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National Health and Medical Research Council
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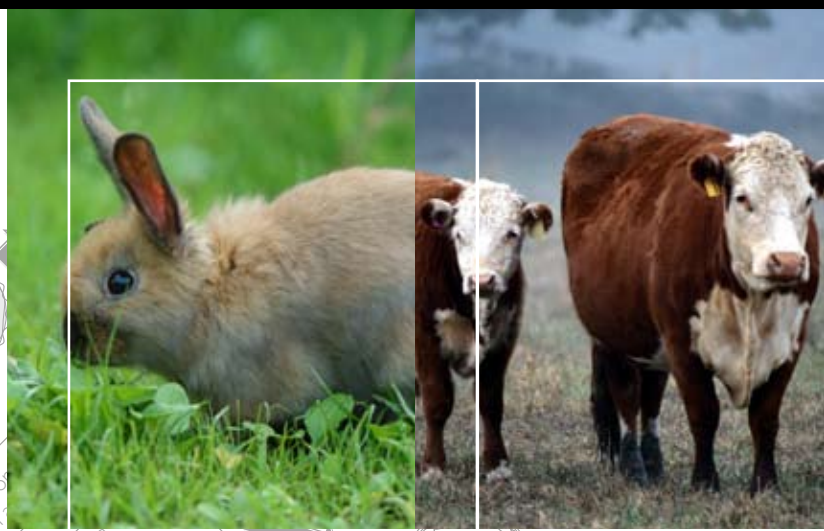
Gene Technology Ethics Committee

ANIMAL WELFARE COMMITTEE

Guidelines for the generation, breeding, care and use of genetically modified and cloned animals for scientific purposes



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FOREWORD

This publication was produced by the NHMRC's Animal Welfare Committee (AWC) in response to requests for assistance by Animal Ethics Committees and investigators who are involved in the genetic modification and cloning of animals for scientific purposes. They should be read in conjunction with the *Australian code of practice for the care and use of animals for scientific purposes*.

The guidelines set down standards for the welfare of genetically modified and cloned animals. In particular they highlight the special ethical and welfare issues related to the creation and use of genetically modified and cloned animals.

The AWC is most appreciative of the informed and constructive comments received as a result of the formal consultation process. They acknowledge the thoughtful contributions from the Gene Technology Ethics Committee, particularly Dr Vaughan Monamy who ably assisted them throughout the process.

CONTENTS

INTRODUCTION

The guidelines should be read in conjunction with the *Australian code of practice for the care and use of animals for scientific purposes* (the *Code*) and the *National Framework for the Development of Ethical Principles in Gene Technology*.

They have been produced by the National Health and Medical Research Council's (NHMRC's) Animal Welfare Committee (AWC) as introductory material to assist investigators, Animal Ethics Committees (AECs), animal technicians and the broader community when they consider research projects involving the generation and use of genetically modified and cloned animals of all species. These include laboratory, agricultural, companion animals and wildlife developed and used in research.

The guidelines:

- assist in the consideration of the use of and impact on animals produced by genetic modification including random (chemical) mutagenesis and cloning
- do not focus on the specifics of reproductive technology
- should assist investigators, AECs and animal carers in maximising the care and welfare of animals in specific research projects
- may assist AECs with operations of standard operating procedures (SOPs) used for genetically modified animals
- should be considered by AECs when reviewing SOPs associated with the production of genetically modified animals
- are designed in part to be a tool for reflection and to focus and stimulate discussion on relevant issues

Detailed consideration of each application by individual AECs will still be essential in the consideration of the research application, and will be facilitated by reference to the appendices. The responsibilities for ongoing care and welfare monitoring for approved projects are outlined in later sections.

DEFINITIONS OF TERMS USED IN THE CONTEXT OF THE GUIDELINES

Animal: any live non-human vertebrate, that is, fish, amphibians, reptiles, birds and mammals, encompassing domestic animals, purpose-bred animals, livestock, wildlife and also cephalopods such as octopus and squid.

Animal welfare: an animal's quality of life based on an assessment of an animal's physical and psychological state as an indication of how the animal is coping with the ongoing situation as well as a judgment about how the animal feels.

Blastocyst: a stage in early embryonic developments in which the cells form a sphere with a fluid-filled cavity in the centre.

Breeding stock: a breeding colony of animals whose phenotype has been well described and accepted by an AEC.

Chimera: an animal produced experimentally by combining cells of different genetic origins. In mouse genetics, targeted mutations produced in embryonic stem cells are recovered by breeding chimeric mice resulting from the mixture of embryonic stem cells with a genetically distinct blastocyst.

Clone: a genetic copy of another living or dead animal. It is not a twin derived by the fertilisation of an egg by a sperm (see *Somatic cell nuclear transfer* and *Cloning*).

Cloning: in its usual sense, cloning refers to the propagation of genetically exact duplicates of an organism by means other than sexual reproduction. The term cloning has been assigned to the reproductive technology of somatic cell nuclear transfer. Progeny obtained from somatic cell nuclear transfer are genetic near copies, not genetic replicas, of the somatic cell donor.

DNA: deoxyribonucleic acid is present in almost all living cells and contains information coding for cellular structure, organisation and function.

Electroporation: introduction of DNA into cells by means of electrical pulses.

Epigenetic: changes in gene expression that occur without changing the DNA sequence of genes.

Exogenous: refers to a gene taken from an organism and introduced into the DNA of a target animal.

Gene expression: the process by which the genetic information or blueprint in genes is transformed into the structure and function of an organism.

Genotype: the genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms.

Genetic modification (of animals): the use of any technique for the modification of genes or other genetic material but not including the use of natural processes such as sexual reproduction.

Heterozygous: describes the situation where cells or organisms carry two different versions of a given gene, one from each parent, at the corresponding site on chromosomes.

Homozygous: describes the situation where cells or organisms carry the same versions of a given gene, one from each parent, at the corresponding site on chromosomes.

Hybrid: an organism that is the offspring of genetically dissimilar parents or stock, especially offspring produced by breeding animals of different breeds or species.

Imprinting: refers to chemical marks on the DNA from the dam and sire so that only one copy of a gene (either the maternal or paternal gene) is activated. The chemical mark on the DNA is usually methylation and imprinting is a form of epigenetic inheritance.

In vitro: a term applied to studies conducted outside a living organism in an artificial environment, such as a test tube.

Knock-in: the introduction by gene targeting of DNA sequences at a specific site.

Knock-out: a mutation in which the target gene is inactivated.

Microinjection: also called pronuclear injection, is when DNA is injected into the nucleus of a single cell embryo using a very fine needle.

Mutation: a permanent transmissible change in the genetic code. It can be an insertion or deletion of genetic information, or an alteration in the original genetic information. Mutations can be caused by many factors including environmental insults such as radiation and mutagenic chemicals.

Neonate: a newborn animal.

Notifiable low risk dealing (NLRD): NLRDs are dealings with genetically modified organisms that have been assessed over time by the Office of the Gene Technology Regulator (OGTR) as posing low risks provided certain risk management conditions are met.

Oocyte: a female germ cell that is in the process of growing into an egg.

Phenotype: the observable physical, behavioural, physiological or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.

3Rs: *Reduction* in the number of animals used, *Replacement* of animals with other methods and *Refinement* of techniques used to reduce the impact on animals when animals are used for scientific purposes.

Re-program, reprogramming: refers to processes where the genetic material in body cells, which is geared to express the particular characteristics of the differentiated tissue from which it comes (eg muscle or nervous tissue), is returned to a state where it can once again differentiate into various tissue types.

Scientific purposes: all those purposes which aim to acquire, develop or demonstrate knowledge or techniques in any area of science including teaching, field trials, environmental studies, research, diagnosis, product testing and the production of biological products.

Somatic cell: any cell of an animal other than a reproductive cell.

Somatic cell nuclear transfer: the technique of inserting a nucleus of a somatic cell from one of the body's tissues, other than a germ cell, into an egg that has had its nucleus removed.

Standard operating procedures (SOP): detailed description of a standardised procedure. Appropriately applied SOPs may facilitate the preparation of proposals sby investigators and must be approved by the AEC before implementation.

Targeted mutagenesis: (see *Knock-out*).

Transgene: the gene(s) transferred into another organism.

Transgenic: refers to an organism containing a transgene.

Transgenesis: incorporation of a transgene.

Vector: a vehicle such as a modified plasmid, virus or DNA molecule, capable of being replicated and bearing cloning sites for the introduction of foreign DNA, which is used to introduce foreign DNA into host cells.

Wildlife: free living animals of native, non-indigenous or feral species including captive-bred animals and those captured from free-living populations.

Xenotransplantation: the transplantation of living organs, tissues or cells from one species to another.

SCOPE

The information in this document is intended to cover the generation and use of genetically modified and cloned animals for scientific purposes. These include laboratory animals such as mice, rats and rabbits, agricultural animals, horses, and companion animals. Mice are the most frequently genetically modified and cloned animals used in biomedical research. Other species may be used for the study of xenotransplantation, the production of pharmaceutical proteins and for increasing agricultural productivity.

Genetically modified and cloned animals may have specific welfare needs that will continue throughout their lifespan and into subsequent generations.

