Home Office

Standard

Genetically Altered Zebrafish Protocols

May 2022

Version 1

Contents

Standard GA Zebrafish Protocols

Note: these General constraints are added automatically to all licences.

General constraints

Please note, constraints on procedures involving anaesthesia, surgery, substance administration and withdrawal of fluids apply to all protocols.

Anaesthesia

Induction and maintenance of general or local anaesthesia, sedation, or analgesia to mitigate the pain, suffering or distress associated with the performance of other regulated procedures is indicated using the following codes in protocols:

- AA no anaesthesia
- ABL local anaesthesia
- AB general anaesthesia with recovery
- AC non-recovery general anaesthesia
- AD under neuromuscular blockade

General anaesthesia

If authorised in this licence and unless otherwise specified, all animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Uncommonly animals that fail to do so or exhibit signs of pain, distress or of significant ill health should be humanely killed unless a programme of enhanced monitoring and care is instituted until the animal fully recovers.

Surgery

If authorised in this licence and unless otherwise specified:

- Surgical procedures should be carried out aseptically, to at least the published Home Office minimum
- In the uncommon event of post-operative complications, animals will be humanely killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. Minimally inflamed wounds without obvious infection may be re-closed on one occasion within 48 hours of the initial surgery. In the event of recurrence, NVS advice will be followed
- Peri and post-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS
- All animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Uncommonly animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be humanely killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers

- Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) should be humanely killed

Administration of substances and withdrawal of fluids

If authorised in this licence and unless otherwise specified, administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes, and frequencies that of themselves will result in no more than transient discomfort and no lasting harm using published guidelines on minimal severity.

Protocol 1: Obtaining zebrafish gametes

Title

Obtaining zebrafish gametes

Protocol details

Briefly describe the purposes of this protocol

Ensure that you state any relevant regulatory guidelines.

To obtain eggs or sperm for experimental use*, in vitro fertilization* or germplasm freezing*.

**Delete as applicable*

Given the controls and limitations in place, what is the highest severity that an animal could experience in this protocol?

Mild

What proportion of animals will experience this severity?

All animals.

Why are you proposing this severity category?

Obtaining eggs or sperm from fish can cause transient discomfort.

Locations where this protocol can be carried out

Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?

Select all that apply.

Select objective(s)

Animals used in this protocol

Zebra fish

Which life stages will be used during this protocol?

Select all that apply

- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered fish for use in this protocol may be obtained from:

- This licence: Protocol X (Breeding and maintenance of genetically altered zebrafish (Mild));* or
- This licence: Protocol X (Breeding and maintenance of genetically altered zebrafish (Moderate));* or
- other projects with authority to breed and maintain genetically altered fish of that type and to provide them for use on other projects.*

**Delete as appropriate*

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

Yes

Describe any procedure that may have been applied to these animals, and why you are choosing to re-use them.

Typically, fish will have been used for gamete collection previously. Fish previously used in this protocol will be allowed to recover for at least 2 weeks after sperm or egg collection before re-use.

What is the maximum number of animals that will be used on this protocol?

X (Note this will be the maximum and not estimated number)

What is the maximum number of uses of this protocol per animal?

For example, if some animals will go through this protocol three more times after their first use, the number of uses will be four. If no animals will go through this protocol more than once, enter '1'.

X *(this will be more than 1 if you are re-using fish for gamete collection)*

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

Which general types or strains will you be using and why?

Genetically altered fish XXX for XXX.

Do you expect any of these GAAs to show a harmful phenotype with welfare consequences?

No

Steps

Step 1 (mandatory)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Gametes are obtained from anaesthetised fish by applying gentle pressure on, or stroking the sides of, the fish (AB/AC).

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Step 2 (optional)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Killing by a schedule 1 method.

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Fate of animals

What will happen to animals at the end of this protocol?

Select all that apply

- Killed

Will you be using non-schedule 1 killing methods on a conscious animal?

No

- Kept alive

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

Fish will be briefly anaesthetised. Gametes should be released readily. No attempt will be made to force gamete release.

Describe the general humane endpoints that you will apply during the protocol.

These will be in addition to the endpoints stated for each step.

Type and depth of anaesthesia will be carefully selected and monitored as advised by the Named Veterinary Surgeon.

