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Genome Resource Banking of Biomedically Important Laboratory Animals

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Abstract

Genome resource banking (GRB) is the systematic collection, storage, and re-distribution of biomaterials in an organized, logistical, and secure manner. Genome cyrobanks usually contain biomaterials and associated genomic information essential for progression of biomedicine, human health, and research. In that regard, appropriate genome cryobanks could provide essential biomaterials for both current and future research projects in the form of various cell types and tissues, including sperm, oocytes, embryos, embryonic or adult stem cells, induced pluripotent stem cells, and gonadal tissues. In addition to cryobanked germplasm, cryobanking of DNA, serum, blood products, and tissues from scientifically, economically and ecologically important species has become a common practice. For revitalization of the whole organism, cryopreserved germplasm in conjunction with assisted reproductive technologies (ART), offer a powerful approach for research model management, as well as assisting in animal production for agriculture, conservation, and human reproductive medicine. Recently, many developed and developing countries have allocated substantial resources to establish genome resources banks which are responsible for safeguarding scientifically, economically and ecologically important wild type, mutant and transgenic plants, fish, and local livestock breeds, as well as wildlife species. This review is dedicated to the memory of Dr. John K. Critser, who had made profound contributions to the science of cryobiology and establishment of genome research and resources centers for mice, rats and swine. Emphasis will be given to application of GRBs to species with substantial contributions to the advancement of biomedicine and human health.

Keywords

Genome resources; Cryopreservation; Animal models

1. Introduction

During the past three decades, there have been profound advances in development of assisted reproductive technologies (ART), including superovulation, embryo transfer (ET), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and *in vitro* embryo culture. Furthermore, cryopreserved gametes (*i.e.* sperm, oocytes), embryos and gonadal tissues (*i.e.* ovarian tissue) have become an integral part of ART [1,2]. Furthermore,

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scientists have used powerful genomic tools to manipulate the genome in a targeted and predictable fashion to create genetically modified (GM) laboratory animal models (*e.g.* mouse, rat, pig, drosophila, and zebrafish) using techniques such as pronuclear DNA microinjection, viral gene delivery [3,4], homologous recombination in embryonic stem (ES) cells [5], ethylnitrosourea (ENU) [6], and somatic cells nuclear transfer (SCNT) [7]. Furthermore, ART, in combination with cellular and genomic tools, have been widely used for the creation of GM animals (transgenics and knockouts) which have made profound contributions to progress in biomedical research, in studies ranging from basic gene function analysis to comparative studies of numerous human disorders. Most recently, Zinc Finger Nuclease (ZFN) technology [8-11], and transcription activator-like effector nucleases (TALEN) technology have collectively provided a solid foundation for easy and efficient production of GM laboratory animal models to study human diseases [12-14]. To date, thousands of new mutant strains of mice, rats, zebrafish and several of swine, non-human primate models, have been developed [15-20].

The availability of the human genome sequence has created an unprecedented need for relevant animal models to identify gene functions and to test new therapeutic strategies aimed at alleviating human disease. In addition, the genomes of several animal species, including the mouse, rat, cat, dog, swine, rhesus monkey, and zebrafish, have been sequenced. To this end, GRB for these animal models have comparative advantages for their optimal utility by the biomedical community. Germplasm cryobanking has been successfully used in almost all livestock species, including those in the families *bovidae*, *suidae*, and *phasianidae*, but with varying degree of success [21,22]. Initial advances made in ART of livestock and rodent species has also helped preserving domestic cats and some wildlife species [23,24]. In humans, it is now common to freeze semen and embryos and to test the donor for pathogens and genetic defects prior to distribution. With advancements in genomic tools and successful adaptation of ART to aquatic species, the numbers of transgenic aquatic species (*i.e.* zebrafish) that are used in genomic research have substantially increased [25,26].

In addition to mice and rats, many dogs, pigs, cats, sheep, non-human primates (NHP) and fish are widely used to better understand genetic and physiological mechanism involved in human health and disease [1,27-29]. There are cases where particular model species are more appropriate or more suitable to study genetic, molecular, cellular and physiological bases of human disease and health. Since there are obvious disadvantages of particular animals (*i.e.* mice and rats) mimicking human disease conditions, scientists have explored alternative animal models such as NHP, pigs, and cats to take advantage of similarities in physiology [28,30]. An animal model for a human disease is often created by either genetic engineering or many years of selective breeding. In either case, the process is resource-intensive, including creation and genetic and phenotypic characterization of these unique animal models. However, once a particular model has been developed, their optimal utilization is often limited due to the lack of necessary equipment, expertise, and knowledge in a given institute. Thus, in-depth studies to elucidate phenotype in GM animal models require widespread distribution.