The guidelines apply to:

- institutions, AECs, investigators and animal facility staff undertaking or overseeing the production of new strains of genetically modified animals, the breeding and care of genetically modified and cloned animals and the use of genetically modified and cloned animals for scientific or teaching purposes
- all proposed research and teaching projects that include the use of genetically modified and cloned animals
- existing projects which use genetically modified animals should be reviewed by the AEC at the time of their next annual report for consistency with these guidelines
- all genetically modified and cloned animals, whether generated at the institution or acquired from an external source
- animals with natural mutations which are then selected for breeding purposes to perpetuate the mutation.

In addition they:

- apply to studies involving the release of genetically modified and cloned animals into the environment up to the point of release, if applicable. In considering the application, the AEC must take into account the ultimate use and future husbandry of genetically modified and cloned animals at the end of the project. AECs should note that research involving genetically modified animals must have the appropriate approval from the Office of the Gene Technology Regulator (OGTR) before work commences
- include the production of hybrid animal species, such as those that may arise in a program to preserve an endangered species by cross species nuclear transfer. They also apply when the nucleus used for transfer is derived from somatic cells that are already genetically modified
- cover projects involving the generation of genetically modified and cloned animals, including their breeding and monitoring procedures.

The maintenance of newly-generated genetically modified animals and clones including those from an outside source, should be considered as a scientific purpose at least until detailed information regarding the phenotype of the animals and any adverse welfare effects of the modification has been documented by the investigator and approved by an AEC for a change from a scientific purpose to a breeding unit. There is an ongoing responsibility to recognise the additional welfare implications of developing and maintaining a new strain and this includes both surrogate and donor animals.

Once a general understanding of the phenotype and welfare implications of the genetic modifications is established and the phenotype report completed (see Appendix 3), AEC consideration must be sought for the colony to be monitored in a routine way, as set out in the *Code*.

AIMS

The potential impact of genetic modification on the welfare of animals raises special concerns. General principles for the use of all animals including those with genetic modification are outlined in the *Code*. The subject of these guidelines is how best to manage the potential impact of genetic modification and cloning on the welfare of animals and the associated monitoring requirements.

These guidelines promote consideration of the 3Rs (Replacement, Refinement and Reduction), and discuss the appropriate husbandry, record-keeping and identification of phenotypes as they apply to genetically modified and cloned animals.

In producing the guidelines the aims of the AWC were to:

- highlight some of the specific roles and responsibilities of AECs in considering applications to produce or use genetically modified and cloned animals and in monitoring approved projects
- highlight specific ethical and welfare issues involved in the generation of genetically modified and cloned animals
- facilitate a nationally consistent approach to record keeping and reporting on genetically modified and cloned animals
- clarify responsibilities to identify welfare and care issues in newly developing strains
- describe the husbandry practices for genetically modified animals
- define the requirements for the recognition of a newly developed strain as an established breeding colony
- apply the 3Rs to the generation, care and use of genetically modified and cloned animals.

The guidelines indicate the expectations of the AWC for the welfare of genetically modified and cloned animals and animals produced by chemical and spontaneous mutation where it may affect the fitness of animals in their environments. They provide advice on practical aspects of caring for those animals, including record keeping. To achieve consistency with the Gene Technology Act 2000, the AWC worked closely with the OGTR.

BACKGROUND

A) GENETIC MODIFICATION

Genetic modification occurs naturally. Examples of spontaneous mutation have been observed in virtually all species and mutated animals (eg immuno-compromised mice) have been used for many years in biomedical research.

The genetic code can also be altered through human intervention and the process of induced mutation means that investigators can investigate gene functions. Transgenesis and targeted mutagenesis allow the study of over-expression or under-expression of specific genes. Altered genes must be predictably transmitted to offspring for either a spontaneous or an induced mutation to be useful in research.

The term transgenesis refers to insertion of exogenous DNA into cells, typically fertilised eggs. Normally DNA is inserted into cells using microinjection, electroporation or certain non-pathogenic viruses. The inserted DNA successfully incorporates into the chromosomes of only a small percentage of embryos. The DNA incorporates at different genetic locations and a different number of copies of the DNA may incorporate in different embryos. Thus, each embryo has the potential to become a unique transgenic animal even though the same quantity and type of DNA was injected into genetically identical fertilised eggs. Not all modified fertilised eggs develop into live born transgenic animals. Embryo loss can occur at every step from injection through to gestation and delivery.

Targeted mutagenesis refers to a process whereby a specific gene is made non-functional (knocked-out) or made functional (knocked-in). The generation of a targeted mutation requires several steps in the laboratory. The specific gene is identified, cloned and modified to make it, for example, non-functional. The modified gene is attached to another DNA sequence called a vector and introduced into embryonic stem (ES) cells by electrical or chemical methods. These ES cells are cultured in special media that permits identification of ES cells incorporating the manipulated gene. Commonly ES cells incorporating the manipulated gene are injected into a blastocyst. Blastocysts containing genetically modified ES cells are then implanted into the uterus of a surrogate mother.

Some injected blastocysts develop into viable embryos and chimeras are born. Animals can carry transgenes and targeted mutations but unless the modified DNA is incorporated into germ cells (unfertilised eggs or sperm), the animal is unable to transmit the genetic modification to its offspring.

B) CLONING

Cloning refers to somatic cell nuclear transfer where a nucleus from a body tissue cell is placed into an enucleated egg cell. The resulting cell is implanted into the uterus of a surrogate mother where pregnancy may continue to term and a near copy of the donor animal is produced.

C) RANDOM MUTAGENESIS

Random or chemical mutagenesis refers most commonly to a process where male animals are injected with a chemical mutagen, exposed to radiation or other environmental factors to cause mutations in their spermatozoa. The resultant progeny are screened for mutations relevant to the aim of the project.

In cloning and random mutagenesis, loss of embryos may occur at every stage of the process.

REGULATION OF THE USE OF GENETICALLY MODIFIED AND CLONED ANIMALS

Genetically modified and cloned animals are subject to State and Territory government animal welfare legislation applicable to animals used for scientific purposes, in addition to the *Code*. They may also be subject to the requirements of the OGTR under the *Gene Technology Act 2000*. This Act prohibits dealings with genetically modified organisms (GMOs) unless they are:

- covered by a GMO licence or
- a notifiable low risk dealing or
- an exempt dealing or a dealing listed on the GMO Register.

Investigators intending to undertake work with GMOs and who are unsure of the regulatory requirements should contact the OGTR to ensure they have the appropriate approval before they begin work.

ETHICAL AND WELFARE ISSUES RELATED TO THE GENERATION AND USE OF GENETICALLY MODIFIED AND CLONED ANIMALS

A) ETHICAL ISSUES

Viewpoints regarding animals vary in the community and relate to the particular intrinsic values that individuals apply to animals. Most acknowledge that animals have some intrinsic value and that more sentient species, including humans, have particular intrinsic value. These guidelines reflect the provisions within the *Code* that the use of animals in research can occur if justified and approved through the AEC process. The intrinsic value of animals is acknowledged and is reflected in their conditional use for scientific purposes: their welfare must be safeguarded and any harm to them must be minimised.

Weighing the predicted value of research against potential effects on animal welfare is of particular relevance when genetic modification and cloning is involved. For example:

- many individual animals are generated in order to derive a smaller number with the desired genetic makeup
- in addition to concerns about the large number of animals used, there is also the tension between Reduction and Refinement because there is a higher than normal culling rate in the breeding programs
- the integrity of animals may be affected by genetic modification and cloning in that the nature of the animal and how it interacts with other individuals and its environment may be altered
- the impact of genetic modification on population robustness and wellbeing may be important for particular species of animals
- the unpredictability of phenotypic expression may be considered to be a combination of the likelihood and consequence of an adverse animal welfare impact.

While there is a need to balance the cost to the animal and the benefit of the research, the possibility of adverse impacts on animals or the environment may be regarded as unacceptable even if there is a significant research benefit. As in all animal research, the role of the AEC, guided by its diverse membership, is to assess if a threshold of acceptability is reached.

These expected (and unexpected) adverse impacts have major ethical implications for all species. The production of these changes imposes significant responsibility on investigators and institutions for each animal's subsequent welfare and for the welfare of succeeding generations of genetically modified animals. Genetically modified farm animals destined for general production use require the health, vigour and lifetime fitness necessary to cope with environments beyond those encountered within research institutions.

In the generation of genetically modified animals, many animals are used to produce the few animals that lead to a genetically modified strain. For example, hundreds of fertilised eggs from many super-ovulated females have to be injected to obtain animals that harbour and express properly the introduced gene. Further, only some of these will be able to transmit the integrated gene through the germ cells to enable the establishment of a transgenic line. Similarly, for targeted mutagenesis in mice, many chimeric animals may be required in order to select the correctly modified individuals used to generate the breeding colony.

This increase in the number of genetically modified animals has created a dilemma for AECs in complying with the principles of the 3Rs, particularly Reduction, as set out in the *Code*. Genetic modification contributes to the Refinement of the experimental process but at the same time results in an increased number of individual animals used. There is a clear tension between Refinement and Reduction that is a challenge for AEC members to resolve. Each proposal must be assessed on its individual merits.

B) WELFARE ISSUES

Genetic modification of animals may impact adversely on welfare both during the generation of a new strain and its subsequent utilisation. Impacts on welfare may be due to the:

- techniques used to produce and monitor the genetic modification(s)
- expression of the modified or deleted genes
- position of the modified gene in the genome
- action of unpredicted factors in gene expression and interactions between the gene products
- disruption to physiological processes of the whole animal or
- poor fit between the new strain and its environment.

