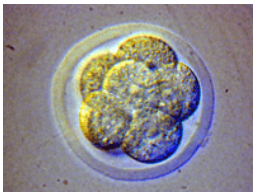
Mouse Embryo Cryopreservation Facility



Lluís Montoliu, CNB, 8 March 2012

Mouse Embryo Cryopreservation Facility

- Why a mouse embryo cryopreservation facility?
- The cryopreservation team
- The cryopreservation lab
- Our tools, equipments and resources
- What do we offer to CNB scientists?
- Spanish node of European Mouse Mutant Archive
- INNOTEK Platform
- How to contact us?

Mouse Embryo Cryopreservation Facility – INNOTEK Platform
Spanish node of EMMA
http://www.cnb.csic.es/~criocnb/

Why a mouse cryopreservation facility?

- To cryopreserve mouse lines generated/used by researchers that are no longer in use, therefore clearing some space in the animal facility, reducing the overall number of mice and optimizing the always limited resources.

- To ease the reception and rescue mouse lines sent by external parties (companies/international projects/scientific collaborators) available as frozen embryos or sperm.

- To ease the shipment of mouse lines generated/used by scientists to external Research Centres avoiding problems usually associated with health certificates.

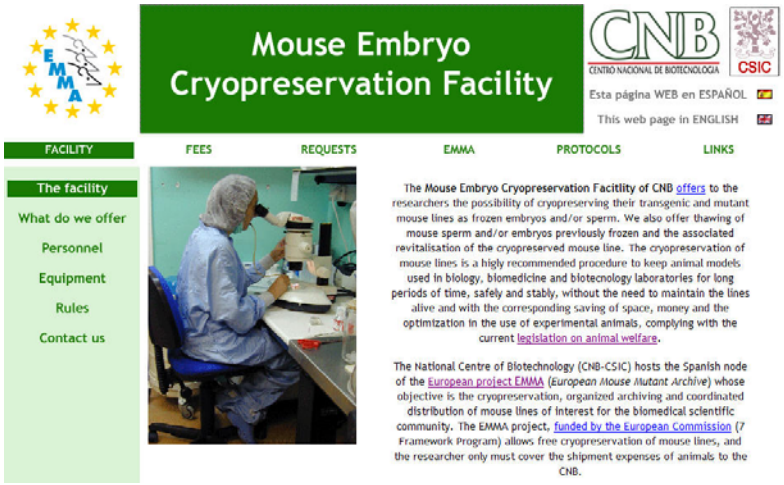
- To preserve, indefinitely, and securely, at a very low cost, mouse lines generated/used by researchers.

- To comply with current legislation on animal welfare, particularly the concept of the 3-Rs: refining, replacing and reducing animal research, whenever and wherever possible.

The cryopreservation team at CNB

Mouse Embryo Cryopreservation Facility – INNOTEK Platform
Spanish node of EMMA
http://www.cnb.csic.es/~criocnb/

The cryopreservation facility website



www.cnb.csic.es/~criocnb/

The cryopreservation lab

Laboratory B15 – Ground Floor

Mouse Embryo Cryopreservation Facility – INNOTEK Platform
Spanish node of EMMA
http://www.cnb.csic.es/~criocnb/

The cryopreservation lab

Laboratory B15 – Ground Floor

Our tools, equipments and resources

Controlled-rate freezing unit (BioCool BC-IV-40)
Stereo-microscopes with additional cold light systems
(Leica MZ8 y MZ9, Nikon SMZ 2T)
CO2 Incubator (Sanyo MCO 17AI)
Fridge-freezer
Thermostatized water-bath
Thermostatized hot plate
Microcentrifugue
Vortex-type shaker
Precision Balance
pH-meter
Liquid nitrogen containers for storage
(Air Liquide GT-21, GT-35, ESPACE 150, TP-100)
Liquid nitrogen containers for shipments
(MVE SC2-1V, SC 4/2 V, SC20/20)
Telstar Shipper Container
computers
Mouse cage isolators (B60, Bell Isolation Systems)
Desktop Thermosealer ME-300 HI (Mercier Co.)
Mini-Incubator K-MINC-1000 (Cook)
Stereo Microscope (Leica M125) with digital camera and software
Styropor box hard-cased dry ice chest (Thermosafe)
Label Printer for Straws (Brady)

Mouse Embryo Cryopreservation Facility – INNOTEK Platform
Spanish node of EMMA
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Our tools, equipments and resources

Three isolator units, purchased with funds from:

ciberer — CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED DE ENFERMEDADES RARAS — **1x**

EMMA — **1x**

excelencia UAM CSIC — **1x**

What do we offer to CNB scientists?

