Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 4

Projects with a primary purpose of: Maintenance of colonies of established genetically altered animals, not used in other procedures

Project Titles and keywords

1. **Breeding, Archiving and rederivation of genetically altered or natural mutant animals**
    - Genetically modified and harmful mutant strains

2. **Breeding & Maintenance of genetically modified zebrafish**
    - Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish

3. **Stock Centre and Cryopreservation Program**
    - Cryopreservation, breeding, genotyping

4. **Production of transgenic mice**
    - CAGE, Germline, piRNAs

5. **Breeding and maintenance of mutant or genetically altered animals**
    - Breeding, genetically altered, mutant

6. **Breeding and Maintenance of Gal-deficient Swine**
    - Swine, heart valves, cardiovascular

7. **Production, Breeding & Cryopreservation of GM Mice**
    - Genetically modified, embryo sperm cryopreservation

8. **Breeding mutant or GA mice and embryo production**
    - Genetically altered, embryo, breeding

Project 1	**Breeding, Archiving and rederivation of genetically altered or natural mutant animals**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To maintain and breed colonies of genetically modified and harmful mutant strains of animals for scientific research, to archive and preserve embryos or sperm from colonies that may not currently being studied, and to create specific pathogen free colonies which are recommended to improve animal welfare and support high quality research.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will continue to allow the breeding, importation and maintenance of genetically altered and mutant animals at our institution, including the option to clean up lines and archive non-required colonies. This is a continuation of a service licence to support biomedical research which has been in place for over 20 years. Genetically altered animals provide valuable models that contribute to scientists understanding of a wide range of biological processes and diseases. The introduction of embryo transfer will allow us to accept animals previously unacceptable on health grounds,	

	increasing scientists' access to a wider range of models. It will also reduce animal use by removing the need to keep breeding and maintaining colonies which are currently not required. Use of live animals for archiving embryos and subsequently rederiving them is a well tried and tested way of obtaining specific pathogen free animals. Animals will only be bred when required, minimising overproduction and wastage. The rodent is the lowest order of vertebrate that can be used for this project. A mammalian model is required as it is similar in many ways to human anatomy, physiology and disease. The techniques are well established and the limitations and potential problems are generally well known.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 6000/ year Rats 245/ year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No animals with genetic disabilities exceeding mild severity will be bred on this licence. There are no adverse effects associated with breeding but adverse effects may occasionally occur during tissue sampling for genotyping. These effects are minimised by the use of anaesthesia, both local and general where necessary. The anaesthetics chosen will be appropriate for the species and the nature and duration of the procedure. Anaesthesia will be carefully and regularly monitored to ensure that an adequate depth is maintained throughout the procedure. Where animals are allowed to recover, the recovery will be monitored carefully and supervised until the animal is able to ambulate, feed and drink normally. Deaths resulting from anaesthetic or surgical complications are most uncommon (<1%), and will be minimised by ensuring correct dosing of anaesthetics and by good maintenance of body temperature, e.g. careful monitoring to ensure adequate anaesthetic depth and use of heated pads.

	Post-surgical infections will be minimised by good sterile and surgical techniques. Analgesics will be used before and after surgery and any animals experiencing significant pain or problems after surgery will be culled in consultation with the Named Veterinary Surgeon.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because of the nature of the licence there are no alternatives other than to use live animals to generate more live animals. Use of live animals for archiving embryos and subsequently rederiving them is a well tried and tested way of obtaining specific pathogen free animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding programmes can be tightly controlled in accordance with the needs of the end user. Good working knowledge of the lines being bred allows optimum use of those animals. The ability to freeze down and transfer embryos will reduce animal use by removing the need to keep redundant colonies. Rederivation will allow access to animals of high health status minimising experimental variation caused by disease. This will give more consistent results and therefore less animal wastage.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The animals will be cared for by dedicated animal technologists who have the expertise and skills required in the breeding of the animals and are able to asses any welfare problems that may occur at an early stage and determine appropriate end points in consultation with the NACWO and NVS. No animals with genetic disabilities exceeding mild severity will be bred on this licence. We have personal licencees experienced in a wide range of techniques including staff members trained in embryo manipulation and vasectomy. The modern facilities meet all aspects of the Home Office Codes of Practice.