An understanding of the nature and extent of possible impacts resulting from genetic modification, and an appreciation of the uncertainty and low predictability of effects are required for making decisions on the ethics of animal use as described in the *Code*. Experience has shown so far that most genetically modified animals do not show any changes to structure or function and operate normally within their environment. Therefore, decisions are made based upon incomplete but evolving knowledge.

GUIDELINES

1. THE ROLE OF AECS IN ASSESSING PROPOSALS TO GENERATE, MAINTAIN AND USE STRAINS OF GENETICALLY MODIFIED AND CLONED ANIMALS

AECs reflect on a range of ethical issues in their assessment of the acceptability of proposals involving the generation, breeding, care and use of genetically modified and cloned animals for scientific purposes.

AECs need to be aware that although many genetically modified animals are clinically normal, there is the potential for genetic modification to result in phenotypes that lead to unexpected distress. A welfare problem may appear only after the project is at a relatively advanced stage or in second or later generations.

A checklist to assist AECs in addressing applications for genetically modified and cloned animals has been provided at Appendix 1.

1.1 GENERATION OF GENETICALLY MODIFIED ANIMALS

The initial generation of a modified animal raises specific concerns of acceptability in terms of animal welfare and ethical issues. This acceptability has limits. In Australia, for example, the *Prohibition of Cloning Act 2002* prohibits the placing of:

- a human embryo or human embryo clone into an animal
- an animal embryo in the body of a human for any period of gestation.

The process of generating transgenic animals has a welfare cost quite apart from the potential negative impact of the subsequent phenotype. For example in the case of mice, young females are injected with hormones, induced to superovulate, mated, and then killed after 10 hours in order to flush out the single cell embryos. These embryos are then injected with the transgene. After overnight incubation, the embryos which have survived the injection are surgically implanted into a surrogate female (see Figure 1). The *Code* requires appropriate aseptic technique, post operative analgesia and care of these surrogate mothers.

Loss of embryos may occur at every phase of the process.

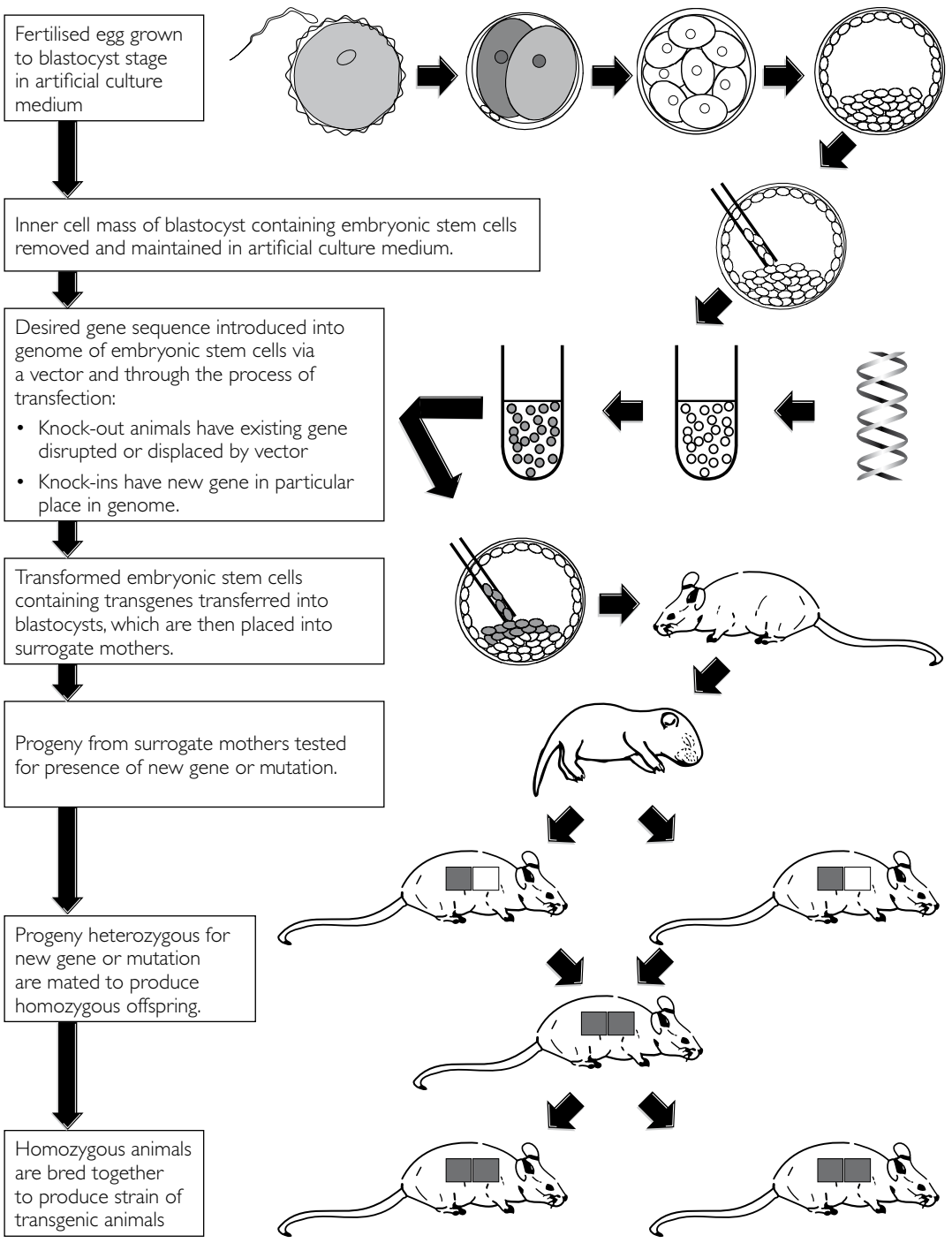

Figure 1. Illustrative flow chart for the production of genetically modified (knock-inand knock-out) animals

The purpose of the proposed generation of genetically modified animals is balanced against hypothetical or potential effects. It is assessed in the context of monitoring and safeguards for the health and welfare of the generated animals, the involved parents, and donor and surrogate animals.

1.2 GENERATION OF CLONED ANIMALS

In general, somatic cell nuclear transfer or cloning merges two processes. One is the culture in an artificial environment of somatic cells from a desired donor. The other is the collection of unfertilised egg cells (oocytes) from the ovaries of females that have been treated with hormones to cause multiple ovulations. Nuclei are removed from these oocytes by micro-suction and these enucleated oocytes become receptacles for the transfer of nuclei from cultured somatic cells. Importantly, enucleated oocytes retain factors that can reset, re-program or de-differentiate DNA in the nucleus from the somatic cell so that an embryo can commence growth (see Figure 2).

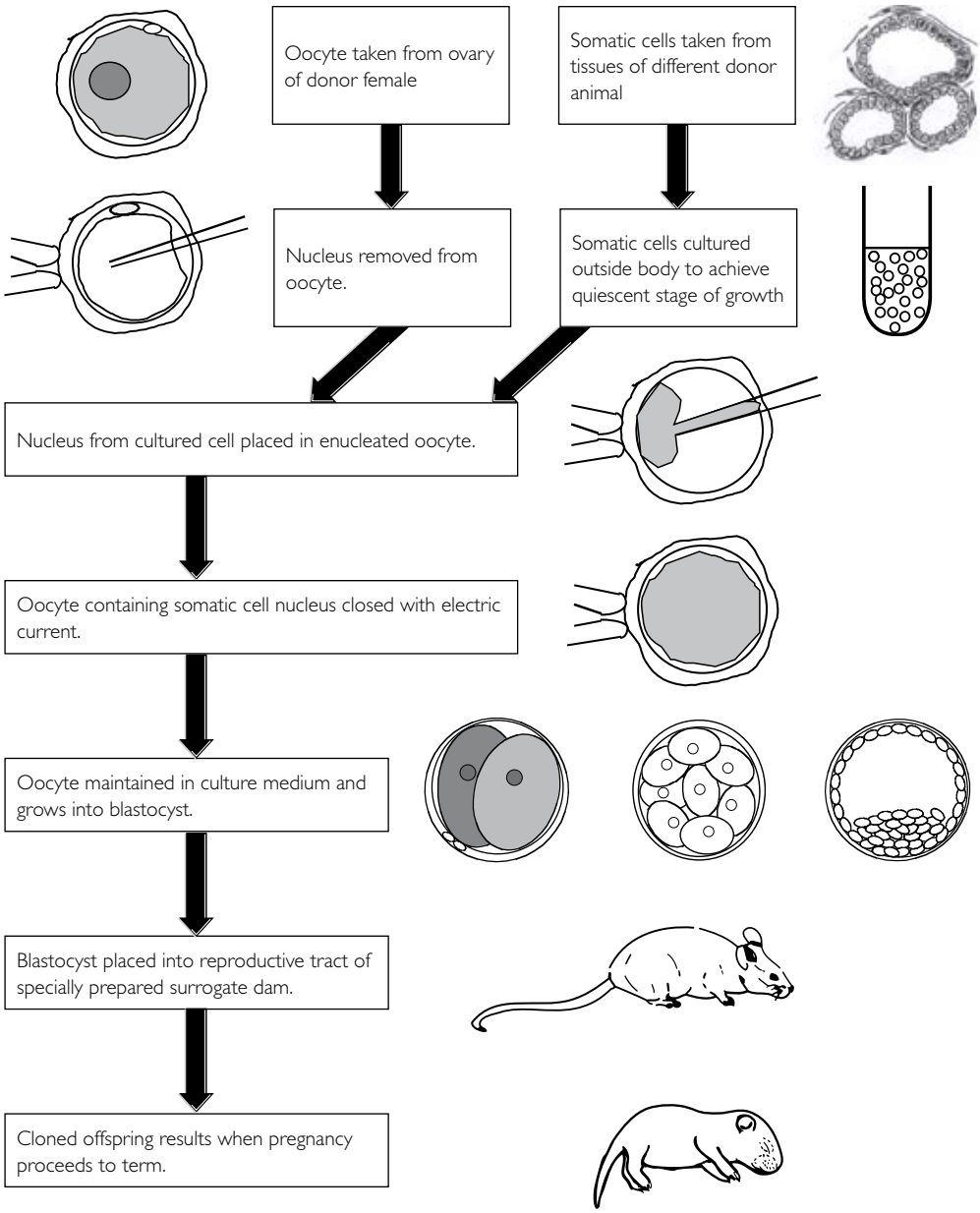

Figure 2. Illustrative flow chart showing production of genetically modified (cloned) animals