Transportation of live animals nationally or internationally has many logistical problems, not only due to animal welfare, but also risks associated with the disease outbreaks, prolonged quarantine, and the cost of transporting live animals. In this context, although some minimum risks are involved, transportation of cryopreserved germplasm, somatic cells or ES cells in an effort to resuscitate strains of interest has numerous advantages over transportation of live animals. However, reanimation of certain lab animal strain depends on development of optimal germplasm cryopreservation protocols and their subsequent post-thaw use with the aid of sophisticated ART. Thus, there is an enormous need for effective

cryopreservation protocols and reliable biotechnologies to screen cryopreserved germplasm, thereby ensuring high-quality, pathogen-free distribution of biomaterials collected from these unique animal models. To date, national, international, continental, or regional GRB have been established for various biomedically important organisms, including *Arabidopsis thaliana*, *C. elegans*, *drosophila*, zebrafish, mice, rats, swine, cats, and rhesus monkeys. This review will focus on genome banking for scientifically important laboratory animals, including the mouse, rat, pig, zebrafish, and some other species (including the cat, dog and NHP).

2. Importance of genome resource banks

Increasing the number of research animal models and their effective sharing among the biomedical community would more rapidly bring novel insights into disease pathogenesis and ultimately facilitate development of effective therapeutic strategies to patients [31]. Traditionally, this has been accomplished by maintenance of live breeding colonies and their distribution. However, the expenses required for live animal colony maintenance when they are not presently needed has put an enormous burden on investigators and funding agencies. Overall, the ability to cryobank the genome of animal models is required to safeguard unique genotypes from several potential problems, including: (a) genetic drift, (b) genetic instability, (c) genetic contamination, and (d) loss due to disease or catastrophic disasters to housing facilities. In addition, preservation of germplasm enhances management efficiencies by: (a) saving animal room space by allowing discontinuation of low-volume strain breeding colonies, (b) reducing workload for staff, (c) facilitating shipment of genotypes by allowing distribution of frozen germplasm rather than live animals, and (d) supporting ownership and patent claims.

There are some obvious benefits of these genome centers to both requesting and donating investigators. For a requesting investigator, these include: a) access to unique models not commercially available; b) specific pathogen free health status; and, c) genetic quality control. Some of the benefits of GRB to the donating investigator are that it: a) fulfills requirements of granting agency (*i.e.* NIH) obligations to share biomedical research resources; b) reduces animal housing resources; c) reduces per diem costs; d) creates a cryopreserved archive; and, e) eliminates the direct shipment of animal models to multiple requesting investigators. Given their scientific, economic or ecologic importance and the time and financial resources used during their development, it is imperative to secure these animal models via cost-effective means for current and potential future studies. Cryopreservation has enabled long-term storage of important animal models, so that these models no longer need to be maintained as live colonies when they are not in use. Furthermore, GRB would protect existing animal models from natural (*i.e.* hurricane, tornado or flood) and man-made or accidental disasters (*i.e.* fire). In addition, a GRB also facilitates global exchange of animal genetics, due to its cost effectiveness, as well as helping management of potential disease transmission and other health concerns. Probably one of the best lessons learned was from a fire-related loss which occurred in 1995 and killed approximately 700 pigs at the University of Wisconsin swine research farm. Research animals used in studies of organ transplants, nutrition and bone development were lost due to fire. In addition to millions of dollars in financial losses, this unfortunate event has caused an abrupt halt to 25 y of cross-breeding research, with implications for human ailments, including the hereditary link to the detrimental effects of cholesterol.

Transportation of live animals between institutions requires consideration of the risk of disease transmission from donor animals. In this regard, ART currently has an important role in animal colony management with the use of cryopreserved embryos or sperm. To date, ART have been developed and are available for some biomedically important species

such as the mouse, rat, pig, domestic cat, and NHP. Currently, instead of live animal transportation, there is a growing interest for receiving animal models in the form of cryobanked germplasm, with the subsequent application of ART to establish a live animal colony. Reanimation by frozen-thawed embryos is the most commonly used method to eradicate pathogens from infected rodent colonies. In addition to embryos, cryopreserved sperm can also be used in combination with IVF or ICSI to generate embryos, which can then be transferred to clean recipients to generate live animals.

