Sharing and archiving of genetically altered mice:

Opportunities for reduction and refinement

A report of the RSPCA Resource Sharing Working Group (RSWG)

Members of the RSWG

Nicola Osborne (Convenor)
Research Animals Department, RSPCA, Wilberforce Way, Southwater, West Sussex RH13 9RS (to whom all correspondence should be addressed)

Ian Jackson (Chair)
MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU

David Cox / Tony Peatfield
Medical Research Council, 20 Park Crescent, London W1B 1AL

Martin Fray
MRC Harwell, Frozen Embryo and Sperm Archive, Harwell Science and Innovation Campus, Oxfordshire OX11 0RD

Jane Hurst
Faculty of Veterinary Science, University of Liverpool, South Wirral CH64 7TE

Maggie Leggett
Formerly of the Biotechnology and Biological Sciences Research Council, Polaris House, North Star Avenue, Swindon SN2 1UH

Kathleen Mathers
MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA

Christine Nicol
School of Veterinary Science, University of Bristol, Langford House, Langford, Avon BS40 5DU

Vicky Robinson
National Centre for the Replacement, Refinement and Reduction of Animals in Research, 20 Park Crescent, London W1B 1AL

Ian Rosewell
Cancer Research UK, London Research Institute , Clare Hall Laboratories, Potters Bar, South Mimms, Hertfordshire EN6 3LD

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Introduction

The number of genetically altered (GA) mice used in scientific procedures within the UK and internationally has risen significantly over the last 15 years and continues to do so. 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 the application of the principles of the 3Rs.

With this in mind, the RSPCA set up a working group, in association with the MRC, BBSRC, and NC3Rs to discuss how archiving and sharing of GA lines can provide the opportunity for reduction and refinement. For the purposes of this report, archiving is defined as the storage of frozen (cryopreserved) mouse 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 the animals with other scientists. This report provides an overview of current 'best practice'; which will need to be reviewed and updated as scientific understanding and knowledge develops.

1. Why archive and share resources?

As more novel GA lines are produced, and their use becomes more widespread, the sharing of such animals and the use of archive facilities is becoming an increasingly important means of both reducing and refining animal use.

The benefits of archiving are four-fold. It:

1. enables reduction of animal use by minimising the number of GA lines maintained on the shelf
2. provides some insurance against loss of valuable stocks caused by adverse events such as environmental disasters, disease outbreaks, genetic drift, and breeding failure
3. facilitates the sharing of resources, which in itself provides more opportunity for reduction and minimises the need of researchers to replicate research, or reproduce resources
4. is a refinement avoiding the need for the live transportation of animals.

Archiving should be part of every establishment's GA breeding/colony management programme, not only to optimise 3Rs practices locally, but also to facilitate the sharing of GA lines which will expand these 3Rs benefits more widely. There might also be a financial saving, which, although it should not be the main factor, may enhance the overall benefit.

2. What to archive and when

The exact timing of what and when to archive will vary between research programmes and establishments, however this decision should be an integral part of the project planning process for all GA lines.

2.1 What to archive

Both embryos and sperm can be successfully cryopreserved and recovered, whilst ovarian tissue is not routinely frozen because it requires careful selection of the source animal and recipient.
The decision of whether to use embryos or sperm depends on a number of factors, including breeding performance. If there is a need to preserve the genomes of both parents (for example an inbred line or strain on a complex background), then it is essential to use embryos. However, in most cases, the preservation of only the male genome is sufficient and the development of sperm freezing protocols brings with it some significant advantages. Table 1 summarises the advantages and disadvantages of both approaches.

Table 1

Cryopreservation of embryos	Cryopreservation of sperm
Pros	**Pros**
Well established protocols published and in use across many centres.	Simple, fast and cheap freeze technique that requires no special equipment and little training.
Recovery, or rederivation is more straightforward and less time consuming than with frozen sperm.	Recovery becoming easier with advances in freezing, IVF and ICSI techniques.
Dissemination of stocks is relatively easy.	
Viability testing of frozen embryos can be easily performed prior to culling the remaining animals.	Requires fewer animals to freeze down sufficient stocks than embryo freezing.
Success is not particularly strain dependent.	
Cons	**Cons**
Requires significantly more animals to freeze down sufficient stocks compared with sperm freezing.	Recovery or rederivation requires IVF and so uses more animals than recovery from frozen embryos.
Typically requires specialised equipment and skilled personnel.	Protocols are less well established.
	Success is strain dependent.
	Viability testing of frozen sperm requires recovery by IVF before embryo transfer and so uses more animals than embryo freezing.

Note: The cryopreservation of ovarian tissue is not routinely conducted because of the advantages associated with freezing sperm and embryos. However, the archiving of ovarian tissue remains an option when a GA line is a poor breeder, has a short lifespan, or is likely to be of limited usefulness or demand, and this option makes best use of stock animals that are available.

Deciding what to archive will depend upon whether it is a new GA line or an established line, as well as on local facilities and the availability of particular expertise.