Somatic cells are maintained for a period in nutrient poor culture medium to induce a state of metabolic quiescence before their nuclei are transferred and oocytes sealed with an electric current. Resulting embryos are cultured for seven days either in artificial media or in the ligated uterine tubes of a specially prepared female animal. After this period

of culture, resultant blastocysts are transferred to the uterus of a surrogate female where pregnancy may proceed to term. Appropriate aseptic technique, post operative analgesia, and care of all survival surgery patients are mandatory, as required by the *Code*.

There can be variations to the process just described. The genetic hazards associated with cloning result from imperfect reprogramming and epigenetic effects.

1.3 SCOPE OF IMPACT ON THE ANIMALS

AECs should approve only those projects for which the generation or use of genetically modified and cloned animals has been justified on the basis of predicted benefits of the research and the impact on the welfare of the animals. This requires detailed consideration of the ethical aspects of the proposal.

The assessment will take into account the number of animals required to be generated for specific research and the number of unsuccessful, discarded or wasted animals. These numbers should be estimated by investigators at the outset, and should be reported by the investigators in the annual reports of approved generation projects. Data should be available to the AEC for their consideration in resolving the tension between Reduction and Refinement. Ideally, data should be collated in an electronic database within the institution, and potentially made available in a de-identified form to other institutions.

AEC approval for the generation of new strains of genetically modified animals should, in the first instance, cover the period from the start of the process until data on the mortality, morbidity and population health of the new strains are available. These data must be included in reports to the AEC as a basis for monitoring the progress of the project and determining if it may continue.

Intensive monitoring of the animals is required in the initial generation phase (see Appendix 2).

1.3.1 Monitoring of animals

Each project involving genetic modification or cloning will have unique features that require attentive monitoring and assessment of the welfare of the animals across the various stages of the project. The purpose of monitoring is to make and analyse routine observations in order to detect deviations from norms of health and wellbeing in animals and to signal the need for responses to distress. The purpose of assessment is to characterise the nature of deviations or distress detected by monitoring so that interventions can be appropriate.

Monitoring should include steps for actively seeking to identify adverse effects on the animal's welfare and should include the involvement of staff members who regularly care for the animals and have responsibility for their husbandry (see appendices). Because many genetically modified animals have no conspicuous phenotypic changes, the intensity and nature of monitoring, and the frequency of the reporting process, will need to be tailored to the particular circumstance. Specialised assessment may be required to characterise behavioural changes and changes in the form and function of different body systems.

Whole of life monitoring is necessary to fully understand the physiological and welfare implications of the particular modification, as the expression of genes could occur at any stage of the animal's life. Notwithstanding this principle, intervention is clearly essential to avoid suffering should it become apparent.

1.3.2 Collection of relevant data on the outcome of the genetic modification and cloning

The criteria for determining the data required should be sufficiently broad to cover anticipated and unexpected outcomes and to assess genetic change through appraisal of appropriate controls. Data should include but not be limited to mortality rates, cause of unexpected death and age at which it occurs and success rate of achieving the desired phenotype. This includes the generation of genetically modified and cloned animals as well as the subsequent breeding program. Any adverse effect must be documented and consideration be given to performing full post-mortem examination (with histology) and suitable clinical pathology. This information must be included in the reports to the AEC.

The sale or transfer of animals at an early stage of the generation of a new strain and the accumulation of data about the new phenotype can only occur if the recipient of the animals accepts the responsibility for completing the phenotype assessment after receiving data collected to date from the supplier.

1.3.3 Provision of full phenotype description to the AEC regarding welfare issues at designated stages

The frequency of reporting must be sufficient to inform the AEC in its decisions regarding refinement to, or continuation of, the project. It is important that the justification of the work is re-assessed by the AEC as the work progresses.

The stages may include the welfare of the mother during pregnancy and parturition, the first generation of genetically modified offspring, and that of subsequent generations. The final report on the project should include a phenotype report and must include a statement on the potential benefits and any detriments observed.

Where the initial stages of genetic modification of animals are performed by a third party, the reporting process will follow the provisions outlined in the *Code* for projects involving more than one AEC.

1.3.4 Subsequent breeding for scientific procedures

A phenotype report will have been completed in the initial generation of the genetically modified animal (see Appendix 3). This should be provided by the investigator to the AEC at the time of the request to use these animals. In the light of this information, predictable welfare issues can be anticipated and managed. Monitoring is still required, particularly for longer term health and welfare issues which may arise.

Proposals to produce genetically modified and cloned animals which are likely to suffer severe or prolonged distress, such as certain models of human disease, must not be approved unless the AEC is satisfied that the proposed management of such distress, through the provision of specialist care, early intervention and appropriate end points, will result in humane treatment of the animals.

The proposals must include detailed information on the expected outcome(s) of the genetic modification (without exception for commercial applications) and the steps that will be taken to minimise the impact on the welfare of the animal caused by changes to gene function. Any adverse events must be dealt with and promptly reported to the AEC in accordance with the *Code*. Unanticipated adverse outcomes must also be reported.

Projects using animals may be performed only if their use is justified by weighing the predicted scientific or educational value of the project against the potential effects on the welfare of the animals.

2. REPORTING REQUIREMENTS OF INVESTIGATORS

2.1 PHENOTYPE REPORT AND ASSESSMENT OF WELFARE

Comprehensive records of the phenotype of genetically modified and cloned animals are important for contributing both to high standards of animal welfare and to scientific knowledge. The phenotype requires description when new genetically modified and cloned animals are under development.

The phenotype report should accompany animals whenever they are transferred between laboratories or institutions. It should include information about the number of animals affected, nature and time of onset of adverse effects and any effective means of alleviating the distress that they cause to the animals. All transport of genetically modified animals must meet OGTR and International Air Transport Association (IATA) regulations.

The phenotype report should compare genetically modified and cloned animals to their unmodified counterparts and could include morphological, pathological, behavioural, physiological, biochemical and immunological profiles, growth rate and food and water intake. An example of a phenotype report is at Appendix 3.

2.2 FINAL REPORT ON THE GENERATION OF NEW GENETICALLY MODIFIED AND CLONED ANIMALS

A final report on the generation of a new strain of genetically modified animal must be approved by the AEC before the strain can be regarded as breeding stock, as outlined in the *Code*. This should be a stand alone document and not simply embedded within a new research proposal.

Similarly, a final report is required when genetically modified and cloned farm animals are submitted for release for commercial development and use. This report should describe the health, vigour, production performance and lifetime fitness of the genetically modified and cloned animals. It should also specify the measures that are in place to identify these animals and their succeeding generations within their production environments and to monitor and report on their continuing health, fitness and production performance.

The maintenance of lines of genetically modified and cloned animals for scientific purposes requires AEC approval and ongoing monitoring.

3. REDUCTION, REFINEMENT AND REPLACEMENT

When the use of genetically modified and cloned animals is contemplated, investigators should consider the use of:

- genetically modified embryos *in vitro* rather than implanting them in recipient animals (Reduction)
- testing the effects of genetic modification on cell and tissue differentiation in ES cells *in vitro* including using genomics and proteomics and other emerging technologies (Reduction and Replacement)
- pilot studies as mandatory to demonstrate that the proposed genetically modified and cloned animals are fit for the scientific purpose (Reduction)
- homozygous breeding pairs in order to reduce the number of animals born that do not have the desired mutation or are infertile (Reduction)
- heterozygote breeders in situations where homozygote animals experience unacceptable morbidity, mortality or chronic disease (Refinement)
- improved techniques in the collection of tissues, determination of genotype and individual animal identification (Refinement)
- inducible knock-out or knock-in systems so that the expression of phenotype can be controlled and expression only induced for a specific study period (Reduction and Refinement)
- strategies which maximise the use of surplus animals in accordance with the requirement in the *Code* (Reduction and Refinement)
- complementary approaches such as informatics, microarrays, cell, tissue and organ cultures, population studies, lower organisms and embryos (Reduction, Refinement and Replacement).

