Home Office

NON-TECHNICAL SUMMARY

# Breeding of Transgenic rodents for supply of tissues to the client

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research

**Key words**

Tissues, Supply

| Animal types | Life stages |
|---|---|
| Mice | adult, juvenile, neonate, embryo, pregnant |

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

## Objectives and benefits

**Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.**

**What's the aim of this project?**

To provide a service to clients by breeding Genetically Altered animals and maintenance of the colony for research purposes for supplying the tissues.

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

By breeding animals with a gene mutation specific to the disease of interest, tissues can be harvested from dead animals and used in in-vitro (in the laboratory) experiments to assess likely drug effect. The Project Licence holder will provide experience in breeding genetically altered animals and thus a centralised service for the client. The supply of tissue will be matched to the client's requirements (who do not have the training, expertise and facilities necessary to breed the animals required for their research).

The animals will be bred and housed at the site where tissues are harvested. This means there is no transport of live animals from breeder to point of use, thus reducing the lifetime stress experienced by the animal.

**What outputs do you think you will see at the end of this project?**

The output for the PPL holder is to ensure we meet the demands of the client for supply of animals/tissue and match our expertise of breeding rodents with the clients laboratory expertise.

Tissues from the mice produced in this project will be used by researchers to advance their understanding of neurodegenerative diseases (i.e. diseases of the nervous system such as Parkinsons Disease), and support the client's aim to develop drugs to treat these diseases.

The client's data will contribute to the better understanding of human neurodegenerative disease and longer term the drugs are aimed at improving patient outcomes.

Data will be discussed at lab meetings, and disseminated at scientific meetings as presentations and posters and peer reviewed publications.

**Who or what will benefit from these outputs, and how?**

Neurodegenerative disease affects millions of people and animals worldwide. The research associated with this project will help understand the role of genes, and the mechanisms of action taking place in these neurodegenerative diseases. This in turn will help in the development of medicines to treat them.

Some of the mice used in this project do not have the specific gene of interest, it has been deleted. This gene is normally expressed in a subpopulation (a small and specific type) of brain cells. This project will further the understanding of this genes' role in neurodegeneration.

**How will you look to maximise the outputs of this work?**

Throughout this project, current journals and data will be reviewed to ensure that the best techniques are being used to meet the targets of this project.

Collaboration of the animal care team and the research staff means that matings will only be set up to match the scientific demands of the research teams. The colony will be managed by an experienced member of the animal care team and they will ensure the breeding is carried out efficiently, to match the demand.

Through monthly colony reviews of the breeding performance of the strains and any issues experienced (e.g. litter sizes, unexpected deaths, etc.) production of animals can be further matched to demand to avoid over production.

Throughout and at the end of the research programme data obtained from the use of the tissues supplied will be published in journals and posters. Data will be shared at scientific meetings.

**Species and numbers of animals expected to be used**

- Mice: 1000

# Predicted harms

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**Explain why you are using these types of animals and your choice of life stages.**

We are using all life stages of the animals under this project. Adult animals are bred to produce further animals for future breeding stock and tissue collection from animals that are killed. Animals which carry the gene modification required by the client are not expected to show any deviation from normal health and wellbeing.

Animals, particularly rodents, are in widespread use in biomedical and veterinary science and mice in particular are invaluable for finding out the function of genes and what drives a wide variety of biological, physiological and pathological processes.

**Typically, what will be done to an animal used in your project?**

Mice, with changes already made to their genes (genetic material), will be purchased and used to create the breeding colony. They will be bred and maintained in Individually Ventilated Cages (IVCs). IVCs are special cages which provide the animals with filtered clean air, so the mice remain healthy and are of a constantly high health status. Animals will be killed using an approved humane method, and the tissue collected as soon as the animal is dead.

When fresh whole blood is required for an experiment, animals may be exsanguinated (anaesthetised and then a fatally large volume of  blood withdrawn from their body while they are under anaesthetic).

When tissues are required with no blood contamination, animals will be terminally anaesthetised and their whole blood replaced with a buffer (such as saline) or a fixative (such as paraformaldehyde) to fix the tissue for later experimentation.

