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NON-TECHNICAL SUMMARY

Cryopreservation, Breeding and maintenance of genetically altered mice as a service

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Cryopreservation, Re-derivation, Breeding, Rodents, Health status

Animal types	Life stages
Mice	adult, juvenile, embryo, neonate, pregnant, aged

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?

Maintain a high-health status of rodent colonies as required by many ongoing scientific projects, using the practice of embryo re-derivation.

Preserve and archive important genetically altered rodent lines by cryopreservation to ensure prevention against loss as well as to avoid unnecessary breeding.

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?

Animal models remain indispensable tools for studying disease biology and physiological processes. One of the reasons the mouse remains a valuable role model for such study is the ability to manipulate its genome. Therefore, transgenic and gene targeted mice will continue to be required.

Where possible, we would import genetically altered (GA) lines into our facility from sources that provide high health status animals devoid of pathogens. However, this is not always possible as many academic Institutions both national and internationally do not maintain high health status animals compared with those housed in our facilities. Such animals before entering our main experimental facility are cleaned by embryo re-derivation. Once the health status of imported animals becomes comparable with existing animals, breeding and experimental work can start. In addition, rather than import live animals from other sources that cause travel stress to animals, we will have the ability to import frozen genetic material (sperm or embryos) and produce a line of interest by using non-conventional breeding practices, such as surgical embryo transfer in suitable female mice.

Value of cryopreservation of sperm and embryos. Generation of a bank of frozen embryos and/or sperm from all modified lines would reduce breeding of mice and ensure a repository is available for future use. Cryopreservation of GA animal lines contribute significantly towards implementing 3Rs, but especially reduction.

The present project license that provides the above services to the scientific community is held by a senior academic and will expire in January 2021. Specifically, these services include provision of breeding high health status genetically altered (GA) mice by conventional breeding or by embryo transfer and cryopreservation of genetic material from the lines that need to archived or are still needed by the research community.

Several research groups who are familiar with my work in the field have indicated that Animal Facility management should continue to provide these services through a dedicated project licence for a further 5 years.

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

1. Maintain a high-health status of rodent colonies as required by many ongoing scientific projects in Leeds by the practice of embryo re-derivation

2. Preserve and archive important genetically altered rodent lines by cryopreservation to ensure prevention against loss as well as to avoid unnecessary breeding

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

We can provide the skills, knowledge and equipment required to efficiently produce transgenic mice with the minimum of animal wastage.

Cryopreservation service. Archiving frozen embryos and/or sperm from all GA lines would reduce breeding, and therefore the number of excess animals being produced. It will also ensure a repository is available for future use to guard against the potential loss of a line which would be difficult to replace.

Maintaining a high animal-health status. Required for many scientific projects by preventing any import of pathogens into the animal facility by the practice of embryo re-derivation.

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

The staff members providing the Transgenic Service are familiar with the mouse lines we commonly use and are highly experienced in the techniques involved in transgenic mice production and the breeding of GA mice. The skills and methods are continually being improved and optimized. No individual lab would be able to devote an equivalent amount of time to increasing efficiency and troubleshooting.

Species and numbers of animals expected to be used

- Mice: No more than 14,300 rodents will be used over the 5 year project time frame. This is broken down into 13,000 (max) undergoing mild severity and 1,300 (max) undergoing moderate severity procedures.

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.

The production of GA mice is technically difficult. We can provide the skills, knowledge and equipment required to efficiently produce transgenic mice with the minimum of animal wastage. 1.

Cryopreservation service. Archiving frozen embryos and/or sperm from all modified lines would reduce breeding, and therefore the number of excess animals being produced. It will also ensure a repository is available for future use to guard against the potential loss of a line which would be difficult to replace. 2. Breeding and maintenance of GA lines. 3. Maintaining a high animal-health status. Required for many scientific projects by preventing any import of pathogens into the animal facility by the practice of embryo re-derivation.

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

Vasectomy

Under general anaesthetic the vas deferens will be exposed via the scrotal approach. Each duct will be severed, resected, sutured and/or cauterised, the wound closed, and the animal allowed to recover

Embryo recipients

Surgical or non-surgical Implantation of genetically modified embryos into the oviduct or uterus of pseudo pregnant female, the animal allowed to recover, give birth, the litter is kept but the female is killed by a Schedule 1 method

Superovulation

Administration of substances by intraperitoneal injection (e.g. gonadotrophin followed about 48 hours later by luteinizing hormone. Maximum volumes 0.5 ml of each) All mice are killed by a Schedule 1 method within 7 days of the initial injection and oocytes/embryos are harvested post-mortem

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

All surgical procedures will be carried out using sterile techniques to minimise the potential risks of infection to the animal. All animals will be given pain relief before any surgery to avoid pain or potential discomfort. Animals will be either transferred onto other PPL’s to be used in further projects, or humanely killed once they reach their set end point.

