National Centre
for the Replacement
Refinement & Reduction
of Animals In Research

Sharing & archiving of genetically altered mice

Opportunities for reduction and refinement

Pioneering Better Science

Original version – A report of the RSPCA Resource Sharing Working Group, 2009.

Current version – Produced by The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) Breeding and colony management working group (**see www.nc3rs.org.uk/colonymanagement**), November 2021.

Contributors

Prof Ian Jackson (Chair) – MRC Human Genetics Unit, University of Edinburgh

Mr Brendan Doe – CRUK Cambridge Institute

Dr Ellen Forty – NC3Rs

Dr Martin Fray – Mary Lyon Centre (MRC Harwell)

Ms Sarah Hart-Johnson – Francis Crick Institute

Dr Natalia Moncaut – CRUK Manchester Institute

Dr Esther Pearl – NC3Rs

Dr Michelle Stewart – Mary Lyon Centre (MRC Harwell)

Ms Hannah Wardle-Jones – CRUK Cambridge Institute

Contents

Introduction

The use of genetically altered (GA) animals has become a mainstay of biomedical research, with new technical approaches (such as CRISPR/Cas9) accelerating the pace at which new animal models become available.

This raises scientific, ethical and logistical issues, in terms of the generation, breeding, maintenance and use of these animals, together with challenges in terms of applying the principles of replacement, reduction and refinement (the 3Rs).

With this in mind, the NC3Rs established a working group, including international experts on breeding, managing and archiving colonies, to define best practice in breeding and colony management.

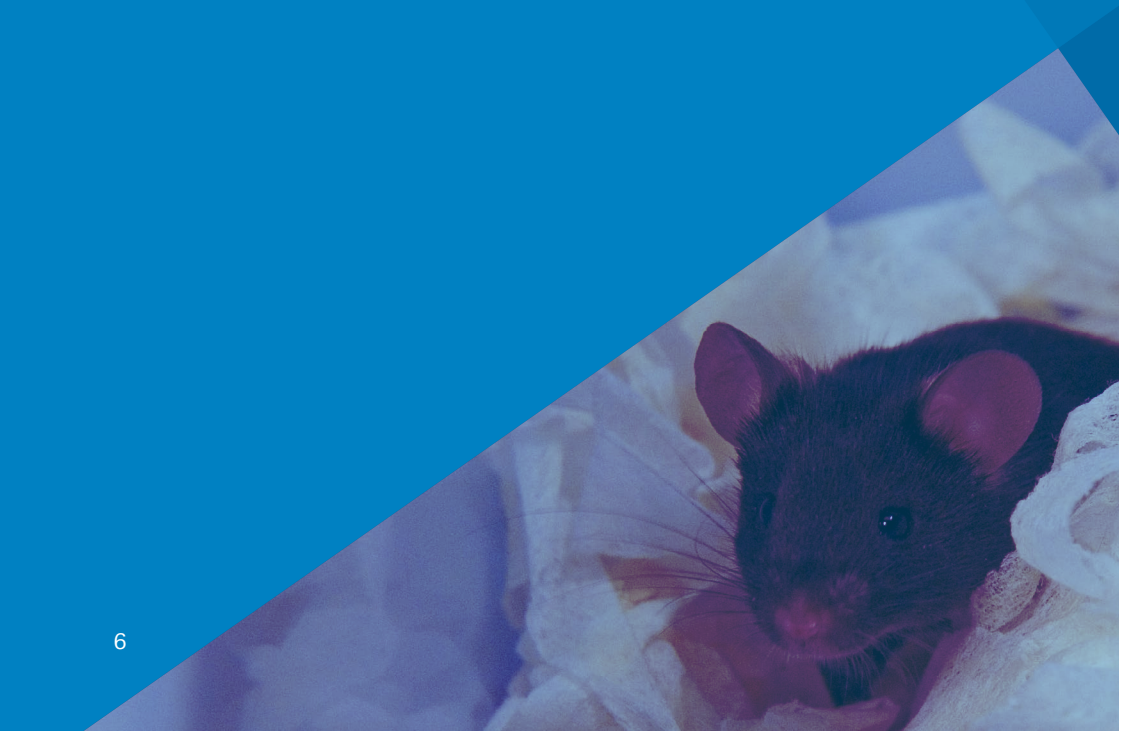
As part of this agenda, this document, originally written in 2009 by the RSPCA (in association with the MRC, BBSRC, CRUK and the NC3Rs), has been updated to reflect how current best practices in the archiving and sharing of GA strains can provide an opportunity for reduction and refinement.

