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# Sharing mutations: Biobanks are still required in the post-CRISPR/Cas9 era

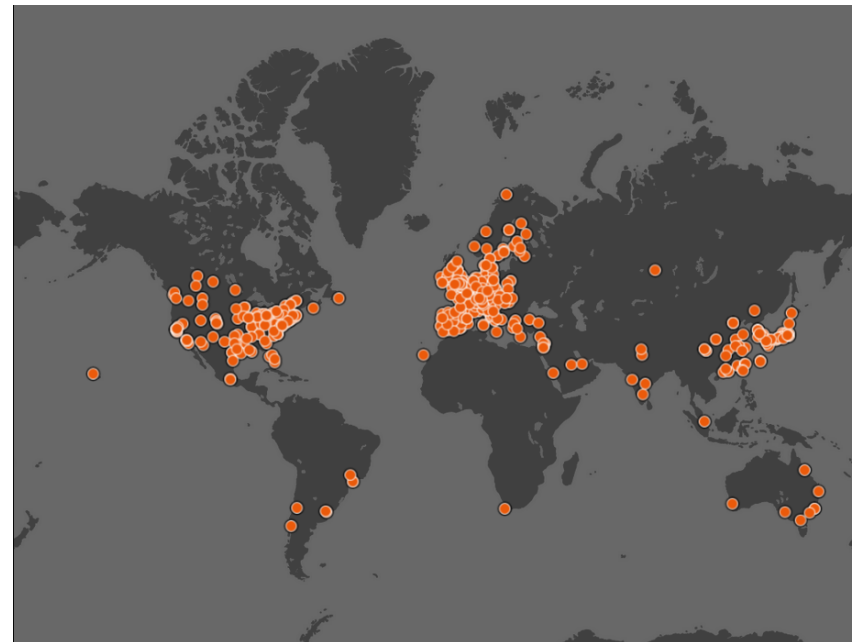
Martin D. Fray

MRC Harwell Institute, UK



# European Mouse Mutant archive

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# What is a biobank?

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- Banks make a return on investments



- High quality reagents = high quality science
- Not a dumping ground



# Sharing resources through biobanks

- More collaborations
- Higher profile and citation index
- Cost benefit
- Quid pro quo
- Grace periods permit publication
- Beneficial rights are retained
- Biobanks offer **free** archiving

**Archiving**  
Safe guard your mouse lines against loss and ensure a co-operative future for mouse research

	<b>Free of Charge</b> Allowing your funding to be better spent within your research group. The only costs are in shipping the mice to our facility.
	<b>2 year Grace Period</b> Offered to enable publication of your work before the line is made publicly available
	<b>Secure your Mouse Lines</b> Cryopreserved sperm and embryos are viability tested ensuring each mouse stock can be resurrected when required.
	<b>Publicly Available</b> Mouse lines are publicly available for distribution via <a href="https://www.infrafrontier.eu/">https://www.infrafrontier.eu/</a> and <a href="http://www.mousebook.org/">http://www.mousebook.org/</a> .
	<b>Protect your IP via an MTA exchange</b> MTAs are signed electronically via our MTA portal. This is both straightforward and secure, protecting your IP rights.
	<b>Acknowledged in Resulting Publications</b> Ensuring that your work is recognised throughout the scientific community

Further information at <https://www.infrafrontier.eu/> or contact [fesa@har.mrc.ac.uk](mailto:fesa@har.mrc.ac.uk)



# Know thy mice!

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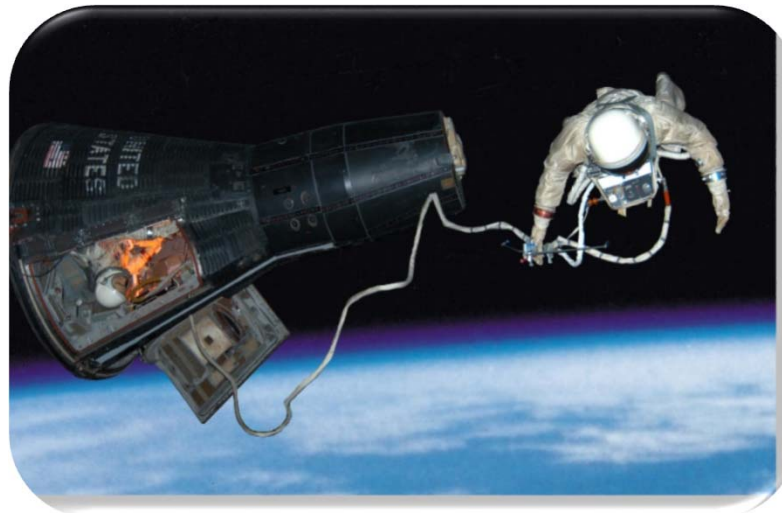
- Biobanks distribute QCed reagents
- Use correct nomenclature
- Health status is controlled
- Identical material is distributed



