Home Office

NON-TECHNICAL SUMMARY

Colony Management, Production & Preservation of GA animals as a service & associated support

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

colony management, cryopreservation, GA production, rederivation, Monoclonal Antibodies

Animal types	Life stages
Mice	adult, juvenile, embryo, neonate, pregnant
Zebra fish	embryo, neonate, juvenile, adult
Xenopus laevis	embryo, adult, neonate, juvenile

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 centrally managed breeding colonies of Genetically Altered (GA) and Wild-type(Wt) mice, zebrafish & xenopus, and associated procedures such as new GA line production at a high and consistent standard to the scientific community at this Institute.

A retrospective assessment of these aims will be due by 11 July 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

The Institute holds multiple genetically altered mouse, zebrafish and Xenopus colonies live at any one time for the diverse portfolio of work undertaken in the field of medical research. There are ~700 new lines created, or imported each year and a similar number are archived off the shelf or for distribution to collaborators. Providing this service centrally and in a demand-matched breeding mode, supported and managed by specialised technical experts is administratively efficient and has proved welfare benefits.

Our main outputs will be

• Centralisation of common use lines ensuring minimisation of waste as well as best standards of care

• Production of sophisticated and refined GA models for the research here,

• A Cryopreservation service to archive rodent & aquatic species, ensuring lines are backed up and animal numbers are reduced,

• Rederivation of all imported lines to ensure infection free mice at the highest health status, allowing distribution between units, and allowing stability & characterisation of the microbial population, reducing phenotypic & scientific variation.

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

This is a service licence and as such we expect to have maintained our colonies of mice and fish in an efficient manner, to have exceeded best practice in colony management, to have created ~100 novel sophisticated GA lines and maintained them at the highest health status. To have cryopreserved >2000 lines, and rederived all lines entering the Institute to the highest health status. To support us in this we have a colony management team whose focus is on implementing best practice and analysing practices across the Institute to enable us to exceed our performance year on year. For example, we have an comprehensive mouse database and analyse the data through Power BI reports looking at PEI, mortality rates, trios v pairs, mating age etc.

We also expect to have worked to refine the techniques we use, such as optimising surgical techniques, taken on new technologies to make our work more efficient and enable us to reduce animal numbers. To have trained staff and scientists in best practice, and shared that through the use of journals, presentations and technical forums. We are working on a CRACKIT challenge currently looking at embryo culture techniques for improved outcomes.

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

Providing this service centrally and in a demand-matched breeding mode, supported and managed by specialised technical experts is administratively efficient and has proven welfare benefits. The protocols covered by this licence will benefit animal users across the establishment, all of whom will have their own PPLs with benefits clearly described in the field of medical research. We have a policy where we don't allow duplicates of any colony, and keep all colonies at the highest health status. As a consequence we can minimise waste and use spare stock for cryopreservation. We also encourage use of cryopreserved stock to manage a line, many Cre lines for example can be maintained from the freezer, and then an IVF carried out by our skilled transgenic team as and when the line is needed, minimising overall animal use and waste.

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

We will disseminate best practice through training courses, journals, presentations and technical forums.

Species and numbers of animals expected to be used

- Mice: 350,000
- Xenopus laevis: 2000
- Zebra fish (Danio rerio): 45,000

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.

This is a service licence for the Institute for mouse, zebrafish and Xenopus users. By making these services available centrally we can ensure reduced numbers of animals and best practice in welfare. These animals & life stages are the most appropriate for the breeding and production of GA animals.

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

Most of the animals used on this licence will be of a mild severity and be bred for use in other experimental project licences.

Where new GA lines are produced, cryopreserved or rederived, an animal might undergo superovulation - a mild procedure involving an injection with hormones on 2 occasions causing it to produce a higher number of embryos than is normal before being mated and killed. A very small number (<5%) might undergo a repeat procedure if they failed to mate, and the NVS deemed the animals' welfare to allow it.

Alternatively an animal might be mated with a sterile male to render it "pseudopregnant" and then undergo a moderate surgical procedure of 5- 10 minutes under analgesia and anaesthesia whereby genetically altered embryos are implanted. The mouse will then carry to term as normal, and give birth to a litter of GA mice.