Although there is a focus on mice, as the most frequently used animal in biomedical research, the concepts in this guidance can be applied to all common purpose-bred laboratory species.

For the purposes of this document, archiving is defined as the storage of frozen (cryopreserved) embryos or gametes which preserves the genetic stock and eliminates the need to maintain the stock as live animals. These cryopreserved resources are often the best means of sharing GA strains with other scientists, while avoiding the welfare concerns related to transporting live animals. This document is intended to provide an overview of current best practice and includes a comprehensive list of resources for more detail on each topic.

1 | Why archive and share resources?

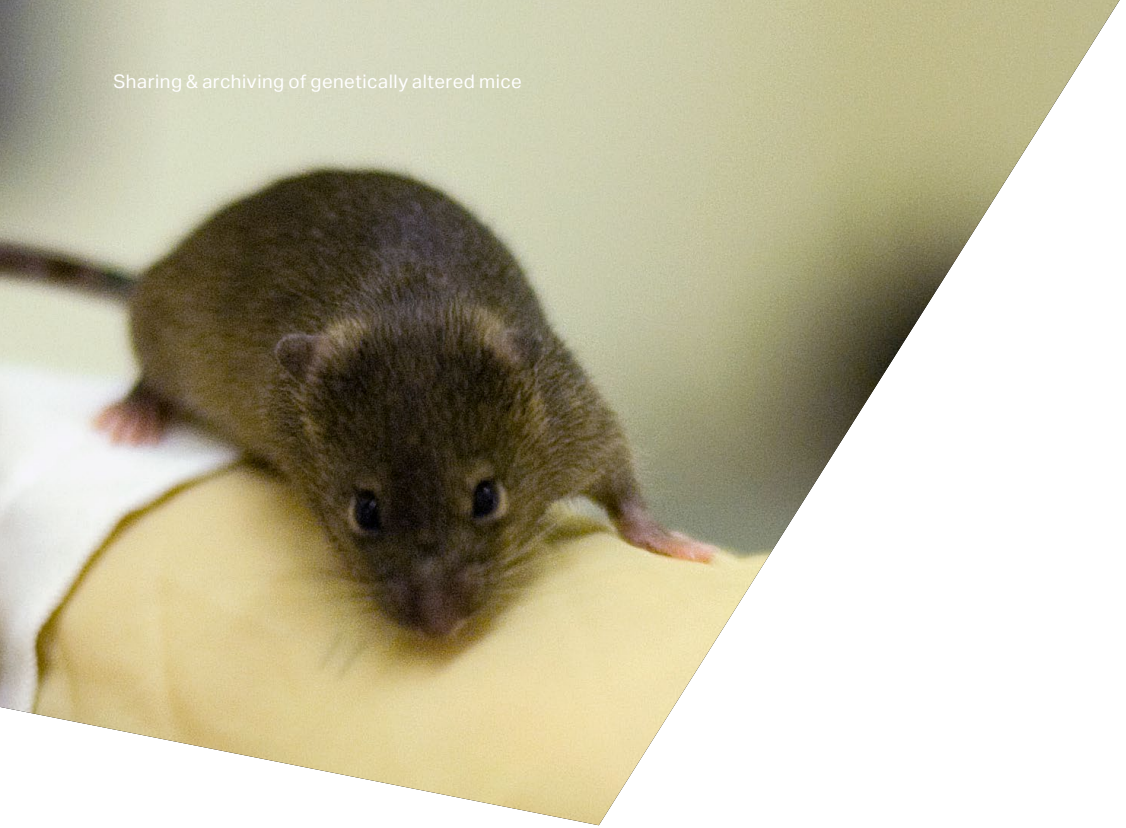
Archiving should be part of every institution's GA breeding/colony management programme, not only to optimise 3Rs practices locally, but also to facilitate the sharing of GA strains which will expand these 3Rs benefits more widely.