As the numbers of newly generated GM research models are increasing almost exponentially each year, these animals cannot be utilized to their full potential due to lack of formal production, distribution, and characterization. In addition, at least in some cases, due to limited financial or laboratory resources, a single research laboratory may not be sufficient to realize the full potential of these animal models. Generally GM models are generated by individual investigators who may not have the physical or financial resources to produce adequate numbers or to distribute other investigators. To this end, distribution of these lines via cryopreserved germplasm provides significant advantages for the most efficient use of the created lines. One of the challenges is the logistics of post-thaw reanimation of animal colonies which are often received as sperm and embryos by the receiving institution. Effective utilization of germplasm or tissues by a requesting investigator highly depends upon their skill set and equipment to perform such procedures. Some of the ART (AI, IVF and ET) can be performed in a basic laboratory setting, whereas others (e.g. ICSI and SCNT) also require more expensive systems and highly trained personnel. Thus, centers which are capable of performing such sophisticated reproductive, genomic, disease diagnostic, bioinformatics procedures are needed to distributed high-quality animal models to investigators nationally and internationally.

3. Responsibilities of genome resource banks

Effective germplasm, ES cells, and tissues distribution systems, as well as resource and data sharing plans, are crucial components of federally and privately funded projects, due to both rapid progress in research and the need for dissemination of animal models and associated information. A typical laboratory or institute responsible for creating or developing the animal models are usually not well qualified for world-wide distribution of unique models due to inadequate administrative, laboratory, and vivarium-related resources. In general, primary responsibilities of a GRB are to: a) improve techniques for cryopreservation of germplasm; b) improve methods for genomic, diagnostic and ART, which would be used during reanimation of a particular animal model; c) develop improved methods for detection and elimination of microbial pathogens, diagnostic tools for diseases and their phenotypic characterization, bioinformatics support, health monitoring, colony management genotyping and karyotyping, strain acquisition, cryobanking, cataloging/distribution/database/and website management; and d) providing consultation services (including workshop organization; Fig. 1).

Regardless of the species involved, GRB would receive existing mutant animal models with an appropriate material transfer agreement, cryobank their germplasm, DNA, or ES/somatic cells in an effective way, including appropriate labeling and storage conditions for potential future use. Cryopreserved sperm, embryos and ES cells are the preferred method for maintaining and distributing large numbers of GM rodent models when they are not in use or when there is a need from other investigators with similar interests. All of these activities need to be organized under the auspices of high-quality infrastructure. Through appropriate research efforts, the centers also would advance research and technologies to optimize the utility of animal models for biomedical research. The GRB should also confirm the described genetic modification before distribution to other investigators. It is also important

to maintain an inventory of these cryobanked biomaterials on searchable, user-friendly, computer databases accessible via the World Wide Web and make them readily available to the scientific community. When a particular transgenic mutant animal model is requested, one means by which the requesting investigator's request could be fulfilled is for the centers to reconstitute the line using appropriate ART and then ship the resulting breeding pairs to the requesting investigator after appropriate health monitoring (using molecular serologic tests). It is understood that the GRB would perform on-going health monitoring to assure maintenance of a pathogen-free status of the repository. An alternative approach, arguably a more beneficial aspect of the GRB, would be for the GRB to ship pre-screened pathogen-free cryopreserved germplasm/ES cells. Of course, this approach would rely upon the receiving institute having the ability to perform the necessary ART to reconstitute the animal model in their own animal facilities. These approaches would eliminate introduction of potential infectious diseases and expedite the progress of research projects.

The GRB would act as a repository and distribution center for high-quality, pathogen-free germplasm, ES cells, tissues, or live animals. Optimal methods to efficiently and effectively cryopreserve germplasm from a wide variety of laboratory animals with various genetic backgrounds are needed to provide assurance that reconstitution of a given strain will be possible after it is placed into a repository. The GRB would eliminate the requirement of individual laboratories to maintain and distribute animal models, thereby ensuring the highest-level of quality and uniformity of genetic and microbiological monitoring. In addition, GRB should develop new technologies to improve the handling of animal models including advances in ART, GM, cryobiology, genetic analysis, phenotyping, and infectious disease diagnostics. These centers would be viewed as having the necessary expertise for outside investigators to consult with concerns regarding cryobiology, reproduction, breeding, genetic, and health monitoring, and provide web-based instructions and organize workshops to better inform the biomedical community regarding routinely used techniques and future technology development.

