

# JAX<sup>®</sup> Sperm Cryopreservation *A Customer's Perspective*

*The following communication from Peter Mombaerts, M.D., Ph.D describes his use of The Jackson Laboratory's Sperm Cryopreservation service to cryopreserve and distribute over one hundred unique mouse strains. Dr. Mombaerts was formerly at The Rockefeller University in New York where he was Professor and Head of Laboratory of Developmental Biology and Neurogenetics. He is now with the Max Planck Institute of Biophysics in Frankfurt, Germany where he is Director of the Department of Molecular Neurogenetics.*

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## **Public availability of published mouse strains from my laboratory, through The Jackson Laboratory, Bar Harbor, Maine, USA.**

A new technique has recently been developed at The Jackson Laboratory (JAX) for the cryopreservation of mouse sperm. The technique is much improved and applicable to strains that are commonly used in gene targeting and transgenesis. A JAX field team came to my laboratory at The Rockefeller University in January 2007, and froze sperm from 129 of our published and unpublished strains, using the new 'sperm cryo' technique. Through this initiative, today 80 of our published strains are publicly available through JAX, and the remaining strains will become publicly available one by one, after publication. This personal communication describes the following aspects of the sperm cryo initiative in my laboratory: history, how to locate a strain online, levels of strain availability, advantages of a third-party distribution scheme, cryorecovery fee, caveats, timing of availability for strains that are not yet published, outlook, credits, and contact information at JAX. This initiative was motivated by the growing complexity of our mouse colonies and the numerous requests for our strains. It is also related to the closing of my laboratory at The Rockefeller University, and its relocation to the Max Planck Institute of Biophysics in Frankfurt, Germany.

### **History**

Gene targeting technology, which was developed by Mario R. Capecchi, Sir Martin J. Evans, and Oliver Smithies, the 2007 Laureates of the Nobel Prize in Physiology or Medicine, has been so successful in biomedical research that many laboratories face great logistical and financial obstacles in maintaining large numbers of gene-targeted strains over a period of years and shipping them in a timely fashion to requesters after publication. JAX has recently developed a new technique of sperm cryopreservation that is likely to facilitate enormously the preservation and distribution of mouse strains generated in academic laboratories. The new technique is an order of magnitude more efficient than current techniques for strains such as C57BL/6 and 129, which are typically used in gene-targeting projects.



A team from JAX (Jane Farley, Shannon Byers, and Kurt Christiansen) froze sperm samples from 129 of our strains on site, over a four-day period in January 2007. This collection consists of 116 strains with gene-targeted mutations, 8 transgenic strains, and 5 strains with genomic alterations produced by chromosome engineering (which is based on gene targeting). The JAX cryoteam applied a new and much improved sperm cryo technique, which will soon be published. To evaluate the quality of the cryopreservation process for each individual strain, back at home JAX made an attempt to recover each strain by in vitro fertilization (IVF). All but two strains were recovered successfully. For one strain that failed, the sperm donors were too old; for the other strain, there is no explanation, and another experiment with new sperm donors will be conducted. The genotypes of the cryorecovered mice have been confirmed by us, and are in the process of being confirmed formally by JAX.

## How to locate a strain online

Among the 127 successfully cryopreserved strains, the published strains (80 today) can be located online, as follows:

1. Go to <http://jaxmice.jax.org/info/index.html>
2. Under 'Find JAX® Mice', click on '2. JAX® Mice Database', to initiate a query.
3. For 'Strain Name/Common Name', leave drop-down box at 'contains', and enter 'Mom', which refers to my last name. Click 'Search database', for a query. Strains are identified with a stock number, for example, stock no. 006790 (or stock no. 006790) for the H knockout (Fuss *et al.*, Cell 2007), and with the strain name B6;129P2-Del(14)3Mom/MomJ.
4. Standardized strain names were not included in our papers prior to Fuss *et al.*, Cell 2007, which was published on July 27, 2007. JAX assigns standardized nomenclature to mouse strains, according to the rules and guidelines established by the International Committee on Standardized Genetic Nomenclature for Mice: <http://www.informatics.jax.org/mgihome/nomen/index.shtml>