There are multiple benefits of archiving mouse strains. These include:

- Reducing the number of GA strains maintained on the shelf at any one time, minimising the overall number of animals used.
- Providing some insurance against loss of valuable stocks or strains caused by adverse events such as environmental disasters, disease outbreaks, genetic drift, transgene silencing and breeding.
- Facilitating strain sharing, providing further opportunities for reduction in animal use, and minimising the need for researchers to duplicate strains or models.
- Refining the process of strain sharing by avoiding the need to transport live animals, by shipping cryopreserved gametes or embryos instead. *
- Preventing unnecessary use of tick-over (maintenance) colonies.
- Minimising genetic drift within a strain when used appropriately, maximising data reproducibility by maintaining genetic integrity of the strain over time and ensuring that strains available for sharing are well characterised.

*It is important to note that although shipping gametes or embryos eliminates live transport, animals will be used to rederive the strain at the receiving facility. Rederivation should be done using the most refined and up-to-date methods and by staff that are appropriately trained and competent.



- Avoiding wasting animals at the end of a project or when projects need to be closed down quickly, for example due to staff shortages. This is particularly true for inbred colonies when sperm, embryos and oocytes can be cryopreserved for later use in a range of applications (e.g. microinjections, QC tests), in addition to strain recovery.
- Managing strains with welfare concerns; archiving the strain can reduce the length of time it is maintained as a live colony.

Cryopreservation and maintenance costs vary considerably from institution to institution. Nevertheless, archiving can have financial benefits, avoiding or reducing cage and genotyping costs, and decreasing the staff resource required to maintain animals.

2 | What to archive and when

Institutions need to develop a comprehensive archiving strategy. Cryopreserving novel strains soon after genotype and phenotype characterisation brings benefits such as securing the strain against uncontrolled genetic drift and safeguarding against problems with breeding, disease or transgene silencing.

When establishing a colony there may be surplus mice from breeding not required for the maintenance of the colony, which could be of a suitable genotype to be used for cryopreservation (e.g. archiving heterozygotes produced during the establishment of a homozygous colony).

Both embryos and sperm can be successfully cryopreserved, and live strains recovered with relative ease on a routine basis. The decision of whether to cryopreserve embryos or sperm depends on a number of factors, including breeding performance of the strain, genotype complexity, local expertise and the availability of specialised equipment. More detailed information on deciding whether to archive sperm or embryos can be found in the archiving best practice page of the NC3Rs Breeding and Colony Management resource (see www.nc3rs.org.uk/archiving). Links to well-established protocols for cryopreservation of different materials from different species can be found in Appendix 2.

If local storage capacity is not a limitation, all unique GA mouse strains should be part of the cryopreservation programme, including strains carrying the same genome modification but on a different genetic background. Tissues and DNA from unique strains should also be archived, as these can be used for quality control and comparison with material from future rounds of recovery of the strain. In addition, they can be useful for addressing specific questions before, or instead of recovery.

Before taking an important strain off the shelf ensure that the strain has either been cryopreserved (and check that the quality control is sufficient) or that the strain is available elsewhere on the required background (see Appendix 2 for online repository databases).

Ideally a strain should be cryopreserved at the following checkpoints:

1. When a new GA strain has been generated – archiving a new strain should only happen after a basic allele quality control has been performed (see www.nc3rs.org.uk/archiving), to ensure that the desired genotype has been achieved.
2. When a new strain has been generated by crossing different strains together (also consider cryopreserving intermediate steps if a complex breeding plan is required to achieve the desired strain).
3. When a strain has been backcrossed onto a particular genetic background.
4. To replace cryopreserved stocks when depleted. For example, after thawing stocks and refreshing strains.
5. To avoid unnecessary tick-over when a colony will not be used for experiments for an extended period of time.

3 | How to archive

There are two main approaches to archiving mouse strains: archiving in-house or archiving through a centralised facility such as the European Mouse Mutant Archive (EMMA), the Centre for Animal Resources and Development (CARD) or the Mutant Mouse Resource and Research Centre (MMRRC).

In-house facilities will require well-funded resources and a robust quality control programme to ensure that cryopreserved embryos and gametes are of high enough quality to enable the consistent recovery of live born offspring.

All these requirements are integral to a centralised repository, which are often available at no cost to depositors. Centralised repositories also remove the burden associated with setting up frequent shipments when sharing GA mouse strains that are in high demand. In the UK these services are provided by the MRC Harwell Archive (see archive.har.mrc.ac.uk) which acts as the UK node for EMMA.