4. Mouse genome resource banking

The laboratory mouse has been used to study a vast array of diseases, including hematopoiesis, autoimmunity, cancer, cardiovascular disease, cystic fibrosis, dermatological disorders, developmental defects, metabolic defects, and neurological disorders [32]. Thus, there is no doubt that mice have been the most widely used laboratory animals by the biomedical community, due to their extremely well-characterized genetics and also the availability of numerous transgenic and ES cell lines. The National Institutes of Health (NIH) established a mouse and rat embryo cryopreservation program in 1979 to assist the NIH Animal Genetic Resources to develop and manage laboratory animal models [33]. Induced Mutant Resource at the Jackson Laboratory [34] has also achieved tremendous success in helping the biomedical community as a reliable mouse resource. However, due to a substantial increase in the numbers of GM mouse models and associated demands, these repositories were not adequate to meet the needs of the scientific community. Therefore, in 1999 a geographically-dispersed consortium, the Mutant Mouse Regional Resources Center (MMRRC) [35], was established by the support of NIH National Center for Research Resources (NIH-NCRR) to receive and distribute scientifically valuable mouse models in the USA and abroad. This consortium currently consists of four centers, namely the University of Missouri-Columbia [36], the University of California-Davis [37], the University of North Carolina-Chapel Hill [38], and The Jackson Laboratory-Bar Harbor [34]. The MMRRC distributes and cryopreserves scientifically valuable GM mouse strains and mouse ES cell lines with potential value for the genetics and biomedical research communities. The MMRRC is a national network of breeding and distribution facilities, plus an information coordinating center, serving as NIH's premier repository of spontaneous and

induced mutant mouse lines and cell lines. Strains of mice are often maintained in a cryopreserved state, unless there is enough demand for maintenance of a live colony. The MMRRC responds to the needs of production and distribution to provide an uninterrupted regional supply of GM mice to investigators for comparative genomic studies. The MMRRC facilities have the capacity to resuscitate the strain at site and also offer cryopreserved germplasm and ES cells for resuscitation at the recipient institution on demand. In addition to the MMRRC, there are also other small mouse repositories in the U.S and major mouse repositories in other countries such as Japan (RIKEN BioResource Center, Kumamoto University) [39], the United Kingdom, and Italy (European Mouse Mutant Archive). The listing of the centers for each centers in the U.S and other countries are given in Table 1. A searchable online database International Mouse Strain Resource (IMSR) was used to gather the data [40].

5. Rat genome resource banking

Although transgenic mice are important models for human diseases, there are some cases in which rats are superior rodent models. The laboratory rat is one of the most commonly used experimental animals, not only because of its relatively bigger size, but also because it offers the best functionally characterized mammalian model system [41]. They are better suited for microsurgery, cell and tissue transplantation, in vivo functional analyses and studies that require multiple sampling. Rats are a model organism for analyzing several important biomedical traits, such as cardiovascular diseases, metabolic disorders, autoimmune diseases, cancer susceptibility, renal diseases, neurological disorders, transplantation studies, etc. For instance, the rat is often the preferred model in the case of polygenetically-controlled diseases. The diversity of rat models is composed of many genetically different inbred strains derived from some outbred stocks [42] and continues to be extended by strains derived from colonies with spontaneous mutations, as well as rats established by special breeding programs. To study polygenetical traits, there are a number of consomic and congenic strains for various quantitative trait loci which have already been developed, or are under development [43,44].

To better use these research models, several national and regional genome resources banks have been established in the USA, Japan, Germany and Czech Republic, which currently serve the rat biomedical community. These include the NIH-NCRR funded Rat Research and Resource Center (RRRC) [45] located at the University of Missouri-Columbia; European Rat Resources Centre (ERRC) [46] in Hannover, Germany; National BioResource Project for the Rat (NBRP) [47] in Japan; and the European Rat Tools for Functional Genomics (EURATools) [48] in the Czech Republic (Table 2). The main goals of the rat GRB are to: (1) provide the biomedical community with a repository and distribution center for valuable rat strains, and (2) shift the burden for maintaining and distributing unique rat models from investigators to a national resource center. Currently, these centres/institutions have cryopreserved and live rat lines received through active recruitment of valuable rat models and donations from investigators who have created models. Upon importation into these centers, gametes and embryos are cryopreserved to protect against future loss of the model. Furthermore, models in these centers are available for distribution as live animals, tissues, or cryopreserved embryos, sperm and newly developed rat ES cell lines.