# Genetic anchor points

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- Point of production
- Crossed to new genetic background
- Publication



# Reproducibility – vital to progress

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PERSPECTIVE

## The Economics of Reproducibility in Preclinical Research

**Leonard P. Freedman<sup>1\*</sup>, Iain M. Cockburn<sup>2</sup>, Timothy S. Simcoe<sup>2,3</sup>**

**1** Global Biological Standards Institute, Washington, D.C., United States of America, **2** Boston University School of Management, Boston, Massachusetts, United States of America, **3** Council of Economic Advisers, Washington, D.C., United States of America

\* [lfreedman@gbsi.org](mailto:lfreedman@gbsi.org)

- Irreproducibility costs \$28B/year in USA alone
- 36.1% from biological reagents and reference materials
- [PLoS Biol13\(6\):e1002165.doi:10.1371/journal.pbio.1002165](https://doi.org/10.1371/journal.pbio.1002165)



# Arguments still apply post CRISPR

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- Need to educate new users:

C57BL/6NTac-Gnb4<sup>em2(IMPC)/H</sup>

Standardisation & genetic background

~100 point mutations/gen (Lynch et al 2010)

~£2,500/year to maintain colony

Time to generate, plus time to confirm edit





# Obligations on biobanks

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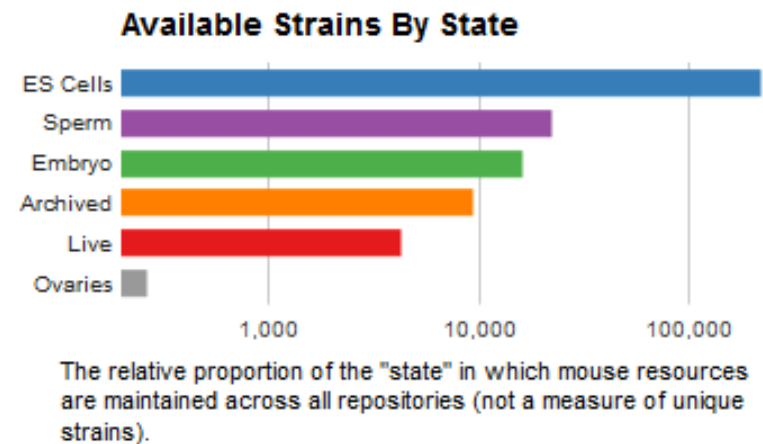
[www.findmice.org](http://www.findmice.org)