- Cryopreserving mouse lines
- Freezing embryos and/or sperm from mouse lines
- Thawing frozen embryos and/or sperm from mouse lines
- Rescueing mouse lines
- Importing / exporting frozen mouse embryos/sperm
- Storage of mouse lines (embryos/sperm) in liquid nitrogen

Mouse Embryo Cryopreservation Facility – INNOTEK Platform
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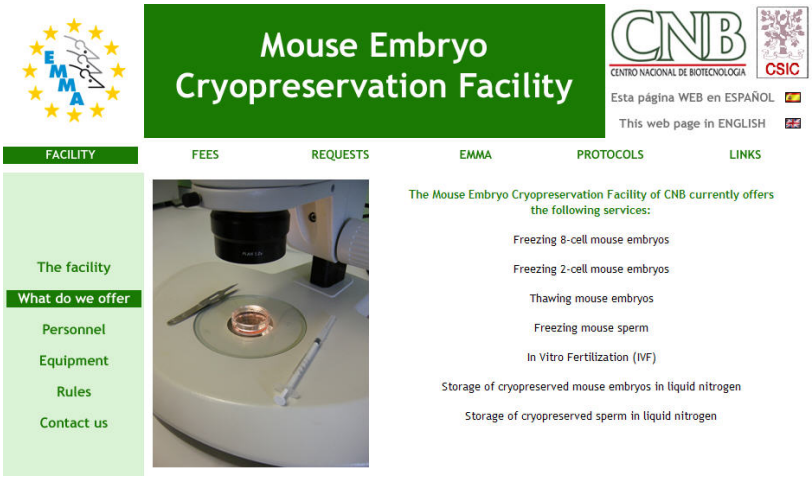
Do we have to freeze embryos or sperm… or both?

- Cryopreserved MOUSE EMBRYOS carry the full genome and will develop, upon thawing and transferring to suitable recipients, into mouse individuals, rescueing exactly the mouse line that was initially cryopreserved.

Cryopreserved MOUSE SPERM will require in vitro fertilization techniques with suitable mouse oocytes in order to rescue a mouse line. The mice rescued might not be genetically identical to the individuals used to cryoperserve the mouse line.

- MOUSE EMBRYOS are good for preserving mouse lines
- MOUSE SPERM is good for preserving mouse gene alleles, genetic mutations
- Current world archives are moving from embryos to sperm
- Ideally, whenever possible, embryos AND sperm should be cryopreserved

What do we offer to CNB scientists?



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Methods available at the cryopreservation facility

- Ultrarapid freezing of mouse 8-cell embryos
- Vitrification of mouse embryos
- Freezing sperm (standard, JAX, CARD methods)
- In vitro Fertilization (IVF)
- Freezing mouse 2-cell IVF-derived/8-cell embryos by controlled-rate freezers
- Embryo transfer to mouse recipients
- Genotyping
- Handling mouse / embryos / sperm importations/exportations

