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This publication is no longer current and is not being updated.

Use of Standard Genetically Altered (GA) zebrafish (Danio rerio) breeding protocols

1. Purpose and general principles

These protocols will cover the needs of most users who carry out routine breeding and maintenance (B&M) of most GA zebrafish, or create new GA lines.

They are intended to be copied into PPL applications without change, avoiding the need for users to devise their own protocol text.

Inspectors will usually be able to accept the standard protocol wording, without asking for additional information.

Fish become “protected animals” under ASPA from when they become capable of free feeding. This is species and temperature dependent but zebra fish reared under standard conditions at 28^{o}C will generally be protected from 5 days post fertilization (5dpf) and this threshold life stage has been used throughout the protocols. If 5dpf is not considered the age at which fish become capable of free feeding, the applicant must provide details of when this stage is reached.

2. Addition of non—standard or experimental procedures

Altering the standard wording is likely to lead to additional work for the applicant, and may result in requests for additional information from Inspectors. Wherever possible the standard protocol wording should be used, unchanged.

We have included a standard “creation of founders” protocol. This avoids the need for an “in vitro manipulation” step on the breeding protocol.

Users should not add “non-B&M” experimental techniques to the standard B&M protocols (e.g. experimental imaging, surgery or phenotyping assessments). When required, these procedures should be specified on separate experimental protocols that specify “Continued use” from the breeding protocol, and they must be explained in the project plan.

Where non-standard procedures are required for the B&M of specific strains or circumstances, or (exceptionally) where phenotyping has to be carried out prior to the final experimental protocol, a bespoke protocol will need to be created. This can use the standard wording, where applicable. The project plan must include a clear explanation why it is necessary to include the use of such procedures in the B&M protocol.

3. Severity classification and controls

Breeding should always be carried out under the lowest appropriate severity protocol. It is expected that most zebrafish B&M can be carried out under a “Mild” severity protocol.

If, exceptionally, a moderate or severe B&M protocol is needed, it should name the strains or groups of strains involved. It must give specific adverse effects and humane end points that are appropriate to a breeding protocol (rather than experimental end points.)

Do not use “just in case” higher severity protocols. The standard “Mild” protocol ensures that fish do not suffer more than mild adverse effects, but also provides for short-term maintenance of fish that are showing unexpected adverse effects, whilst the advice of the Home Office Inspector is obtained. (This provision should only be used if it is sought to keep the fish alive for scientific reasons).

There must be clear explanation and justification if it is sought to maintain zebrafish showing moderate or severe adverse effects on a B&M protocol. Full use should be made of refinement strategies, before considering this – e.g. only using breeding stock until an age before the onset of known adverse effects, or cross-breeding non-harmful heterozygote parents to generate experimental stock.

In line with good 3Rs practice, GA zebrafish breeding numbers should be matched to experimental use. Wherever possible, zebrafish not required for use should be humanely killed before the known age of onset of adverse effects.

It is unacceptable to maintain GA zebrafish suffering adverse effects as “stock”. Fish needed for use should be transferred to the experimental protocol on which the harmful phenotype is required, as soon as possible.

Analgesia should be provided following painful procedures, including fin clipping.

4. Moderate and Severe protocols

We cannot provide a standard Moderate or Severe breeding protocol, as these should be tailored to the actual genetic alterations involved. A template for moderate / or severe B&M is included (Protocol 2), showing where information is needed. Delete this if not required.

In Moderate or Severe protocols the specified adverse effects, control measures and humane end points should relate to the specific strains being bred and maintained.

The adverse effects and control measures should be replicated on any continuation experimental protocol, with any necessary modifications to detail different harms e.g. where more advanced adverse effects are expected in experimental fish.