



# INFRAFRONTIER

mouse disease models

## Centralised mouse repositories – Common and specialised services provided by major mouse repositories

Martin Hrabé de Angelis, Helmholtz Zentrum München

INFRAFRONTIER / IMPC Repository Workshop, Munich, May 9th



# Mouse Repositories – more than freezers and cages

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# Common activities of repositories

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- Evaluation of mouse strains
- Importation and cryopreservation
- Distribution (including provision of protocols, health certificates, MTA, CoU, customs)
- Quality control programs  
(for health status and genetic purity)
- Customer service and technical support
- Facilitate sharing: Public interfaces providing access to resources and data



# Value-added research services of repositories

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- Breeding services (speed congenics and contract breeding)
- Disease diagnostics or histopathology
- Genomic services such as genotyping and mapping
- Model development by targeted mutagenesis, transgenesis and converting ES cells into mice
- Phenotyping services
- Axenic services
- Intracytoplasmic sperm injection services (ICSI)
- GEMM-ESC archive: Archive of embryonic stem cells (ESCs) derived from validated genetically engineered mouse models (GEMM) of cancer



# Value-added services of repositories – Axenic service

- Production and maintenance of germ-free mice
- EMMA workshop in preparation: Axenic mice and gnotobiology





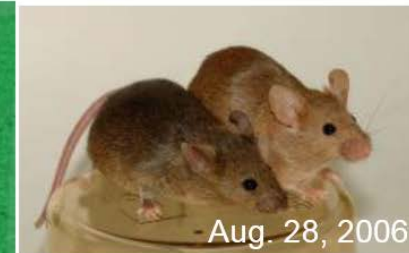
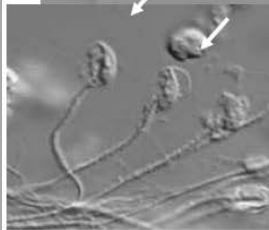
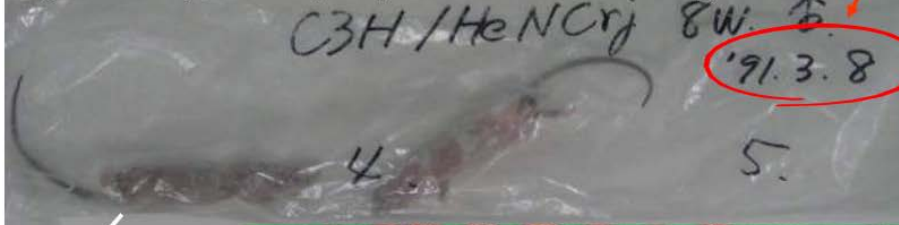


# Value-added services of repositories – ICSI

## Retrieval of sperm from frozen mouse bodies

Mice frozen at  $-20^{\circ}\text{C}$  for 15 years

(provided by Dr. Iwaki, Tokyo Jikei Medical University)

