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RESEARCH ARTICLE

Why use a biobank? Sharing mutations, gene editing and navigating the COVID-19 pandemic

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ABSTRACT

Cryopreservation is seen as a key aspect of good colony management, which supports the drive towards improvements in animal care and the implementation of the 3Rs. However, following the advent of gene editing technologies, the generation of new rat and mouse models is quicker and cheaper than ever before. This has led some to question the future value of biobanks around the world. In the following commentary we argue that the need to cryopreserve rat and mouse strains and distribute them from well-funded repositories is as strong as it has ever been.

Repositories should not be considered as simply collections of redundant model organisms. Biobanks have a vital role to play in good experimental design. They distribute identical, quality controlled mutant strains to the community and eliminate the need to recreate mice. Archived material provides a check point in the development of new strains of rodents that minimises genetic drift and breeding failures. Cryopreservation also makes resource sharing easier and cheaper, and improves animal care by eliminating the need for live animal shipments. Furthermore, routine cryopreservation of valuable strains protects them from unforeseen events, such as the SARS and COVID-19 pandemics, which were accompanied by the very real prospect of immediate lab closures and/or severe disruption to courier services, rendering live animal shipments non-viable.

Introduction

From the latter part of the 20th century to the present-day tens of thousands of genetically altered rats and mice have been generated to study mammalian genetics and the causes of human disease. This number continues to grow in the wake of the gene-editing revolution and large-scale gene knockout programmes, such as the International Mouse Phenotyping Consortium, described by Brown et al[1]. Researchers who had no previous experience of working with mice are now able to obtain sophisticated models and even make them with relative ease using gene editing or similar technologies. Researchers that make or otherwise obtain genetically altered (GA) mice will invariably not have the desire or resources to keep them indefinitely as breeding colonies. Even when colonies are maintained for extended periods they will undergo genetic drift. Lynch et al[2] estimated that ~100 mutations arise in every generation. Thus, even after a few generations a line will be genetically distinct from the original parents unless carefully backcrossed onto animals maintained under a genetic stability programme. In addition, rodents may pick up pathogens, be subject to breeding failure or be inadvertently contaminated with the wrong genetic background, the end result being that animals studied from later generations may be very different from the ones that were described when they were originally generated. This problem may be compounded by sharing mutant strains with colleagues who have little experience of working with animals. Without diligent colony management, if left unchecked any one of these scenarios would contribute to the non-reproducibility of biomedical data, which is estimated to cost $28B annually in the USA alone, as reported by Baker[3] and Freedman et al[4]. A practical solution to these problems is to keep meticulous breeding records and to cryopreserve all rat and mouse strains as early as possible in their history.

Rodent biobanks have existed for over 50 years but the principles they work under have not changed a great deal in the intervening years. Biobanks are still important to the biomedical community. In fact, the COVID-19 pandemic proved just how important they were as labs scrambled to freeze down the models they were working on. Furthermore, during this period the safest way to exchange rodent strains was as frozen materials because the courier networks could not reliably support live-animal shipments.

Since the 1970s it has been possible to cryopreserve mouse and rat embryos and, more recently, robust generic procedures have been developed for sperm freezing and *in vitro* fertilisation (IVF) recovery. These technologies are widely available to the scientific community through bespoke laboratories set up by institutions to meet their local needs, as well as public repositories such as the National BioResource Project for the Rat in Japan (NBRP-Rat: www.anim.med.kyoto-u.ac.jp/NBR/), the Rat Resource & Research Center (RRRC: www.rrrc.us), the European Mutant Mouse Archive (EMMA: www.infrafrontier.eu) and the Mutant Mouse Resource & Research Centers (MMRRC: www.mmrrc.org).

Archiving provides the opportunity for a genetic anchor point, allowing researchers to return to defined materials. Obvious genetic anchor points could include: a) the point of production; b) the point at which the mutation is crossed onto a new background; or c) publication of the mutation or model. Ideally, sufficient material would be frozen from the same generation to meet expected future demand. Another issue that drives the use of biobanks is the need to find alternatives to long-distance shipments of live animals, particularly over international borders. Many biobanks have taken the decision to promote the exchange of embryos/germplasm over and above live-animal shipments. As a consequence, repositories are seeing a steady fall in the percentage of strains being sent out as live animals.