Fish that do not return to normal swimming behaviour within 30 minutes after removal of the anaesthetic will be killed by a schedule 1 method.

Any fish that develop infection or exhibit any abnormal behaviour on recovery from anaesthesia will be killed by a schedule 1 method.

Fish will be immediately killed by a schedule 1 method if they show signs of suffering that is greater than minor and transient or in any way compromises their health or wellbeing (for example fish that do not grow, behave, swim and feed normally).

Experimental design

What outputs are expected to arise from this protocol?

For example, test results, phenotypic information, or products.

Eggs or sperm.

Will this protocol generate quantitative data?

No

Protocol justification

Why is each type of animal, experimental model, and/or method selected for this protocol:

a) the most appropriate scientific approach?

These gamete collection methods are well described in the literature.

---

b) the most refined for the purpose?

Gamete collection is not expected to cause more than mild and transient distress and no lasting harm.

For each model and/or method, what is the scientific need for the expected clinical signs?

No clinical signs are expected due to the phenotype.

Why scientifically do the animals need to suffer to this degree?

No clinical signs are expected.

Why can't you achieve your scientific outputs with an earlier humane endpoint, or without animals showing any clinical signs?

N/A

Will you be administering substances for experimental purposes?

No

Protocol 2: Breeding and maintenance of Genetically Altered Zebrafish (Mild)

Title

Breeding and maintenance of Genetically Altered Zebrafish (Mild)

Protocol details

Briefly describe the purposes of this protocol
Ensure that you state any relevant regulatory guidelines.

To produce genetically altered (GA) zebrafish.

Given the controls and limitations in place, what is the highest severity that an animal could experience in this protocol?

Mild

What proportion of animals will experience this severity?

Up to all animals. Some/all* fish will likely be assessed as sub-threshold.

** Edit as appropriate*

Why are you proposing this severity category?

Fish produced under this protocol are not expected to exhibit any harmful phenotype.

Locations where this protocol can be carried out
Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?
Select all that apply.

Select objective(s)

Animals used in this protocol

Zebra fish

Which life stages will be used during this protocol?

Select all that apply

- Embryo and egg
- Neonate
- Juvenile
- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered fish for use in this protocol may be obtained from other projects with authority to breed and maintain genetically altered zebrafish of that type and to provide them for use on other projects.

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

No

What is the maximum number of animals that will be used on this protocol?

X (*This is the maximum and not estimated number)*

What is the maximum number of uses of this protocol per animal?

For example, if some animals will go through this protocol three more times after their first use, the number of uses will be four. If no animals will go through this protocol more than once, enter '1'.

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

Which general types or strains will you be using and why?

GA fish strain XXX for XXX.

Do you expect any of these GAAs to show a harmful phenotype with welfare consequences?

No

Steps

Step 1 (mandatory)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

The production, hatching and maintenance of genetically altered fish by male/female pairing for natural spawning (AA) *

OR

The production, hatching and maintenance of genetically altered fish by in vitro manipulation of gametes or zygotes, blastulae, embryos and/or fertilisation (generation of founder stock). (AA)*

**Delete as applicable*

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Step 2 (optional)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Tissue sampling for genetic analysis by one of the following:

- biopsy of caudal fin (AB)
- swab of surface mucus (AA, AB)
- fin clipping of larva at over 5 dpf (AA)
- micro-abrasion of embryos (e.g., by ZEG machine) (AA)

Rarely, due to technical problem in analysis, a second fin clip or skin swab will be taken.

The least invasive sampling method to obtain the smallest sample commensurate with achieving the scientific objectives will be used, and consideration will be given to sampling at the earliest life-stage possible.

- Where the transgene product is tagged with a fluorescent protein, identification by fluorescent microscopy or other non-invasive imaging technique (AA/AB).

Following tissue sampling, fish may be singly housed for up to 72 hours pending the results of genotyping

---

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Step 3 (mandatory)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Maintenance to the age of 18 months.

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Step 4 (optional)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Killing by a schedule 1 method

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Fate of animals

What will happen to animals at the end of this protocol?

Select all that apply

- Killed

Will you be using non-schedule 1 killing methods on a conscious animal?

No

- Kept alive
- Continued use on another protocol in this project*

Please state the relevant protocol.

Following any identification of genetic status, genetically altered fish produced under the authority of this protocol may be supplied to protocols X and Y in this project.