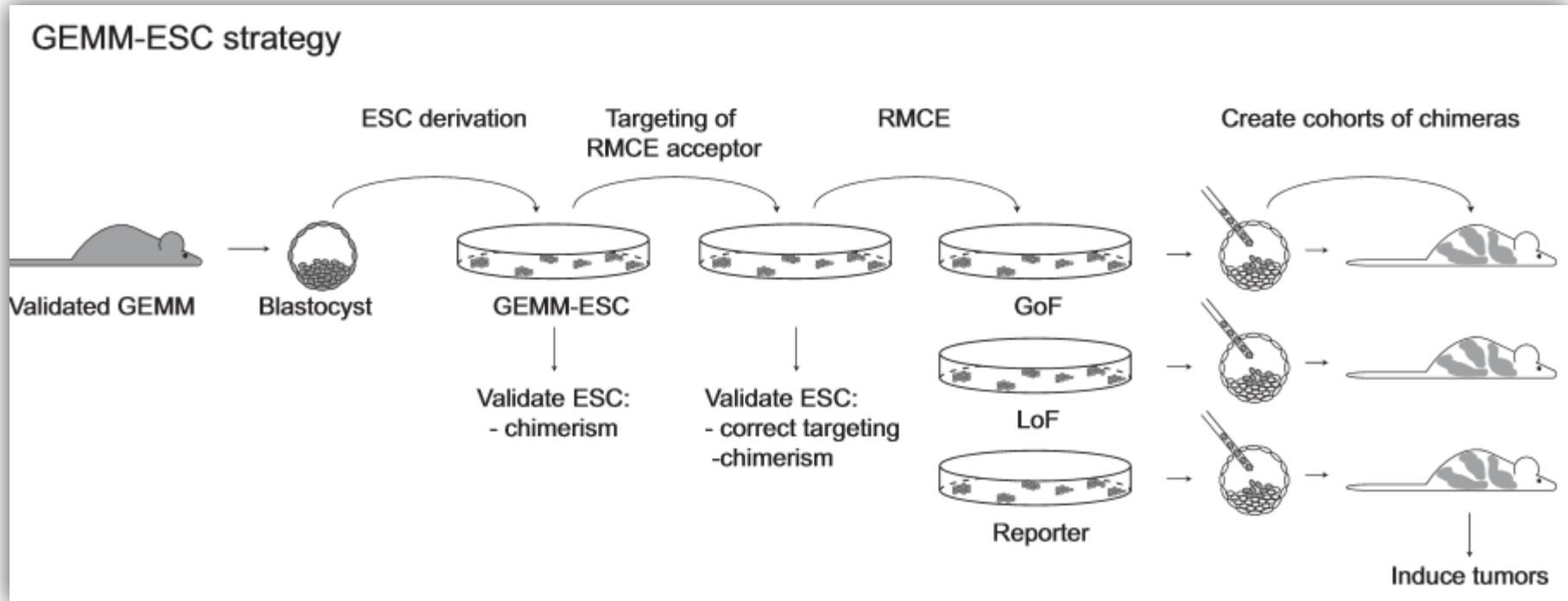


Strain of male	No. oocytes that survived injection	No. (%) oocytes cleaved	No. embryos transferred	Recipient females		No. (%) implant	No. (%) offspring
				No. used	No. pregnant		
BALB/c-nude	106	81 (76.4)	81	6	4	42 (51.9)	17 (21.0)
C3H/He	108	97 (89.8)	97	7	4	34 (35.1)	12 (12.4)

Ogonuki et al., PNAS 103: 13098-13103, 2006



# Value-added services of repositories – GEMM-ESC archive





# Technology development

## Seminal contributions to sperm cryopreservation

### Conserving, Distributing and Managing Genetically Modified Mouse Lines by Sperm Cryopreservation

G. Charles Ostermeier<sup>1,2,3</sup>, Michael V. Wiles<sup>1,3</sup>, Jane S. Farley<sup>2</sup>, Robert A. Taft<sup>2\*</sup>

<sup>1</sup> Technology Evaluation and Development, The Jackson Laboratory, Bar Harbor, Maine, United States of America, <sup>2</sup> Reproductive Sciences, The Jackson Laboratory, Bar Harbor, Maine, United States of America

BIOLOGY OF REPRODUCTION 78, 546–551 (2008)  
Published online before print 28 November 2007.  
DOI 10.1095/biolreprod.107.065359

### Methyl-Beta-Cyclodextrin Improves Fertilizing Ability of C57BL/6 Mouse Sperm after Freezing and Thawing by Facilitating Cholesterol Efflux from the Cells<sup>1</sup>

Toru Takeo,<sup>3</sup> Takayuki Hoshii,<sup>3</sup> Yuki Kondo,<sup>3</sup> Hiroshi Toyodome,<sup>4</sup> Hidetoshi Arima,<sup>4</sup> Ken-ichi Yamamura,<sup>5</sup> Tetsumi Irie,<sup>3</sup> and Naomi Nakagata<sup>2,6</sup>