Zebrafish generally breed naturally, or a small percentage might be used for a procedure whereby their eggs are collected under analgesia and anaesthetic (administered in their water) by gently but firmly stroking the belly before putting them back to recover.

Xenopus normally would lay naturally, but on occasion for collection of sperm may undergo a hormone injection and Females also undergo superovulation to produce more eggs than they would naturally.

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

Most animals in this PPL are not expected to undergo more than transient discomfort. In the case of surgery, the animals will experience mild pain, are expected to make a rapid and unremarkable recovery. Animals will be monitored daily and in the event of post-operative complications, animals will be killed unless, in the opinion of the Named Veterinary Surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. Analgesic agents will be administered as required.

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)?

~70% of mice are expected to be subthreshold and 17% mild, 6%of mice are expected to be moderate (surgical) and 6% of mice are expected to be severe - because they were found dead with no prior clinical signs

~74% of fish experience mild severity, 23% severe and 2 % moderate.

~97% of Xenopus experience mild severity

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

- Killed
- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 11 July 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

This is a service licence to produce and maintain genetically altered animals and aquatic species to the Science here. All Projects using our animals will justify their work in detail with regards to medical research.

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

N/A

Why were they not suitable?

N/A

A retrospective assessment of replacement will be due by 11 July 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 numbers estimated here are based on the likely level of work we will experience, knowing the data from the last 5 years. Demand is reasonably stable, and the Institute has a regular supply of new animal users who will use this service.

The number of animals used in each protocol is based on experience and we are constantly working to reduce those numbers. For example in the production of GA animals, we know how strains will respond to hormone treatment and how many embryos we can get from each animal. We also know the average success rates of different procedures and ensure our staff are trained to maintain and improve on those numbers. An example would be we use ~10 mice treated with hormones to collect in excess of 300 embryos. These are genetically manipulated and will be transferred into ~10 females, and ~40 pups will be born. We have recently refined this process to use hyperovulation where possible, which in some strains ~doubles the number of eggs collected, halving the number of animals we use.

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

This has all been done on experience, working to best practice guidelines in the field and maintaining those high standards. These are reviewed by our AWERB procedures regularly.

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

The number of animals used in the pursuit of this PPL's objectives will be kept to a minimum through training of staff to high standards, and monitoring success rates, such that benchmarks are set for staff to improve on. We have an excelllent mouse colony management database, which is already allowing us much better control in tracking our animal usage, health concerns, the genetic background and modifications of our mice, and also any phenotypic concerns. we have detailed Power BI reports that look at multiple aspects of our breeding and animal use. This allows us to both refine the husbandry and care of our animals and keep numbers to a minimum – the allele tracking has already allowed us to identify 10s of colonies where there were duplication, and savings have been made. Our databases keep track of all our work, monitor the embryo output of strains, success rates of constructs and ES cells, transgenic and embryo transfer rates, allowing us to recommend best practice, put limits on animal use and spot problems as they occur and investigate them.

We have a new colony management service who coordinate this effort and also work with all groups using animals to refine & optimise breeding. Animals are only bred if user requirement has been

established, and the breeding programme is subject to regular review to optimally meet anticipated demand. Spare animals are made available for use on other scientific projects. Colony plans and breeding schemes are regularly reviewed to ensure minimal wastage.

Unnecessary production or import of GAA is avoided by searching cryobanks and databases. Examples of resources available include: NC3R's mouse database, Animal Welfare Management Discussion Group (AWMDG), Mouse locator, PubMed, the Jackson laboratory, and various Cre databases.

Cryopreservation helps to reduce animal numbers removing strains no longer in use and allowing maintenance via smaller live colonies. We track cryopreservation survival rates and IVF fertilisation rates so we can use the most appropriate route for each strain, keeping animal numbers to a minimum. Cryopreservation of rodent strains is relatively common, but by implementing a service for preserving frogs and fish as well, we can extend the advantages to these species as well.