Research and Innovation at the cryopreservation facility

MTG

The JAX method

OPEN ACCESS Freely available online PLoS one

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

G. Charles Ostermeier[1,2*], Michael V. Wiles[1*], Jane S. Farley[2], Robert A. Taft[2*]

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

PLoS ONE | www.plosone.org 1 July 2008 | Volume 3 | Issue 7 | e2792

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

MBCD
GLN
GSH

The CARD method

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

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

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

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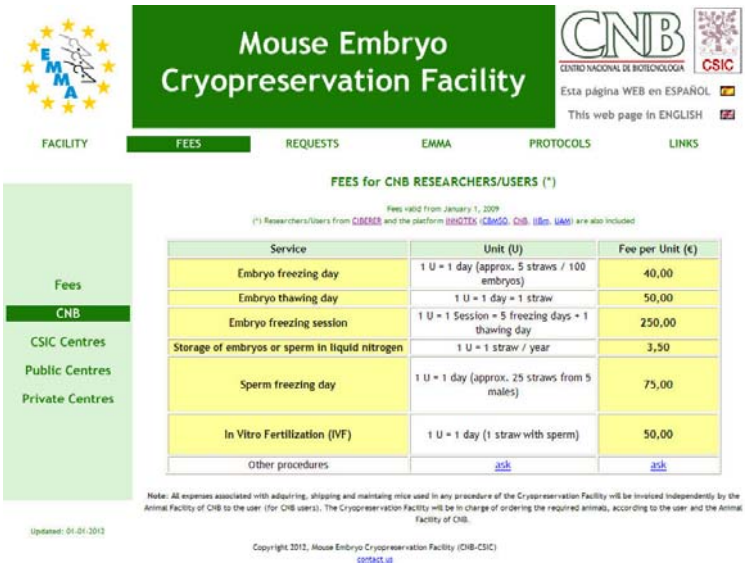
CNB cryopreservation facility fees



CNB CENTRO NACIONAL DE BIOTECNOLOGIA excelencia UAM CSIC

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EMMA
Mutant mice for the scientific community

European Mouse Mutant Archive

CNB CENTRO NACIONAL DE BIOTECNOLOGIA

CNB-CSIC hosts the Spanish node of EMMA since 2009

www.emmanet.org

Objectives of EMMA

European Mouse Mutant Archive

- The central European repository for mutant mouse strains
- Access to mutant mouse lines for the scientific community
- Dissemination of knowledge

EMMA is supported by the European Commission FP6/FP7 Research Infrastructures Programmes

European Commission

www.emmanet.org

EMMA procedures

European Mouse Mutant Archive

- **Supported by EU**
- Cryopreservation costs for submitted mouse lines is **free** for scientists (submitters only pay shipment costs)
- Submissions are **evaluated** by a external scientific committee
- Submitted mouse lines are **freely available** to other researchers
- Two years **grace period** is accepted, accompanying **MTA document** is also accepted
- Access to cryopreserved mouse lines requires the payment of a **repository fee** (1100 Euros for embryos/sperm, 2400 Euros for live mice), plus shipment costs

www.emmanet.org

EMMAservice: 2009-2012

European Mouse Mutant Archive

CNR/Monterotondo
CNR Campus "A. Buzzati-Traverso", Monterotondo/Rome, Italy

CNRS/CDTA
Centre de Distribution, de Typage et d'Archivage animal, Orléans, France

MRC/MGU
Mammalian Genetics Unit, Harwell, UK

Karolinska Institutet
Karolinska Institutet, Stockholm, Sweden

FCG/IGC
Instituto Gulbenkian de Ciência , Oeiras, Portugal

HMGU/IEG
Institute of Experimental Genetics, Munich, Germany

EMBL/EBI
European Bioinformatics Institute, Hinxton, UK

GIE-CERBM/ICS
Institut Clinique de la Souris, Illkirch/Strasbourg, France

Sanger Institute
Wellcome Trust Sanger Institute, Hinxton, UK

CNB/CSIC
Centro Nacional de Biotecnología, Madrid, Spain

FLEMING
BSRC "Alexander Fleming", Vari/Athens, Greece

IMG
Institute of Molecular Genetics, Prague, Czech Republic

UOULU/BCO
Biocenter Oulu, Oulu, Finland

VETMEDUNI/BIAT
Biomodels Austria, Vienna, Austria

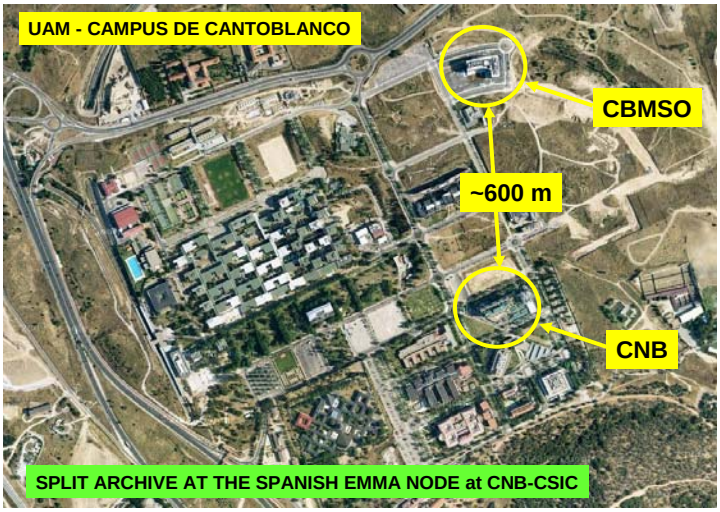
European Commission

7FP: 14 Partners (2012)

www.emmanet.org

CNB-CSIC Madrid EMMA node

European Mouse Mutant Archive



www.emmanet.org EMMAinf meeting Monterotondo 14.12.2007

Who can submit mice to EMMA ?