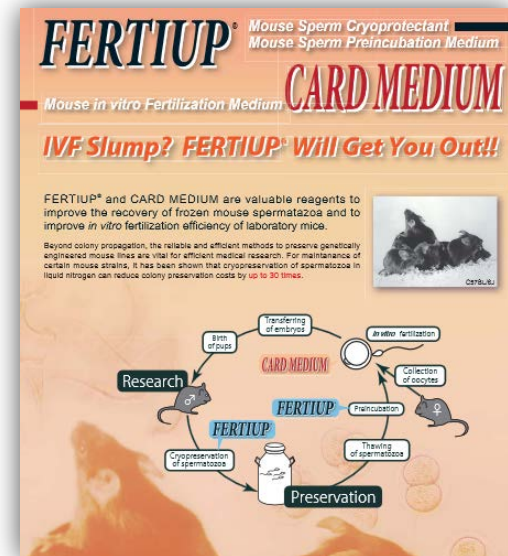
Department of Clinical Chemistry and Informatics,<sup>3</sup> and Department of Physical Pharmaceutics,<sup>4</sup> Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan  
Division of Developmental Genetics,<sup>5</sup> Institute of Molecular Embryology and Genetics, and Division of Reproductive Engineering,<sup>6</sup> Center for Animal Resources and Development, Kumamoto University, Kumamoto 860-0811, Japan

BIOLOGY OF REPRODUCTION 85, 1066–1072 (2011)  
Published online before print 20 July 2011.  
DOI 10.1095/biolreprod.111.092536

### Reduced Glutathione Enhances Fertility of Frozen/Thawed C57BL/6 Mouse Sperm after Exposure to Methyl-Beta-Cyclodextrin<sup>1</sup>

Toru Takeo and Naomi Nakagata<sup>2</sup>

Division of Reproductive Engineering, Center for Animal Resources and Development, Kumamoto University, Kumamoto, Japan







## Further developments:

- Cold transportation
- Vitrification of oocytes
- ICSI using freeze dried sperm
- Efficient derivation of iPS cells from mutant lines
- Targeting in mutant iPS cells
- etc



# Repositories develop and share technologies

Springer book on cryopreservation technology will be published in 2014



## EMMA Cryopreservation Workshop

An EMMA cryopreservation technology-development workshop was held on 7-8 May, 2012 at the CSIC (Consejo Superior de Investigaciones Científicas) main campus. It emerged as an initiative from the EMMA technology-development working group and was organized by EMMA and the CSIC. At the workshop an extraordinary group of experts in the cryopreservation field brainstormed and discussed in depth the latest technological advances in cryopreservation, including sperm and embryo cryopreservation, updated in vitro fertilization (IVF) methods and related techniques as ovary cryopreservation, laser-assisted and piezo-driven intracytoplasmic sperm injection (ICSI), transportation of frozen material and other technical and logistic challenges relevant to the operation of current mouse embryo/sperm archives.

### Organizers

- Martin Fray (Mary Lyon Centre, MRC, Harwell, UK)
- Michael Hagn (Institute of Experimental Genetics, HMGU, Munich, Germany)
- Lluís Montoliu (National Center of Biotechnology, CNB-CSIC, Madrid, Spain)

### List of invited speakers

- Sue Bath (Melbourne, Australia)
- Martina Crispo (Institut Pasteur, Montevideo, Uruguay)
- Xiang Gao (Model Animal Research Center, Nanjing University, Nanjing, P.R. China)
- Marina Gertsenstein (Toronto Centre for Phenogenomics, Toronto, ON, Canada)
- Carlisle Landel (Transposagen Biopharmaceuticals, Inc., Lexington, KY, USA)
- Kent Lloyd (Mouse Biology Program, University of California, Davis, CA, USA)
- Peter Mazur (The University of Tennessee, Knoxville, TN, USA)
- Keiji Mochida (RIKEN Bioresource Center, Tsukuba-shi, Ibaraki, Japan)
- Naomi Nakagata (Center for Animal Resources & Development, Kumamoto University, Japan)
- Lauryl Nutter (Toronto Centre for Phenogenomics, Toronto, ON, Canada)
- Atsuo Ogura (RIKEN Bioresource Center, Tsukuba-shi, Ibaraki, Japan)
- Marcello Raspa (EMMA-CNR, Monterotondo/Rome, Italy)
- Stuart Read (The Australian National University, APF, Canberra, Australia)
- Jorge Sztein (CMB Cryopreservation and Assisted Reproduction, NIAID-NIH, Rockville, MD, USA)
- Rob Taft (The Jackson Laboratory, Bar Harbor, ME, USA)
- Toru Takeo (Center for Animal Resources & Development, Kumamoto University, Japan)
- Michael Wiles (The Jackson Laboratory, Bar Harbor, ME, USA)