6. Swine genome resource banking

Laboratory mice and rat models have provided tremendous knowledge about human physiology and disease which have led to the development of pharmaceuticals and cell-based therapies. However, they are not always a superior model for particular human diseases, due to obvious differences between rodents and man. Some of the shortcomings

include body size, physiological differences such as life span, digestive system, cellular metabolism, endocrine and reproductive functions. Swine share more anatomic and physiologic characteristics with humans and have made important contributions to almost every field of human medicine. As regard to use of animals in research, swine are one of the most suitable larger species due to lesser emotional bonding as compared to cats and dogs. Thus, pigs are likely to be less debated as research animal model than either cats or dogs. As an experimental animal model, swine have many advantages, since they have a favorable reproductive capacity with a short gestation period, large litter size, and a polyestrus cycle. Furthermore, swine anatomy and physiology are similar to humans, especially in the cardiovascular, pulmonary, skeletal, and digestive systems. These similarities have made the swine the primary species of interest as organ, tissue, and cell donor species for xenograft transplantation procedures. For example, pigs with unique genotype were selected because of their physiological and pathological similarity to patients who suffer from atherosclerosis [49] and hypercholesterolemia [50]. The creation of genetically altered pigs for potential use in clinical xenotransplantation is now technically feasible as precise genetic modifications in the pig genome are also achievable [29].

Recently the development and increased use of swine strains (*i.e.*, inbred strains, various hybrid lines, and transgenics, knockouts) in biomedical research by investigators around the world generated increased demands for specific-pathogen free quality swine models. Genetic manipulation through SCNT is currently very successful in creating swine models of human diseases, including cystic fibrosis and retinal degeneration [51,52]. The only national swine resource and research center (NSRRC) was established at the University of Missouri-Columbia in 2003 (with the support of NIH-NCRR) to develop the infrastructure for biomedical investigators to have access to critically needed swine models of human health and disease [45]. The NSRRC is a well-integrated and coordinated program with active investigators who effectively utilize swine models to generate new information relevant to the resource. In addition, since NSRRC is expected to maintain swine strains for xenograft transplantation procedures, it accommodates the specific needs for isolation, growth, preservation, expansion, and distribution of germplasm, cells, tissues and organs from swine models. Thus, the NSRRC serves as a central resource for reagents, information and training related to use of swine models in biomedical research.

7. Non-human primate genome resource banking

An animal model with high resemblance to humans is crucial for the ultimate goal for development of medications as well as determination of the efficacy and safety of medications and therapeutic procedures. To this end, there is no doubt that NHP are the closest species to humans, due to their genetic, anatomic and physiological similarities [53]. Some neurological disorders such as Alzheimer's and Parkinson's, and Huntington diseases only occur naturally in humans. The utilization of NHP transgenic technology has opened a new era for animal modeling in biomedicine, which accelerates development of the most appropriate animal models and increases understanding of human diseases, as well as development of therapies for human patients. Thus, effective exchange of cryopreserved NHP biomaterials among leading researchers via NHP GRB should hasten development of effective medications to cure human diseases.

The increasing demand for genetic materials from NHPs created a need for establishing centralized genetic resources to facilitate sharing of genetic materials. In 1999, a NIH-NCRR funded NHP Reagent Resource was established at Harvard University to develop and produce monoclonal antibodies for *in vivo* depletion of immune cell subpopulations in NHP [54]. Furthermore, recently, The National Primate Research Centers (NPRCs) established working groups for developing GRB to facilitate collaboration among NHP researchers [55].

In this regard, the national NHP DNA bank was established for macaques, baboons, chimpanzees, marmosets and vervet monkeys to facilitate colony demographic comparisons, cross species comparisons and technology development [56]. Recently, transgenic [15,19,20], stem cell [57,58], cloning [59], ART [60], and cryopreservation [61,62] technology have become available for NHP species. Clearly, these techniques collectively will promote biomaterial sharing for transgenic NHP and ultimately play a crucial role in bridging the gap between rodent models and humans.

8. Zebrafish genome resource banking

Since the beginning of the 1980s, the zebrafish has been extensively used as a model organism for investigating mammalian genetics and development. Zebrafish are easy to breed and maintain, and they have a rapid development and high spawning productivity. Furthermore, since zebrafish embryos are transparent, they are genetically tractable by biomarkers, making them particularly suitable for cell biology studies. There have been growing numbers of studies which demonstrated the power of the zebrafish for modeling human diseases [63,64]. During the last two decades, using large-scale ENU and insertional mutagenesis screens, researchers have created several thousand mutant strains [65]. These zebrafish models have provided important resources for identifying causative mutations in early embryonic defects and diseases. Formal zebrafish GRB have been established in the USA (International Resource Center) [66] and Japan (National BioResource Project) [67,68] (Table 3).

