The Infrafrontier RI
Lluís Montoliu (CNB-CSIC & CIBERER-ISCIII)

www.infrafrontier.eu
Providing unique scientific resources, services, and expertise to advance the understanding and treatment of human diseases using rodent models.
Access to physical resources, analytical platforms, knowledge and data

- Model Development
- Systemic Phenotyping
- Archiving & Distribution
- Training, Expertise
- Scientific Outreach
- Data

www.infrafrontier.eu
Promoting responsible and reproducible research

Promoting the 3Rs
- Replacement
- Reduction
- Refinement

Minimising duplication by providing centralised high-quality resources
INFRAFRONTIER Resources and Services

Model Development ➔ Systemic Phenotyping ➔ Archiving / Distribution

www.infrafrontier.eu

Distributed research infrastructure – one face to the customer
A European Research Infrastructure for the generation, phenotyping, archiving and distribution of mammalian models

A consortium of leading European research institutions - **29 partners in 15 countries**

+ Canada, Israel
Infrafrontier GmbH

2013
The European Mouse Mutant Archive (EMMA)

- Open access to more than 7708 mouse lines
- High-quality health controlled mice with defined and verified genotype
- EMMA ships globally
EMMA: current members

- CNR/IBC
  Istituto di Biologia Cellulare, Monterotondo, Italy
- CNRS/CTDA
  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
  Biomedical Sciences Research Center Al. Fleming, Athens, Greece
- OULU
  University of Oulu, Oulu, Finland
- BIAT
  Vetmeduni Vienna, Biomodels Austria, Vienna, Austria
- IMG
  Institute of Molecular Genetics, Prague, Czech Republic
- TAU
  Tel Aviv University, Tel Aviv, Israel
- NKI
  Netherlands Cancer Institute, Netherlands, Amsterdam

16 nodes / 13 countries

www.infrafrontier.eu
EMMA Shipping Destinations

>700 unique destinations (cities) worldwide
Systemic phenotyping and mouse clinics

Mouse clinics offer a **whole-system screen** covering more than 500 parameters in different disease areas and organ systems.

Added benefit of obtaining **maximum information** using a **minimum amount of animals**.
Publications related to INFRAFRONTIER / EMMA

- 2574 publications; both from strain submissions and strain requests
- 49 Cell publications
- 43 Nature Genetics publications
- 82 Nature publications
• **International Mouse Phenotyping Consortium**

• Systemic phenotyping of knock-out mice for all ~20,000 protein coding genes → **comprehensive catalogue of gene function**

• Phenotyping data and mice are made **openly available** to the biomedical community

• **INFRAFRONTIER** is the largest distributor of IMPC lines worldwide - **350 shipments per year**
We’re Celebrating Our 10 Year Anniversary!

The IMPC is celebrating 10 years of research and mouse knockout production. We are launching a series of thematic workshops starting on October 25th, 2021 and recently hosted the Celebratory Conference on Mouse Genetics and Genomic Medicine.

Read more on our upcoming workshops

Search All 7824 Knockout Data...

What you need to know about IMPC data

www.mousephenotype.org

7824 total knockout genes phenotyped
IMPC Data Release 15.1 Notes

IMPC
- Release: 15.1
- Published: 11 October 2021

Statistical Package
- OpenStats
- Version: 1.4.0

Genome Assembly
- Mus musculus
- Version: GRCm38

Summary
- Number of phenotyped genes: 7,824
- Number of phenotyped mutant lines: 8,457
- Number of phenotype calls: 90,010

Data access
- Ftp interface: ftp
- RESTful Interfaces: APIs

www.mousephenotype.org
INFRAFRONTIER Therapeutic Areas

Collection of INFRAFRONTIER resources and services related to a therapeutic area in one page
INFRAFRONTIER resources for

Covid-19 research
Cancer research
Rare Diseases research

EMMA strains

Search EMMA strains
Search strains...