Some animals that will be used for future breeding may be anaesthetised and have a small tissue sample taken for genetic analysis to ensure they are of the correct genotype to provide the correct offspring for future experiments.

**What are the expected impacts and/or adverse effects for the animals during your project?**

Animals are not expected to exhibit any harmful characteristics or abnormalities as we are breeding well established strains.

**Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per animal type)?**

Mice: Mild 100%

**What will happen to animals at the end of this project?**

- Killed

# Replacement

**State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.**

**Why do you need to use animals to achieve the aim of your project?**

Fresh tissue is required by the client to run in vitro experiments to further the understanding of what causes disease and to test how drugs work. For the research that will be undertaken on these tissues there is not currently any appropriate substitute available.

**Which non-animal alternatives did you consider for use in this project?**

Before this work was instigated a laboratory technique called NETSseq has been used on human tissue to identify the areas which we would like to investigate further. Now that this work has been completed on human tissue, the only way to continue this work is to use living tissue.

Non-animal sources of tissue are not available to further this work, and so our client needs animal tissues for scientific research purposes. It is not possible to accurately prove on a computer how these complex biological processes will work, only work with fresh tissue will do this. Theories and ideas can

be generated about how diseases spread and drugs work, but only live tissue work will prove these ideas are correct, before a medicine can be developed in further subsequent projects.

A literature search has been completed by the client and shown this work has not previously been undertaken, and the answers to their questions are not available.

Our client has previously completed testing using cultures of cell lines in dishes. The results of this work gave positive data. To further their research animal tissues are now required for the next stage of research in the laboratory.

A search using the ECVAM database has been completed, but no alternative to animal tissue testing has been identified to carry out this work.

**Why were they not suitable?**

Alternatives such as fruit fly, worms and zebrafish, which have been modified to remove or change the genes of interest to our client are not available.

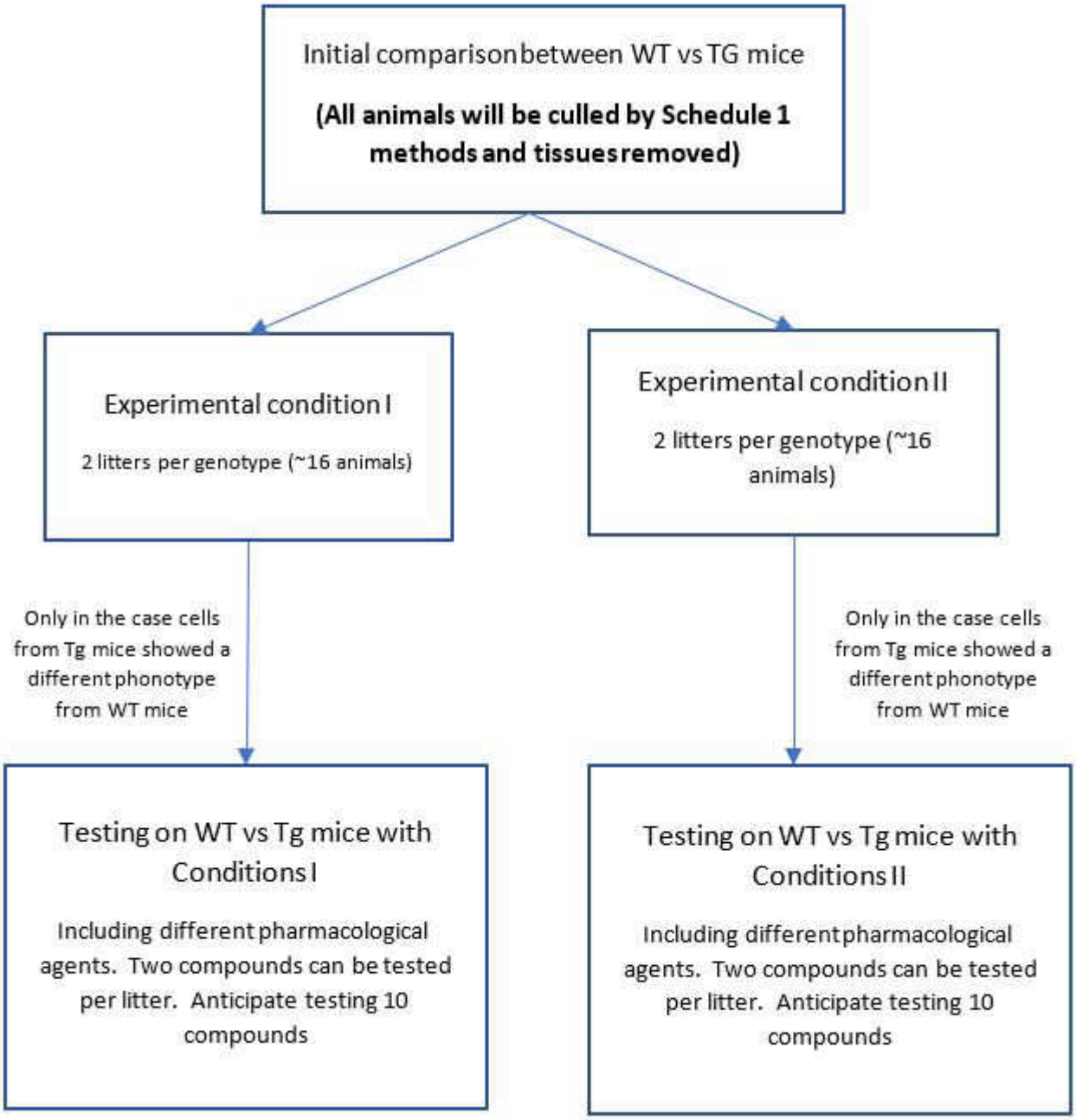
# Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