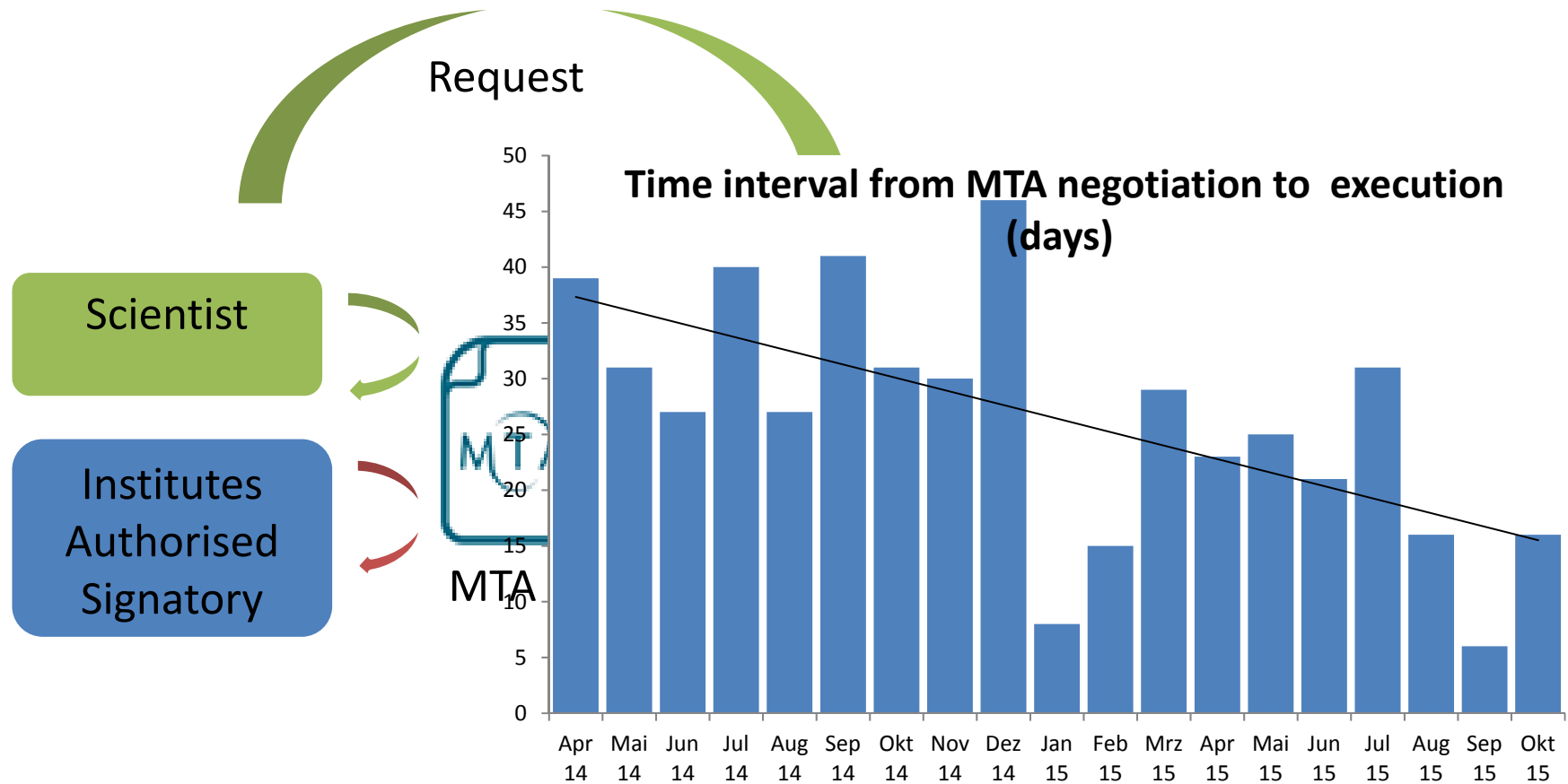
# What is possible?