Browse EMMA strains
Collections  Human Diseases

Resources and Services

Deposit mice
EMMA archives your mouse mutant free of charge

Order mice
Access to 8000 mouse mutants and research tools

Axenic service
INFRAFRONTIER produces germ-free mice free of charge

NKI GEMM-ESC archive
Rapid target gene validation in complex cancer mouse models

Mouse production and phenotyping
INFRAFRONTIER open calls

Training and consulting
Hands-on courses in cryopreservation and phenotyping

Procedures & Knowledgebase

News & Events

Information on service fees, legal issues, health monitoring and animal welfare

Validated laboratory protocols and a comprehensive list of relevant publications

UQxh – a new player in mitochondrial complex III disorders

More news and events

www.infrafrontier.eu
Due to the current coronavirus (COVID-19) situation, there may be delays in providing the EMMA services. While traffic restrictions and other measures taken to slow down spreading of the virus are in force, work at EMMA nodes is impaired considerably. Therefore, delivery times will be longer than usual.

Nevertheless, please go ahead and place your orders, so that we can already organize the paperwork with you. Even this could take longer, as people at EMMA nodes or at the involved authorities and carriers might not be able to always go to work, while social distancing is advised. The situation is beyond our control and we will resume all activities whenever our institutions/governments will authorize us to do so.

Thank you for your understanding.

Nervous System
### Multiple sclerosis mouse models in Infrafrontier

<table>
<thead>
<tr>
<th>EMMA ID</th>
<th>Disease name / DOID</th>
<th>Gene symbol</th>
<th>Common strain name(s)</th>
<th>(International) Strain Designation</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM:00444</td>
<td>multiple sclerosis / DOID:2377</td>
<td>Tg(Gfap-TNF^<em>)K21Gki TgK21 [CBA;B6-Tnfraf1a1m18t Tg(Gfap-TNF^</em>)K21Gki/Fimm]</td>
<td>STOCK Tnfraf1a1m18t Tg(Gfap-TNF^*)K21Gki/Fimm</td>
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<td></td>
</tr>
<tr>
<td>EM:00143</td>
<td>multiple sclerosis / DOID:2377</td>
<td>Prf1 Pp(KO)</td>
<td>NOD.B6-Prf1^tm1562/Cnmm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM:07765</td>
<td>multiple sclerosis / DOID:2377</td>
<td>Prf1</td>
<td>A/J.Rko-/+ or A/J.Rko-/+ A.B6-Prf1^tm1562/Elat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Showing 1 to 3 of 3 entries
INFRAFRONTIER Trans-national Access Calls

• One of our main avenues to support biomedical research
• INFRAFRONTIER has published **25 trans-national access calls**
• Selection of projects based on **scientific excellence**
• **More than 400 projects** have been supported
• Recent calls:
  – **Precision model development** (mice, rats)
  – Derivation of **germ-free mice**
  – Late-onset phenotyping for **ageing research**
  – Specialised phenotyping (**metabolic, behavioural, inflammatory**)  
  – **COVID-19 Therapeutics Pipeline**
New TA – COVID-19 Therapeutics Pipeline

- BSL3 pipeline and COVID-19 models
- Test therapeutic candidates
- Further analysis on collaboration

**STANDARD Access Unit: Clinical monitoring, scoring and viral load**

- Mice monitored daily for morbidity (body weight), mortality (survival) and scored for clinical symptoms (weight loss, eye closure, appearance of fur, posture, and respiration).
- Viral titers in the lung and brain of infected mice (PFU, TCID50,qRT-PCR) at day 2 and 5 post infection.
The EMMA-Infrafrontier Spanish node at CNB
EMMA-Infrafrontier Services provided from CNB

- Depositing mouse lines into EMMA
  - Depositing requests evaluated by external committee
  - Approved requests are cryopreserved for free
  - Depositor covers shipping costs
  - Cryopreserved mouse lines freely available to everyone
  - Grace period possible (up to 2 years), MTAs accepted

