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This publication is no longer current and is not being updated.

E. Protocols

The term “protocol” is used to describe a single or a series of regulated techniques applied for a particular experimental or other scientific purpose to a protected animal. In most cases a protocol will involve all regulated procedures applied to the animal until the animal is killed or released from the controls of ASPA. Depending on the complexity of your work you may need one or several protocols.

Protocols must specify **what** type and number of animals are proposed to be used and **what** procedures will be performed. In the adverse effects section you must specify **what** the effect of the regulated procedures on the animal will be, **what** measures will be taken to prevent or control the adverse effects, **what** humane endpoints will be applied and **what** the fate of the animal will be.

- Each protocol should cover one complete sequence of procedures carried out on an animals from start to finish of the experiment, study or production process where possible
- Similar sequences of procedures with similar adverse effects should be grouped together in a single protocol
- Alternative or optional steps should be identified
- Refer to Home Office guidance/advice on use, re-use and continued use, re-homing and setting free, use of wild-caught animals and on categorisation of severity

In each protocol, details of any planned use of anaesthesia, analgesia and other pain relieving methods must be included.

The summary table will be created automatically on ASPeL when you complete the protocols. Please copy the section below for additional protocols.

Summary

Protocol Number	Title	Severity Category	Species	Estimated Numbers	GA	Life Stage
1	Breeding and maintenance	Mild	*Zebrafish*		yes	all ages
2 [*delete as appropriate*]	Breeding and maintenance of.....	Moderate	*Zebrafish*		yes	all ages
3	Obtaining Zebrafish gametes	Mild	*Zebrafish*		yes	adult
4	Generation of founders	Mild	*Zebrafish*		yes	embryo

Protocol Number: 1

Title	Severity category
Breeding and maintenance of Genetically Altered Zebrafish	Mild

Species

Species of animal	Fish - Zebrafish
Are some of these animals genetically altered?	Yes
Estimated numbers over the duration of the project	
Life stage of the animals	All ages, to 18 months of age

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the proposed use now represents 'continued use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and other advice on use, re-use and continued use.

Continued use

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

- Protocol 4 of this project (Generation of founders (F0 generation)); or
- Other projects with authority to breed and maintain genetically altered zebrafish of that type and to provide them for use on other projects.

Re-use

N/A

Protocol steps

- List numbered and broadly chronological steps starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment
- It is accepted that the order of steps may be varied according to scientific need
- Protocols may contain optional steps, but should include at least one step which will always be undertaken. (How you will use optional steps must be clearly explained in the programme of work)
- List alternative techniques e.g. dosing routes, as lists for clarity
- Indicate where one or more steps may be repeated within an experiment, e.g. a cross-over study
- Use anaesthetic codes but remember that administration of anaesthetics (and analgesics) are themselves regulated procedures and should, therefore, be specified
- Do not include technical detail in the description of procedures. Instead use principles & performance measures, e.g. 'light emitting substances' instead of 'luciferin'
- Specify limits and controls only where they impact directly on animal welfare, e.g. 'intramuscular injection (max volume 0.1ml, no more than two injections at 48 hr interval)'. In all other cases use performance criteria
- If appropriate indicate the method of killing, i.e. Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC)

Protocol steps

Aim: To produce genetically altered (GA) zebrafish.

1. Breeding of genetically altered fish by male/female pairing for natural spawning (AA)

2. (Optional) Tissue sampling for genetic analysis by one of the following:

   - Swab of surface mucus (AA, AB)
   - Biopsy of caudal fin (AB)
   - Unregulated techniques such as fin clipping of embryos at <5 dpf where the injury is expected to be fully healed by 5dpf
   - Rarely, due to a technical problem in analysis, a second fin clip or skin swab may be taken.