In this article we put forward an argument in support of the continued cryopreservation of mutant rats and mice in the gene-editing era. The need to share resources, as well as to ensure reproducibility, are issues that are as relevant to CRISPR lines as they are for mutant strains created using more traditional techniques.

Archiving in the Current Era

Gene manipulation through CRISPR/Cas9 mediated gene editing has proven itself to be reproducible in any laboratory that has access to a competent molecular biologist and a skilled microinjection technician. Gene editing has the potential to generate a whole host of coisogenic mutations, on any selected background, thus eliminating the need for extensive backcrossing. This versatility makes gene-editing technologies enormously attractive to biologists.

Unravelling this complex genetic landscape requires extensive quality control (QC) analysis of F1 animals in order to confirm which alleles they carry, (Codner et al[5]; Mianné et al[6];). This expertise may not be easily accessible to all laboratories that use gene-editing technologies and valuable resources will be wasted if the animals are not fully characterised before use. However, it is not common

practice to archive the F0 founders because they are often mosaic and carry a uniquely complex set of genome modifications (Yen et al[7]). Genotyping and sequencing these animals will provide an indication of the genotypes to be expected in their offspring and on occasion it may be appropriate to cryopreserve a valuable F0 founder, such as a male that is predicted to transmit an allele of interest and has become unwell, or that has failed to breed successfully. To avoid confusion later on, it is advisable to store the F0 samples using a distinct coding system, away from any well-characterised material derived from the same project that may be frozen for long-term storage.

It is likely that pedigrees arising from gene-editing projects will inherit unique mutant profiles, in addition to the original objective. Recognising the potential value of these bystander alleles is important because they may provide useful information on gene function when studied as part of an allelic series. When it is practical to do so, germplasm/embryos from these pedigrees should be cryopreserved as distinct mutant strains. Occasionally, two F0 founders will give rise to exactly the same mutation. If this happens, it is again advisable to cryopreserve the progeny as two separate stocks because some off-target effects may have also been generated, as was reported by Iyer et al[8]. Such off-target events will be specific to the individual founder, making the lines genetically distinct.

Strain Descriptions and Nomenclature

The importance of accurate sample records cannot be overemphasised. This extends to ownership, conditions of use, and licensing conditions, all of which when handled properly actually facilitate, rather than hinder, sharing between collaborators. But most of all it is critical to accurately record strain and allele nomenclature, a point nicely summed up by Taft et al[9], who commented that if researchers are to generate high-quality science using mouse models they need to take care to 'Know Thy Mouse'. Whether the alleles are described in a publication, a presentation, or on a website, the nomenclature used should accurately reflect the naming conventions laid down by the International Committee on Standardized Genetic Nomenclature for Mice (www.informatics.jax.org/mgihome/nomen/index.shtml). This allows investigators to clearly distinguish between different alleles and to understand the relationship between those alleles and their genetic background. An example of a common error is the confusion between C57BL/6N and C57BL/6J. These mice have a similar appearance but they have been separated by nearly 70 years of breeding and have accumulated numerous mutations that have a profound effect on their phenotypes (Simon et al[10]).

Once characterised, new strains should be registered with the appropriate reference resource; e.g., the international database resource for the laboratory mouse, Mouse Genome Informatics (MGI: https://www.informatics.jax.org/) or the Rat Genome Database (RGD: www.rgd.mcw.edu/). Although often seen as a difficult area to navigate, the nomenclature rules are quite logical and the MGI/RGD staff are always on hand to provide advice, when necessary. Furthermore, allele names can be registered well in advance of publication and kept private until required.

In brief, CRISPR alleles are named after the relevant targeted gene followed by the superscript 'em' to signify that the allele is an endonuclease-mediated mutation. A serial number, and the laboratory code indicating where the allele was made, are also included: e.g., Tlr2^{em2H}.