European Mouse Mutant Archive

Anyone **owning** a mouse strain (having generated the mouse line)

Anyone **having** a mouse strain generated by a third party that has issued a **permission** to submit the mouse line to an archiving repository

Example: you can't submit Jackson lines to EMMA

www.emmanet.org

Which mice can be submitted to EMMA ?

European Mouse Mutant Archive

Any **new** genetically modified mouse (transgenic, knockout, knockin, knock-down), induced mutant, spontaneous mutant,…

Any **old / known** genetically modified mouse (transgenic, knockout, knockin, knock-down), induced mutant, spontaneous mutant,… in a **new mouse genetic background**

www.emmanet.org

Why submitting mice to EMMA ?

European Mouse Mutant Archive

1) Because archiving/cryopreserving mouse lines in EMMA is **FREE OF CHARGE**

2) Because there is **NO loss of intellectual propertly rights** potentially associated with the archived mouse lines

3) Because the use of standard operating protocols (SOPs) ensures consistently **high standards**

4) Because EMMA allows and provides a **centralized access** to mutant mice of interest

www.emmanet.org

Why submitting mice to EMMA ?

European Mouse Mutant Archive

5) Because EMMA **distributes mutant mouse lines of interest for the scientific community**

6) Because all mice obtained through EMMA have **SPF-FELASA conditions / quality**

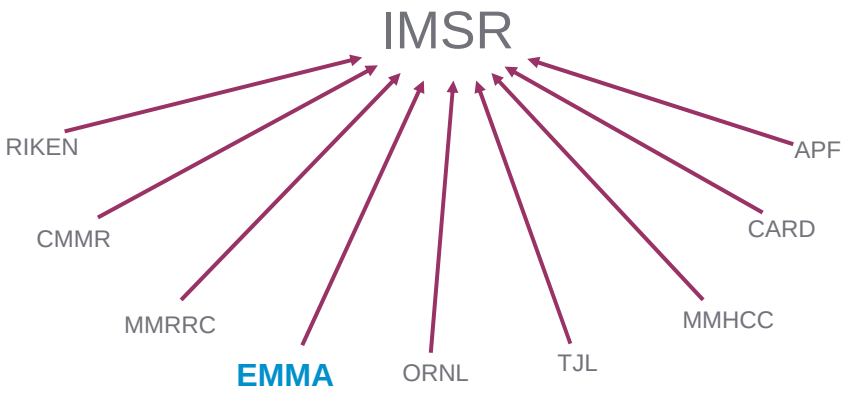
7) Because it **contributes to the progress and development of the scientific community** through sharing animal models

8) Because using EMMA **increases the visibility** of mouse lines and triggers their use and **potential collaborations**

www.emmanet.org

EMMA in the Global network of repositories

European Mouse Mutant Archive



IMSR: **International Mouse Strain Resource**: **www.findmice.org**

www.emmanet.org

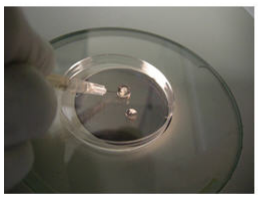
New agreement CNB and CNIO

European Mouse Mutant Archive

The CNB joins forces with CNIO to distribute mouse strains essential for basic biomedical research

Monday, 05 March 2012

The Spanish National Centre for Biotechnology (CNB) just signed an agreement for the cryopreservation and the distribution of embryos and sperm from transgenic and mutant mouse strains generated at the Spanish National Cancer Research Centre (CNIO). Thanks to the **European Mouse Mutant Archive** (EMMA), mice from CNIO's researchers will be **available to qualified biomedical researchers**.