3. Alternatively imaging for assessment of genetic status:

   - Fluorescent microscopy or other non-invasive imaging technique (e.g. where the transgene product is tagged with a fluorescent protein) (AA, AB)

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

5. Fish not used on other protocols will be killed by a Schedule 1 method.

Fate of animals not killed at the end of protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol

Fate of animals not killed at the end of protocol	
Yes	**Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
Yes	**Kept alive at the licensed establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
No	**Discharge from the controls of the Act by setting free to the wild or by re-homing.** Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in section 17A(3) & (4) will be met.

Details

Continued use: Following any identification of genetic status, genetically altered fish produced under the authority of this protocol may be supplied to other protocols in this project, or to other projects with authority to use genetically altered fish of this type.

Wild type offspring that have suffered no more than mildly during the course of procedures, and which are not suffering or likely to suffer as a result, may be kept alive in accordance with Project licence Standard Condition 11 for prospective re-use.

Expected adverse effects, refinement controls and humane end-points

For the series of regulated procedures described above, both as a whole and as component steps:

Describe the likely adverse effect(s) and the expected incidence in the different animals used

- Explain how animals will be monitored for the onset or development of adverse effects
- Set out the refinement measures and other controls you will adopt to prevent adverse effects from occurring or to minimise their severity
- In all cases specify practicable and realistic humane end-points

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

Expected adverse effects, refinement controls and humane end-points

Overall adverse effects

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

- 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 by a schedule 1 method, or in the case of individual fish of particular scientific interest, advice will be sought promptly from the assigned Home Office Inspector.

Humane endpoint: 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.)

Anaesthesia

Type and depth of anaesthesia will be carefully selected and monitored in consultation with the NVS.

Humane endpoint: 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.

Genotyping:

The site and amount of tissue removal will be such that there is no compromise to normal swimming. Following fin clipping for genotyping in >5dpf fish peri-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS.

Humane endpoint: Any fish exhibiting any abnormal behaviour will be killed by a schedule 1 method.

Infections can result from fin clipping (<1%) or from damage to scales or loss of mucous surface from swabbing. The procedure will be carried out using sterile equipment.

Humane endpoint: Fish that develop signs associated with infection will be killed by a schedule 1 method.

Protocol Number: 2

Title	Severity category
Breeding and maintenance of GA Zebrafish	Moderate

Species

Species of animal	Fish - Zebrafish
Are some of these animals genetically altered?	Yes
Estimated numbers over the duration of the project	
Life stage of the animals	All ages (up to 18 months of age)

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the proposed use now represents 'continued use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and other advice on use, re-use and continued use.

Continued use

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

- Protocol 1 of this project (Breeding and maintenance of GA zebrafish)
- Protocol 4 of this project (Generation of founders (F0 generation)); or
- Projects with authority to breed and maintain genetically altered fish of that type and to provide them for use on other projects.

Re-use

N/A

Protocol steps

- List numbered and broadly chronological steps starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment
- It is accepted that the order of steps may be varied according to scientific need
- Protocols may contain optional steps, but should include at least one step which will always be undertaken. (How you will use optional steps must be clearly explained in the programme of work)
- List alternative techniques e.g. dosing routes, as lists for clarity
- Indicate where one or more steps may be repeated within an experiment, e.g. a cross-over study
- Use anaesthetic codes but remember that administration of anaesthetics (and analgesics) are themselves regulated procedures and should, therefore, be specified
- Do not include technical detail in the description of procedures. Instead use principles & performance measures, e.g. 'light emitting substances' instead of 'luciferin'
- Specify limits and controls only where they impact directly on animal welfare, e.g. 'intramuscular injection (max volume 0.1ml, no more than two injections at 48 hr interval)'. In all other cases use performance criteria
- If appropriate indicate the method of killing, i.e. Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC)

Protocol steps

Aim: To produce Genetically Altered (GA) zebrafish.