4. ANIMAL HUSBANDRY

Most husbandry procedures could be covered by AEC approved Standard Operating Procedures (SOPs). The technical competence of staff performing these procedures should be assessed by the AEC.

4.1 DETERMINATION OF GENOTYPE OF GENETICALLY MODIFIED AND CLONED ANIMALS

Genotyping is usually essential to confirm that an animal possesses the specified genetic modification. Screening of newborn or weaned animals by genotyping is performed on DNA prepared from a small sample of tissue. Due recognition must be given to the distress accompanying the tissue collection process. For this reason a minimal amount of tissue should be sampled in order to cause least discomfort to the animal or alternative sources of DNA such as buccal mucosa or faeces should be considered.

Consideration must be given in the assessment of the technique proposed, developmental age of the animal and whether there exists a need for anaesthesia. In this regard a balance must be struck between the benefits of anaesthesia and the stress associated with its administration and the animal's recovery from this procedure. It is an obligation of the investigator to justify the chosen method to the AEC.

The following points apply to mice which predominate as genetically modified and cloned research animals.

4.2 TAIL BIOPSY OF MICE

Verification of DNA transmission in genetically modified mice is often achieved using DNA extracted from a tail biopsy. Prompt analysis allows mice to be genotyped prior to weaning. Weaning may occur between three to four weeks of age depending on the strain.

In the mouse, the terminal tail innervation and ossification occurs between two to four weeks of age. If used, tail sampling of mice younger than three weeks of age may be performed without anaesthesia. For animals over three weeks of age, a short acting anaesthetic such as isoflurane must be used. The amount of tail removed must be the minimum required, and precautions must be taken to achieve haemostasis.

Alternatives to tail biopsies for Polymerase Chain Reaction (PCR) analysis should be encouraged and promoted wherever possible. For instance, when the PCR technique has been optimised the amount of tissue required can be minimised, allowing other types of samples to be taken such as saliva, hair or faeces. The use of tail biopsy techniques in mice for genotyping requires specific justification and AEC approval.

4.3 BLOOD COLLECTION

Under certain circumstances the collection of blood is necessary for the assessment of phenotype and genotype of genetically modified and cloned animals. Various techniques are available which bring with them different considerations regarding volume which can be taken, whether anaesthesia is appropriate and the aesthetics of particular techniques.

4.3.1 Retro-orbital bleeding

The collection of retro-orbital blood should be discouraged and only used if no other means is practicable. If it is necessary to use this method, with or without anaesthesia, there is an obligation on the investigator to justify the use of this technique to the AEC. An approved SOP may be appropriate in this circumstance. In making a deliberation, the AEC must consider the competency of the individual staff involved, the exploration of alternative methods and the opportunity to adopt another method if one becomes available.

4.3.2 Other blood collection methods

Investigators should be aware that the collection of blood from the saphenous, tail, or sub-mandibular vein (cheek pouch technique) is possible in adult mice. Blood may be able to be collected using a scalp vein needle.

4.4 IDENTIFICATION OF INDIVIDUALS

Permanent methods for identification of individual mice include ear notching, ear tagging, tattooing, freeze marking, toe clipping and micro-chipping. Some of these techniques may be able to be used to collect tissue for genotyping if sufficient can be sampled and if PCR optimisation has been achieved. Other means of identification apply to larger species.

4.4.1 Identification of neonatal mice

Some methods of identification in small animals can cause welfare problems such as acute pain and mismothering as a consequence of handling. Where possible, consideration should be given to the use of methods which allow temporary but accurate identification of very young animals. Permanent identification of selected genotyped animals can then take place at a later date.

Methods for temporary identification are best suited to cage mates of the same age. They are not appropriate for large groups of animals. They include dying a patch of fur with food colouring, which lasts between one and two weeks or clipping a patch of fur on the back or side which lasts from one to four weeks, depending on the stage of the hair cycle at which clipping occurs.

4.4.2 Toe clipping of mice

Toe clipping (removal of the terminal phalanx of a digit) of weanling and older mice is not recommended and should only be used as a method of identification when no other method such as tattooing, ear tags or subcutaneous implantation of a microchip is feasible.

Use of toe clipping without anaesthesia should only be performed on hairless neonates up to two weeks post-partum when the developmental elements required for pain perception are relatively undeveloped.

Tissue obtained by toe clipping (or ear notching) for the purpose of identification, may also be used for genotyping.

Use of toe clipping must be justified to the AEC, conducted only by people who have had appropriate training and carried out in accordance with an approved SOP.

5. DETERMINATION OF THE PHENOTYPE OF GENETICALLY MODIFIED AND CLONED ANIMALS

There is a need for investigators and AECs to be aware of welfare issues raised by abnormal or unexpected effects of genetic modification and cloning. In the process of generating genetically modified and cloned animals there is a possibility that a phenotype with adverse welfare implications may occur. Whilst this is relatively uncommon, the animals need to be carefully and systematically monitored so that any consequences can be described and addressed.

Animals need to be monitored for sufficient time to enable adverse phenotypes to become apparent. In some cases adverse effects of genetic modification and cloning may only be apparent at a particular stage of an animal's life history or may only emerge when the animal is placed in particular conditions. Adverse effects should be looked for actively and data gathering should involve all people with responsibility for the husbandry of the animals.

Phenotypic defects may be the desired consequence of genetic modification. The investigator creating a mouse strain should be allowed to explore all reasonable avenues to characterise the strain while collecting the population genetics information. For example, some genetically modified animals may have no overt phenotypic changes but have significant pathological changes. Valuable information may be gained during the phenotypic characterisation. This can be an important part of the scientific process and investigators need to outline the value of this information, which may add weight to the justification for projects under consideration by AECs.

5.1 GENETIC BASIS FOR ADVERSE PHENOTYPES

Adverse phenotypes can occur through any of the following mechanisms for genetically modified and cloned animals:

5.1.1 Genetically modified animals

Adverse phenotypes may result from:

- Insertional mutations where random insertion of transgene may lead to unexpected interactions with pre-existing genes or where a transgene may be introduced into the DNA sequence of a pre-existing gene and destroy its function.
- Problems with the expression of transgenes. The level, site and timing of expression of transgene are uncontrollable and may differ each time transgenic animals are constructed. In addition, product of the transgene may be foreign to the transgenic animals and have unexpected effects. Finally, the transgene product may not be foreign to the animals but may be excessive and overtax mechanisms for maintaining physiological stability.

5.1.2 Cloned animals

Adverse phenotypes may result from:

- Epigenetic effects where alterations in DNA function occur without changes in DNA sequence. These effects can result from chemical changes to DNA (such as methylation) and may underlie imperfect reprogramming and defects in imprinting.
- Imperfect reprogramming where transferred somatic cell nuclei fail to revert to a state where they can express the capacities and potentials for developing an individual organism in an ordered manner. Errors in reprogramming can affect any gene and thus lead to the wide range of anatomical and functional disorders associated with cloning.
- Defects in imprinting where the chemical marks that distinguish between DNA from the female and that from the sire lead to differences in gene expression and may cause problems.
- Somatic mutations whereby the DNA in the transferred somatic cell nuclei is damaged, for example by radiation or may contain viral DNA.
- Histocompatibility differences in mice have caused problems for the acceptance of embryos by surrogate females. Histocompatibility refers to the degree that individual animals accept tissue grafts from one another.

5.2 MONITORING GENETICALLY MODIFIED ANIMALS

Accurate and systematic record keeping is essential to characterise the phenotype of a new strain of animal. The record sheets and phenotype report in the following appendices are provided as **examples only** of how relevant observations can be recorded. The record sheets are for use in the various phases in the development of new strains of genetically modified animals. They are designed to reflect the different frequency of monitoring which is required at different stages of development.

The record sheets can be tailored by investigators and AECs to make them more appropriate to the species involved and the strain being developed and to account of the body of knowledge applicable to monitoring and phenotyping (see Additional Reading). The objective is to monitor the animals comprehensively to detect and address any adverse welfare impact and, ultimately to be able to complete the detailed phenotype report on that strain. Information for the phenotype report will be derived from monitoring and specialised assessment of behaviour and the form and function of organ systems.

It is anticipated that monitoring will be non-invasive and aimed primarily at the detection of failure to thrive and the normal physical attributes and behaviours. Any deviation from general health may indicate abnormalities arising from genetic modification which require investigation and assessment and more frequent monitoring. In most cases genetically modified animals used in research demonstrate no overt phenotypic deviation and basic monitoring is sufficient.

The development of a database of genetically modified animal is encouraged for projects which make use of previously developed strains of animals. The establishment of a database containing phenotype reports for strains of genetically modified animals would assist:

- with access and use of these animals
- AECs in their appraisal of applications
- investigators to satisfy AEC requirements to provide detailed information on the phenotype of the animal they propose to use.

Appendix 1

Appendix 1 provides a checklist to assist AECs in addressing applications for genetically modified and cloned animals. Applications for genetically modified and cloned animals must still be subjected to all the conditions required by the Code. However some additional points for consideration may include:

- is this a new genetically modified strain being developed?
- what numbers are required?
- have permits from OGTR been obtained?
- what genetic modification is being proposed?
- what special care is required?
- what monitoring systems will be put in place?

- how often is reporting required?
- what techniques will be used for genotyping?
- what techniques will be used for the generation of the genetically modified or cloned animal?
- will a phenotype report be developed?
- when is a new strain a normal breeding colony?
- will the information be published?