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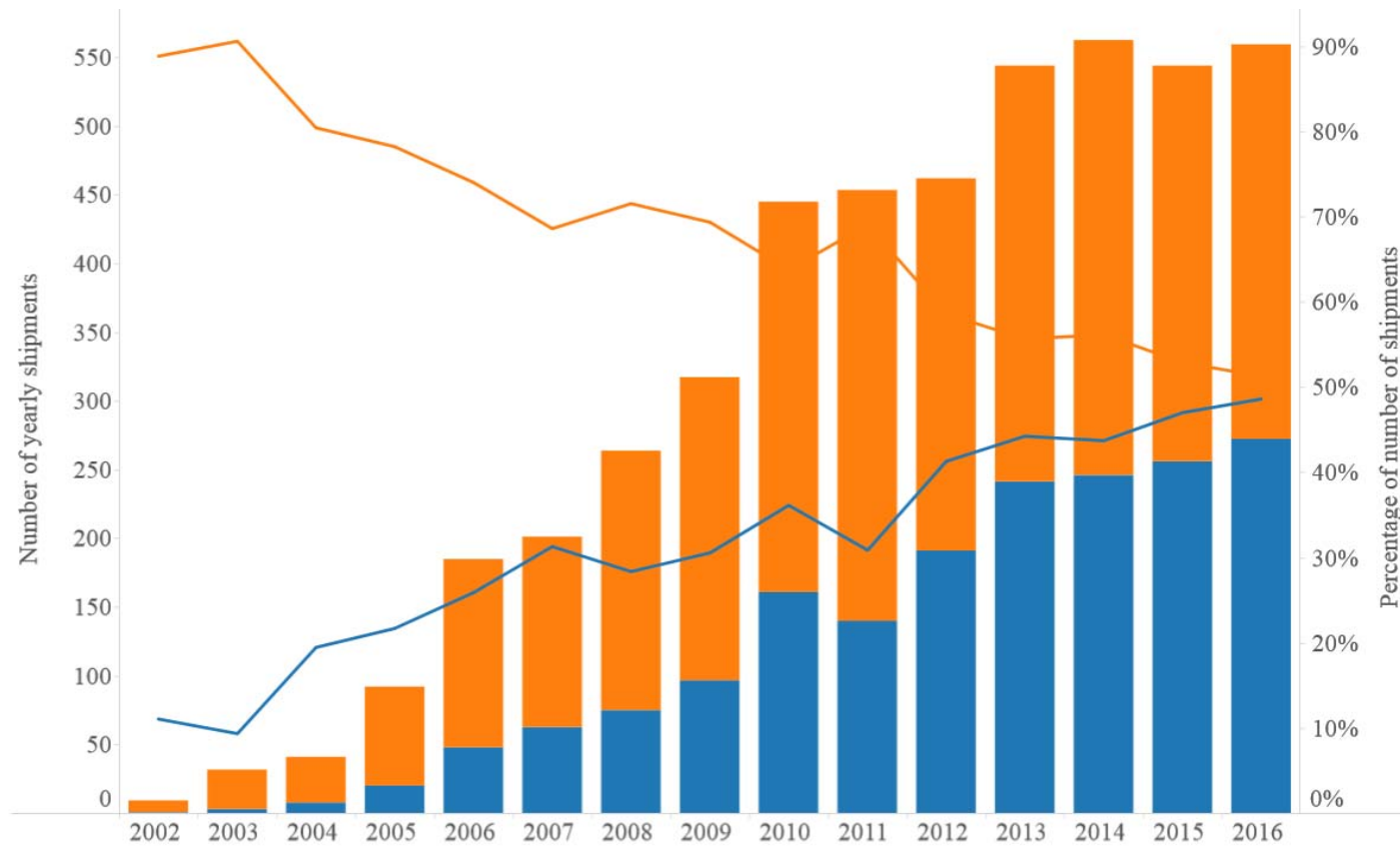
- >7000 IMPC strains in biobanks
- Service time 2-3 weeks frozen
- Rederived live mice 3-4 months



# Document processing



# Animal care: live or frozen

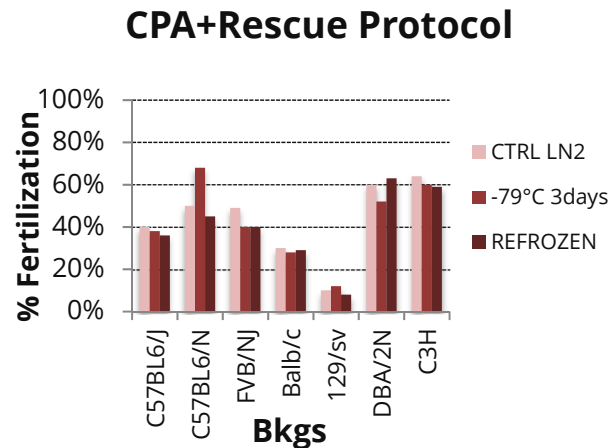
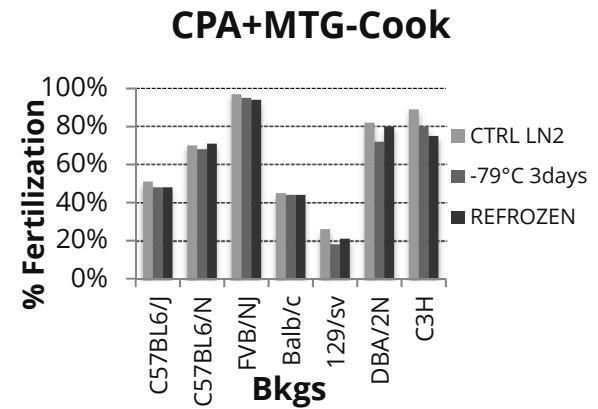
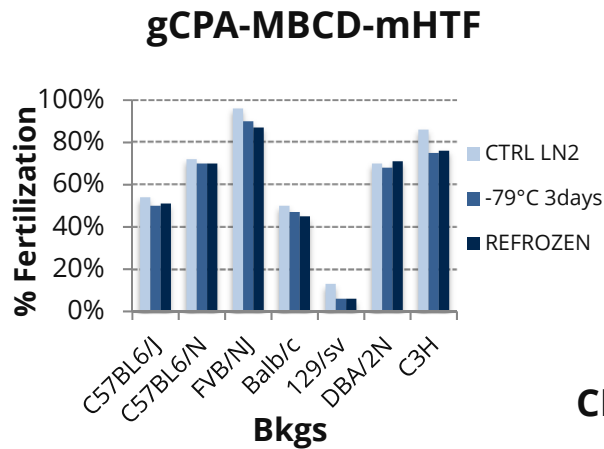