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)?

The majority of the work will be under a mild severity limit with very little likelihood of any adverse effects to the animals. Any procedures that are carried out under moderate rated protocols have very little chance of adverse effects.

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

- Killed
- Used in other projects

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?

In some scientific projects it is not possible to replace the use of rodents. When investigating disease and developmental processes, the complexity of a whole organism cannot always be recapitulated using alternative in vitro systems. The tissue, cell and molecular interactions involved in such complex processes cannot be examined in their entirety in vitro.

The genetically altered mice generated and bred under this PPL will be investigated for phenotypes and processes that cannot be examined in any other way. All animals bred on this PPL are destined for use in another PPL and the case for that particular model will have been made, and approved, separately in that PPL and by that institute's Animal Welfare and Ethical Review Body.

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

It is not possible to replace the use of rodents

Why were they not suitable?

As we need to freeze sperm or embryo to archive lines, this is only possible using live rodents.

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?

Using estimates from numbers used under the previous licence and in discussion with users of the facility, data from cyropreservation and embryo transfers that is stored on our filemaker database.

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

Looking at breeding data provided by our database, HO returns and data from cyropreservation and embryo transfers that is stored on our filemaker database. The breeding data is used to work out

breeding calulation which uses litter size, litter interval and how much stock that needs to be prodcue, which will give an idea of how many breeders need to be set up.

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

The numbers proposed in this programme of work are based on reasonable estimates of generating the required GA mouse lines for the facility over the next 5 years. The advantage of using a centralised transgenic service is that it will decrease the overall number of mice used to generate GA lines. The reasons for this are:

- The availability of highly skilled workers will ensure the lowest number of animals possible will be used.
- Central coordination of animal stock production allows the most efficient use of breeding stock. Excess mice or embryos generated for one project can be used in other transgenic projects.
- Sharing of sterile male mice between projects requiring generation of pseudo pregnant females.
- Making use of our expertise in the areas of sperm and embryo freezing to archive all mutant lines, such that a stock of live mice for each line are not required to ensure lines are not lost.
- The strains that are being used in the facility have been chosen because they are the most efficient for production of transgenic mice.
- New techniques are continually tested and adopted to reduce animal usage significantly.

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.

The majority of the work will be under a mild severity limit with very little likelihood of any adverse effects to the animals. Any procedures that are carried out under moderate rated protocols have very little chance of adverse effects. However, all surgical procedures will be carried out using sterile techniques to minimise the potential risks of infection to the animal. All animals will be given pain relief immediately after any surgery to avoid pain or potential discomfort. Animals will be either transferred onto other PPL’s to be used in further projects, or humanely killed once they reach their set end point.

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

Most of the rodents being used have to be at breeding age (6-8 weeks) to carry out this work, with the exception of the superovulated females which are 4 weeks+.

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

The availability of highly skilled workers will ensure the lowest number of animals possible will be used.

Equipment is being used that will maximise the efficiency of transgenic production. Such equipment is costly and could only be purchased by a central service unit.

Central coordination of animal stock production allows the most efficient use of breeding stock. Excess mice or embryos generated for one project can be used in other transgenic projects. Sharing of sterile male mice between projects requiring generation of pseudo pregnant females.

Making use of our expertise in the areas of sperm and embryo freezing to archive all mutant lines, such that a stock of live mice for each line are not required to ensure lines are not lost. The strains that are being used in the facility have been chosen because they are the most efficient for production of transgenic mice.

New techniques are continually tested and adopted to reduce animal usage significantly. we are hoping to do futher training on the Subcuticular Stitch and Wound Glue method shown on MRC course, which would replace the need for autoclips on the embryo transfer females and further training on nonsurgical transfers at the moment it would need to be a mixture of both surgical and non surgical methods so we could compare the data.

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

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Behringer R. et al., (2014) Manipulating the Mouse Embryo: A Laboratory Manual, 4th ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

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

The NC3r's is sent out in a monthly newsletter, looking out for training sessions which would help implement and improve my work and practises.