9. Genome resource banking for other mammalian species

There has been great progress in the development of germplasm cryopreservation and related ART for utilization of GRB in a few species where genomic databases are rapidly becoming [69] available (e.g., cats, dogs) either directly, or for closely related counterparts that can serve as appropriate models [70-72]. Besides the animal species covered above, domestic cats and dogs are valuable research models for the study of genetic disease in humans, providing critical insight into pathology and treatment options, including emerging gene therapies. However, maintenance of research cat and dog models can be expensive and challenging due to disease manifestations that confound reproduction. Studies of the domestic cat have contributed to many scientific advances [73,74]. The reasons for the use of cats as experimental animal model include their diverse genetics, neurobehavioral complexity, intermediate size, high breeding capacity, and modest housing costs. In addition to the cat models with hereditary diseases, it is now possible to introduce genes of interest to create transgenic cat models for human diseases [75,76]. For example, both humans and cats are affected by pandemic AIDS lentiviruses that are susceptible to species-specific restriction factors. Thus, transgenic cat production and subsequent biomaterial sharing would benefit human and cat health, potentially developing ways to confer protection from epidemic pathogens.

Conversely, the dog has been the most prevalently used species in transplantation research. Due to long existence with humans, dogs are also affected from similar diseases and thus important models for studying human diseases. Many commonly inherited human diseases including diabetes, asthma, epilepsy and cancer are because of complex interactions between multiple genes and environmental factors [77]. With the recent improvement in ART and genomic modifications [78-84], dogs are expected to have important roles in the study of human diseases. The physiology, disease presentation and clinical manifestations of the dog are much more similar to human when compared with various other model organisms such as mice and rats. Some of the hereditary canine diseases have an equivalent human disease, including cardiomyopathies, muscular dystrophy and prostate cancer. The primary goal of

GRB involving cats or dogs would provide a functional genome resource for propagating, managing, and preserving cat or dog disease models (as well as rare felids and canids). Potential benefits include more productive use of research models, improving animal welfare, expanding usefulness of underutilized models, improving logistics, and providing long-term preservation of valuable cat and dog models.

10. Cryopreservation

Cryopreservation of germplasm is of critical importance for operation of animal and cell resource centers. Improved methods to efficiently and effectively cryopreserve germplasm are critical to provide assurance for reconstitution of a given strain when needed.

10.1. Sperm

Freezing and long-term storage of haploid genome as sperm could most efficiently preserve newly created animal models. Compared to the limitations in the number of oocyte/embryo collected from a single female, several thousand offspring can be theoretically obtained from just a single male's sperm. Sperm collection is relatively simple, does not require gonadotropin administration or mating prior to collection, and requires only a few animals. In addition, post-mortem sperm collection is also quite simple. Consequently, cryopreservation of spermatozoa offers an economic alternative to the cryopreservation of embryos, especially for strains such as transgenics and induced mutants, including those produced by homologous recombination and introduction of null alleles (*i.e.* knockout). Cryopreservation of sperm from such strains is especially efficacious, as thawed sperm can be used to fertilize oocytes by IVF or AI from a selected common inbred, or hybrid, strain for production of offspring to re-establish a breeding colony for the strain. Transgene or mutant carriers can be identified among the progeny by relatively efficient genotyping methods and homozygotes can then be produced, if possible and desired, by mating two heterozygous carriers.

Regardless of post-thaw motility, sperm freezing allows reanimation of the mouse, rat, pig, and cat, with the use of either AI or IVF if decent motility is achieved, or with ICSI when post-thaw sperm motility is extremely low. Today, the most successful used mouse sperm freezing protocol utilizes raffinose pentahydrate and skim milk. This protocol can be easily performed in most laboratories because it does not require controlled freezing machine and very easy to perform and effective in many inbred mouse strains, including C57BL/6J, BALB/cJ, 129S1/SvImJ, and FVB [85]. Although mouse sperm cryopreservation protocols have been established for quite some time, it took approximately an additional decade to develop effective freezing protocols for rat sperm; they include simple compounds such as Tris (hydroxymethyl) aminomethane, egg yolk, lactose monohydrate and Equex Stem [86,87]. Rat sperm freezing is also very easy to perform and obviates controlled freezing machines. The zebrafish genome is also mostly successfully cryobanked via sperm in the presence of Ginsburg Fish Ringers solution, methanol and skim milk [88]. Currently AI with cryopreserved semen has become available to dog owners worldwide, and the demand for services to freeze semen is increasing [84]. In spite of methods having been reported to produce reasonable success for sperm cryopreservation, there is still room for improvement for reliable preservation of all animal models. The economic advantages of sperm preservation over other germplasm preservation will continue to be the driving force to develop more reliable sperm preservation methods in the future.