3.1. Centralised cryopreservation facilities

Centralised facilities provide **free** embryo and sperm cryopreservation services, plus long-term storage, on the condition that archived strains can be made available to the scientific community. The intellectual property (IP) remains with the originator and, where necessary, a grace period (e.g. a two-year grace period for EMMA) can be applied which allows investigators to prevent sharing of their mouse strains before their data has been published. An example of a centralised facility is EMMA (www.infrafrontier.eu) comprised of 16 archiving centres across Europe.

3.2. In-house cryopreservation facilities

Many institutions that generate and breed GA mice have in-house cryopreservation and storage facilities which retain strains for use locally. To fully achieve the benefits of cryopreservation it is important that local cryopreservation and archiving facilities are effectively managed. This includes specific staff training and rigorous record keeping.

3.2.1. Staff training

Centralised facilities employ highly trained staff that are familiar with the latest techniques and best practices. The high throughput in these laboratories ensures that the staff remain competent in key skill areas. High staff competence minimises the number of animals used and the impact and stress of specific archiving and rederivation procedures on the animals involved.

When setting up archiving facilities for the first time, or when new skills are required, it is essential to seek training and guidance. National repositories also act as centres of excellence for training, hosting courses on embryo handling, rederivation and cryopreservation techniques. For example, a range of cryopreservation courses are offered across Europe by the EMMA partners (see bit.ly/3yTxaG2).

3.2.2. Record keeping

All archiving facilities need a good inventory system to ensure that the archive's contents are easily accessible. The inventory records should include a unique identifier for each sample, details of the freeze/thaw methods, the contents of each straw or vial (i.e. embryos or sperm), the genotype of the animals providing the embryos/sperm and details of the quality control tests performed.

The records should include genetic information on each mouse strain to ensure future users fully understand how the strain was made, the breeding history and current genetic background, the genotyping protocol to be used and whether there are any breeding or welfare concerns associated with the strain (see Appendix 1 and the mouse passport described in Wells *et al*., 2006). Collecting these details at the time a strain is cryopreserved will make the process of rederivation and further breeding much more efficient and reproducible.

To avoid confusion, mouse strains should be named according to standardised nomenclature that is recognised globally. The International Committee on Standardised Genetic Nomenclature for Mice regularly reviews and updates the naming systems in light of new developments in the field, for example the advent of CRISPR/Cas9 gene editing. Further information and advice on the use of standardised nomenclature is available at: www.informatics.jax.org/mgihome/nomen.

The Mouse Genome Informatics (MGI) team at the Jackson Laboratory keep a comprehensive list of all registered allele, gene and strain names at: www.informatics.jax.org.

4 | How to share

Few would argue that the products of publicly funded research, such as GA strains, should not be accessible to the wider scientific community, and that sharing cryopreserved material avoids the stress induced by transporting live animals.

However, whilst archiving is relatively straightforward, there are both real and perceived barriers to sharing GA strains. Some of these are resource driven, such as staff time, the cost implications of maintaining and disseminating cryopreserved stocks in-house, and the need to keep records and databases up to date.

A perceived barrier is the need to retain IP rights. When archives are maintained in-house, IP rights are readily managed through appropriate Material Transfer Agreements (MTAs), and the conditions of the MTA are checked before strains are shared. The same protection can also be applied when using centralised facilities such as EMMA. These centralised services ensure that fully executed copies of the originator's MTAs are exchanged before a strain is distributed. This mechanism operates whether or not a strain was originally submitted with restricted access, and ensures all beneficial rights are retained by the originator.

A significant barrier to sharing is the dissemination of knowledge on what is available to share. Researchers can take a range of steps to ensure that their GA strains are quickly and easily accessible to the wider community. This includes registering GA strains and alleles with MGI (www.informatics.jax.org) which will also confirm the identity of the originating laboratory. As discussed earlier in this document, depositing strains with centralised repositories such as EMMA enables the strain to be cryopreserved free of charge, the only cost is that of shipping the animals or tissue.

When sharing mouse strains, to facilitate reproducible research, it is important that the information contained in the mouse passport is fully updated (see 3.2.2 and Appendix 1). This also highlights the benefits of acquiring strains directly from a centralised repository where genetic drift is more likely to be controlled and the background characterised.