1. Breeding of genetically altered fish by male/female pairing for natural spawning (AA)

2. (Optional) Tissue sampling for genetic analysis by one of the following:

   - Swab of surface mucus (AA, AB)
   - Biopsy of caudal fin (AB)
   - Unregulated techniques such as fin clipping of embryos at <5 dpf where the injury is expected to be fully healed by 5dpf
   - Rarely, due to a technical problem in analysis, a second fin clip or skin swab may be taken.

3. Alternatively imaging for assessment of genetic status:

   - Fluorescent microscopy or other non-invasive imaging technique (e.g. where the transgene product is tagged with a fluorescent protein) (AA, AB)

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

5. Fish not used on other protocols will be killed by a Schedule 1 method.

Fate of animals not killed at the end of protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol

Fate of animals not killed at the end of protocol	
Yes	**Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
Yes	**Kept alive at the licensed establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
No	**Discharge from the controls of the Act by setting free to the wild or by re-homing.** Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in section 17A(3) & (4) will be met.

Details

Continued use: Following any identification of genetic status, genetically altered fish produced under the authority of this protocol may be supplied to other protocols in this project or to other projects with authority to use genetically altered fish of this type.

Wild type offspring that have suffered no more than mildly during the course of procedures and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11 for prospective re-use.

Expected adverse effects, refinement controls and humane end-points

For the series of regulated procedures described above, both as a whole and as component steps:

Describe the likely adverse effect(s) and the expected incidence in the different animals used

- Explain how animals will be monitored for the onset or development of adverse effects
- Set out the refinement measures and other controls you will adopt to prevent adverse effects from occurring or to minimise their severity
- In all cases specify practicable and realistic humane end-points

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

Expected adverse effects, refinement controls and humane end-points

Please note: For breeding protocols classified greater than mild severity, each genetically altered line or type of line must be specified, with details of appropriate monitoring and end points for that particular strain, or group of strains.

Example of text to describe the adverse effects that are expected to result from the genetic alteration:

Overall adverse effects

Adverse effects of genetic alterations

(1) Strain 1 / Fish showing {....} type of genetic modification.

Offspring are expected to show the following clinical signs:

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

Humane endpoint: 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 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 detail of adverse effect*....} until reaching {....}dpf
- {*give detail of any other strain-specific adverse effect*}

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

(2) Strain 2.....

(3) Strain 3.....

Anaesthesia

Type and depth of anaesthesia will be carefully selected and monitored in consultation with the NVS.

Humane endpoint: 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.

Genotyping:

The site and amount of tissue removal will be such that there is no compromise to normal swimming. Following fin clipping in >5dpf fish peri -operative analgesia will be provided; agents will be administered as agreed in advance with the NVS

Humane endpoint: Any fish exhibiting any abnormal behaviour will be killed by a schedule 1 method.

Infections can result from fin clipping (<1%) or from damage to scales or loss of mucous surface from swabbing. The procedure will be carried out using sterile equipment.

Humane endpoint: Fish that develop signs associated with infection will be killed by a schedule 1 method.

Protocol Number: 3

Title	Severity category
Obtaining Zebrafish gametes	Mild or Moderate *(Delete as appropriate)*

Species

Species of animal	Fish - Zebrafish
Are some of these animals genetically altered?	Yes
Estimated numbers over the duration of the project	
Life stage of the animals	adult

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the proposed use now represents 'continued use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and other advice on use, re-use and continued use.

Continued use

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

- Protocol 1, 2 of this project, or
- Other projects with authority to breed and maintain genetically altered fish of that type and to provide them for use on other projects.

Re-use

Fish that have been kept alive and maintained under the supervision of the NVS may be re-used in this protocol provided they meet the criteria set out in ASPA Section 14. Fish previously used in this protocol will be allowed to recover for at least 2 weeks after sperm or egg collection before re-use.