10.2. Oocytes

To date, genome cryobanking via oocytes has been much less attractive than sperm and embryo banking in almost all biomedically-important species [89,90]. This is mostly

because of limitations regarding the number of oocytes obtained from mammalian species as well as low success in survival rate after freezing and thawing. In addition, it should be noted that it is possible to use sperm for colony rescue via ICSI even though they lose most of their structural (membrane and acrosome integrity) and motility functions. However, this is not the case for oocytes as most of cellular and sub cellular structures are extremely vulnerable to cryopreservation induced damages. Although adequate means for the cryopreservation of preimplantation embryos are available for the laboratory mouse and rats [33], methods for cryopreserving oocytes for many mammalian species are generally less successful. Mature oocytes have several, unique characteristics contributing to this recalcitrance [90]. The large intracellular water volume, cortical granules, and meiotic spindle all make them sensitive to the stresses imposed during freezing. Zebrafish egg cryopreservation would be useful in aquaculture or biodiversity conservation, but it is also still far from being successful due to structural barriers (*i.e.* yolk) for CPA permeation and chilling sensitivity [91,92].

10.3. Embryos

As compared to sperm, embryo collection procedure is a substantial undertaking for most large animal models (swine, monkeys, dog, cats) and somewhat in rodents. However, it is often required for banking of animal models with complex genetic backgrounds (*i.e.* having multiple mutations). Successful means for the cryopreservation of preimplantation embryos by slow cooling (~ 0.5 °C/min in the presence of 1.5 M dimethyl sulfoxide (DMSO)) are available for the laboratory mouse and rats [33,93,94]. In these protocols, DMSO or other permeating cryoprotectant (ethylene glycol) and non-permeating (sucrose) cryoprotectant are commonly used to bank rodent embryos. Rall and Fahy [95] described an alternative approach for the cryopreservation of mouse embryos by direct vitrification. Vitrification is a process of solidification whereby an aqueous solution (40-50% CPA) does not crystallize during cooling; instead, the viscosity of the solution increases with decreasing temperature, resulting in formation of an amorphous glass-like solid. This method of cryopreservation has been successful for embryos of animal models such as swine, NHP, and cats [96-99]. Similar to oocytes, zebra fish embryos cannot currently be cryopreserved due to their large size, high lipid content, multi-compartmental structure and high sensitivity to chilling [92].

10.4. Ovarian tissues

Ovarian tissue cryopreservation is preferred for mouse and rat lines in which male fertility is reduced or absent, such as the X-autosome translocation mouse models. The advantages of ovary cryopreservation are that it often requires only a few donor females and it overcomes time-consuming and expensive superovulation procedures. Cryopreservation of ovarian tissues from animal models may provide an opportunity for long-term preservation of early stage oocytes. It should be noted that ovary cryopreservation could complement, but not substitute for embryo cryopreservation. Cryopreserved murine ovarian tissue with subsequent orthotopic transplantation into immune deficient or genetically identical mouse recipients has proven to be a successful and cost-effective approach for the purpose of genome banking [100]. Cryopreservation and subsequent transplantation of ovarian tissue is a useful reproductive technique that has been used to rescue transgenic strains with reproductive problems [100-102]. Cryopreservation of zebrafish ovarian tissue mass has also been recently explored [103,104].