For new GA lines:

- wherever practical, all novel or scientifically interesting GA lines should be cryopreserved
- when space is limited all lines showing novel phenotypes, and likely to be in demand by other users, should be cryopreserved either in-house, or using facilities such as FESA (see 3.1)
- tissue and DNA samples should be archived. These can be used for quality control and comparison on subsequent rederivation of the line. In addition, they may be useful for addressing particular questions before, or instead of, rederivations.

For established GA lines:

- any line with specific animal welfare concerns should be prioritised for archiving
- scientific objectives should be regularly reviewed so that GA lines that are presently not required, or are subject to sporadic use, can be archived rather that maintained as 'tick-over' colonies.

2.2 When to archive

Ultimately, all GA lines should be cryopreserved and available to others following publication in the scientific literature. Archiving should be a routine part of scientific programmes that produce GA animals.

For existing GA lines, the health status of the animals should not be seen as an obstacle to archiving, but may be an additional factor to consider. In fact the need to transfer animals from a non-specific pathogen free unit into another specific pathogen free (SPF) building or establishment can provide an ideal opportunity to archive a stock and rederive the line to improve the health status of the colony.

3. How to archive

In the UK there are two main options: to archive in-house or, where local expertise does not permit, to use a centralised facility such as FESA (see below). In-house facilities will require specific staff training (see 3.2.1) and sufficient local demand to ensure that frozen embryos and gametes are of high enough quality to permit the consistent recovery of live born offspring. To facilitate sharing when a GA line is of wide interest and is, or is likely to be, in demand by other users, embryos and gametes archived locally should also be deposited in a central storage and distribution facility, such as FESA, or equivalent elsewhere. It may also be prudent to archive especially valuable or significant lines in FESA in addition to local storage, to serve as a contingency.

3.1 FESA

At present, there is only one publicly funded (not-for-profit) archive facility in the UK – the Frozen Embryo and Sperm Archive (FESA) based at MRC Harwell. FESA, acts as the UK node for the European Mouse Mutant Archive (EMMA – www.emmanet.org). It offers free embryo and sperm preservation of valuable mutant and transgenic mouse lines with long-term storage facilities, on condition that archived lines can be made available to the scientific community for research. FESA is able, where appropriate, to restrict access to GA lines for up to two years to protect the depositors' research and to encourage deposition. Further information on submitting lines to FESA for archiving and dissemination is available at: www.har.mrc.ac.uk/services/fesa/fesa_guidelines

3.2 In-house

Many establishments generating and breeding GA lines have in-house archiving facilities. There are several important factors to consider when establishing new facilities to ensure that the archive is effectively managed These include specific staff training and record keeping requirements.

3.2.1 Staff training

Staff who are competent and well trained will use the minimum number of animals for archiving and rederivation procedures, and minimise the potential for pain, suffering and distress. When setting up archiving facilities, staff need to receive appropriate training to enable them to develop the range of technical skills required to successfully create, cryopreserve, rederive and care for GA lines. Many establishments that routinely archive GA lines can act as centres of excellence for training, but there are also a number of relevant courses available. One such course is the Mouse Embryo and Spermatozoa Cryopreservation Course organised by FESA at Harwell. Further information is available at: www.har.mrc.ac.uk/services/fesa/training.html

3.2.2 Record keeping

All archive facilities need a good inventory so that stored material is easily accessed to enable both recovery of the animals and sharing with other users. The inventory, ideally, should include basic information for each line such as what tissues have been frozen, as well as technical information such as how the GA line was made (construct details, what ES cells or blastocysts were used), and what genetic background the line is on. A complete record will also contain information such as the source colony health status at the time of freezing and any local establishment names for the GA line.
To avoid confusion, the GA line should also be named according to standardised nomenclature. The International Committee on Standardised Genetic Nomenclature for Mice, and the Rat Genome and Nomenclature Committee have been set up to regularly review and update the respective naming systems. Further information and advice is available at: www.informatics.jax.org/mgihome/nomen/index.shtml

Records for each GA line should also include the information appropriate for creating a 'mouse passport' (Wells et al., 2006), for example: the number of back crosses or generations bred; husbandry or welfare refinements; breeding recommendations and phenotype information. This is vital scientific and welfare information for when the line is rederived.

4. How to share

Few would argue that the products of publicly funded research, such as GA lines, should not be accessible to the wider scientific community. However, whilst archiving is relatively straightforward, there are both real and perceived barriers to sharing. Some of these are resource driven, such as the staff time, the cost implications of maintaining and disseminating frozen stocks in-house, and the need to keep records and databases up to date.