Appendix 2

Appendix 2 shows a set of model record sheets for monitoring animals, mice in particular, during the production of new genetically modified strains. They provide for basic observations on mortality, appearance, behaviour and responsiveness of animals and can signal deviations from normal form and function that demand further investigation both for the welfare of animals and the benefit of the research project.

The model record sheets are designed to account for differences in the frequency of monitoring that apply to animals of different ages. They can be modified to suit the investigator, AEC and the particular strain of genetically modified animal under development. One purpose of the record sheets is the collection of information over the number of generations necessary to establish a genetically modified strain. Information in the record sheets can form the basis of reports to an AEC.

Entries made under the category of *Appearance* in record sheets can be expressed as 'no deviation from the norm' or 'deviation from the norm', which may be coded according to the custom of the investigator; e.g. no (N) or yes (Y), (-) or (+), (ü) or (x). Quantitative information may also be recorded, for example, as 'ü3, x3' for a litter of six animals when three animals are normal for an attribute and three animals show deviations from normality. Grades of deviation could be quantified against a standard: for example, the litter of six animals could be recorded as 'ü3, x1, x2' if the deviation is more marked in two of the animals.

Entries into record sheets under categories of *Behaviour, Responsiveness and Nutrition* can be expressed as numbers of animals. These categories have subheadings that refer to grades of behaviour, response or attribute.

Model record sheet 1 applies to the assessment of litters during the first nine days of life when the frequency of monitoring is relatively high. They cover the initial litter from a surrogate dam and subsequent litters from heterozygote parents and homozygote parents as the new strain is being established. Key observations are: physical status, nutritional status, behaviour and responsiveness.

Model record sheet 2 applies to life after weaning up to 52 weeks of age. The frequency of monitoring changes during this period from once every two weeks, to once per month.

Model record sheet 3 is for the breeding performance of genetically modified animals and, except for the heading, is no different from the usual record sheet for breeding.

Model record sheet 4 is designed for attachment to cages where it can be used for keeping a check on the process of monitoring.

Appendix 3

Appendix 3 provides an example of a phenotype report for genetically modified animals. This report should accompany animals whenever they are transferred between laboratories or institutions. It should include information about the number of animals affected, the nature and time of onset of adverse effects and any effective means of alleviating any distress.

ADDITIONAL READING

GENERAL BACKGROUND

References cover the broad issue of genetic modification and animal welfare and are directed towards aspects of public policy.

Adams DB (2006). Advice note for the National Consultative Committee on Animal Welfare *Biotechnology, genetic modification, cloning and animal welfare*. Office of the Chief Veterinary Officer, Department of Agriculture, Fisheries and Forestry, Australia, April 2006. **http://www.affa.gov.au/corporate_docs/publications/pdf/animalplanthealth/animal_welfare/nccaw/biotech_gm_clone_aw_apr06.pdf**

Animal Procedures Committee (2001). *Animal Procedures Committee Report on Biotechnology*. Animal Procedures Committee UK, London. **http://www.apc.gov.uk/reference/biorec.pdf**

Canadian Council on Animal Care (1997). *CCAC Guidelines on transgenic animals*. **http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/TRANSGEN/TRANSGE1.HTM**

Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology (2002). Animal Biotechnology: Science-based concerns. National Research Council of the National Academies Press, Washington DC **http://www.nap.edu/books/0309084393/html/**

Farm Animal Welfare Council UK (2004). Report on Welfare Implications of Animal Breeding and Breeding Technologies in Commercial Agriculture. Farm Animal Welfare Council UK, London. **http://www.fawc.org.uk/pdf/breedingreport.pdf**

German Mouse Clinic. **http://www.mouseclinic.de**

MacKenzie AA (Editor)(2005). Biotechnology applications in animal health and production. Revue scientifique et technique des Office International des Épizooties, Volume 24, Number 1. World Organisation for Animal Health, Paris. **http://www.oie.int/eng/publicat/RT/A_RT24_1htm/**

Monash University. Animal Welfare Committee Policy on use of genetically modified animals Revised 2005 **http://www.monash.edu.au/research/ethics/animal/index.html**

Nuffield Council on Bioethics (2005). The ethics of research involving animals. Nuffield Council on Bioethics, London. **http://www.nuffieldbioethics.org**

Seamark RF (2003). Review on the current status of the extent and use of cloning in animal production in Australia and New Zealand. Food Standards Australia and New Zealand. **http://www.foodstandards.gov.au/_srcfiles/Cloning_Review_Final_June%202003.pdf**

The Royal Society (2001). The use of genetically modified animals. The Royal Society, London. **http://www.royalsoc.ac.uk/displaypagedoc.asp?id=11513**

Welfare of genetically modified animals

References come from scientific journals and deal specifically with welfare.

Anagnostopoulos AV, Sharp JJ, Mobraaten LE, Eppig JT and Davisson MT (2001). Availability and characterization of transgenic and knockout mice with behavioral manifestations: where to look and what to search for. *Behavioural Brain Research* 125:33–37.

Buehr M, Hjorth JP, and Hansen AK (2003). Genetically modified laboratory animals - what welfare problems do they face? *Journal of Applied Animal Welfare Science* 6:319-338.

BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (2003). Refinement and reduction in production of genetically modified mice. *Laboratory Animals* 37:S1-S49.

Dennis MB Jr. (2002). Welfare issues of genetically modified animals. *Institute of Laboratory Animal Resources Journal* 43:100-109.

Jegstrup I, Thon R, Hansen AK and Hoitinga MR (2003). Characterization of transgenic mice - a comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. *Laboratory Animals* 37:1-9.

van der Meer M, Rolls A, Baumans V, Olivier B and van Zutphen LFM (2001). Use of score sheets for welfare assessment of transgenic mice. *Laboratory Animals* 35: 379-389.

Wells DJ, Playle LC, Enser WE, Flecknell PA, Gardiner MA, Holland J, Howard BR, Hubrecht R, Humphreys KR, Jackson IJ, Lane N, Maconochie M, Mason G, Morton DB, Raymond R, Robinson V, Smith JA and Watt N. (2006). Assessing the welfare of genetically altered mice. *Laboratory Animals* 40:111-114.

Physiology of genetically modified animals

References come from scientific journals and deal with aspects of physiology of genetically modified animals without specific mention of welfare.

Gehrmann J and Berul CI (2000). Cardiac electrophysiology in genetically engineered mice. *Journal of Cardiovascular Electrophysiology* 11:354–68.

James JF, Hewett TE and Robbins J (1998). Cardiac physiology in transgenic mice. *Circulation Research* 82:407–15.

Lorenz JN (2001). Considerations for the evaluation of renal function in genetically engineered mice. *Current Opinion in Nephrology and Hypertension* 10:65–9.

Lorenz JN (2002). A practical guide to evaluating cardiovascular renal and pulmonary function in mice. *American Journal of Physiology*: 282:R1565–82.

Williams RS and Wagner PD (2000). Transgenic animals in integrative biology: approaches and interpretations of outcome. *Journal of Applied Physiology* 88:1119-1126.

Phenotype characterisation of genetically modified animals

References come from scientific journals and deal specifically with methods and experience in characterising the physical, physiological and behavioural traits (i.e. the phenotype) of genetically modified animals.

Anagnostopoulos AV, Mobraaten LE, Sharp JJ and Davisson MT (2001). Transgenic and knockout databases: behavioral profiles of mouse mutants. *Physiology and Behavior* 73:675–689.

Arndt SS and Surjo D (2001). Methods for the behavioural phenotyping of mouse mutants. How to keep the overview. *Behavioural Brain Research* 125:39–42.

Bailey KR, Rustay NR, and Crawley JN (2006). Behavioral phenotyping of transgenic and knockout mice: practical concerns and potential pitfalls. *Institute for Laboratory Animal Research Journal* 47:124–131.

Berg AL and Bohlooly Y M (2006). The program for phenotyping of genetically modified animals at AstraZeneca. *Experimental and Toxicologic Pathology* 57:383–384.

Berul CI (2003). Electrophysiological phenotyping in genetically engineered mice. *Physiological Genomics* 13:207–16.

Bilbo SD and Nelson RJ (2001). Behavioral phenotyping of transgenic and knockout animals: a cautionary tale. *Lab Animals* 30:24–29.

Brayton C, Justice M and Montgomery CA (2001). Evaluating mutant mice: anatomic pathology. *Veterinary Pathology* 38:1–19.

Brown MJ and Murray KA (2006). Phenotyping of genetically engineered mice: humane ethical environmental and husbandry issues. *Institute for Laboratory Animal Research Journal* 47:118–123.

Carter DB, Lai L, Park KW, Samuel M, Lattimer JC, Jordan KR, Estes DM, Besch–Williford C and Prather RS (2002). Phenotyping of transgenic cloned piglets. *Cloning Stem Cells* 4:131–145.

Ching KA, Cooke MP, Tarantino LM and Lapp H (2006). Data and animal management software for large–scale phenotype screening. *Mammalian Genome* 17:288–97.

Christensen G, Wang Y and Chien KR (1997). Physiological assessment of complex cardiac phenotypes in genetically engineered mice. *American Journal of Physiology* 272:H2513–24.

Collins KA, Korcarz CE and Lang RM (2003). Use of echocardiography for the phenotypic assessment of genetically altered mice. *Physiological Genomics* 13:227–39.