# Promoting frozen shipments

- Researchers need:
  - Faster service
  - More flexible exchange process
  - Cost incentive
  - More alternatives to live animal transportation



# Dry-ice: Robust & cheap

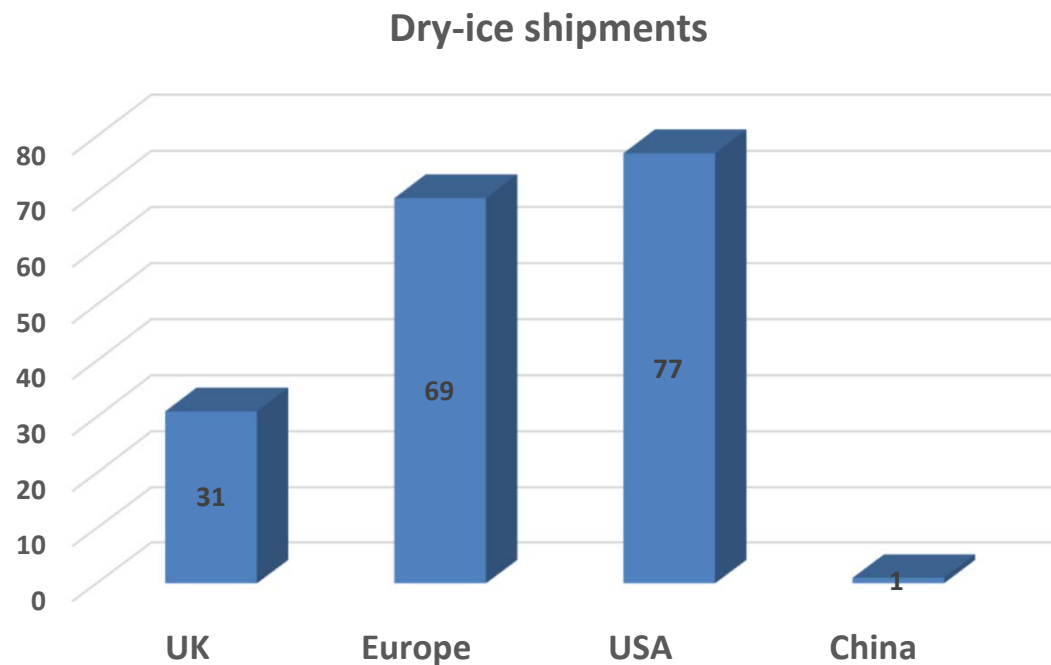


- Safe >2 years
- Can be returned to LN<sub>2</sub>
- Raspa et al (2018) Therio, 107, 41-49



# Sperm shipments on dry-ice

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# Unfrozen materials

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Refrigerated transport boxes