4.1. Centralised repositories

The International Mouse Strain Resource (www.findmice.org) is an umbrella organisation established to coordinate the archiving and dissemination activities of the public archiving centres, providing links to all public archives around the world. In the UK, information on GA strains held or archived by institutions within in-house facilities is accessible to investigators who register with the Mouse Locator Network (mouse-locator.crick.ac.uk).

4.2. UK Mouse Locator Network

The Mouse Locator Network (MLN) is a mechanism by which unique locally held or archived mouse strains can be identified and shared, reducing welfare concerns and costs associated with shipping mice long distances (Burgeon and Rosewell, 2003). The success of this email network relies on participation from all UK institutions with in-house archives of GA strains, as well as a comprehensive local searchable strain database at each institution, in order that strains can easily be located and that requesters have confidence in the technical details of the strain (e.g. the correct mutation, time since the last backcross). There are similar networks in other countries such as Compartir in Spain (Montoliu, 2009).

4.3. Transportation

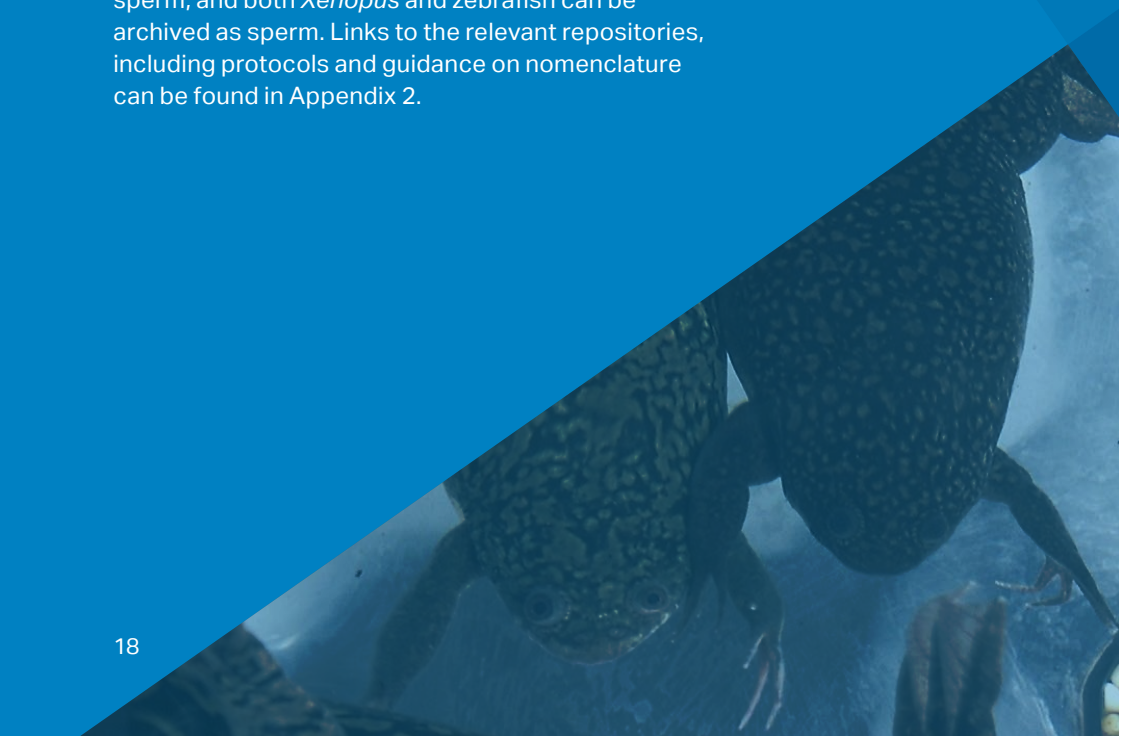
Wherever possible, GA strains should be distributed as fresh or cryopreserved embryos or gametes in order to avoid the transport of live mice, which has notable welfare concerns. Live mice may be subject to quarantine at the receiving institution to protect the health statuses of existing stocks and often require rederivation to ensure that any newly introduced strains are clean (e.g. specific pathogen free) prior to entry into the main housing areas of a facility. However, other factors do influence this, and the optimal means of transportation will also depend on how far the animals need to travel, the mode of transport and the competence of the institution to receive live, fresh or cryopreserved stocks.

If the transport of live mice cannot be avoided then they should only be carried by approved animal couriers, in accordance with LASA Guidelines for the Transport of Laboratory Animals (see Appendix 2).

