

Standard Genetically Altered (GA) Mouse and Zebrafish (Danio rerio) breeding protocols

The standard protocols for GA mouse and zebrafish breeding have been updated. They now include more refined approaches and are formatted according to the new-style ASPeL project licence application.

1. Purpose and general principles

These protocols will cover the needs of most users who carry out routine breeding and maintenance (B&M) of GA mice or zebrafish, or create new GA lines. You can incorporate them into your project licence application with minor edits only where indicated. Inspectors will usually be able to accept the standard protocol wording, without asking for additional information.

Standard protocols have been drafted for **mice**. They can be edited to use with rats or other species, as appropriate.

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°C will generally be protected from 5 days post fertilization (5dpf). This threshold life stage has been used throughout the zebrafish protocols. If your fish do not become capable of free feeding at 5dpf you must include details of when this stage is reached in your application.

2. Non-standard or experimental procedures

Wherever possible use the standard protocol wording, unchanged, except where it is clearly indicated that editing is required. You should not add experimental techniques such as experimental imaging, surgery or phenotyping assessments to these standard protocols. Such experimental procedures should be specified on separate experimental protocols with “continued use” from the B&M protocol.

Where non-standard procedures are required for breeding and/or maintenance of specific strains or in unusual circumstances - or (exceptionally) where phenotyping has to be carried out just prior to the final experimental procedure – you will need to draft a bespoke protocol. This can use the standard wording, as far as is applicable. You will need to justify the inclusion of any non-standard procedures.

Induction of conditional gene expression is not included in the B&M protocol for two reasons:

- i. It is very rarely used for B&M and virtually always forms part of an experiment, so should be specified within an experimental use protocol
- ii. The use of tamoxifen can be associated with adverse effects that are not compatible with a mild severity classification

In the rare situations where GA lines are maintained on a gene induction agent (invariably doxycycline, not Tamoxifen), you should draft a bespoke protocol.

3. Severity classification and controls

Breeding should always be carried out under the lowest appropriate severity protocol. We expect most breeding and maintenance can be carried out under a “Mild” severity protocol.

If, exceptionally, a moderate or severe B&M protocol is needed, you must name or clearly describe the strains or groups of strains needed. You must provide specific adverse effects and humane end points that are appropriate to a breeding protocol. These will usually be different from adverse effects and humane endpoints for experimental protocols. You should consider if it is appropriate to maintain animals showing moderate or severe effects on a breeding protocol.

In line with good 3Rs practice, animal breeding numbers should be matched to experimental requirements. Animals should be killed before suffering adverse effects, or they should be transferred to the protocol on which they will be used and for which the harmful phenotype is required. Keeping “stock animals” suffering adverse effects is not justifiable.

If you intend to keep animals alive on a B&M protocol when they are suffering more than mild adverse effects, you must provide a clear scientific justification. We expect you to make full use of refinement strategies before considering this – for example 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.

Do not include “just in case” higher severity protocols. The standard “Mild” protocol ensures that animals do not suffer more than mild adverse effects. It also provides for short-term maintenance of animals showing unexpected adverse effects whilst the advice of a Home Office Inspector is obtained in the exceptional scenario that you need to keep the fish alive for justifiable scientific reasons.

Analgesia should be provided following painful procedures, in accordance with veterinary advice.

4. Founder stock

For simplicity, we have incorporated creation of founder stock through manipulation of germplasm or blastocysts as an optional step in the B&M protocols. If you will not be doing this, you should delete the sub-step.

Where you are creating founder stock by manipulation of the germplasm or blastocysts, the numbers of animals requested should be based on the number of animals expected to be born – or in the case of fish, attain free-feeding stage - not the number of embryos manipulated.

5. Genotyping

We have included non-regulated biopsy methods on the B&M protocols for clarity.

For rodents, removal of the tip of the tail for genotyping is not usually the most refined method. If it is required, then you should keep a record of the scientific reason and make this record available to the Home Office on request.

6. Moderate and Severe protocols

We cannot provide a standard Moderate or Severe breeding protocol as these must be tailored to the actual genetic alterations involved. The template protocol for moderate / or severe breeding and maintenance shows where additional specific information is needed.

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. You will need to replicate the adverse effects on any continuation experimental protocol, with modifications to detail any different harms required for experimental use.

7. Selection of protocols

If you do not need the Moderate/severe breeding and maintenance protocol or any other protocols for your project, then delete them. Only include what is needed for your project.

8. Transport of sentinels

Do not include the transport of live animals as sentinels to diagnostic laboratories in these standard protocols. We discourage this; tissues should be sent instead. If there is a specific scientific need for live GA sentinels, then follow the guidance provided in the Advice Note on re-homing and setting free.