### Knowledgebase

Protocols



Bibliography



Workshops



EMMA Cryopreservation Workshop



INFRAFRONTIER Repository Management Workshop



Policy documents



Links



### Sponsored by



ISTT [www.transtechsociety.org](http://www.transtechsociety.org)  
International Society for Transgenic Technologies



# Educational and training programs





Technology innovations are passed on  
via cryocourses and manuals

## 2014 training courses

- **MRC Harwell:** EMMA cryocourses in March / September
- **CNR / JAX:** Joint EMMA / JAX cryocourse in October
- **JAX:** Cryocourse, May
- **CARD / ISTT:** Cryocourse in September
- **CARD:** Cryocourses in Japan
- **RIKEN:** Cryopreservation and ICSI courses
- Etc...

**Monterotondo Cryopreservation Course 1999-2014**

INFRAFRONTIER I3 - Cryopreservation training course



**Laboratory Course on  
CRYOPRESERVATION OF MOUSE GERMLASM**

October 20 - 24, 2014

**Consiglio Nazionale delle Ricerche  
Campus "A. Buzzati-Traverso"  
I-00015 Monterotondo Scalo, Rome, Italy**

The Consiglio Nazionale delle Ricerche, EMMA-Monterotondo Campus International Development and The Jackson Laboratory offer a comprehensive course on cryopreservation of mouse embryos, sperm and ovaries. This Course is financially supported by the European Union FP7 Capacities Specific Program, through the Infrafrontier-I3.

**CRYOPRESERVATION COURSE**

The course is offered to teach methods in cryopreservation for banking of various strains of mice used in research, including inbred, transgenic and knock-out strains. Several methods of cryopreservation are now available and because no single method is adequate for all the various strains of mice being developed, a variety of methods are taught. The course is designed primarily as a "hands-on" laboratory program in which participants learn techniques for the cryopreservation of cleavage-stage embryos, spermatozoa and ovaries.

*Techniques include:*

- Embryo "two-step" equilibrium freezing in plastic straws.
- Embryo non-equilibrium "ultra-rapid" cooling or "vitrification" in straws.
- Sperm freezing and recovery of frozen sperm by in vitro fertilization.

In addition, general principles of cryobiology, development of inventory databases for individual programs, and adaptation of long-term storage systems and cryogenic equipment for different situations will be presented and discussed.



## Federation of International Mouse Resources

The Federation of International Mouse Resources (FIMRe) is a collaborating group of Mouse Repository and Resource Centers worldwide whose collective goal is to archive and provide strains of mice as cryopreserved embryos and gametes, ES cell lines, and live breeding stock to the research community.

[www.fimre.org](http://www.fimre.org)



- No funding was obtained for FIMRe
- At present FIMRe is in a 'frozen' state
- Possible 'reanimation' needs to be discussed



Panel discussion





## **SciColl International** unlocking collections, expanding research



SciColl is an independent, global consortium devoted to promoting the use and impact of object-based scientific collections across disciplines, including archaeology, biology, biomedicine, earth and space sciences, technology and others.