The specific details of individual mutations will be captured when the allele is registered with the reference laboratory and is assigned a unique MGI or RGD identification number. Strain descriptions are captured in a similar way and, where appropriate, will include details of the genetic background used for the initial targeting and subsequent crossing experiments, plus a 'backslash' and the laboratory code indicating which repository the strain can be obtained from: e.g., C57BL/6NTac-Tlr2^{em2H}/H.

Consistency in naming across the different media formats will ensure that the correct allele details are presented to the community and that the same allele is distributed to all end users.

Approaches to Cryopreservation

Embryo freezing, such as that described in Whittingham et al[11] and Wilmut[12] has been a standard laboratory technique since the 1970s, although sperm freezing, historically, has not been so reliable. However, advances in technology mean that simple and robust methods are now available to freeze mouse and rat sperm and to perform IVF recoveries using sperm collected from all common genetic backgrounds (Takeo and Nakagata[13]; Takeo et al[14]). Details of these protocols are freely available on many repository websites; e.g., www.rrrc.us/Protocols/, https://www.infrafrontier.eu/ and www.card.medic.kumamoto-u.ac.jp and are easy to follow. What is more, many of the reagents can be either made in house or purchased commercially.

Embryo freezing is still the method of choice when freezing complex strains, such as those carrying multiple mutations, for which access to homozygous embryos will eliminate the need for extensive breeding to recreate the model. However, embryo freezing is generally more time consuming and expensive, and uses more animals than sperm freezing. For these reasons, embryo freezing has, for the most part, been superseded by sperm freezing in many central repositories.

Sufficient sperm can be harvested from a single male to provide 8–10 aliquots. Although it is advisable to freeze sperm from multiple males in order to safeguard specific stocks, freezing of sperm from individual males can be beneficial. For example, freezing sperm from single males can be used to fix intermediate crosses when developing a line as an insurance against loss. This may be of particular relevance for models generated by genome editing, in which multiple on-target mutations can be generated from a single experiment. Rather than discard potentially valuable alleles, sperm from mice with bystander mutations can be quickly banked at minimal impact, as there is little benefit in performing extensive QC checks on sperm frozen from such mice.

Regardless of whether embryo- or sperm-freezing methods are being used, the same degree of diligence must be employed when archiving principal, well-characterised on-target mutations. Sufficient material needs to be frozen to guarantee that the mutation can be recovered at a later date. This dictates that embryos and/or sperm are harvested from multiple animals and carefully controlled for quality. A reserve of 200, or more embryos split between ~10 straws/vials will provide sufficient material to protect the mutant line and to share with numerous collaborators. Similarly, freezing sperm from 2–5 males will provide sufficient material for between 16 and 50 recovery attempts. Embryos and sperm can be pooled before freezing, ensuring that a single QC test will confirm the ability to recover the mutation from any sample.

Will You Be Able to Recovery the Model of Interest?

Cryopreservation allows stringent QC systems to be established, thus ensuring the integrity and longevity of the material available for retrieval and distribution. Whether CRISPR alleles are being cryopreserved as bespoke projects or as part of large-scale programmes, as is the case for the International Mouse Phenotyping Consortium (IMPC: www.mousephenotype.org/), it is advantageous to re-genotype animals at the point of archiving. This is easily achieved with GA animals from which post-mortem tissue samples can be collected for genotyping at the time of embryo and sperm harvesting. This is a simple precaution, which ensures that the animals used for cryopreservation carry the expected allele/s and that no error occurred during selection. The use of generic assays; e.g., for LacZ/Cre, may be appropriate for some models but care should be taken when interpreting the data in case the reporter has become uncoupled from the targeted sequence. Generic assays, therefore, may not provide complete verification of a strain's identity.