By centralising the provision of animals, we can provide a demand matched supply and reduce wastage. We can use any spare animals to cryopreserve embryos for our GAA production service, or for the production of rodent serum. We use datasheets, genetic monitoring and microbiome monitoring to enable us to track our animals in as many ways as possible to manage any harm to them or possible change to their phenotypes – thus allowing us to keep numbers to a minimum and welfare high.

A retrospective assessment of reduction will be due by 11 July 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

We use mice, zebrafish and Xenopus in this project.

All communally bred mice bred under the PPL are bred under the mild protocol baring one strain (severe). Occasionally there will be need for the moderate protocol when importing new lines. The methods we use in the production of new GA animals are all refined to reduce suffering to a minimum. The Zebrafish and Xenopus models are also mild.

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

This service PPL by its nature requires the use of animals, and will result in GAA being made available for use in most of the PPLs used at the Institute, for which the benefits are clearly described within each PPL and will be published via the scientific groups holding these PPLs. Much of the work covered here requires further justification through an application process, and if it is felt that alternatives are available, the work will be turned down.

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

We make every effort to refine our procedures wherever possible, and there is a definite culture of care within the service with many BRF and GeMS technicians presenting on 3Rs work at animal welfare meetings.

We continue to instigate many changes to our Surgical techniques, taking tips from the LASA Surgical technical forums and from the LASA recommendations for aseptic surgery and have worked closely with the vet to ensure our pre, peri and post-operative care is the best we can offer. We have extensively used non-surgical embryos transfer over the past 5 years, particularly in our rederivation work. There are still higher rates of success for surgical, and both methods have their advantages and disadvantages and are used dependent on the stage and manipulation applied to the embryo.

Breeding is mild for the most part by keeping lines with moderate or severe phenotypes breeding heterozgously wherever possible, and using them before a phenotype appears – but there will be occasions where for experimental need, the homozygous line needs to be maintained.

Cryopreservation is encouraged, as a means of reducing the welfare issues involved in animal shipment as well as to take animals off the shelf and reduce waste, moving towards managing colonies from the freezer. We also freeze a very high volume of lines through sperm cryopreservation, reducing the procedures needed using animals to archive a strain.

We keep up to date of new genetic tools be they new gene editing techniques such as endonucleases or novel inducible, conditional, spatial specific and binary systems which reduce the severity of phenotypes in GAA to pass on to users and to update the general use lines we have available. Our transgenic rates are assessed and improved upon through a variety of minor refinements, and come in above average in an international survey of transgenic services, and have contributed to a large range of research.

New environmental enrichment products are trialled continuously by animal care staff, and there is an active training and development programme for animal care staff ensuring best practice.

We use ear clips as standard for genotyping, and are working with a collaborator on faecal samples for repeat samplingand ensure all our staff are trained in best practice and constantly search for methods to refine our techniques. New GA mice undergo welfare assessment, and passports are used where mice are transported to ensure continued high standards of welfare. Our mice undergo a genetic stability program to ensure we know the background and regularly refresh our lines to minimise drift and phenotypic variation. We also monitor the microbiome for that purpose.

With regards to antibody work we always choose the least severe method, and keep abreast of new adjuvants to minimise side effects.

We rarely use vasectomy since we moved to a strain with sterile males – the Prm1EGFP line.

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

We work with the LASA guidelines for Aseptic Surgical technique, NC3RS and RSPCA guidelines in the transport of animals, maintenance ad production of GA animals, cryopreservation & archiving as well sa those published by expert working groups and published in Lab Animals (such as those on health monitoring and gene editing)

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

Our staff all participate actively in improving their skills sets and receive mandatory Home Office training, IAT At qualifications from our onsite accredited team as well as attending local inductions, refresher and best practice sessions. We have regular attendance at seminars workshops and conferences in the field of Lab animal science, colony management and GA production & maintenance. These are reported back to a wider group through our animal technical forums as well as through the department seminar series, the NIO's newsletter and through out technician training team. keeping abreast of advances through journals, email discussion groups and technical forums as well as the useful NC3RS technician hub and GA resources ensures we are at the forefront of new advances in the 3Rs.

A retrospective assessment of refinement will be due by 11 July 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?