This nomenclature is based on the locus (for instance, the M71 olfactory receptor is *Olf151*, and M72 is *Olf160*) and a consecutive number (for instance, tm1Mom for targeted mutation #1 from the donating investigator Mombaerts.) It may not always be easy to locate a strain based on the

'common name' that is used exclusively in our papers prior to Fuss *et al.*, Cell 2007. Enter the query in the box within the blue-shaded bar 'Search the JAX® Mice database on a keyword'. For instance, when typing in 'OMP-GFP', the strain name turns out to be B6;129P2-*Omp<sup>tm3Mom</sup>*/MomJ, stock no. 006667. The ID of the PubMed reference (PMID) that describes a strain for the first time can also be used; for instance, typing in '17662950', the PMID for Fuss *et al.*, Cell 2007, reveals stock no. 006790.

5. If all else fails, give JAX a call at 1 (800) 422-6423. You can talk to Customer Service and Technical Information representatives for assistance.

There are many strains at JAX from my graduate work with Susumu Tonegawa at MIT, and these also carry the designator 'Mom': T-cell receptor (Tcr) knockout mice and Recombination Activating Gene-1 (Rag1) knockout mice. (Most of these strains are backcrosses or crosses that were made after I shipped mice to JAX in 1993.) Two 'Mom' strains are from my postdoctoral work with Richard Axel at Columbia University: OMP-tau-lacZ (stock no. 006594) and P2-IRES-tau-lacZ (stock no. 006595). The remainder of the 'Mom' strains were generated in my laboratory at The Rockefeller University since 1995.

## Levels of strain availability

There are various levels of availability. It is important to realize that these levels are dynamic. Availability can change from one day to another - users should check the database on a regular basis if they are eager to obtain a strain.

- The highest level is 'Repository-Live', such as for stock no. 004946, the OMP-synapto-pHluorin mice (Bozza *et al.*, Neuron 2004). In early 2004, we deposited this strain as live mice. This strain has been available live since 2004 due to sufficient and regular demand. In the year 2007, this strain has thus far been requested 15 times. A homozygous mouse is priced today at \$87.30.
- Another level is 'Under Development for Distribution Colony': the strain has been cryorecovered, and the colony is being expanded to make live mice available for distribution. An example today is stock no. 006790, our H knockout. If you are interested in such a strain, you can register your interest by clicking on 'Strain Interest Form', here: <http://jaxmice.jax.org/jaxmice-cgi/jaxmicedb.cgi?objtype=interest&scode=006790>. The interest expressed by prospective buyers is used by JAX to gauge the speed and extent of colony expansion.

*Peter Mombaerts (left) tours the JAX® Sperm Cryopreservation facility with Rob Taft of The Jackson Laboratory.*



Filling out the 'Strain Interest Form' places the potential buyer in a queue on a first-come, first-served basis, but does not bind the requester to buying mice when they become available. The requester receives an email when the colony is sufficiently developed for shipping, and at that time, can decide to make a purchase. Thus, buying mice after registering interest is optional.

- Another level is 'Repository-Cryopreserved', such as stock no. 006653, the  $\Delta V1rab\Delta$  strain, which lacks a cluster of V1R genes on chromosome 6 as a result of chromosome engineering. Once upon a time, this strain was available in the repository as live mice, but today it is back in a cryopreserved form.
- The lowest level is 'Under Development for Cryopreservation Repository', such as stock no. 006632, VRi2-IRES-tauGFP. These strains are at early stages of the cryorecovery process. Today, there are just a handful of our strains in this category.
- Information about our unpublished mouse strains is not available online, for obvious reasons. Nearly all our relevant unpublished mouse strains have been or will soon be deposited as cryopreserved sperm with JAX. After a cryorecovery check and confirmation of genotype, these strains will remain dormant for months or years, until publication. Meanwhile, I can give JAX permission to release an unpublished strain to a specific investigator on an individual basis, for instance in the context of a collaboration.

It is possible to select a subset of our strains according to level of availability by changing the drop-menu of 'Standard Supply' in the query form.