5 | Other species

Although there is a focus on mice, this guidance applies to all common laboratory animal species and guidelines and repositories are available for rat, zebrafish and *Xenopus*.

It is possible to archive rats using embryos and sperm, and both *Xenopus* and zebrafish can be archived as sperm. Links to the relevant repositories, including protocols and guidance on nomenclature can be found in Appendix 2.



6 | Checklist for cryopreservation

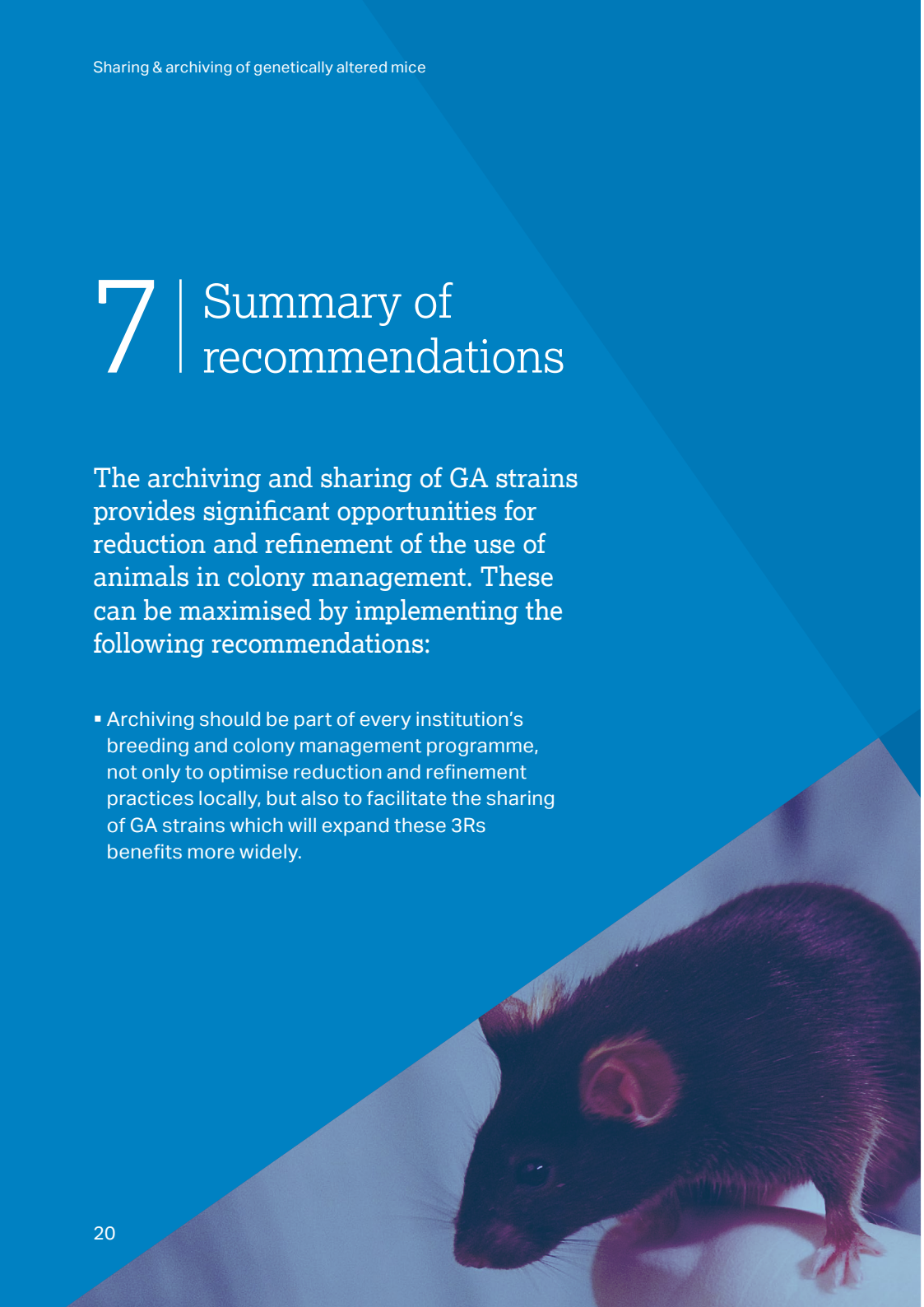
When cryopreserving and archiving a strain:

- Confirm the genetic modification(s) of the strain through specific genotyping assays and re-genotype the mice providing the gametes (e.g. males for sperm cryopreservation, both males and females for embryo cryopreservation) at the point of cryopreservation.
- Update the mouse passport to include the genetic background and generation details of the cryopreserved material, link to the relevant health screens, and detail the cryopreservation and thawing protocols.
- Quality control the cryopreserved material (e.g. culture to blastocyst stage, see www.nc3rs.org.uk/archiving).
- Store cryopreserved stocks at two or more locations (ideally different sites) for security.