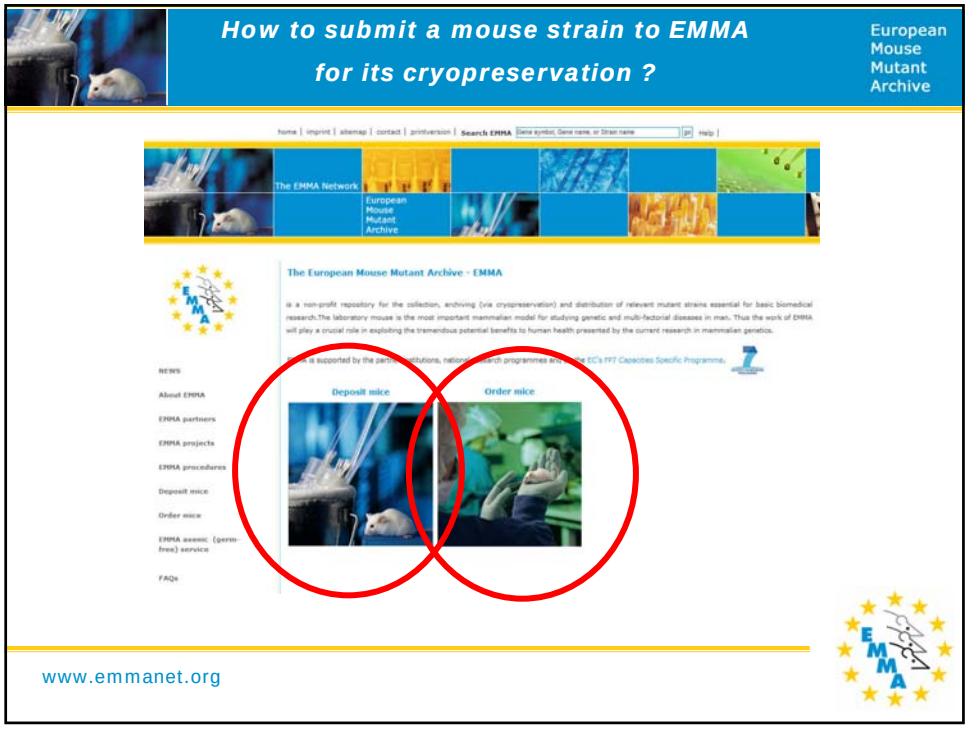


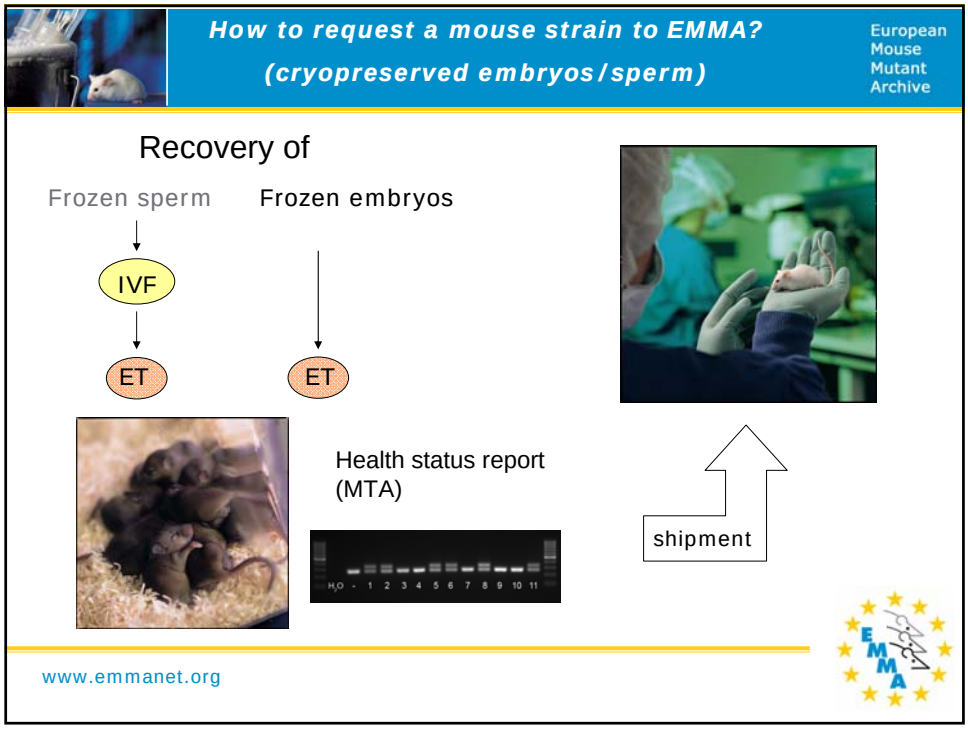
In January 2009 CNB joined, coordinated by **Lluís Montoliu**, the EMMA network as its Spanish node; collecting, archiving via cryopreservation and distributing relevant **mutant strains essential for basic biomedical research**. From now on, strains with relevance in oncology generated at the CNIO will be also in this non-profit repository available for life scientists.