Protocol steps

- List numbered and broadly chronological steps starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment
- It is accepted that the order of steps may be varied according to scientific need
- Protocols may contain optional steps, but should include at least one step which will always be undertaken. (How you will use optional steps must be clearly explained in the programme of work)
- List alternative techniques e.g. dosing routes, as lists for clarity
- Indicate where one or more steps may be repeated within an experiment, e.g. a cross-over study
- Use anaesthetic codes but remember that administration of anaesthetics (and analgesics) are themselves regulated procedures and should, therefore, be specified
- Do not include technical detail in the description of procedures. Instead use principles & performance measures, e.g. 'light emitting substances' instead of 'luciferin'
- Specify limits and controls only where they impact directly on animal welfare, e.g. 'intramuscular injection (max volume 0.1ml, no more than two injections at 48 hr interval)'. In all other cases use performance criteria
- If appropriate indicate the method of killing, i.e. Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC)

Protocol steps

Aim: To obtain eggs or sperm for experimental use, *in vitro* fertilization or sperm freezing

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

Fish may be killed by a Schedule 1 method

Fate of animals not killed at the end of protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol

Fate of animals not killed at the end of protocol

No **Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.

Yes	**Kept alive at the licensed establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
No	**Discharge from the controls of the Act by setting free to the wild or by re-homing.** Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in section 17A(3) & (4) will be met.

Details

Once fully recovered from anaesthesia, fish that have suffered no more than mild severity as result of this procedure and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11 of this licence.

Expected adverse effects, refinement controls and humane end-points

For the series of regulated procedures described above, both as a whole and as component steps:

Describe the likely adverse effect(s) and the expected incidence in the different animals used

- Explain how animals will be monitored for the onset or development of adverse effects
- Set out the refinement measures and other controls you will adopt to prevent adverse effects from occurring or to minimise their severity
- In all cases specify practicable and realistic humane end-points

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

Expected adverse effects, refinement controls and humane end-points

Overall adverse effects

Adverse effects of genetic alteration in the case of moderate phenotypes ??

Anaesthesia

Type and depth of anaesthesia will be carefully selected and monitored as advised by the NVS.

Humane endpoint: 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.

Gamete Collection

Massaging the abdomen of fish could cause scale loss leading to a breach in the epidermis and/ or dermis leave the fish vulnerable to infection (<1%) or could cause compression damage to internal organs (< 1%). Gametes should be released readily; therefore, no attempt should be made to force gamete release'

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

Protocol Number: 4

Title	Severity category
Generation of founders (F0 generation)	Mild

Species

Species of animal	Fish - Zebrafish
Are some of these animals genetically altered?	Yes
Estimated numbers over the duration of the project	
Life stage of the animals	All

If the animals have been used, bred or surgically prepared under the authority of this or any other project licence, briefly describe what has been done to them and indicate whether the proposed use now represents 'continued use' or 're-use' - refer to the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and other advice on use, re-use and continued use.

Continued use

Re-use

Protocol steps

- List numbered and broadly chronological steps starting with the first preparative step and ending with the death of the animal or the last regulated procedure in the experiment
- It is accepted that the order of steps may be varied according to scientific need
- Protocols may contain optional steps, but should include at least one step which will always be undertaken. (How you will use optional steps must be clearly explained in the programme of work)
- List alternative techniques e.g. dosing routes, as lists for clarity
- Indicate where one or more steps may be repeated within an experiment, e.g. a cross-over study

- Use anaesthetic codes but remember that administration of anaesthetics (and analgesics) are themselves regulated procedures and should, therefore, be specified
- Do not include technical detail in the description of procedures. Instead use principles & performance measures, e.g. 'light emitting substances' instead of 'luciferin'
- Specify limits and controls only where they impact directly on animal welfare, e.g. 'intramuscular injection (max volume 0.1ml, no more than two injections at 48 hr interval)'. In all other cases use performance criteria
- If appropriate indicate the method of killing, i.e. Schedule 1 or non-Schedule 1. Give brief details of non-Schedule 1 methods e.g. perfusion fixation (AC)

