Project 2	**Breeding and maintenance of genetically modified and mutant mice**	
Key Words (max. 5 words)	Mice, ógenetically modifiedô, Breeding, mutant	
Expected duration of the project (yrs)	5	
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)	Producing genetically modified animals for research allows scientists to understand the mechanisms by which a disease may arise, how to treat the disease or related symptoms and how current or future drugs may work to treat that condition. Studying the difference between genetically modified mice and conventional mice will allow scientists to know what characteristics or disease states that

Project 3	**Discovery of healthy lean gene mechanisms**
Key Words (max. 5 words)	Genes, healthy leanness, obesity, diabetes
Expected duration of the project (yrs)	5

Purpose of the project (as in

project)?	One new gene identified by this research is already being tested with a targeted medicine in human diabetic patients. Development of animal models with altered lean gene levels in this project will help us understand how these medicines work and help improve how effective they are in living animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use various strains of commonly studied laboratory mouse because they share substantial common biology with humans in health and disease. We expect to use 1600-1800 mice over the 5 year period, many of which will be generated by our genetically-altered mouse breeding programmes.
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?	We will study the impact of lean genes on obesity-causing and ageing processes that impact metabolic health. To do this we will use diets and genetically altered mouse models that make the mice prone to obesity, or diabetes, or the effects of ageing. We carefully monitor the mice in these studies to prevent exceeding moderate severity levels. Indeed we need to understand how lean genes affect metabolism before major symptoms occur.. In ageing we study the mice at defined middle-aged and old-aged points before ónatural deathô through ageing becomes common for that strain. Mice are humanely euthanized once we have gathered the necessary metabolic information
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Metabolism is a highly integrated physiological processes that reflects complex interactions between the brain (e.g. appetite) the adipose tissue (storage of excess fat), muscle (calorie usage), liver (calorie storage and integration), etc. Because of this there is no way to replace the insight that investigating lean gene effects in whole animals provides. Ultimately we must understand what such manipulations would do in living humans. However, we use clonal cell models of several tissue types to test key hypotheses before animal experimentation is considered. For example, cultured fat cells have been used in our research to show that one lean geneô prevents defects in release of healthy fat cell hormones. Similar approaches are being taken with cultured human

	graded dose-finding protocol. The introduction of new non-invasive, low stress procedures for body fat mass determination allows us to minimise suffering while maximising the amount of information obtained from each animal We will use new home cage chambers that allow us to follow metabolism in real-time without interfering with the animal (Indirect calorimetry measures oxygen used/CO2 respired). This removes the need for metabolic cages that have grid features in most studies. We follow a path of progressive method development and refinement. For example, for nutrient metabolism exploratory methods such as oral administration of glucose with blood sampling are used to test for major effects of gene alteration on broad outcomes such as ódoes diabetes improveô Only then if an effects is clear are in depth methods used, such as using infusions of labelled nutrients (e.g. glucose) and tracking what happens to them in a living animal are employed to work out mechanisms of health change. The use of any invasive (e.g. surgical) techniques are discussed with colleagues performing similar work locally and across the country. Monitoring systems will be tailored to each model and strict humane endpoints will be applied to minimise suffering. Training and good practice is encouraged through group meetings and regular discussions with the NVS and key staff.

	factors or components of a signalling pathway) work at a molecular level.
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 main reason for this work is to provide basic understanding of fundamental mechanisms. However, knowledge of how decisions of cell fate are reached, including the genes involved, and the properties of stem cells within these systems, will ultimately allow them to be controlled or introduced in a beneficial way for treatment of human and animal disease and trauma. Our project is also likely to benefit diagnosis of genetic disease, to inform clinical treatment, and ultimately to increase the range of options available for treatment. These statements are based on our track record. For example, genes that we discovered and/or studied such as *SRY*, *SOX9*, *AMH*, *DAX1*, *SOX3*, *FOXL2*, are now routinely examined to diagnose the underlying cause of disorders of sex differentiation, where this knowledge helps counsel patients and inform clinical care. AMH, which we first described as being expressed in the postnatal ovary in mice, is now routinely used to determine ovarian function in assisted conception. *SOX2* and *SOX3* are now screened in disorders affecting CNS, pituitary and sensory system development, and it was our work that directly led to patients with anopthalmia due to mutations in *SOX2* now being managed for the pituitary defects that always accompanies the eye problems. *SOX2*, which our work showed was necessary for pluripotency, is one of the critical genes used to derive patient specific iPS cells, which are just beginning to be used in trials to treat, for example, macular degeneration. *SOX2*, *SOX4* and *SOX9* are also beginning to be used diagnostically in some forms of cancer where their overexpression is correlated with prognosis.
What species and approximate numbers of animals do you expect to use over what period of time?	We mostly work with mice because of the powerful techniques and knowledge available for this species in terms of genetics, embryology, cell biology and behaviour, but also as they have relevance to the human situation. Over the last 5 years we used a total of approximately 40,000 and we anticipate similar numbers will be used during the 5-year duration of this PPL. For some types of experiment we may use rats (probably no more than a few hundred), because their larger brain size and more complex behaviour is more appropriate for the types