**How have you estimated the numbers of animals you will use?**

The colony mice have already been developed by a commercial supplier. Following delivery the colony will be expanded by conventional mating in which either one male and one female are put together (pair mating) or with one male placed with two females (trio matings). The numbers of animals required are estimated on the expectation that 25% of the offspring will have the required genes necessary for our client's research. The colony will be maintained by an experienced staff member and will only be bred on receipt of a 'breeding form' and the 'study form', which meets the purpose proposed on this Project Licence.

Since establishment the colony has been further refined to breed as Homozygous. This means that 100% of the animals bred can be used, and the controls used will be commercially sourced wildtype mice. Having a 100% Homozygous colony also removes the need to genotype the off spring, except when carrying out quality control checks.




**What steps did you take during the experimental design phase to reduce the number of animals being used in this project?**

Our aim will be to produce mice which only the genetic makeup required for our client's experiments.

Reference documents used include: Efficient Breeding of Genetically Altered Animals (Home Office Website).

Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (Home Office).

Guidance on the Breeding of GA Animals (NC3Rs Website).

ARRIVE Guidelines (NC3R's Website).

Experimental Design Assistant (NC3R's Website).

Since the initiation of this project it has now been possible to establish a homozygote colony carrying the genetic material of interest. This means that all animals produced can now be used for experiment, and not only 25% as previously stated.

**What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?**

Mouse colony Management:

The production of Genetically Altered Animals (GAA), including breeding programs will be controlled centrally by experienced members of staff with the assistance of a dedicated animal tracking system (this is computer software that records every animal in the colony). This will enable animals to be easily tracked for many things, including how many breeders in any given line, stock levels and colony organisation. We will only maintain the colony and breed on demand to minimise wastage.

Both sexes of animal will be used to produce tissues for our client's research. A commercial genetic analysis company will be used for DNA analysis (i.e. to find out the genes each mouse is carrying) providing rapid results (typically within 72 hrs) and before mice are weaned from their mums. This means we do not keep any animals that are not required any longer than is absolutely necessary. The vet who visits the animals will be actively involved in providing advice about the breeding of the mice and will provide advice about the health and welfare of the mice.

Also the facility has an experienced member of staff who will lead the maintenance of the GA animal colony. Prior to the agreement to commence each project, a form will be completed and assessed. This will be used to help us to determine the cohort size and plan the breeding.