- Continued use on other projects*

**Delete as applicable*

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

GA fish may be grown and maintained until they reach a maximum of 18 months of age.

The amount of tissue removed for the purpose of genotyping will be the minimum required from the most appropriate site and such that normal behaviour is not compromised.

Named Veterinary Surgeon advice will be followed regarding the use of analgesia following fin clipping for genotyping in over 5dpf fish.

Describe the general humane endpoints that you will apply during the protocol.

These will be in addition to the endpoints stated for each step.

Some fish may have the potential to develop a harmful phenotype after a certain age, but in all cases will be killed before reaching that age and before onset of clinical signs, unless moved on to another protocol as continued use for a specific purpose.

Fish exhibiting any unexpected harmful phenotypes will be killed, or in the case of individual fish of particular scientific interest, advice will be sought promptly from a Home Office Inspector.

Fish will be immediately killed if they show signs of suffering that is greater than minor and transient or in any way compromises their health or wellbeing (for example fish that do not grow, behave, swim and feed normally).

Fish that develop signs associated with infection will be killed by a Schedule 1 method.

Any fish exhibiting abnormal behaviour following genotyping will be killed by a Schedule 1 method.

Type and depth of anaesthesia will be carefully selected and monitored in consultation with the Named Veterinary Surgeon. Fish that do not return to normal swimming behaviour within 30 minutes after removal of the anaesthetic will be killed by a Schedule 1 method.

Animals are not expected to die because of any authorised genetic alteration. A small number of animals, living beyond when they become capable of independent feeding, may suddenly and unexpectedly die having shown no preceding clinical signs indicative of impending death. Unless otherwise indicated, such deaths, should they occur, are unlikely to be related to the genotype. However, as per the published ASRU Advice Note on Severity Assessment of GA animals, should the mortality rate (age-matched) of the genetically altered strain rise beyond that present in the background source breeding colony, this will be reported under PPL standard condition 18.

Experimental design

What outputs are expected to arise from this protocol?

For example, test results, phenotypic information, or products.

Genetically altered fish.

Will this protocol generate quantitative data?

No

Protocol justification

Why is each type of animal, experimental model, and/or method selected for this protocol:

a) the most appropriate scientific approach?

Genetically altered fish are required for XXX.

---

b) the most refined for the purpose?

Fin clips and swabs will only be taken if more refined genotyping methods are not suitable scientifically.

For each model and/or method, what is the scientific need for the expected clinical signs?

No clinical signs are expected.

Why scientifically do the animals need to suffer to this degree?

No clinical signs are expected.

Why can't you achieve your scientific outputs with an earlier humane endpoint, or without animals showing any clinical signs?

N/A

Will you be administering substances for experimental purposes?

No

Protocol 3: Breeding and maintenance of Genetically Altered Zebrafish (moderate)

Title

Breeding and maintenance of Genetically Altered Zebrafish (moderate)

Protocol details

Briefly describe the purposes of this protocol
Ensure that you state any relevant regulatory guidelines.

To produce genetically altered (GA) zebrafish.

Given the controls and limitations in place, what is the highest severity that an animal could experience in this protocol?

Moderate

What proportion of animals will experience this severity?

X% of strain XXX.

Why are you proposing this severity category?

Fish produced under this protocol are expected to exhibit a harmful phenotype.

Locations where this protocol can be carried out
Select all that apply.

Select establishment(s)

Which of your objectives will this protocol address?
Select all that apply.

Select objective(s)

Animals used in this protocol

Zebra fish

Which life stages will be used during this protocol?

Select all that apply

- Embryo and egg
- Neonate
- Juvenile
- Adult

Will any animals coming on to this protocol be classed as 'continued use'?

'Continued use' describes animals that are specifically genetically altered and bred for scientific use or animals that have had procedures applied to them in order to be prepared for use in this protocol.

Yes

How did these animals start their use?

Describe the procedures that have been applied to animals that will continue their use on to this protocol.

Genetically altered fish for use in this protocol may be obtained from:

- this licence: Protocol X (Breeding and maintenance of GA zebrafish (mild)); or
- other projects with authority to breed and maintain genetically altered fish of that type and to provide them for use on other projects.

Will you be re-using animals on to this protocol?

'Re-use' describes using animals again for a new experiment when you could equally use a naïve animal to get the same results.