The submission and distribution of mouse mutant strains to and from the **EMMA archive** is subject to legally binding conditions. The documents stating the conditions for submission and distribution of EMMA strains are available in their web page. EMMA-maintained lines are supplied to qualified researchers as a service to the scientific community at large, solely for research purposes and not for commercial use. The legally binding EMMA conditions further state that the recipient individual, laboratory or institution may not transfer or sell these mice or their progeny to any third party outside the recipient institution. Any already existing Material Transfer Agreements will remain in full force and effect.

The mouse strains imported into the EMMA facilities are subject to quarantine in cage isolators. Live colonies are obtained upon cleansing by embryo derivation and maintained in specific-pathogen-free (SPF) conditions. In addition, as a special service, EMMA provides the re-derivation of selected mouse strains into the germ-free status.

www.emmanet.org


How to submit a mouse strain to EMMA
for its cryopreservation ?
European
Mouse
Mutant
Archive
The EMMA Network
European
Mouse
Mutant
Archive
The European Mouse Mutant Archive - EMMA
Deposit mice
Order mice
www.emmanet.org


How to request a mouse strain to EMMA?
(cryopreserved embryos/sperm)
European
Mouse
Mutant
Archive
Recovery of
Frozen sperm
Frozen embryos
IVF
ET
ET
Health status report
(MTA)
shipment
www.emmanet.org

How to request a mouse strain to EMMA?
(cryopreserved embryos/sperm)

European Mouse Mutant Archive

Obtaining mouse strains from EMMA is not free

However, EU has implemented in these projects:

EMMA TRANSNATIONAL ACCESS APPLICATIONS

Limited number of mouse strains distributed for free upon receiving and evaluating expression of interests in open calls that are launched each year

Calls and application forms are regularly available at the EMMA website

www.emmanet.org

CNB cryopreservation facility or EMMA node?

Cryopreservation through EMMA procedures is OPTIONAL

CNB and external scientists can choose :

A) EMMA Service
free, mouse lines available through EMMA database*
(*) Grace period and MTA can be included

B) Cryopreservation Facility at CNB
paid service, mouse lines private

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Spanish node of EMMA
http://www.cnb.csic.es/~criocnb/

The INNOTEK Platform

SCIENTIFIC-TECHNOLOGICAL PLATFORMS IN SUPPORT OF RESEARCH AT THE INTERNATIONAL CAMPUS OF EXCELLENCE UAM-CSIC

INNOTEK

Advanced technologies for the generation and characterization of animal models

www.cnb.csic.es/~innotek/
innotek@cnb.csic.es

The INNOTEK Platform

CNB	Animal Experimentation Facility Responsible: Angel Naranjo
	Transgenic Facility CNB-CBMSO Responsible: Belén Pintado
	Mouse Embryo Cryopreservation Facility Responsible: Lluis Montoliu
	Histology Facility CNB-IIBm Responsible: Lluis Montoliu
CBM	Animal Experimentation Facility Responsible: Javier Palacín
	Transgenic Facility CNB-CBMSO Responsible: Belén Pintado
	Genomics Facility Responsible: Mª Begoña Aguado
iibm	Animal Experimentation Facility Responsible: Fernando Núñez
	Image and Spectroscopy by High Field Magnetic Resonance Facility: SIERMAC Responsible: Sebastián Cerdán
	Non-Invasive Neurofunctional Evaluation Facility: ENNI Responsible: Isabel Varela-Nieto
	Histology Facility CNB-IIBm Responsible: Lluis Montoliu
UAM	Animal Experimentation Facility-Veterinarian Laboratory Responsible: Carmen Fernández Criado


CNB Mouse Cryopreservation Facility
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www.cnb.csic.es/~criocnb/