- Requesting mouse lines from EMMA
  - Access to EMMA archive requires payment of repository fee
  - Frozen sperm/embryos: 1,600 Euros + shipping costs
  - Live mice: 3,200 Euros + shipping costs

www.infrafrontier.eu
Equipment: **ISOLATOR UNITS**

**for EMMA procedures**
(allow safe importation of mice from different research centres, irrespective of their health status, without interfering with mice kept at the CNB animal house)
CNB-CSIC Madrid EMMA node

 bells B60 isolator
(three units)
EMMA procedures at CNB - I

• Cryopreservation of mouse strains
  – Cryopreservation of mouse embryos, sperm, oocytes
  – Genotyping (single embryos) and quality control

• Rescue of mouse strains from frozen material
  – Thawing of mouse embryos, sperm, oocytes
  – In vitro fertilization and embryo transfer
  – Genotyping and quality control

wwwuser.cnb.csic.es/criocnb/
EMMA procedures at CNB - II

- **Importing mouse lines**
  - Receiving frozen mouse embryos (LN$_2$)
  - Receiving frozen mouse sperm (LN$_2$ and dry ice)
  - Receiving refrigerated mouse embryos (+4/+8$^\circ$C)
  - Receiving refrigerated mouse epididymes (+4/+8$^\circ$C)
  - Receiving live mice

- **Exporting mouse lines**
  - Shipping frozen mouse embryos (LN$_2$)
  - Shipping frozen mouse sperm (LN$_2$ and dry ice)
  - Shipping refrigerated mouse embryos (+4/+8$^\circ$C)
  - Shipping refrigerated mouse epididymes (+4/+8$^\circ$C)
  - Shipping live mice

wwwuser.cnb.csic.es/criocnb/
The CARD cold transportation kit
• Cryopreserved mouse sperm and embryo simples kept in LN2 tanks totally immersed
• Backup samples stored at a second independent location in Campus
CNB-CSIC Madrid EMMA node

SPLIT ARCHIVE AT THE SPANISH EMMA NODE at CNB-CSIC
The CARD revolution

Nakagata’s Lab

- HTF-like medium with reduced glutathione added (GSH)
- Preincubation medium with MBCD added

KYUDO and Cosmo Bio Co., Ltd. Japan (www.cosmobio.co.jp)
FREEZE

THAW

SPERM CRYOPRESERVED

IVF

EMBRYOS CRYOPRESERVED

QC OK

FREEZE

THAW

RESCUE
EMMA procedures at CNB - III

- Rederivation of mouse strains (into SPF zone)
- Generation of knockout mice from ES cell clones (TNAs)
- Generation of genome edited (CRISPR) mice (TNAs)

  - Advice / experimental design / guide RNA selection
    - Gene disruptions
    - Gene deletions/inversions
    - Gene substitutions/mutations (ssDNA oligos)

  - Electroporation of mouse embryos
  - Embryo transfer into pseudopregnant females

www.user.cnb.csic.es/criocnb/
Infrafrontier and CRISPR licensing
EMMA Infratontier mouse lines at CNB

EMMA lines in total: 7708
EMMA lines at CNB: 543

Pie chart showing:
- 379; 70% not requested
- 164; 30% requested
Number of EMMA mouse strains at CNB requested "N" times

Number of EMMA mouse strains

164 mouse lines requested
Impact of INFRAFRONTIER on ANIMAL WELFARE

• Number of animals “saved” (not bred, not born, not used) because they are cryopreserved

• ~50 animals/year needed to maintain a colony

• ~ 7,000 mouse lines archived in EMMA-INFRAFRONTIER

• Up to 350,000 mice per year
BEFORE: to cryopreserve ONE mouse line

We required 8-10 males from the mouse line to be cryopreserved

We crossed the males 1:1 once per week during 5-6 weeks
REDUCTION

TODAY: to cryopreserve ONE mouse line

We require 2-5 males from the mouse line to be cryopreserved

We obtain oocytes from 2-5 females for IVF

IVF and Cryopreservation methods extremely refined (CARD, Univ. Kumamoto)

From 60-70 mice to 4-10 mice in 1 day per ONE line to be cryopreserved
Blastocyst genotyping for quality control of mouse mutant archives: an ethical and economical approach

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Cryopreservation 
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