**Film Analysis:**

9.10. The films can then be randomised before the aggression levels are scored by hand. On a separate excel file, randomise the name of each aggression film and give it a new number (e.g. “WT1” to “8”; “Mutant 1” to “2”) etc. Films should be analysed by two independent observers. The accuracy of their results can be judged using the Pearson correlation.

9.11. The films can be replayed in LabWatcher programme. Select LabWatcher from the VideoTrack menu. Open an AVI and reduce the playback speed to half to make analysis more accurate. The films should be manually annotated; press keyboard buttons to record the number of bites, tail thrashes and pushes against the mirror. Choose “Execute” from the Experiment menu and input a name for the experiment – e.g. WT 1 _analysis. Press the background and then start buttons.

9.12. The results should be exported into Microsoft Excel and the data points analysed. For aggression, the average time spent being aggressive (complete number of bites, thrashes and pushes) in a five- or ten-minute time-period can be compared for each treatment group or genotype.

9.13. Plot the data as a histogram, and use appropriate statistical tests to compare the different groups (either a Student’s -test, or ANOVA followed by an appropriate post hoc test).

**10.0 Supporting Information:**

**11.0 History Review:**

**12.0 Emergency Procedures:**