No

What is the maximum number of animals that will be used on this protocol?

X *(This is the maximum and not estimated number.)*

What is the maximum number of uses of this protocol per animal?

For example, if some animals will go through this protocol three more times after their first use, the number of uses will be four. If no animals will go through this protocol more than once, enter '1'.

1

Genetically altered animals (GAA)

Will this protocol use any genetically altered animals?

Yes

Which general types or strains will you be using and why?

For example: We require genetically altered fish strain X that over expresses/does not express gene X for the purpose of Y.

Do you expect any of these GAAs to show a harmful phenotype with welfare consequences?

Yes

Why are each of these harmful phenotypes necessary?

For example: To determine whether gene X plays a role in the development of disease X, we will create fish that either don't express the gene or overexpress the gene which leads to the clinical findings {...}.

How will you minimise the harms associated with these phenotypes?

Ensure that you include any humane endpoints that you will use.

Some lines may be embryonic lethal or lethal before adulthood (e.g., XXX), and such lines will be made as conditional knockouts or be maintained as heterozygotes.

Steps

Step 1 (mandatory)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

The production, hatching and maintenance of genetically altered fish by male/female pairing for natural spawning (AA) *

OR

The production, hatching and maintenance of genetically altered fish by in vitro manipulation of gametes or zygotes, blastulae, embryos and/or fertilisation (generation of founder stock). (AA)*

*Delete as applicable

Is this step optional?

No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

Yes

What are the likely adverse effects of this step?

State the expected adverse effect, including the likely incidence, and the anticipated degree and duration of suffering.

For example:

Strain 1

Offspring are expected to show the following clinical signs:

- overtly normal up to {....} dpf
- progressive development of {give details of adverse effect....} until reaching {....} dpf
- {give details of any other strain-specific adverse effect}

Breeding stock are {not expected to show clinical signs* / expected to show the following clinical signs*} (*delete as applicable):

- overtly normal up to {....} dpf
- progressive development of {give details of adverse effect....} until reaching {....} dpf
- {give details of any other strain-specific adverse effect}

How will you monitor for, control, and limit any of these adverse effects?

If adverse effects can't be prevented, how will you attempt to ameliorate their initial signs?

{Provide details}

What are the humane endpoints for this step?

This would be the point at which you would kill the animal to prevent further suffering.

Offspring will be killed before {....} dpf, or at the onset of clinical signs if earlier, unless required for experimental use when they will be transferred as continued use to protocol {...}

Breeding stock will be killed before {....} dpf or at the onset of clinical signs if earlier.

Step 2 (optional)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Tissue sampling for genetic analysis by one of the following:

- biopsy of caudal fin (AB)
- swab of surface mucus (AA, AB)
- fin clipping of larva at over 5 dpf (AA)
- micro-abrasion of embryos (e.g., by ZEG machine) (AA)

Rarely, due to technical problem in analysis, a second fin clip or skin swab will be taken.

- Where the transgene product is tagged with a fluorescent protein, identification by fluorescent microscopy or other non-invasive imaging technique (AA/AB).

Following tissue sampling, fish may be singly housed for up to 72 hours pending the results of genotyping

---

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Step 3 (mandatory)

Describe the procedures that will be carried out during this step.
Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Maintenance to the age of 18 months.

Is this step optional?
No

Do you expect this step to have adverse effects for the animals that are more than mild and transient?
Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

Yes

What are the likely adverse effects of this step?
State the expected adverse effect, including the likely incidence, and the anticipated degree and duration of suffering.

Add details for each strain or type of GAA. For example:
Strain X will show the following clinical signs: overtly normal up to 4 weeks of age;
- progressive {....} until reaching .{....} . at 15 weeks of age.

How will you monitor for, control, and limit any of these adverse effects?
If adverse effects can't be prevented, how will you attempt to ameliorate their initial signs?

Add details for each strain or type of GAA. For example:
Strain X: Breeding stock will be only allowed one breeding cycle and offspring will be killed or transferred to an experimental protocol before the age of {x weeks}. During that time, they should show no more than XXX.

What are the humane endpoints for this step?
This would be the point at which you would kill the animal to prevent further suffering.

Add details for each strain or type of GAA. For example:
Strain X: Breeding stock will be killed after the first litter is weaned, and before onset of {...}. Offspring will be killed before 4 weeks of age or at the onset of clinical signs if earlier unless required for experimental use when they will be transferred for continued use.