7 | Summary of recommendations

The archiving and sharing of GA strains provides significant opportunities for reduction and refinement of the use of animals in colony management. These can be maximised by implementing the following recommendations:

- Archiving should be part of every institution's breeding and colony management programme, not only to optimise reduction and refinement practices locally, but also to facilitate the sharing of GA strains which will expand these 3Rs benefits more widely.



- Archive novel GA mouse strains as cryopreserved embryos and/or sperm in order to:
    - Safeguard stocks in the case of unplanned events.
    - Remove unused breeding stocks from the shelf (avoiding tick-over colonies).
    - Minimise genetic drift.
    - Facilitate sharing.
    - Avoid transportation of live animals.
    - Potentially minimise costs.
    - Archive GA stocks locally and/or centrally in an accessible repository.
- Maintain good records of archived stocks (see Appendix 1), and include:
    - An inventory of contents.
    - Information on the stocks (including quality control and cryopreservation protocols).
    - An updated mouse passport for each strain containing welfare and scientific information.
- Register stocks with MGI when appropriate.
- All institutions using and/or creating GA strains should be accessible through the UK Mouse Locator Network or local equivalent.
- Distribute GA strains as cryopreserved material when possible.

Appendices

Appendix 1: Information required when archiving or sharing GA mouse strains.

This table has been generated from information in the mouse passport guidelines (Wells *et al*., 2006) and the strain submission form for EMMA (see 'Submission form' at **bit.ly/3DQu0Xc**).

List of information required when archiving or sharing GA mouse strains

Category	Details to record
Genotype	Strain name (following www.informatics.jax.org/mgihome/nomen, plus local laboratory name).
Genotype	Gene(s) genetically altered and approach used (include MGI identifier for the gene(s) and allele(s) where possible).
Genotype	Current genetic background.
Genotype	Breeding history (number of generations backcrossed and/or number of sib-matings).
Genotype	Details of genetic alterations: ▪ Include method of creation (e.g. chromosomal anomaly, gene trap, induced mutation, spontaneous mutation, targeted mutation, endonuclease mediated, undefined). ▪ Also include affected gene/allele/transgene, ES cell line/mutagen/plasmid construct where relevant AND original genetic background (include the MGI identifier where possible).
Phenotype	Phenotype including welfare concerns and behavioural characteristics (e.g. aggression, maternal behaviour, also include advice to control welfare impacts).
Phenotype	Phenotypic description of mice homozygous for the genetic alteration.
Phenotype	Phenotypic description of mice heterozygous or hemizygous for the genetic alteration.
Breeding/Welfare	Viability of homozygous mice.

Category	Details to record
Breeding/Welfare	Fertility of homozygous, heterozygous or hemizygous mice.
Breeding/Welfare	State if homozygous matings are possible/required.
Breeding/Welfare	Breeding and reproduction details (breeding strategy, frequency and size of litters, mortality, breeding life span).
Breeding/Welfare	Husbandry requirements (e.g. diet, housing system, environmental conditions, enrichment).
Breeding/Welfare	State if mice are immunocompromised.
Breeding/Welfare	Details of phenotype or welfare concerns that may affect breeding.
Breeding/Welfare	Health status (current status when records are updated for archiving or sharing).
Characterisation	Details of the genotyping protocol (e.g. PCR protocol including primer and reaction details, or other method such as Southern blotting) for the strain.
Characterisation	Details of phenotyping (e.g. coat colour).
Additional	Reference to literature if the strain has been characterised and published.
Additional	MTA requirements.
Additional	IP rights or patents related to the strain.
Additional	Details of the original producer of the strain.