Material that has been frozen in order to provide long-term protection for a valuable model should always be subject to a viability test to confirm that the mutation can be recovered. Ideally, these viability tests should be performed before the colony is removed from the shelf. The test itself may take the form of thawing an aliquot of embryos, culturing them to the blastocyst stage and genotyping them (Scavizzi et al[15]). Alternatively, the embryos can be transferred into recipient females, followed by characterisation of the progeny. The same principles apply to frozen sperm, with the exception that an IVF recovery will need to be performed first. Data collected from the viability tests can be used to calculate the number of mutant animals that could be recovered from the frozen resource and a decision can be made as to whether or not more material needs to be frozen.

Despite the lengths biobanks go to in order to QC the materials they export, the recipient scientists also need to play their part. And it is still advisable for recipient scientists to take independent steps to ensure the materials they import will perform in their studies as they expect them to, as reported by Birling et al[16] and Gofflot et al[17].

Resource Sharing and Repositories

Repositories have been likened to a bank. To quote Lloyd et al[18] "Just as a bank makes returns on investments, repositories add scientific value and utility to deposited mouse lines: they increase reliability through curation, preservation, genetic QC, protection from pathogens and more." In recognition of this truism, publishing houses and funding bodies are encouraging investigators to use public repositories and to share their models freely with the wider scientific community. This should be done as a matter of principle; sharing resources not only fosters an environment of cooperation and collaboration but also conserves resources. Rats and mice cost money to generate, maintain and characterise and every effort should be made to

minimise the need to remake mutant strains. On top of this, the cost of maintaining a colony that is not part of an active research programme could easily cost several thousand pounds per year, in addition to the time spent overseeing the colony. These costs will accumulate year-on-year and will quickly dwarf the investment in the occasional recovery from liquid nitrogen.

There are numerous options open to researchers who have developed a GA strain to the point where full archiving is appropriate. At this stage the researcher could use a commercial service or their own in-house cryo-laboratory, if one is available. Both of these options will be accompanied by service fees. A cost-efficient alternative would be to offer the lines to one of the centrally funded public repositories which offers a 'free-of-charge' archiving service.

Sharing rat and mouse lines in this way has given the scientific community open access to a huge resource via a global network of large, securely funded biobanks, such as the RRRC(www.rrrc.us), NBRP-Rat (www.anim.med.kyoto-u.ac.jp/NBR/), EMMA (https://www.infrafrontier.eu/), The Jackson Laboratory (JAX: https://www.jax.org/), MMRRC (https://www.mmrrc.org/), The Institute of Resource Development and Analysis Center for Animal Resources and Development (CARD: www.card.medic.kumamoto-u.ac.jp/card/), the Canadian Mouse Mutant Repository (CMMR: http://www.cmmr.ca/) the Australian Phenomics Network (APN: www.australianphenomics.org.au), and the RIKEN BioResource Research Center (RIKEN-BRC: www.web.brc.riken.jp/en/). These repositories can offer a pre-publication 'grace' period (usually two years) to encourage early deposition of mutant strains, during which time details of the mice are not made public. Repositories also ensure that the resources they hold are fully credited to the depositors and they only release QC-verified materials. This QC verification extends to the release of genotype-confirmed animals on a defined genetic background.

Many laboratories do not have individuals with the skills to handle frozen embryos/ germplasm, and biobanks can and do step in to provide a rederivation service for their local communities. Custom repatriation and rederivation of strains gives investigators access to the many 1,000s of valuable strains held in laboratories and biobanks around the world. This, in turn, helps to reduce the number of live-animal shipments. Searching of these public resources is simplified by resources such as the International Mouse Strain Resource (IMSR: www.findmice.org). This is an umbrella organisation with a database that displays the strain details of mice held in public repositories, as well as some that are held in large breeding houses and individual laboratories.

It should be noted that, if the community is expected to embrace the use of central biobanks, it is imperative that the repositories continue to work together to improve their services and to ensure that turnaround times are acceptable. Once all the documentation is in place, it is quite possible to dispatch frozen materials or animals (taken from breeding colonies) within 2–3 weeks. But it may take 3–4 months to deliver strains that need to be recovered from biobanks. These timescales compare very favourably with the generation/characterisation of INDELS using CRISPR/Cas9 but are considerably shorter than the average time needed to generate, and QC verify more complex alleles. However, where the system often falls down is the process of signing off on all the documentation, including material transfer agreements and purchase orders. Here, all parties can assist the transactions by processing the necessary documentation promptly.