Moreover, most tissues develop in a complex way in three dimensions over time in a carefully orchestrated manner, and require vasculature and innervation to operate. Therefore, although some aspects of certain

	for it to become a widely available anti-cancer therapy. Chief amongst these are the need for the further development of genetically engineered immune cell therapy so that: (i) it can be used to treat a greater range and number of cancer patients; (ii) it will be more effective at completely eradicating cancer in treated patients. Therefore, the objectives of this project are: (i) The further development of genetically engineered immune cell therapy to enable it to be used to treat a greater number of cancer patients; (ii) To identify ways of enhancing the anti-cancer efficacy of engineered immune cell therapy so that it is a more effective therapy in treated patients.
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 primary benefit of this project will be the further development of genetically engineered immune cell therapy, thereby enabling a greater number of cancer patients to be treated with this promising therapy. Furthermore, this project is expected to lead to the identification of strategies that can be used to enhance the anti-cancer efficacy of genetically engineered immune cell therapy.
What species and approximate numbers of animals do you expect to use over what period of time? In the context of what you	This project will only use mice that have been specifically bred for research purposes. We estimate that we will use up to 960 mice per year.

needle insertion. (iii) Limited does of radiation of mice to partially deplete their own immune cells prior to the transfer of cancer-targeted genetically engineered immune cells. High doses of radiation can cause sickness in mice but we will use lower doses that are very well tolerated. In addition to the

Project 3	Immuno -modulatory and Inflammation Research
Key Words (max. 5 words)	autoimmune disease Immune system, modulation
Expected duration of the project (yrs)	

	that the immune system is responding as predicted.
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)?	

	responsiveness, weight loss >20%) will be killed humanely. Anaesthetics and analgesics will be used to reduce the discomfort induced by any surgical procedure. The use of analgesics may impact the disease progression and interfere with the outcome of potential new medicines being tested. Carprofen is routinely administered prior to and for one day post surgery as an analgesic. This substance is a non-steroidal anti-inflammatory agent and hence its long term use may interfere with the biological systems involved in these studies and may inadvertently affect the study outcomes. Rats and mice with arthritis will display swelling of the limbs however, they are expected to still be able to move around their cage, interact with cage mates and display normal feeding and grooming behaviours. Should any animal not be able to display these behaviours it will be killed humanely.. The investigation of the new medicines should reduce any suffering experienced by the animals. At the end of studies the animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All work using animals will be preceded by studies using human and/or animal isolated blood, organs, tissues or cell lines. The immune systems of rodents and humans are

the use of minimum numbers of animals	statistician to ensure that we are using the minimum

Project Title (max. 50 characters)	The biological significance of DNA methylation
Key Words (max. 5 words)	Rett Syndrome, epigenetics, mouse models
Expected duration of the project (yrs)	5

Purpose of the project (as in Article 5)[7]

Basic research	Yes	
Translational and applied research	Yes	
Regulatory use and routine production		No
Protection of the natural environment in the interests of the health or welfare of humans or animals		

prospective therapeutic substances via injections into the brain. This is done under anaesthesia and using adequate analgesia to limit post-operative

Explain how you will assure the use of minimum numbers of animals	calculations using our previous published data on ageing and age-related disease and will be the minimum numbers of mice required to identify mice that are long-lived and protected from the diseases of ageing. Our breeding programme and experimental designs will be streamlined and will employ where possible longitudinal studies on the same mice (including non-invasive studies) which will reduce the overall numbers of mice required to reach the scientific end-points.
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.	Our studies will be performed in mice. They are the lowest mammalian vertebrate group with the necessary characteristics in which ageing and ageing related diseases have been characterised. The mouse is the primary mammalian species in which gene manipulation is undertaken thus permitting the generation of genetic models for study. Mice are also well suited for use in longevity studies because of their relatively short lifespan and small size which both carry economic and practical benefits. There is a wealth of pre-existing information about ageing in mice and they are a well-established model for testing genetic interventions that might alter lifespan and the diseases of ageing. Mouse models have proved in many cases to be excellent models for the understanding of both human physiology and disease. To minimize harm to animals we have established and validated in our previous work monitoring protocols for mice as they age which identify at an early stage any potential welfare costs and allow us to intervene to minimize adverse effects. All animals will be housed in groups where possible with appropriate environmental enrichment and husbandry undertaken according to current 'best practice' at our Institution.