<table>
<tr><td>Project 2</td><td colspan="2">Breeding & Maintenance of genetically modified zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td></td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Under this licence, we want to breed and maintain established genetically modified zebrafish lines and provide tissue samples and embryos for experimentation.

The scientific work supported falls into the following areas:

(a) Blood formation (Haematopoiesis)
Blood stem cells (BSCs) are used in the clinic to treat patients with blood disorders. Their low abundance in the bone marrow and the lack of immune-matched BSC donors leaves a third of all patients without an adequate source of BSCs. The differentiation of BSCs from more abundant cell sources would boost numbers and allow the generation of patient-matched BSCs. To achieve this goal in the future, we require a better knowledge of the molecular programme that drives BSC formation. Under a separate licence, we are generating genetically modified lines that express reporter genes or carry mutations in genes potentially</td></tr>
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	involved in blood formation. Once established, these transgenic lines will be bred and maintained under this licence. This licence will provide embryos for further experimentation and allow us to breed genetic variants to homozygosity or cross them onto new genetic backgrounds. These experiments will help unravel the molecular programme that controls BSC formation during embryogenesis and their maintenance during juvenile and adult life. These lines will also help us to trace cells' precursors during development. (b) Neurodegeneration and Neuronal stem cells (NSCs) Neurodegeneration in old age and acute neuronal damage have devastating consequences on the quality of life and threaten to overwhelm modern healthcare systems. Learning more about the molecular details of neurodegeneration and the formation, maintenance and fate of NSCs will help us develop new therapies in the future. Experiments on neuronal stem cell programming involve the analysis of genetic variants and the use of transgenic reporter lines. Neurodegeneration experiments involve laser-mediated cutting of neuronal tracts and studying the role of certain genes and gene products in transgenic zebrafish lines. (c) Therapeutic bacteria The rise of multi-resistant bacteria makes it necessary to develop new strategies to combat bacterial infections. In vitro studies suggest that predatory bacteria like Bdellovibrios offer an alternative to antibiotics. Their usefulness and safety shall be tested in zebrafish. The use of transgenic embryos in which immune cells are stained will allow us to observe interactions between the pathogen, the predator and the immune cells of the host.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The knowledge gained in the blood project will improve our chances to generate BSCs from more abundant cell types in culture, and open up the possibility to provide patients with patient-matched or patient-specific BSCs for transplantation therapy. Information derived from the neurodegeneration and NSC projects may help identify new targets for therapeutic interventions in neurodegenerative diseases and in patients

	with acute neuronal injuries. Predatory bacteria could replace some of the antibiotic therapies that struggle to deal with increasingly multi-resistant bacterial pathogens.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish Danio rerio; experience tells us that we will need to generate and/or maintain about 15,000 animals in 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Breeding of gene variants to homozygosity or onto new genetic backgrounds may cause behavioural and physical abnormalities in the offspring. Some of the genetic variants may cause a phenotype during early stages of embryonic development. Larvae that display such defects would be humanely killed before they reach free-feeding stage. In other cases, defects may be observed later or when the genetic variant is crossed onto a different genetic background (frequency variable and dependent on the gene in question). Defects may also occur as a consequence of general anaesthesia (<1%) and fin clipping for genotyping (<1%). The latter may cause persistent infections. Squeezing the abdomen for egg and sperm isolation may cause skin damage (<1%) or inner organ compression (<1%). Fish will be checked at least daily and fish with normal or mild phenotypes will be maintained.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	BSCs can neither be generated nor maintained in culture outside the body. Thus, there is no non-animal experimental system that would allow us to study BSC formation. The molecular details on neurodegeneration and NSC biology are likely to be influenced by the cellular environment. While in vitro studies can provide ideas and suggestions, in vivo assays are required to confirm the in vitro findings. Predatory bacteria have been shown to efficiently deal with Gram-negative bacteria in vitro. If we want to develop a therapy based on these predators, their usefulness and safety needs to be tested in vivo. Zebrafish embryos offer an