## Breeding Request Sheet

### Strain Licence details:

| | |
|---|---|
| Project licence no. | |
| Procedure no. | |
| Severity band | |
| Responsible Person | [Name; email, phone] |
| Additional Operators | [Name; email, phone] |
| | |

### Breeding details:

| | |
|---|---|
| Breeding Study Number | Insert study log number |
| Study Purpose | |
| Parent genotypes | |
| Genotype required | |
| Start date | Select start date |
| End date | Select end date |
| | |
| Number of litters required/genotype | |
| Frequency if more than one litter required | |
| Gender to be used under study | |
| Age of animals to be used under study | |
| Request submission date | Select start date |
| Approval date | Select end date |
| Approved by | |

**Study Title**

**Licence details:**

| | |
|---|---|
| **Project licence no.** | |
| **Protocol no.** | L |
| **Severity band** | Mild |
| **Responsible Person** | [Name; email, phone] |
| **Additional Operators** | [Name; email, phone] |
| | |

**Animal details:**

| | |
|---|---|
| **Species/Strain** | |
| **Sex** | Choose an item. |
| **Weight/Age** | |
| **Total no of +/+ requested** | Insert number of mice to be used |
| **Total no of -/-requested** | Insert number of mice to be used |
| **Total no of -/+ requested** | Insert number of mice to be used |
| **Arrival date** (if applicable) | To be completed by Agenda |
| **DoB** | To be completed by Agenda |

**Study details:**

| | |
|---|---|
| **Study Number** | Insert study log number |
| **Study Purpose** | Eg. Electrophysiology on acute brain slices |
| **Start date** | Select start date |
| **End date** | Select end date |
| **Tissue/Live animals required** | Eg. Live |
| **Room number** | Eg. 418DR |

**Animal Fate:** The animals will be killed using a schedule 1 method

| | |
|---|---|
| **Request submission date** | Select start date |
| **Approval date** | Select end date |
| **Approved by** | |

# Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare**

**costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

Genetically altered and non-genetically modified mice will be used as the basis of the colony and the colony will be maintained using conventional breeding methods. The client requires mice that have had their genes modified so that they can investigate what causes neurodegenerative diseases and to test drugs to combat these diseases. Therefore the genetic modifications in the mice will be those relevant to this type of research. However, none of the animals are expected to suffer any pain or disease from either the breeding methods used or the changes made to their genetic material.

**Why can't you use animals that are less sentient?**

Our client currently needs juvenile and adult animal tissue for scientific use in their laboratory to investigate the genetic cause and treatment of neurodegenerative diseases. Neurological tissue from mammalian sources is needed to represent what is happening in the target species for therapies (man and animals). These mechanisms are likely to be different in species such as fruit flies, worms and fish, making them unsuitable.

**How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?**

The breeding protocol on this licence does not allow any animals to undergo any procedure that would cause the animal's health to be anything other than normal. To minimise suffering experienced by the mice, they will be monitored and humanely killed if they show any changes in their behaviour or health. Our animal unit is proactive with enrichment e.g. fun tunnels and nesting material or similar products. Tunnel handling or cup handling practice when working with the animals is followed in the unit rather than lifting animals by the tail. This will reduce stress to the animals. For genotyping the least invasive method will be used. This means the method that causes the least pain, suffering or distress (harm) to the animal will be chosen above any other method available to obtain the required tissue sample. Animals will be group housed wherever possible, with the exception of animals separated only for welfare reasons e.g. males fighting.

**What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?**

Wherever possible, animals will be group housed to minimise stress in social species. It might be necessary to separate animals due to fighting which can result in a single housing for welfare reasons. However, stud males in particular, separated from active mating may have to be single housed as re-grouping is not practical.

We will refer to the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (Home Office) for the welfare and ensure best working practice.

**How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?**

We will refer to the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (Home Office) for the welfare and ensure best practice for the housing and care of our mice.

We and our client will consult the NC3Rs Guidelines and monitor publications where such practices are published (NC3Rs/LASA/IAT Websites) for best practice.

We will ensure that our client is aware of the breeding of their animals via a weekly report and ensure the animals bred are used within the initial agreed timeframe. Any deviation from this timeframe will be communicated immediately so that the breeding colony can be increased or reduced so animals are not wasted. We will only breed on demand to minimise wastage. Technicians looking after the colony are empowered to question the colony manager if they suspect any unusual request on the colony.