SciColl is an initiative developed under the OECD's Global Science Forum (GSF) as international interdisciplinary coordinating mechanism for object-based scientific collections

- Memorandum of Understanding among Scicoll members
- Membership flexible (national, institutional or consortia)
- Financial contribution to Scicoll depends on collection staff size (e.g 10 – 50 people, 4000€ p.a.)
- Scicoll's budget of approximately €500,000 per year will be provided by member contributions
- Scicoll Secretariat hosted by the Smithsonian Institution in Washington DC



# Coordination and collaboration - IMSR

## International Mouse Strain Resource (IMSR)

[Search](#)[Repositories](#)[Participate](#)[Glossary](#)[Contact Us](#)[About Us](#)[Deposit Strains](#)

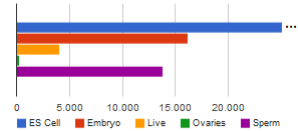
### Welcome to the IMSR

The IMSR is a searchable online database of mouse strains, stocks, and mutant ES cell lines available worldwide, including inbred, mutant, and genetically engineered strains. The goal of the IMSR is to assist the international scientific community in locating and obtaining mouse resources for research. Note that the data content found in the IMSR is as supplied by strain repository holders.

For each strain or cell line listed in the IMSR, users can obtain information about:

- Where that resource is available (Repository Site)
- What state(s) the resource is available as (e.g. live, cryopreserved embryo or germplasm, ES cells)
- Links to descriptive information about a strain or ES cell line
- Links to mutant alleles carried by a strain or ES cell line
- Links for ordering a strain or ES cell line from a Repository
- Links for contacting the Repository to send a query

Available Strains by state



Search for:

[Hide Options](#)

Strain State:

Any  
ES Cell  
embryo  
live  
ovaries

Strain Type:

Any  
closed colony  
coisogenic strain  
congenic strain  
consomic or chromosome substitution strain

Repository:

Any  
APB (Australian Phenome Bank) Australia  
CARD (Center for Animal Resources and Development) Japan  
CMMR (Canadian Mouse Mutant Repository) Canada  
EM (European Mouse Mutant Archive) Germany  
EMS (Dr. Elizabeth M. Simpson, Ph.D.) Canada  
HAR (MRC Harwell) UK  
JAX (JAX Mice) USA  
KOMP (The Knockout Mouse Project) USA

[View Repository Reports.](#)



All regions and repositories are selected by default; to limit your search to a specific region, click on the map, or select one or more specific repositories from the select list.

Mutations:

- |  |   |   |   |  |                                     |  |                                |   |
|--|---|---|---|--|-------------------------------------|--|--------------------------------|---|
| <input type="checkbox"/> chemically induced mutation | <input type="checkbox"/> chromosomal aberration | <input type="checkbox"/> deletion                   | <input type="checkbox"/> duplication          | <input type="checkbox"/> gene trap         | <input type="checkbox"/> insertion  | <input type="checkbox"/> inversion     | <input type="checkbox"/> other | <input type="checkbox"/> radiation induced mutation |
| <input type="checkbox"/> reciprocal translocation    | <input type="checkbox"/> recombinase(cre/loxp)  | <input type="checkbox"/> robertsonian translocation | <input type="checkbox"/> spontaneous mutation | <input type="checkbox"/> targeted mutation | <input type="checkbox"/> transgenic | <input type="checkbox"/> transposition |                                |   |



## Coordination and collaboration - IMSR

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- **IMSR funded by funded by grant LM009693 from the National Library of Medicine of the NIH, 2008 - 2011**
- **Current funding?**
- **Long term funding and possible further developments of IMSR should be discussed**



**Panel discussion**



# Coordination and collaboration – Agreement for International Recovery of Cryopreserved Mouse strains of Repositories

- Agreement covers exchange of specific strains among repositories
- Numerous bilateral agreements in place
- At present no exchange of resource collections (e.g. IMPC mice) among repositories
- Discuss application of Houston Principles



Panel discussion

## AGREEMENT FOR INTERNATIONAL RECOVERY OF CRYOPRESERVED MOUSE STRAINS FROM REPOSITORIES

This Agreement for International Recovery of Cryopreserved Mouse Strains from Repositories (hereinafter "Agreement"), effective April 10th, 2007 (hereafter "Start Date"), by and between RIKEN Tsukuba Institute a non-profit corporation duly organized and existing under the laws of Japan with its administrative offices at 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan (hereinafter "RIKEN") and the GSF – National Research Center for Environment and Health GmbH, a non-profit corporation duly organized and existing under the laws of Germany with its administrative offices at Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany (hereinafter "GSF").