### Advantages of a third-party distribution scheme

There are multiple important advantages of distributing published mouse strains through JAX, instead of through an academic laboratory such as mine:

1. JAX does not inform the donating investigator about the identity of the requesters, neither pro-actively nor retro-actively. Requesters do not need to explain for which experiments they request a strain, and will not feel obliged to return a favor of some sort to the donating investigator. The anonymity and no-strings-attached aspect of this scheme of distribution through a third party thus lowers the psychological threshold for studying published strains from other laboratories.
2. There is no need for a Material Transfer Agreement (MTA) between the requester and The Rockefeller University. Neither The Rockefeller University nor I and my co-authors receive licensing fees or royalties for non-commercial use of the mice. Any commercial use of our strains will require a license from The Rockefeller University.
3. JAX verifies the genotype of the mice before they are shipped to the requester. The genotyping protocol is available online with the JAX® Mice Data Sheet.
4. JAX offers requesters more than one breeder pair. JAX may even offer all the mice that are produced by cryorecovery, if no one else needs them.
5. JAX provides a health certificate with the mice shipped to the requester. Typically the health status of JAX mice is superior to that of animal facilities in academic institutions.

6. NIH regards JAX as an approved vendor, such that the mice can be delivered directly to the requester's mouse room, instead of passing through quarantine. This direct route results in substantial savings of time and money.
7. As the strain is stored as sperm, the numbers of mice that are shipped can be scaled, for additional cost. A 'speed expansion' service is available, by which many mice of the same strain are born within a narrow timeframe by IVF. This service enables the generation of a cohort of mice with essentially the same date of birth, which is otherwise difficult to accomplish by conventional breeding. Such service may facilitate aging research. Furthermore, JAX can set up breeding colonies to provide investigators with a dedicated supply of mice. JAX can also perform customized breeding protocols and create special crosses.
8. JAX can supply the strains upon request as frozen embryos. For the foreseeable future, JAX will not distribute samples of cryopreserved sperm, because the technique of cryorecovery by IVF is not straightforward and not widely established.
9. The JAX® Mice Data Sheet has all relevant information for published mouse strain, in a convenient format, including the primary reference, the genotyping protocol, and additional information that is not in the literature. With time, this Data Sheet will be the most reliable source of information about a given strain. We already use this online information routinely in my laboratory. The Data Sheet is dynamic: corrections and edits can be made easily between JAX and me.
10. Importantly, JAX is unlikely to lose any strains, whereas academic laboratories tend to do so with time when strains are no longer actively used. (In fact, during preparations for the sperm cryo session in early 2007, I found out that we had lost a few published strains with gene-targeted mutations.) Moreover, academic laboratories typically have no systematic 'backup' of their strains for protection in case of a disaster in their animal facility. Straws of sperm are stored under liquid nitrogen at two locations in Bar Harbor, and an off-site location. JAX has agreements for exchanging live mice and cryopreserved samples with distribution centers in Europe and in Japan.

## Cryorecovery fee

JAX currently distributes ~1,100 strains with targeted mutations. In a given year, ~50% of the strains that are purchased, are requested only once. JAX cannot maintain all strains continuously as live mice due to cost and space constraints. If a strain is purchased approximately five times in a given year, and in sufficient numbers, the likelihood is high that the strain will be kept 'on the shelf', and that live mice will be continuously available. That is the case with our OMP-synapto-pHluorin mice from deposit in 2004 to now. If a strain is either purchased only occasionally or insufficient interest is expressed via the Strain Interest Form, the strain is taken 'off the shelf'. The donating investigator is notified by email in advance that the strain will be taken off the shelf. For cryopreserved strains, a cryorecovery fee of \$1,900 is charged, with a delivery time of ~16 weeks. JAX acknowledges that this fee, which is necessary to cover the costs, can be a financial impediment, and hopes to reduce this cryorecovery fee in the future for strains that can be cryopreserved as and recovered from sperm. Meanwhile, the more the repository is used by the community, the greater the chance that a strain will remain on the shelf. For now, market forces determine the costs for the user, and the balance between availability of a given strain as on versus off the shelf.

## Caveats

Strains cryorecovered from sperm are provided to users as heterozygous for a targeted mutation or hemizygous for a transgene, because oocytes used for IVF are from wild-type mice, typically C57BL/6J. Cryopreservation of mouse oocytes is not yet feasible.

Sperm is collected from only two males, and in a few cases from just a single male. As most of our strains are not inbred, selection of 1-2 individuals creates a genetic bottleneck. It is thus possible that the phenotype may differ from what is published, due to changes in genetic background. I invite users to contact me without hesitation if the phenotype they observe is different.