A perceived barrier is the need to retain intellectual property (IP) rights. When archives are in-house, IP rights are readily managed through appropriate Material Transfer Agreements (MTAs). The same protection can also be maintained when using centralised facilities such as FESA. These centralised services ensure that fully executed copies of the depositors MTAs are exchanged before a line is distributed. This mechanism operates whether or not a line 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 several steps to ensure that their GA lines are quickly and easily accessible. This includes registering GA lines with free online searchable databases such as the International Mutant Strain Resource (IMSR – www.informatics.jax.org/imsr/index.jsp), which is also listed by the Federation of International Mouse Resources (FIMRe – www.fimre.org). FIMRe is an umbrella organisation set up to help coordinate the archiving/dissemination activities of the public archiving centres and it provides links to all public archives around the world. In addition, information on GA lines held or archived by UK establishments within in-house facilities should be accessible through the UK Mouse Locator Network (http://bioinformatics.cancerresearchuk.org/mouse_locator/mouse_locator.html).

4.1 UK Mouse Locator Network

The Mouse Locator Network (MLN) is a mechanism by which locally held or archived mouse lines can be identified and shared (Burgeon and Rosewell, 2003). It is an e-mail network to which requests for GA lines are posted and disseminated. Since there are a large number of UK establishments using and creating GA lines, it is vital that all in-house archives are connected and represented on the UK MLN. For the network to function effectively, each establishment needs to have a representative(s) who can quickly and easily identify whether the establishment has the GA line of interest, and correspond promptly with the requester. It is also essential that all establishments that create or use GA mice have a local searchable database/list of the GA lines present within the facility and/or archived. For further information about the MLN e-mail: locator@cancer.org.uk or contact Ian Rosewell (ian.rosewell@cancer.org.uk) or Laurence Bugeon (l.bugeon@imperial.ac.uk).

4.2 Transportation

Wherever possible, GA lines should be distributed as fresh or frozen embryos or gametes in order to avoid the welfare problems associated with the transport of live mice. However, other factors do have a bearing on this, and the optimal means of transport will also depend on how far the animals need to travel, the mode of transport and the competence of the establishment to receive live, fresh or frozen stocks. If the transportation of live mice cannot be avoided then they should only be carried by approved animal couriers, in accordance with LASA guidelines (2005) and the latest IATA live animal regulations.

5. Summary of recommendations

The archiving and sharing of GA lines provide significant opportunities for reduction and refinement. These can be maximised by implementing the following recommendations.

- Archiving should be part of every establishment's breeding/colony management programme, not only to optimise reduction and refinement practices locally, but also to facilitate the sharing of GA lines which will expand these 3Rs benefits more widely.
- Novel GA mouse lines should be archived as frozen embryos and/or sperm in order to:
  - safeguard stocks in the event of unplanned events
  - remove breeding of unused stocks from the shelf
  - facilitate sharing
  - potentially minimise costs.
- GA stocks should be archived locally and/or centrally in an accessible repository.
- Good records of archived stocks should be maintained, and include:
  - an inventory of contents
  - information on the stocks
  - a passport for each stock containing welfare and scientific data.
- Stocks should be registered with International Mutant Strain Resource (IMSR), where feasible and appropriate.
- All establishments using and/or creating GA lines should be accessible through the Mouse Locator Network.
- GA lines should be distributed as frozen material when possible.

Appendix 1 - Useful resources

Archive facilities

- **FESA:** www.har.mrc.ac.uk/services/fesa
- **EMMA:** www.emmanet.org

Mouse passports

- **Wells et al.** (2006). Assessing the welfare of genetically altered mice. Laboratory Animals 40, (2) 111–114.

Nomenclature

- **FELASA (2007).** Guidelines for the production and nomenclature of transgenic rodents. Laboratory Animals 41, 301–311.
- **Rules and guidelines:** www.informatics.jax.org/mgihome/nomen/index.shtml

Online databases

- **FESA:** www.har.mrc.ac.uk/mousebook
- **EMMA:** www.emmanet.org
- **IMSR:** www.informatics.jax.org/imsr/index.jsp
- **UK Mouse Locator Network**
  http://bioinformatics.cancerresearchuk.org/mouse_locator/mouse_locator.html
  Burgeon and Rosewell (2003). Mouse locator-UK: a networking tool for academic transgenic research in the UK. Transgenic Research 12, 637.

Protocols

- Robinson et al. (2003). Refinement and reduction in production of genetically modified mice – sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Laboratory Animals 37, Suppl 1.

Training

- Mouse Embryo and Spermatozoa Cryopreservation Course (FESA)
  www.har.mrc.ac.uk/services/fesa/training.html

Transport

- LASA (2005). Guidelines for the care of Laboratory Animals in Transit. Laboratory Animals 39, 1–39.
- LASA Guidelines for the Transport of Laboratory Animals – supplementary information for those transporting animals within or through the UK available at: www.lasa.co.uk/position_papers/publications.asp
- IATA Live Animal Regulations are available at:
  www.iata.org/ps/publications/lar.htm

National Centre for the Replacement, Refinement
and Reduction of Animals in Research

Research Animals Department, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex RH13 9RS
Tel: 0300 1234 555 www.rspca.org.uk/sciencegroup