10.5. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells

It is important to bank ES cells since the mechanisms underlying cellular differentiation, expansion and self-renewal can be studied along with differentiated tissue development and regeneration [105,106]. A very recent development, with potentially profound significance

for clinical therapy, is generation of induced pluripotent stem cells (iPS cells) from somatic cells [107,108]. The generation and use of iPS cells, particularly for autologous stem cell therapy, poses fewer ethical problems compared to the derivation and use of ES cells [109]. However, these technologies depend on effective cryopreservation of ES and iPS cells for wide distribution (nationally and internationally [110]). Animal-derived ES cells or iPS cells from biomedically or agriculturally important animal models are of great interest because their prospective uses in creating genetically modified animal models, but also for in vitro drug and toxicity screening [111,112]. Similar to gametes and embryos, the ability to cryopreserve ES cells is crucial, since they allow for the generation of quality-controlled stocks of cells, transport of cells between investigators, and avoiding the need for expensive and time-consuming establishment and continuous culture when there are not used at particular time point. Fibroblast-derived iPS cells contain low cytoplasmic water content and also have less cellular complexity. Thus, they can be simply cryopreserved in a buffer solution (high glucose DMEM, ES cell-qualified fetal bovine serum-DMEM) containing ~10% DMSO. This can be easily achieved by commercially-available freezing boxes which provide 1°C/min cooling upon placement in -80°C freezers. Current cryopreservation methods can successfully preserve most ES cells or iPS cells, although the success rate varies widely. However, there is a substantial difference between the number of cells potentially needed to either treat patients or run a high-throughput drug screen and the number of cells that can be preserved using a particular technique. Appropriate cryopreservation will eliminate the tendency of ES cells to differentiate post-thaw. In that regard, ES cells that maintain their pluripotency are more likely to contribute to the germ lines of resulting chimeras when implanted into the host blastocyst.

10.6. Somatic cells

With development of the SCNT technique and IPS, cryo storage of embryo derived stem cells (ES) and somatic cells (*i.e.* fibroblast cell) has become a viable option for cryo-resuscitation of laboratory animals. Somatic cells are often very easy to obtain, abundant, and can be effectively expanded. Thus, they offer a very inexpensive source of genome preservation. Furthermore, due to their relative small cell volume, simple cellular structures, and abundance, cryopreservation of both somatic cells and iPS cells can be easily achieved in an appropriate buffer solution containing 1 mol/L DMSO. Freezing can be achieved in a very inexpensive cell freezing boxes using 1°C/min cooling rate. Although SCNT has obvious potential for the reanimation of rare genotypes, its wide application is prevented by the low efficiency in terms of offspring outcomes (<2%) and thus it is not routinely used for reanimation. However, cryostorage of somatic cells for future use, once the procedure of SCNT has improved, is certainly a wise step to be undertaken.

11. Conclusion

In conclusion, there is no single animal model which would allow investigation of all aspects human diseases conditions. Comparative approaches which welcome alternative cell and animal models will ultimately obtain much more meaningful information, with a greater impact on global human and animal health. To this end, increasing the number of model species and optimal management of these research models via GRB would promote rapid advancement of the biomedical and veterinary fields.

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Fig. 1.
Schematic representation of the important components and functions of animal genome resource banks.

Table 1

Transgenic and mutant mouse genome resource banks around the world.

Name of the mouse repository	Country
The Jackson Laboratory	USA
Mutant Mouse Regional Resource Centers	USA
KOMP Repository, University of California-Davis	USA
Oak Ridge Collection at the Jackson Laboratory	USA
Texas A&M Institute for Genomic Medicine	USA
Neuromice.org , Mouse Genome Consortium	USA
National Cancer Institute Mouse Repository	USA
JAX-PGA at The Jackson Laboratory	USA
Kumamoto University	Japan
RIKEN BioResource Center	Japan
National Institute of Genetics	Japan
Oriental BioService, Inc.	Japan
Canadian Mouse Mutant Repository	Canada
European Mouse Mutant Archive	Italy
Wellcome Trust Sanger Institute	UK
Mammalian Genetics Laboratory	UK
Australian Phenome Bank	Australia
Mutant Mouse Models of Human Immunological Disease Network	Greece
National Applied Research Laboratories, Taiwan, R.O.C. (RMRC-NLAC)	Taiwan

Adapted from International Mouse Strain Resource (IMSR) [40]

Table 2

Transgenic and mutant rat genome resource banks around the world.

Name of the Rat Repository	Country	Reference
Rat Research and Resource Center	USA	[45]
European Rat Resources Centre	Germany	[46]
National BioResource Project	Japan	[47]
European Rat Tools for Functional Genomics	Czech Republic	[48]

Table 3

Transgenic and mutant zebrafish genome resource banks around the world.

Name of the Zebrafish Repository	Country	Reference
International Resource Center (ZIRC), the Oregon University	USA	[66]
National BioResource Project-zebrafish	Japan	[67]