	ideal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of fish needed in the breeding programme can only be estimated by experience. Experience tells us that we need a colony of 100 fish (larvae, juveniles and adults) at any one time to ensure the maintenance of the line and to get enough embryos for experimentation. We estimate to keep approximately 60 different lines for 5 years. Overall, we need 50 x 60 x 5 = 15,000 fish. In the experiments performed on embryos, we will apply appropriate statistical tests to use the lowest number of embryos possible that gives us statistically significant data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In haematological research, zebrafish has the potential to replace the mouse in a number of experimental contexts. The optical transparency of its embryo and of the Casper mutant adult allows cell imaging in live animals in experiments that cannot be performed in any other organism. The ease with which compound mutants can be generated allows us to answer questions in zebrafish that cannot easily be addressed in the mouse. The transparency and the availability of superb transgenic reporter lines is also an important argument for using zebrafish for the infection/predation studies and the investigations on neurodegeneration and NSCs. Manipulated embryos provided by this licence (unless transferred to a different licence) will all be humanely killed before they become free-feeding. Larvae in the breeding programme will be checked on days 4 or 5 to ensure that only normal and mildly affected larvae will be maintained beyond free-feeding. These larvae will be checked at least daily.

<table>
<tr><td>Project 3</td><td colspan="3">Stock Centre and Cryopreservation Program</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cryopreservation, breeding, genotyping</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The purpose is to create a centralized stock centre service that is maintained through efficient colony management.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The benefits of this aim are threefold: optimising the numbers of fish being used within the facility; refinement of current husbandry methods by the acquisition, and data collection and application of highly technical skills.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Zebrafish and other small teleost species, in total using Approximately 97,000 zebrafish over 5 years

Approximately 2,000 medaka over 5 years

Approximately 1,000 pearl danio over 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td colspan="3">There are no adverse effects expected, but in the unlikely event there are any adverse effects, these</td></tr>
</table>

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	could include abnormal swimming behaviours and potential adverse phenotypes. The level of severity is mild in all cases. The animals can be of continuous use or be reused in different protocols, and will be culled according to schedule 1 method at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose is to provide a service to those who can justify the requirement of using animals for their projects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The benefit of our aim is to reduce the overall number of animals generated by the users of the facility. The efficient management of a stock centre will ensure that the minimal amount of fish are used within their economic breeding life. The use of cryopreservation will also allow for a minimal amount of animals to be produced, as unused lines can be archived, with no need to regenerate lines continuously. Animals that are generated will be raised according to a protocol that has a high survival rate, reducing the number of animals generated overall.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Zebrafish will be used with this PPL as it is fulfilling a service to those who require and can justify their use. Animals will only be used for the duration of their economic breeding life; animals over 18 months, which are more prone to disease, will not be used and culled according to a schedule 1 method. Animals that are generated will be raised according to a protocol that has a high survival rate; not only does this reduce the number of animals generated, but also reduces unnecessary suffering and fatality. The close monitoring of the fish by the highly skilled staff will ensure that the animals are bred at safe intervals of time, and bred when it is beneficial to their health (preventing eggbound females). Cryopreservation minimizes the necessity for

	generating lines, but does require the use of anaesthetic and handling of the fish, which are stressors. Current techniques do not require the death of the fish and they can be reused throughout their economic breeding life, at safe intervals. These techniques are carried out by highly skilled staff, who are trained in anaesthetizing and handling fish, and are capable of minimizing any stress that may affect the animals.