Step 4 (optional)

Describe the procedures that will be carried out during this step.

Explain where one or more steps are repeated in one experiment, list any alternative techniques within a step (e.g., dosing routes), and include all procedures performed under terminal anaesthesia. When describing the technical aspects of a step, be broad enough to be flexible when the variation does not impact on animal welfare (e.g., use "antibiotic" instead of "penicillin"). Finally, avoid specifying volumes and frequencies when they do not impact on animal welfare.

Killing by a schedule 1 method

Is this step optional?

Yes

Do you expect this step to have adverse effects for the animals that are more than mild and transient?

Do not list uncommon or unlikely adverse effects, or effects from procedures that will cause no more than transient discomfort and no lasting harm. For example, an intravenous injection of a small volume of an innocuous substance.

No

Fate of animals

What will happen to animals at the end of this protocol?

Select all that apply

- Killed

Will you be using non-schedule 1 killing methods on a conscious animal?

No

- Kept alive
- Continued use on another protocol in this project

Please state the relevant protocol.

List relevant protocol(s)

- Continued use on other projects

Animal experience

Summarise the typical experience or end-to-end scenario for an animal being used in this protocol.

Consider the cumulative effect of any combinations of procedures that you may carry out.

GA fish may be grown and maintained until they reach a maximum of 18 months of age.

Breeding stock will only be allowed one breeding cycle and offspring will be killed or transferred to an experimental protocol before the age of {x weeks}. During that time, they should show no more than XXX.

The amount of tissue removal for the purpose of genotyping will be the minimum required from the most appropriate site and such that normal behaviour is not compromised.

Named Veterinary Surgeon advice will be followed regarding the use of analgesia following fin clipping for genotyping in over 5dpf fish.

Describe the general humane endpoints that you will apply during the protocol.

These will be in addition to the endpoints stated for each step.

Any fish showing XXX will be immediately killed by a Schedule 1 method.

Some fish may have the potential to develop a harmful phenotype after a certain age, but in all cases will be killed before reaching that age and before onset of clinical signs, unless moved on to another protocol as continued use for a specific purpose.

Fish exhibiting any unexpected harmful phenotypes will be killed, or in the case of individual fish of particular scientific interest, advice will be sought promptly from the assigned Home Office Inspector.

Genotyping

Fish that develop signs associated with infection following genotyping will be killed by a Schedule 1 method.

Any fish exhibiting abnormal behaviour post genotyping will be killed by a Schedule 1 method.

Fish that show signs associated with infection will be killed by a Schedule 1 method.

Type and depth of anaesthesia will be carefully selected and monitored in consultation with the Named Veterinary Surgeon. Fish that do not return to normal swimming behaviour within 30 minutes after removal of the anaesthetic will be killed by a Schedule 1 method.

Other than that, described in the strain-specific adverse effects above animals are not expected to die because of any authorised genetic alteration. A small number of animals, living beyond when they become capable of independent feeding, may suddenly and unexpectedly die having shown no preceding clinical signs indicative of impending death. Unless otherwise indicated, such deaths, should they occur, are unlikely to be related to the genotype. However, as per the published ASRU Advice Note on Severity Assessment of GA animals, should the mortality rate (age-matched) of the genetically altered strain rise beyond that present in the background source breeding colony, this will be reported under PPL standard condition 18.

Experimental design

What outputs are expected to arise from this protocol?

For example, test results, phenotypic information, or products.

Genetically altered fish.

Will this protocol generate quantitative data?

No

Protocol justification

Why is each type of animal, experimental model, and/or method selected for this protocol:

a) the most appropriate scientific approach?

Genetically altered fish are required for XXX.

---

b) the most refined for the purpose?

Fin clips and swabbing will only be taken if more refined genotyping methods are not suitable scientifically.

For each model and/or method, what is the scientific need for the expected clinical signs?

For example: We need fish { } with genetic alterations XXX in order to ….

Why scientifically do the animals need to suffer to this degree?

For example: We need animals that are showing {clinical signs} in order to { }…

Why can't you achieve your scientific outputs with an earlier humane endpoint, or without animals showing any clinical signs?

For example: We cannot achieve our scientific objectives without animals showing XXX because XXX.

Will you be administering substances for experimental purposes?

No