Protocol steps

Aim: Generation of founders

1. GA embryos will be generated by in vitro manipulation of gametes or zygotes, blastulae, embryos and/or fertilisation. Viable embryos will be raised to generate founders (AA).
2. (Optional) Where possible fish will be screened prior to independent feeding for likely transmission of the mutation/transgene (AA)
3. (Optional) Fish that are potential carriers are grown to an appropriate life stage and are then tested to identify those fish carrying the genetic alteration by :

    - Tissue sampling for genetic analysis by one of the following

        - Swab of surface mucus (AA,AB)
        - Biopsy of caudal fin (AB)
        - Unregulated techniques such as fin clipping of embryos at <5 dpf where the injury is expected to be fully healed by 5dpf

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

    Alternatively

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

    OR

    - By appropriately breeding the potential carriers to enable the identification of the transgene presence in the carrier (AA).

4. Fish expressing a relevant genetic alteration may be transferred to protocol 1 or 2 for breeding and colony maintenance.

5. Any fish not moved to another protocol, or fish in which the desired genetic alteration cannot be detected, will be killed by a Schedule 1 method

Fate of animals <u>not</u> killed at the end of protocol

Indicate the proposed fate of animals which are not killed at the end of the protocol

Fate of animals not killed at the end of protocol	
Yes	**Continued use in another protocol under this or another project licence** - give details below and ensure that you give an appropriate cross reference in the protocol sheet under which the continued use will occur.
No	**Kept alive at the licensed establishment.** Note that any subsequent re-use must be authorised in the relevant project licence.
No	**Discharge from the controls of the Act by setting free to the wild or by re-homing.** Specify below the particular circumstances when animals may be set free to the wild or re-homed and detail how the qualifying criteria set out in section 17A(3) & (4) will be met.

Details

Continued use: Following any identification of genetic status, genetically altered fish produced under the authority of this protocol may be supplied to other protocols in this project or to other projects with authority to use genetically altered animals of this type.

Expected adverse effects, refinement controls and humane end-points

For the series of regulated procedures described above, both as a whole and as component steps:

Describe the likely adverse effect(s) and the expected incidence in the different animals used

- Explain how animals will be monitored for the onset or development of adverse effects
- Set out the refinement measures and other controls you will adopt to prevent adverse effects from occurring or to minimise their severity
- In all cases specify practicable and realistic humane end-points

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

Expected adverse effects, refinement controls and humane end-points

Overall adverse effects

Injected constructs may cause death or developmental abnormality before fish reach the stage of protection. Harmful genetic alterations in embryos may be evident as altered morphology prior to hatching. Embryos will be assessed for morphological phenotypes before the stage of independent feeding and any showing morphological abnormality not required for the scientific purpose would be killed using a schedule 1 method.

Some genetic alterations may result in a harmful phenotype during post-hatching development evidenced as failure of larvae to inflate the swim bladder, difficulty swimming, altered morphology, failure to feed or breathing difficulties. If fish exhibit any of these adverse effects they will be killed immediately by a schedule 1 method.

On occasion (<5%) late onset mutations in more mature stages may lead to lines of fish with mild lordosis (defined as less than 20% curvature of the spine), poor growth /body condition or difficulty swimming.

Humane endpoint: Any fish showing harmful phenotypes will be killed by a schedule 1 method as soon as the phenotype is visible.

Anaesthesia

Type and depth of anaesthesia will be carefully selected and monitored in consultation with the NVS.

Humane endpoint: 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.

Genotyping:

The site and amount of tissue removal will be such that there is no compromise to normal swimming. . Following fin clipping in >5dpf fish peri -operative analgesia will be provided; agents will be administered as agreed in advance with the NVS

Humane endpoint: Any fish exhibiting any abnormal behaviour will be killed by a schedule 1 method.

Infections can result from fin clipping (<1%) or from damage to scales or loss of mucous surface from swabbing. The procedure will be carried out using sterile equipment.

Humane endpoint: Fish that develop signs associated with infection will be killed by a schedule 1 method