Project 4	**Production of transgenic mice**	
Key Words (max. 5 words)	CAGE, Germline, piRNAs	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will produce transgenic mice for two purposes. The first will be to develop a light activated tool kit to target assay and manipulate cells for potential in vivo research. The second will be to use transgenic mice to answer valuable questions about the roles of piRNAs (small RNAs that are essential for successful germ cell development) in germline development and spermatogenesis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The development of techniques described in this license will have a widespread effect on many fields of science, including neuroscience and cancer research, allowing many experiments that so far could only be performed on cell culture to be done in viva, a much more realistic setting. Our work on piRNAs will not only provide others with additional foundations within this vast field, but in the short term we plan to define a molecular basis for the function of piRNAs in the testis, leading to the longer term benefit of characterising the importance of piRNAs and small RNAs in reproductive biology,	

	increasing our understanding of reproductive biology and potentially enhancing the success of fertility programs in mammals. In addition we will publish our findings in peer reviewed journals, thereby sharing with the scientific community so that our data and methods can help others working on similar projects. Our GM mice will also be valuable to the wider community, reducing the need for others to expand animals recreating them.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 4000 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are no expected adverse effects from breeding and maintaining the transgenic animals. We do not anticipate any toxic effects of the transgenes we will be using, however where possible they will be tested in an in-vitro setting prior to making the transgenic mouse. All analysis will be done ex-vivo, so mice will be culled for this purpose, signifying the end of their use. Breeders will be retained so that the transgenic mice may be used under another authorised protocol, or they will be kept alive at the designated establishment
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The ability to enable the targeting of a specific cell population in a heterogeneous tissue can only be validated in an in vivo setting, as the level of complexity within an organ cannot be obtained in an in vitro setting. We will however, carry out preliminary developments as laid out under this project licence in cell culture or on ex-vivo tissue taken from transgenic mice. The work on piRNAs can currently only be done invivo as their function is restricted to germ cells, of which there are no in-vitro systems available. We are attempting to develop a cell culture system for this but its success is currently unclear and limited. To truly test candidate genes we must create invivo settings.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Our in-vitro and ex-vivo experiments reduce the number of animals used in the initial stages of investigation. Experiments will be designed such that the maximum data can be obtained at any given time, reducing the need for constant use of control animals and comparative conditions. Our GM mice will also be bred using an efficient breeding strategy to minimise the number of mice used to obtain the desired qenotype.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to use mice for these projects. These are the least sentient animals that are best suited for this work, as they capture human processes relatively faithfully. In addition methods for developing transgenic mice are well established and they are often the preferred species for both areas involved in this licence, thereby making the GM mice more available to the wider community.

Project 5	**Breeding and maintenance of mutant or genetically altered animals.**	
Key Words (max. 5 words)	Breeding; genetically altered; mutant	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To provide and maintain breeding colonies of genetic altered animals (GAA) and naturally occurring mutant strains of scientific interest; whose offspring will be used under other Project licenses. The use of genetically altered animals allows for specific gene traits to be studies in a complex physiological environment. Some naturally occurring mutant strains of animals have incidences of genetic disorders or conditions such as hypertension breeding these animals allows for the study of the condition in a complex physiological environment. This cannot be achieved by in vitro methods. The project will allow us to acquire and maintain new genetic lines of animals and control the breeding of these animals to the highest standards. The breeding will be managed by trained Animal	