Cranston A, Howard L and Howard CV (2004). Quantitative phenotyping as an efficient means to estimate C–cell number in a knock–in mouse model of MEN2B. *Transgenic Research* 13:339–348.

Crawley JN (1999). Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health sensory functions motor abilities and specific behavioral tests. *Brain Research* 835:18–26.

Crawley JN (2003). Behavioral phenotyping of rodents. *Comparative Medicine* 53:140-146.

Crawley JN and Paylor R (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and Behavior* 31:197–211.

Fitzgerald SM, Gan L, Wickman A and Bergstrom G (2003). Cardiovascular and renal phenotyping of genetically modified mice: a challenge for traditional physiology. *Clinical and Experimental Pharmacology and Physiology* 30:207–216.

Heinrichs SC (2001). Mouse feeding behavior: ethology regulatory mechanisms and utility for mutant phenotyping. *Behavioural Brain Research* 125:81–88.

Johnson JT, Hansen MS, Wu I, Healy LJ, Johnson CR, Jones GM, Capecchi MR and Keller C (2006). Virtual histology of transgenic mouse embryos for high–throughput phenotyping. *PLoS Genetics* 2:e61 [Epub 2006 Apr 28].

Karl T, Pabst R and von Horsten S (2003). Behavioral phenotyping of mice in pharmacological and toxicological research. *Experimental and Toxicologic Pathology* 55:69–83.

Kulandavelu S, Qu D, Sunn N, Mu J, Rennie MY, Whiteley KJ, Walls JR, Bock NA, Sun JC, Covelli A, Sled JG and Adamson SL (2006). Embryonic and neonatal phenotyping of genetically engineered mice. *Institute for Laboratory Animal Research Journal* 47:103–117.

Marston HM, Spratt C and Kelly JS (2001). Phenotyping complex behaviours: assessment of circadian control and 5–choice serial reaction learning in the mouse. *Behavioural Brain Research* 125:189–193.

McKerlie C (2006). Cause and effect considerations in diagnostic pathology and pathology phenotyping of genetically engineered mice (GEM). *Institute for Laboratory Animal Research Journal* 47:156–162.

Murray KA (2002) Issues to consider when phenotyping mutant mouse models. *Lab Animals* 31:25–29.

Pinkert CA (2003). Transgenic animal technology: alternatives in genotyping and phenotyping. *Comparative Medicine* 53:126–139.

Rafael JA, Nitta Y, Peters J and Davies KE (2000). Testing of SHIRPA a mouse phenotypic assessment protocol on Dmd(mdx) and Dmd(mdx3cv) dystrophin–deficient mice. *Mammalian Genome* 11:725–8.

Rao S and Verkman AS (2000). Analysis of organ physiology in transgenic mice. *American Journal of Physiology, Cell Physiology* 279:C1–C18.

Rogers DC, Fisher EM, Brown SD, Peters J, Hunter AJ and Martin JE (1997). Behavioral and functional analysis of mouse phenotype: SHIRPA a proposed protocol for comprehensive phenotype assessment. *Mammalian Genome* 8:711–3.

Schneider I, Tirsch WS, Faus–Kessler T, Becker L, Kling E, Busse RL, Bender A, Feddersen B, Tritschler J, Fuchs H, Gailus–Durner V, Englmeier KH, Angelis MH and Klopstock T (2006). Systematic standardized and comprehensive neurological phenotyping of inbred mice strains in the German Mouse Clinic. *Journal of Neuroscience Methods* [e-pub ahead of print, 28 August 2006].

Spink AJ, Tegelenbosch RA, Buma MO and Noldus LP (2001). The EthoVision video tracking system—a tool for behavioral phenotyping of transgenic mice. *Physiology and Behavior* 73:731–744.

Surjo D and Arndt SS (2001). The Mutant Mouse Behaviour network a medium to present and discuss methods for the behavioural phenotyping. *Physiology and Behavior* 73:691–4.

van der Staay FJ and Steckler T (2001). Behavioural phenotyping of mouse mutants. *Behavioural Brain Research* 125:3–12.

van der Staay FJ and Steckler T (2002). The fallacy of behavioral phenotyping without standardization. *Genes, Brain and Behavior* 1:9–13.

Veasey SC, Yeou–Jey H, Thayer P and Fenik P (2004). Murine Multiple Sleep Latency Test: phenotyping sleep propensity in mice. *Sleep* 27:388–393.

Weninger WJ and Mohun T (2002) Phenotyping transgenic embryos: a rapid 3–D screening method based on episcopic fluorescence image capturing. *Nature Genetics* 30:59–65.

Wurbel H (2002). Behavioral phenotyping enhanced—beyond (environmental) standardization. *Genes, Brain and Behavior* 1:3–8.

Transgenic animals in research on pain

References come from scientific journals and deal with the use of transgenic animals to study the phenomenon of pain, a fundamental consideration in animal welfare.

Holmes FE, Mahoney SA and Wynick D (2005). Use of genetically engineered transgenic mice to investigate the role of galanin in the peripheral nervous system after injury. *Neuropeptides* 39: 191-199.

Lariviere WR, Chesler EJ and Mogil JS (2001). Transgenic studies of pain and analgesia: mutation or background genotype? *Journal of Pharmacology and Experimental Therapeutics* 297: 467-473.

Liang DY, Liao G, Wang J, Usuka J, Guo Y, Peltz G and Clark JD (2006). A genetic analysis of opioid-induced hyperalgesia in mice. *Anesthesiology* 104: 1054-1062.

Mogil JS and Grisel JE (1998). Transgenic studies of pain. *Pain* 77: 107-128.

Mogil JS, Ritchie J, Sotocinal SG, Smith SB, Croteau S, Levitin DJ, and Naumova AK (2006). Screening for pain phenotypes: analysis of three congenic mouse strains on a battery of nine nociceptive assays. *Pain* (2006): in press.

Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH and Wood JN (2004). Nociceptor-specific gene deletion reveals a major role for nav1.7 (pn1) in acute and inflammatory pain. *Proceedings of the National Academy of Sciences of the USA* 101: 12706-12711.

Wilson SG and Mogil JS (2001). Measuring pain in the (knockout) mouse: big challenges in a small mammal. *Behavioural Brain Research* 125: 65-73.

Random (chemical) mutagenesis

References come from scientific journals and deal with random (chemical) mutagenesis as a specific instance of genetic modification.

Cook MC, Vinuesa CG, Goodnow CC (2006). ENU-mutagenesis: insight into immune function and pathology. *Curr Opin Immunol.* 18(5):627-33. Epub 4 Aug 2006.

Guenet JL (2004). Chemical mutagenesis of the mouse genome: an overview. *Genetica* 122:9–24.

Masuya H, Inoue M, Wada Y, Shimizu A, Nagano J, Kawai A, Inoue A, Kagami T, Hirayama T, Yamaga A, Kaneda H, Kobayashi K, Minowa O, Miura I, Gondo Y, Noda T, Wakana S and Shiroishi T (2005). Implementation of the modified–SHIRPA protocol for screening of dominant phenotypes in a large–scale ENU mutagenesis program. *Mammalian Genome* 16:829–37.

Masuya H, Nakai Y, Motegi H, Niinaya N, Kida Y, Kaneko Y, Aritake H, Suzuki N, Ishii J, Koorikawa K, Suzuki T, Inoue M, Kobayashi K, Toki H, Wada Y, Kaneda H, Ishijima J, Takahashi KR, Minowa O, Noda T, Wakana S, Gondo Y and Shiroishi T (2004). Development and implementation of a database system to manage a large–scale mouse ENU–mutagenesis program. *Mammalian Genome* 15:404–11

APPENDIX 1

CHECKLIST TO ASSIST AECS IN ADDRESSING APPLICATIONS FOR GENETICALLY MODIFIED AND CLONED ANIMALS

Applications for genetically modified and cloned animals must still be subjected to all the conditions required by the *Code*. Some additional points for consideration by the AEC for the generation of genetically modified animals include:

1. Is this a new genetically modified or cloned animal being created?

   If yes, has it been done elsewhere? What checks have been made eg literature search etc? Would it be more efficient to outsource this production? If done elsewhere, it would reduce numbers if the genetically modified progeny were purchased from an existing colony as the initial breeding stock would not be required.

2. Detail of the numbers of the breeding stock required to produce the genetically modified progeny

   This will be large in comparison to the number of animals with the correct phenotype or genotype that will be produced and must be included in the justification of the project, that is, the benefit of the research versus the impact on the animals.

3. Have the relevant permits been obtained from the OGTR if required?

4. Does the institution have the appropriate facilities eg PC2 to house these animals?

5. What is the genetic modification that is proposed?

   - If experimental use of an already developed genetically modified strain, a phenotype report should be available that characterises the modification and any known or expected adverse side effects and the care required to address these effects.
   - If a new strain, detail of the expected impact on the animal's phenotype should be included.

6. What special care, if any, is required for these animals?

   For example, if heart failure develops at six months of age as a result of genetic modification, will the animal be killed at five months before this occurs? That is, will there be defined humane end points?

7. What monitoring systems will be put in place to detect any unexpected adverse effects to characterise the phenotype?

Several appendices are provided as example monitoring forms. Which is most appropriate to this case? Monitoring needs to detect events such as adverse impact, increased mortality and failure to thrive but should not be adversely invasive. It should aim to measure physical status, nutritional status and behaviour and should include whole of life monitoring. In the case of a new strain, several generations should be monitored to ensure phenotypic stability. AECs need to balance these factors.