Allele-specific genotyping assays are not available for some strains from my laboratory. In the future, we will develop specific assays, but for the time being, several strains are genotyped with the same sets of PCR primers. There is thus a minor degree of uncertainty about the identity of some strains, for which I take responsibility. Users are encouraged to contact JAX or me if they have reason to doubt the identity of the mice.

There is a minor chance that the strain carries another targeted mutation or transgene from our colony. These issues of strain identity and strain purity are now being resolved by extensive quality control, at JAX and in my laboratory.

### **Timing of availability for strains not yet published**

Beginning with the publication of Fuss *et al.*, Cell 2007 (H knockout, stock no. 006790), I coordinate with JAX on the timing of when the Data Sheet goes 'live' online with the online publication of the paper. A paper appears in PubMed typically a few days after publication, and can then be referenced with a PubMed ID, enabling JAX and me to release the Data Sheet online with this ID. For Hu *et al.*, Science 2007 (GCD-ITG knockin, stock no. 006704), the delay was just a few days. Prospective users must exercise some patience in locating a strain online at JAX, soon after publication. Actual availability and timing of first shipment are exclusively controlled by JAX, which, in turn, bases its assessment of future strain demand on the interest expressed via the online Strain Interest Form.

### **Outlook**

After JAX cryopreserved 127 of our strains in my laboratory, we have cryopreserved or will cryopreserve another ~35 strains, most of which are unpublished. A few strains (such as OMP-tauYFP, OMP-tauCFP) are of the 'unpublishable' category: they do not fit readily into one of our stories. I am thinking of ways to inform the community about these unpublishable strains, which I would then make available for a collaboration.

Going forward, my policy is to make strains that were generated with NIH grant support publicly available through JAX after publication. Sperm samples will be cryopreserved in advance of publication, and the Data Sheet will go live soon after publication. One by one, these strains will thus become publicly available through JAX in due time. I pay for the sperm cryo fees, which are not negligible. For transgenes, it is not possible and not necessary to cryopreserve all lines produced with a given construct, so only the 'key strains', which are representative of the phenotype, are cryopreserved. For gene-targeted mutations, I plan to cryopreserve all strains. By making published strains available through JAX, I will honor indefinitely my duty to share reagents that were generated in part or in total with NIH funds, regardless of whether I continue to receive such grant support.

Those of you who have been involved in the exchange of mouse strains, from the shipping or receiving end, are familiar with the logistics of moving mice from point A to point B in an academic setting. I believe that it is far more efficient and cost-effective for academic laboratories to let experienced centers such as JAX deal with the distribution of their strains. JAX has been in the mouse business for nearly a century, and is here to stay.

### **Credits**

Many people have contributed to the success of this project. I learned about the new technique soon after it was firmly established, during a visit to JAX in August 2006. The new technique has been developed by the Taft and Wiles group at The Jackson Laboratory. This group includes Robert A. Taft, Associate Director of Reproductive Sciences; Michael Wiles, Senior Director of Technology Evolution and Development, Jane Farley, Manager of Reproductive Sciences, and Chuck Ostermeier, Associate Research Scientist and Reproductive Technology Specialist. They readily agreed to dispatch a field team for the first time, recognizing the large number of strains that had to be cryopreserved before I closed my laboratory. Michael Wiles provided advice. Melissa Berry organized the nomenclature and strain information with me. Jane Farley and her team did the IVF. Sue Cook and Kevin Johnson performed genotyping at JAX. I thank members of my laboratory for their cooperation in setting aside mice. Special thanks go to Akiko Ishii, Tomo Ishii, Mona Khan, and Masayo Omura, for meticulous and ongoing quality control in my laboratory with countless genotypings by PCR.

### **Contact information at JAX**

Steve Rockwood, [steve.rockwood@jax.org](mailto:steve.rockwood@jax.org), for the Sponsored Strain Distribution program, <http://jaxmice.jax.org/services/sponsoredistribution.html>.

Our strains are informally and internally referred to at JAX as 'the Mombaerts collection'.

Rob Taft, [rob.taft@jax.org](mailto:rob.taft@jax.org), for the new sperm cryopreservation technique.