	Technicians who are well versed in caring for laboratory animals and best placed to note any deviations from a healthy animal and apply the most appropriate care. By good husbandry and welfare we will produce high quality research animals. This it is planned will lead to fewer animals being required for experimental need and reduce duplication of work, leading to better quality science.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A significant benefit will be that trained Animal Technicians will embrace the ethos of the 3R's and specifically use their skill when maintaining the colonies to ensure the best breeding strategies are applied in order to reduce any over-breeding. A service licence that enables the facility to import livestock for scientific groups will allow the import process to be streamlined through a facility that can manage the differing health status by utilising various holding strategies In the cases where animals have been acquired from Institutions or Countries where the health of the animals are below normal health screen requirements, then by cleaning up these strains we will be able to have high quality animals. This will lead to reduced numbers being required for scientific work, producing higher quality science and reduction of experiment animals required to fulfil experimental requirements.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of the animal will be mice (90%) and the remainder are likely to be rats. It is expected that the project will use no more than 6,000 in any one year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	The bulk of this Project will involve the breeding of GAA rodents, predominately mice. This will involve the natural pairing and rearing of rodents coupled with the need to take small samples to allow their genotypes to be established. The licence also allows for the generation, collection

end?	and subsequent implantation of embryos into recipient mice, this will involve a small surgical procedure. The majority of the mice >95% are expected to experience mild severity and only a very small number may experience a moderate severity of the project. The majority of these mice are expected to be humanely killed at the end of the protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of genetically altered animals allows for specific gene traits to be studies in a complex physiological environment. Some naturally occurring mutant strains of animals have incidences of genetic disorders or conditions such as hypertension breeding these animals allows for the study of the condition in a complex physiological environment. This cannot be achieved by in vitro methods. Mutant strains and GAA mice that will be bred will have been specifically justified within experimental protocols justifying that scientific goals cannot be met in vitro methods.
2. Reduction Explain how you will assure the use of minimum numbers of animals	GAA are by nature a replacement for “standard wild type” strains. By using GAA, a total reduction of animals used to get experimental data will be achieved, as GAA will be targeted to specific research requirements. By using a service PPL to breed GAA this will lead to reduced numbers of other PPL’s having to have breeding of GAA and thus reduce the duplication which will lead to less animals being breed. Animal tissue will be harvested from GA animals, It is planned that single animals may be able to supply multiple tissue samples to groups of users thus reducing the number of animals used.

	Specific consideration will be made regarding the inheritance of the gene of interest in order to maximise the scientific purpose of the offspring born.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species or strain chosen will be selected by the experimental PPL and only once this has been granted will animals be sourced and maintained under this PPL. The project will allow us to control the breeding of these animals by animal technicians well versed in the breeding and husbandry of specialised colonies. The technicians are also experienced in maintaining these animals in either full or part barrier conditions, and also provide dedicated care, through experience, for those animals whose phenotypes may potentially have adverse effects. As we are supplying animals for a number of different project licences, we can use our expertise in allowing supply to reflect demand and thus reduce overproduction of the strains we are responsible for, thus meeting the principles of the three R's and modern ethical considerations. The breeding records obviously play an extremely important part in this process. The use of homozygous colonies will reduce the need for biopsy sampling for genotyping and this refines the number of procedures that will need to occur. Animal numbers will be minimised by breeding to the demands of the research workers who will be working with statistically viable groups of animals as defined in their project licence. Reduction in the number of competitive litter mates (e.g. wild type sibling, who will not be required for research) will reduce the numbers of litters required as removal of these animals will allow parent stock to focus energy on to required offspring thus reducing pre weaning deaths amongst experimental offspring. As will giving specialised diets to pre and post weaners which may be required to help growth and development.

Project Title (max. 50 characters)	**Breeding and Maintenance of Gal-deficient Swine**	
Key Words (max. 5 words)	Swine heart valves cardiovascular	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Globally there are almost half a million heart valve replacement or repair surgeries done annually. Replacement heart valves are either mechanical (metal) or biological, made from animal tissues. Mechanical devices are durable but require life-long anticoagulation (blood thinning) therapy to be given to the patient. Bioprosthetic heart valves (BHV) do not require anti-coagulation, but degenerate in five to 20 years depending on the recipient. BHV degeneration is affected by the process of tissue preservation and by immunological responses to the device. BHVs degenerate most rapidly in younger patients with active immune systems. The scientific aim of this project is to understand the mechanism(s) of BHV degeneration and determine if fixed tissues from pigs engineered to be deficient for abundant carbohydrate antigen to which humans produce antibody might resist	