8. Frequency of reporting to AEC

All adverse animal welfare events are to be reported.

9. Techniques for genotyping

Has the least invasive method been considered? If the less invasive methods are not possible, have the more invasive techniques been justified? For example PCR analysis versus tail biopsy.

10. Techniques for the generation of the genetically modified animal

Are there SOPs in place, are they appropriate and ensure adequate care of all animals involved?

11. Development of a phenotype report for a new genetically modified strain

This is essential and the application should outline a satisfactory method for accomplishing this.

12. Determination of when the new strain becomes a 'normal' breeding colony

This requires AEC approval and factors to consider include:

- is the phenotype and genotype stable and well characterised?
- are there any special care requirements?
- what is the environment that the animals will be exposed to? A phenotype stable in the laboratory may not be in the field.
- what is the impact on the environment into which they will be placed? Will this affect the wild type if breeding occurs?

13. Publication of information of the existence and characterisation of a new genetically modified strain

This is essential to avoid duplication elsewhere.

APPENDIX 2

A SET OF MODEL RECORD SHEETS FOR THE MONITORING OF MICE DURING THE ESTABLISHMENT OF A NEW GENETICALLY MODIFIED STRAIN

Model record sheet 1: Neonatal Assessment (Day 1 - Day 9)

STRAIN _____ ROOM _____ OGTR APPROVAL NO. _____
AEC APPROVAL NO. _____ INVESTIGATOR _____ ANIMAL TECHNICIAN _____
BREEDER NO. _____ DAM ID _____ BORN _____
SET UP LITTER BORN _____ SIRE ID _____ BORN _____
LITTER ID _____ NO. IN LITTER _____

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Mortalities									
Appearance									
Size									
Skin (pink to pale in colour)									
Bodily condition									
Conformation including deformities									
Extremities (pink to pale in colour)									
Behaviour									
Normal wriggling									
Lower than normal wriggling									
No wriggling									
Responsiveness									
Normal response to touch									
Low response to touch									
No response to touch									
Nutrition									
Good milk band									
Poor milk band									
No milk band									
ASSESSOR'S INITIALS									

Supplementary observations and further investigations (including mothering behaviour eg nest building, pup retrieval etc)

_____
_____
_____
_____

Model record sheet 2: Post-weaning to adult assessment (Day 10 - Week 52)

STRAIN _____ ROOM _____ OGTR APPROVAL NO. _____ INVESTIGATOR _____

AEC APPROVAL NO _____ ANIMAL TECHNICIAN _____ BREEDER NO. _____ DAM ID _____

BORN _____ SET UP _____ SIRE ID _____ BORN _____

ID _____ COLOUR _____

	10 days	21 days	4 weeks	6 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	28 weeks	32 weeks	36 weeks	40 weeks	44 weeks	48 weeks	52 weeks
Mortalities																
Behaviour																
Normal intensity of activity																
Lower or higher than normal intensity of activity																
Posture																
Normal posture																
Abnormal posture (e.g. hunched)																
Coat Condition																
Smooth coat																
Ruffled coat																
Responsiveness																
Normal response to touch																
Low response to touch																
No response to touch																
Weight (g)																
ASSESSOR'S INITIALS																

Supplementary observations and further investigations (including weaning and litter size uniformity): _____

_____

_____

Model record sheet 3: Breeding performance

STRAIN _____ ROOM _____
INVESTIGATOR_____ AEC NO _____

Female I.D **Birth date:** **Male I.D** **Birth date:** **Set Up:**

Date	Litter ID	Live Born	Stillborn	Neonatal Deaths	Neonatal Culled	Weaned	F	M	Ave wt (g)	Comments

Model record sheet 4: Example record sheet for scheduled monitoring and assessment of a new genetically modified strain

STRAIN _____ ROOM _____ OGTR APPROVAL NO. _____

AEC APPROVAL NO. _____ INVESTIGATOR_____ ANIMAL TECHNICIAN _____

BREEDER NO. _____ DAM ID _____ BORN _____

SET UP _____ LITTER BORN _____ SIRE ID _____

BORN _____ LITTER ID _____ NO. IN LITTER _____

	Date	Initials of assessor		Date	Initials of assessor
Day 1			8 weeks		
Day 2			12 weeks		
Day 3			16 weeks		
Day 4			20 weeks		
Day 5			Week 24		
Day 6			Week 28		
Day 7			Week 32		
Day 8			Week 36		
Day 9			Week 40		
Day 10			Week 44		
Day 21			Week48		
			Week 52		

This is an example of a card that could be attached to the cage.

APPENDIX 3

Example phenotype report for genetically modified animals

The main purpose of this report is to assist with the monitoring and assessment of the impact of the genetic modification upon the health and welfare of the affected animals. Please provide information consistent with this purpose (ie. detailed descriptions of *in vitro* methodology are not desired). It is a tool to make it easier for an AEC to appreciate the welfare impact of the genetic modification made to this strain of mouse.

Please use lay language or provide glossary definitions.

Project Details

1. AEC Project No.:			
2. Project Title:			
3. Start Date*:		**Finish Date*:**	
4. Chief Investigator:			
Department:			

* Relates to approved project dates.

Animal Details

5. Genetically modified animal species:			
Strain/genetic description:		**Background Strain:**	
Source: (ie. in-house or specified external laboratory source)			
What is the health profile of the source colony? Provide the most recent serology report			

DECLARATION BY CHAIRPERSON OF AEC

I certify that this report has been considered and accepted by the Animal Ethics Committee at the meeting on .....(date)

....

Chairperson's signature AEC Date

..... Please print name

6. How much is known about the biological characteristics/phenotype of this strain?

Indicate by selecting one of the following:

- ☐ Well characterised
- ☐ Partially-characterised/some information available
- ☐ Unknown

GLOSSARY

Word	Lay explanation

7. Genetic alteration:

Briefly describe which gene has been added /deleted/ altered	
Affected organs/tissues: (eg. gene expressed in liver only)	
Is animal health, welfare, breeding or lifespan affected?	
What abnormalities are known to exist (or do you expect) in these animals?	

8. Clinical Observations

Comparison of genetically modified animals with non-genetically modified littermates is desirable.

- Supply a record of clinical observations made on a representative sample of the genetically modified animal(s).
- Minimum period for observation record is 3 months; life-long data to be included where possible. If supplying "average" data, indicate number of animals observed and a measure of the variability of the data.

9. Phenotype

Briefly detail observations which have been made to characterise the genetically modified animal strain (ie behaviour, physiology, reproductive or developmental measures). Your answer to this question should inform the AEC about abnormalities or changes which have a welfare impact.

10. Minimisation of pain or distress

Describe any adverse affects, pain or distress, and/or unexpected mortality, the causes if known and how these problems were resolved. If none this should be indicated.

11. Special husbandry or animal care requirements specific for the new genetically modified animal strain

If these are necessary, please provide details.

12. Humane euthanasia and experimental endpoint criteria

What objective criteria will be used to determine when an animal will be humanely killed or removed from an experimental study prematurely?

CERTIFICATION OF THE CHIEF INVESTIGATOR

- I understand the requirements of legislation and the *Australian code of practice for the care and use of animals for scientific purposes* (2004) governing the use of animals for research and teaching.
- I will continue to conduct the project in full compliance with the aforementioned requirements.

....

Signature of Chief Investigator Date

.....

Please Print Name

The National Health and Medical Research Council

The National Health and Medical Research Council (NHMRC) was established in 1936 and is now a statutory body within the portfolio of the Australian Government Minister for Health and Ageing, operating under the *National Health and Medical Research Council Act 1992 (*NHMRC Act*)*. The NHMRC advises the Australian community, the Australian Government, and State and Territory Governments on standards of individual and public health, and supports research to improve those standards.

The NHMRC Act provides four statutory obligations:

- to raise the standard of individual and public health throughout Australia;
- to foster development of consistent health standards between the States and Territories;
- to foster medical research and training and public health research and training throughout Australia; and
- to foster consideration of ethical issues relating to health.

The NHMRC also has statutory obligations under the *Prohibition of Human Cloning Act 2002* (PHC Act) and the *Research Involving Human Embryos Act 2002* (RIHE Act).

The activities of the NHMRC translate into four major outputs: health and medical research; health policy and advice; health ethics; and the regulation of research involving donated IVF embryos, including monitoring compliance with the ban on human cloning and certain other activities. A regular publishing program ensures that Council's recommendations are widely available to governments, the community, scientific, industrial and education groups. The Council publishes in the following areas:

- Aboriginal and Torres Strait Islander Health
- Aged Care
- Blood and Blood Products
- Cancer
- Cardiovascular Health
- Child Health
- Clinical Practice Guidelines – Standards for Developers – Topics
- Communicable Diseases, Vaccinations and Infection Control
- Diabetes
- Drug and Substance Abuse
- Environmental Health
- Ethics in Research–Animal
- Ethics in Research–Human
- Genetics and Gene Technology
- Health Procedures
- Health Promotion
- Human Cloning and Embryo Research
- Indigenous Health
- Injury including Sports Injury
- Men's Health
- Mental Health
- Musculoskeletal
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- Oral Health
- Organ Donation
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