WHEREAS, RIKEN and GSF (singly "Party" and together "Parties") believe that the common benefit of the international biomedical research community would be served by cooperative arrangements that facilitate the availability, assure the quality, promote the sharing, reduce the costs, and ensure the preservation of genetically defined mice and mouse resources;

WHEREAS, the Parties believe that such common benefit would be especially served by arrangements between non-profit and governmental mouse repositories across national boundaries, since these institutions share common values and missions;

WHEREAS, the Parties have their own cryopreservation facilities that both maintain strains of mice in a state of cryopreserved embryos, gametes and ES cell lines (hereinafter "Cryopreserved Mice"), and have the technical expertise to recover and deliver live mice ("hereinafter "Recovered Mice");

WHEREAS, the Parties agree that the common benefit of the international biomedical community would be served if a biomedical researcher (hereinafter "Investigator") could choose, based on geographical convenience or other considerations, the site of recovery of Cryopreserved Mice for the express purpose of obtaining Recovered Mice.

NOW, THEREFORE, the Parties hereby agree to the following terms and conditions:

1. The Parties agree to provide to one another specific strains of Cryopreserved Mice for the purpose of supplying Recovered Mice requested by an Investigator, which reciprocal undertaking under this Agreement is subject to the following terms and conditions:
  - a. Cryopreserved Mice provided under this Agreement are subject to any use licenses required of for-profit companies and any material transfer agreements required of academic and non-profit institutions, and the Party providing the Cryopreserved Mice is responsible for monitoring that these legal requirements are satisfied prior to transfer of the Cryopreserved Mice to the receiving Party.
  - b. Cryopreserved Mice will be provided by one Party and received by the other Party solely for the purpose of enabling the requesting Investigator to obtain the specific strain of Recovered Mice. The receiving Party shall not be entitled to replenish Cryopreserved Mice stocks without the prior written approval of the providing Party.
  - c. Recovered Mice will be provided to an Investigator on condition that the Recovered Mice will be used only for the internal research purposes of the Investigator.
  - d. If the case may be that the distribution of Recovered Mice to an Investigator is subject to licenses and/or material transfer agreements, the Party providing the Cryopreserved Mice shall be responsible to inform the Party providing the Recovered Mice accordingly, and the Party providing the Recovered Mice shall not distribute Recovered Mice before being informed by the Party providing the Cryopreserved Mice that all necessary legal requirements are satisfied.
  - e. The Parties shall not:



# Houston Principles

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- IMPC strain access critical - proactive strategy to meet community demands for access (international)
  - Tidal wave of requests coming
  - Expectation from community is for open access
- Build upon existing biorepository infrastructures
- Block “monopolies” and enable repositories to redistribute all lines
- Strains generated by IMPC should be archived in multiple archives
- EU (EMMA), US (MMRRC, JAX), China (Nanjing), Japan (RIKEN BRC)



Panel discussion





## news feature

*Nature* **417**, 785-786 (20 June 2002) | doi:10.1038/417785a

### Mouse genetics: Full house

Jonathan Knight<sup>1</sup> & Alison Abbott<sup>2</sup>

**Across the world, animal facilities are overflowing with mutant mice. Jonathan Knight and Alison Abbott consider a logistical nightmare that is reaching crisis point, thanks to the revolution in genomics.**



Full house

## Achievements since 2002

- Capacity development
- More stable funding (e.g. INFRAFRONTIER)
- Technology development
- Coordination and collaboration among repositories
- IMPC and disruptive innovations require push to next level including legal innovations