	calcification and thereby provide a more durable BHV. This project licence will allow the breeding and maintenance of pigs to supply the tissues required to support the in vitro experiments and to maintain a healthy breeding stock.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Development of new technologies to improve the performance of replacement biological heart valves, especially in patients under 60 years of age. If successful the new valves will broaden the available therapies to treat younger patients, giving them a durable device which will not require lifetime anticoagulation and its associated risks. This would have a major impact in developing nations, with endemic level of rheumatic fever a major cause of heart valve dysfunction, where the resources to manage patients on anticoagulation are limited and therefore the treatment of young patients may not be optimal or in many cases is not available at all.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 350 pigs over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Pigs that have been genetically engineered and bred specifically for this project will be used to provide tissue samples for in vitro studies. The pigs are not expected to show any harmful phenotypes and are expected to perform as normal healthy pigs. Animals may undergo a procedure such as mouth swabbing, ear notching, tail tipping or blood sampling for DNA for genotyping this is expected to cause only momentary discomfort. All animals will be killed by humane methods at the end of the procedure.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-animal alternatives available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Care will be taken to ensure the minimum number of animals are bred to maintain the supply of tissues required for in vitro studies and to maintain the breeding programme to avoid inbreeding.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animals will be housed in accordance with the establishments standard operating procedures that are based on maintaining high standards of animal welfare. Staff at the facility are experienced at breeding and farrowing pigs. Local anaesthesia is used during tail tipping or ear notching.

Project 7	**Production, Breeding & Cryopreservation of GM Mice**	
Key Words (max. 5 words)	Genetically modified, embryo sperm cryopreservation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this licence is to provide research scientists a full transgenic service facility to include the production of new Genetically modified mouse lines, a cryopreservation and rederivation service.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our team has a vast amount of experience in the production, breeding and cryopreservation of new mouse models. A new bioengineering facility was recently set up within the Institute. This facility will offer advice and expertise in the design, testing and assessment of the quality of new Embryonic stem cell lines and constructs prior to their use in the generation of new mouse models. This facility, in combination with our experience and expertise, will benefit many scientists across several disciplines. The areas of research we will produce mice for include stem cell biology, haematopoiesis and haematology, cancer biology infection and inflammation. A central service facility for the production of, and cryopreservation of,	

	genetically modified mice ensures that a minimum number of mice are used by avoiding unnecessary duplication of breeding colonies, stud and vasectomised males and keeps wastage to a minimum.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we use we will use in the region of 8000 mice over the 5 year lifespan of this PPL based on our previous experience.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Harmful adverse effects are rare and unpredictable in the production of new GM lines but any animal showing an unwanted harmful phenotype will be killed by a schedule 1 method. Approximately 50% of the total mice used in this project will be superovulated and should not experience more than transient discomfort from 2 injections 48 hours apart. Occasionally over aggressive males may cause injury to females. If this occurs those males will be killed by a schedule 1 method. Good aseptic surgical technique will minimise rare complications that arise following surgery. Mice having undergone surgery (vasectomy or embryo transfer) will be monitored closely post operatively and will be administered analgesia. Any mouse failing to fully recover within 24 hours of surgery will be killed by a schedule 1 method. The majority of animals will be transferred to the end users PPL where HO permission has been granted for their use. Those that are not transferred may be bred under the authority of this PPL and will be killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The analysis of the effect of a gene at early developmental stages in different tissues and the study of interactions between factors requires a whole animal model. Although Zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes, a mammalian model still