Biobanks Don't Just Preserve Germplasm

Well-funded, well-resourced biobanks can offer additional benefits to the scientific community. Specific training in husbandry, colony management, model generation and cryopreservation is often provided by biobanks who have a vast amount of experience at their disposal and are only too happy to share it.

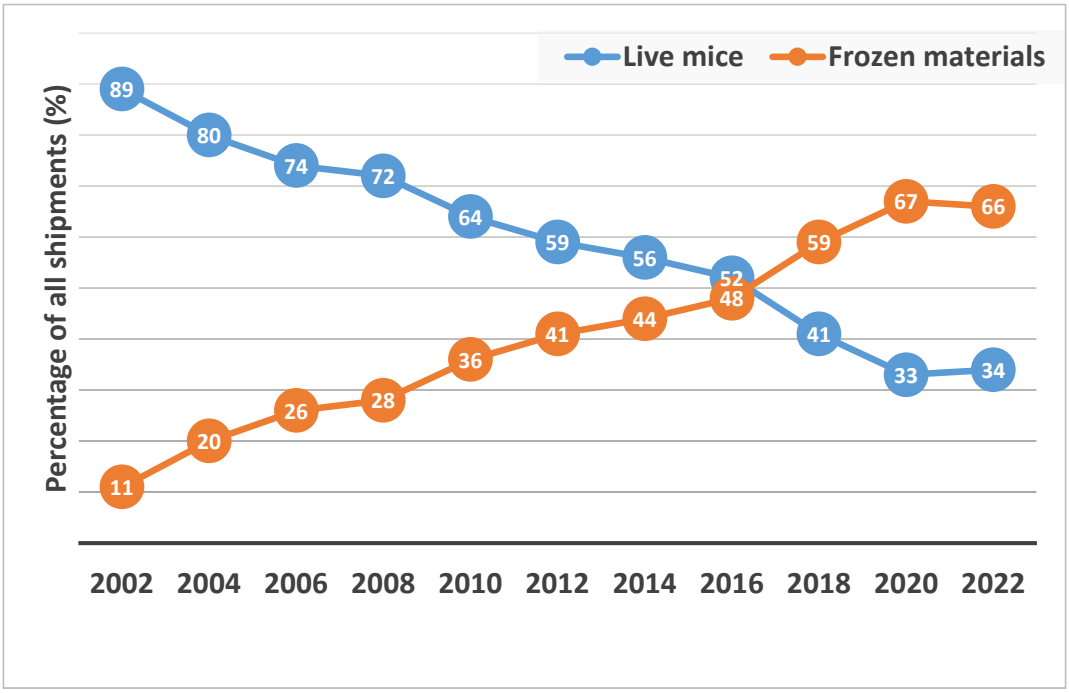

Figure 1. Data from the European Mouse Mutant Archive showing the percentage reduction in live animal shipments from 2002 to 2022.

The resources commonly available within biobanks facilitates *in vitro* recombination of alleles flanked by LoxP or Frt sites. This is easily achieved by treating IVF-derived 2-cell embryos with a cell-permeable Cre enzyme, as reported by Ryder et al[19] or by injecting FlpO RNA into zygotes (unpublished). These simple procedures eliminate the need to segregate the recombinase genes when using global deleter strains that constitutively express recombinase proteins. An added advantage is that these simple techniques eliminate the risk of contaminating models by crossing them with animals on poorly defined genetic backgrounds.

Biobanks also have the capacity to run technology development programmes geared towards applied, rather than discovery, science. In this regard, embryo and sperm cryopreservation procedures have been improved, IVF techniques refined, and more options for shipping embryos and germplasm developed. Examples of this are the use of organ culture media to exchange unfrozen epididymides, and the shipment of sperm on dry ice, which is cheaper than using conventional shippers charged with liquid nitrogen, each of which is helping to lead the community away from its dependence on live-animal shipments.