Appendix 2: Resources

Cited references

Assessing the welfare of genetically altered mice
Wells D J *et al.* (2006).
Laboratory Animals 40(2): 111–114.
www.doi.org/10.1258/002367706776318971

Mouse locator-UK: a networking tool for academic transgenic research in the UK
Burgeon L and Rosewell I (2003).
Transgenic Research 12(5): 637.
www.doi.org/10.1023/a:1025803132451

Compartir: a Spanish academic network initiative for sharing genetically modified mice
Montoliu L (2009).
Transgenic Research 18(6): 829–830.
www.doi.org/10.1007/s11248-009-9281-0

General resources

NC3Rs Archiving best practice
www.nc3rs.org.uk/archiving

Databases for GA mouse strains

International Mouse Strain Resource (IMSR)
www.findmice.org

Mouse Genome Informatics (MGI)
www.informatics.jax.org

European Mutant Mouse Archive (EMMA)
www.infrafrontier.eu

Mouse Locator Network (MLN)
mouse-locator.crick.ac.uk

Repositories for different species

Mouse

- RIKEN BioResource Research Center (RIKEN)
  mus.brc.riken.jp/en/deposit
- Canadian Mouse Mutant Repository (CMMR)
  www.cmmr.ca
- European Mutant Mouse Archive (EMMA)
  www.infrafrontier.eu
- Centre for Animal Resources Development (CARD)
  card.medic.kumamoto-u.ac.jp/card/english
- Mutant Mouse Resource and Research Centre (MMRRC)
  www.mmrrc.org
- MRC Harwell Archive
  archive.har.mrc.ac.uk/index

Rat

- Rat Resource & Research Centre (RRRC)
  www.rrrc.us
- National BioResource Project for the Rat (NBRP)
  www.anim.med.kyoto-u.ac.jp/nbr

Xenopus

- European *Xenopus* Resource Centre (EXRC)
  www.xenopusresource.org
- National *Xenopus* Resource (NXR)
  www.mbl.edu/xenopus
- National BioResource Project (NBRP)
  www.shigen.nig.ac.jp/xenopus/top.jsp

Zebrafish

- European Zebrafish Resource Center (EZRC)
  www.ezrc.kit.edu
- China Zebrafish Resource Center (CZRC)
  en.zfish.cn
- Zebrafish International Resource Center (ZIRC)
  www.zebrafish.org

Guidelines on nomenclature

FELASA guidelines for the production and nomenclature of transgenic rodents
bit.ly/3jVPzxN

Guidelines for nomenclature of genes, genetic markers, alleles, and mutations in mouse and rat
bit.ly/3tt4GSj

Zebrafish Information Network (ZFIN) Zebrafish Nomenclature Conventions
bit.ly/3jPuZz0

***Xenopus* Gene Nomenclature (XGNC) guidelines**
bit.ly/2X5LD4P

Cryopreservation protocols for different species

Cryopreservation protocols from EMMA
bit.ly/3tsHync

Cryopreservation and recovery protocols from CARD
card.medic.kumamoto-u.ac.jp/card/english

Zebrafish sperm cryopreservation and IVF protocols
www.zebrafish.org/wiki/protocols/cryo

***Xenopus* sperm cryopreservation protocol 1**
www.xenopusresource.org/using-frozen-sperm-4

***Xenopus* sperm cryopreservation protocol 2**
www.mbl.edu/xenopus/protocols

Cryopreservation training resources

Infrafrontier training courses
bit.ly/3yTxaG2

MRC Harwell Institute training courses
har.mrc.ac.uk/training/courses

Fish sperm cryopreservation and IVF training courses
www.ezrc.kit.edu/101.php

Guidance related to transport of animals

LASA Guidance on the Transport of Laboratory Animals
bit.ly/3l9j9il

LASA Guidelines for the Transport of Laboratory Animals – supplementary information for those transporting animals within or through the UK
bit.ly/3hddnel

Mouse passport guidelines
bit.ly/3ySnGe7

Acknowledgements

We gratefully acknowledge all members of the Breeding and Colony Management working group for their contributions to this document. We thank the RSPCA for the original version of this document and for their comments on this updated version.

This updated document has been endorsed by the contributors to the original version.

National Centre for the Replacement,
Refinement and Reduction of Animals in Research
Gibbs Building
215 Euston Road
London NW1 2BE

T +44 (0)20 7611 2233
F +44 (0)20 7611 2260
enquiries@nc3rs.org.uk
www.nc3rs.org.uk

Printed on stock containing 100% recycled fibre

November 2021