	remains necessary in order to fully understand the effects of many human genes and their disease-associated mutants and other complex physiological systems that mammals share. The justification for individual experiments will be covered in the end-user's licences. Mouse is the model of choice for genetic modifications modelling human diseases because of the availability and ease of manipulation of mouse ES cells. Where possible, modified embryonic stem cells will be analysed *in vitro* to determine which constructs require analysis in intact animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding colonies to supply the service will maintained at the lowest possible levels and any excess mice will be made available to other users. We will advise users to allow us to cryopreserve lines at the earliest time possible to avoid tick over breeding. Sperm freezing wherever possible will be encouraged over embryo freezing. This will reduce the number of mice required to cryopreserve a line by eliminating the need to superovulate large numbers of female donors and maintain stud males to produce fertilised embryos for freezing. All steps in every process will be carefully monitored to minimise numbers. Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulation of mouse ES cells. Best practice will be used for all procedures and staff will keep up to date with new methodologies and implement new procedures as they arise. Wherever possible constructs and/or manipulated ES cells will be produced and tested in the bioengineering facility in an *in vitro* system before going on to produce new GM lines. Analgesia will be used wherever appropriate.

	Vasectomies will be via the scrotal sac rather than abdomen. Most embryo transfers to date have been carried out using a surgical method. This requires a general anaesthetic and a potential welfare issues which would apply to any animal undergoing a surgical procedure. We will evaluate a non surgical embryo transfer method which would only cause a mild momentary discomfort and has the potential to eliminate the need for anaesthetic and any post surgical complications.

Project 8	**Breeding mutant or GA mice and embryo production**	
Key Words (max. 5 words)	Genetically altered, embryo, breeding	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this licence is to provide a service to other researchers by breeding and supplying genetically altered rodents. We also supply embryos to a group studying human fertility and in vitro fertilization and tissues to groups requiring them for ex-vivo study	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By providing this service centrally we are able to maximise the efficiency of the breeding colonies and thereby keep the number of animals required to a minimum. We are also able to maintain the genetic stability of the strains that we breed by using the best breeding strategies. This ensures that all of the mice stay the same genetically which is beneficial to the science. The animals are then supplied to scientific projects within the institution which have undergone ethical and peer review. The work with the local Fertility Group aims to develop therapies for IVF implantation failure.	
What species and approximate numbers of animals do you expect to use over what period of time?	This project will run for 5 years It is expected that up to 11,750 mice and up to 500 rats will be used including all breeding procedures.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the great majority of the animals the severity of the procedures will be mild. Only mice that have a mild phenotype will be bred under this licence and should not therefore display any adverse effects. The surgical procedure by which mouse embryos are implanted in pseudo-pregnant recipient animals will result in a moderate level of pain and possible adverse effects include wound infection or an adverse reaction to anaesthesia. These will be minimised by using aseptic surgical technique, analgesia and by closely monitoring any animals that undergo surgery. Any animals which cannot be used for scientific research will be humanely killed using a Home Office approved (Schedule 1) method and tissues provided to researchers for ex-vivo projects wherever possible
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The ultimate justification for using animals lies with the end users' Project licence. This will have been subject to ethical, peer and Home Office approval before being granted. It is not possible to replace the use of live embryos because the research requires seeing the affect that a protein has had on a living embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Some mouse models are used across a number of different research projects and Biomedical Services, by controlling the breeding of these models, is able to maximise the efficiency of these colonies and thereby able to keep to a minimum the number of animals required for this work. The number of animals bred will be regularly reviewed through discussion with the end users and the use of a computer database. Animals will only be bred if a requirement has been established and authority for the use of such models has been granted to the end user. The use of superovulation significantly increases the numbers of embryos which can be harvested from each mouse and therefore means that fewer animals are required overall
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The justification for using a particular strain of mice will lie with the end users' Project Licence. We will always use best practice when giving injections or carrying out surgical techniques. Analgesia will be provided prior to surgery Non-surgical methods of embryo transfer will be evaluated and if these methods prove superior to surgical methods they may be adopted as standard.