The trend away from live-animal shipments is illustrated by the data supplied by EMMA[20] (Fig 1). However, when the data are examined in more detail it is clear that there are other forces at play. For example, the distribution data from the National Mouse Archive at MRC-Harwell (EMMA's UK node) it is clear that the COVID-19 pandemic was associated with an accelerated decline in the percentage of mouse strains being shipped as live animals (Fig 2). This is likely to be, in part, due to the repository's ability to handle frozen materials when live animal shipments were impractical, if not impossible. Clearly, the number of live-animal shipments could be reduced still further but the data from the EMMA consortium and MRC-Harwell show that the trend is in the right direction.

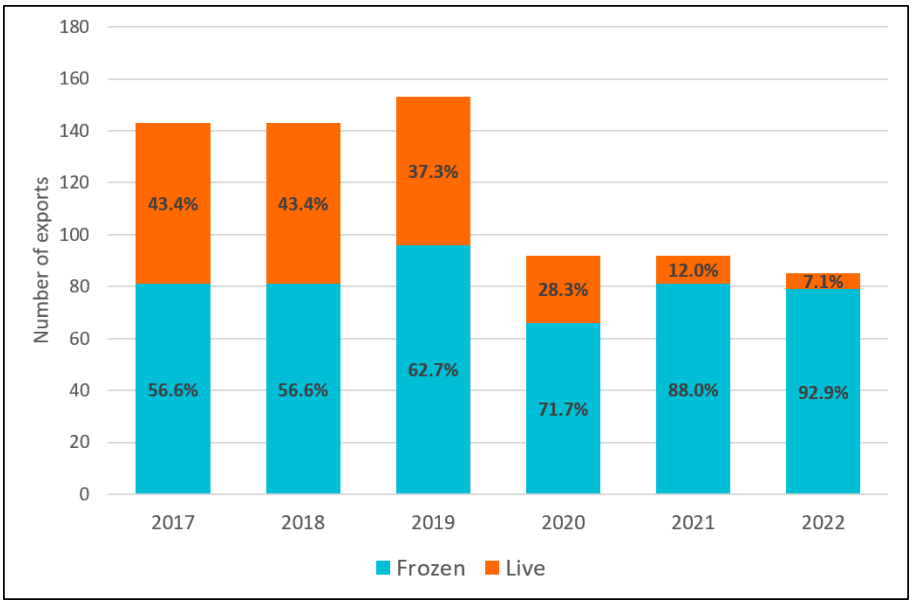

Figure 2. Data showing the percentage reduction in live-animal shipments for the United Kingdom's National Mouse Archive between 2017 and 2022.

Conclusion

This article was written to address the question, 'why do we need biobanks when genetic alterations can be introduced easily using gene-editing techniques.' In this article we have presented an argument for continuing to use biobanks and have highlighted their numerous advantages to the scientific community. Such advantages include the ability to secure GA strains against loss, to minimise genetic drift, and to provide a treasure trove of resources that enables others to build on original findings by returning to genetically defined material that was frozen at specific check points. Added to this, biobanks engage in technology development, help to reduce the number of animals used in research and reinforce our 3Rs credentials by prioritising the shipment of embryos and germplasm.

The relative ease with which gene-editing techniques can be used to generate new and interesting GA mice can be dwarfed by the complexity of the downstream processes required to accurately verify that a defined event has occurred. This alone keeps the pendulum swung firmly in favour of cryopreserving rat and mouse models in the post-CRISPR era. Coupled with this is the imperative to share resources with collaborating scientists who need to work on material that closely reflect the model that was original published. If the community is to address non-reproducibility of biomedical studies, it certainly needs to avoid using surrogate models that originate from a secondary targeting experiment simply because the original model has been lost or is otherwise unavailable. This approach is likely to introduce genetic variability, which will confound experimental findings.

However, if repositories are to fully realise their potential to support the biomedical community, they need to encourage open access to resources, stream line the deposition of new strains and process requests promptly. Efficient archives reduce animal usage, eliminate the need to recreate pre-existing strains and advance research by allowing investigators to use their resources for discovery science rather than model replication.

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