- ✓ Frozen/thawed embryos from archive
- ✓ Epididymides (sperm) in Lifer

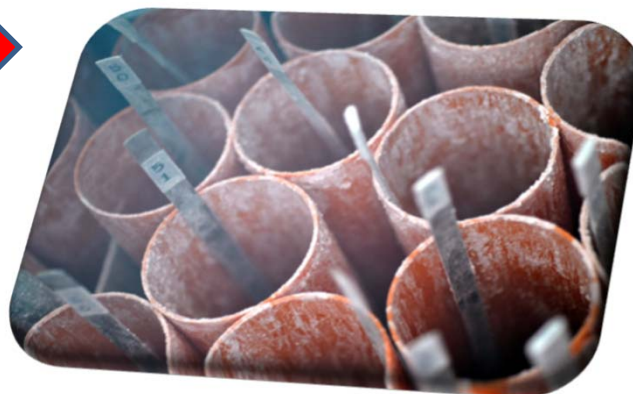




# Animal free banking/exchange

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Unfrozen epididymides  
Unfrozen embryos



Frozen embryos/sperm  
Thawed embryos  
Sperm on dry ice  
Unfrozen epididymides



## Folie 17

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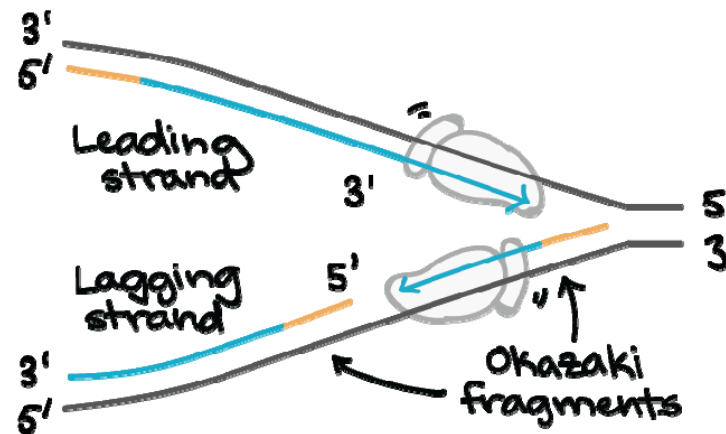
**MF4**

Martin Fray; 15.11.2017

# Biobanks still have currency

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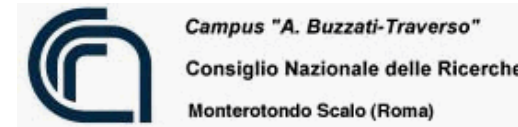
Advance research by allowing leading investigators to use their resources for discovery science rather than on model replication



# Acknowledgements

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- Marcello Raspa – CNR
- Ferdinando Scavizzi - CNR
- Lluís Montoliu – CNB
- Susan Marschall – HMGU
- Toru Takeo – CARD
- Mo Guan – MRC
- Rajia Soininen - Oulu
- Brendan Doe – WTSI
- Sabine Fessle – HMGU
- Auke Boersma – VUW
- Philippe Andre – ICS
- Zuzana Khorshidi - KI
- EMMA - Technical working group



Spanish National Biotechnology Centre



**HelmholtzZentrum münchen**  
German Research Center for Environmental Health

Center for Animal Resources and Development  
The CARD logo is a colorful, stylized graphic of the letters "CARD" in a circular arrangement, with each letter in a different color (C: green, A: blue, R: red, D: yellow).





# Unfrozen epididymides

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Group (n=3)	No. females used	No. embryos used in IVF	No. 2-cells produced	Fertilisation rate (%)
<b>72hrs In Lfor -unfrozen</b>	<b>36</b>	<b>1011</b>	<b>751</b>	<b>74.55 ± 1.53</b>
<b>Lfor- frozen/thawed (24hour)</b>	<b>15</b>	<b>426</b>	<b>356</b>	<b>85.69 ± 4.13</b>
<b>Lfor- frozen/thawed (48hour)</b>	<b>15</b>	<b>572</b>	<b>320</b>	<b>54.24 ± 7.19</b>
<b>Lfor- frozen/thawed (72hour)</b>	<b>15</b>	<b>683</b>	<b>115</b>	<b>17.19 ± 2.74</b>
<b>No Lfor treatment control</b>	<b>18</b>	<b>605</b>	<b>427</b>	<b>66.05 ± 7.70</b>



# Turn-around-time: 2016

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- Frozen (n=75)
  - Order to MTA sign-off ~31.4 days
  - MTA to shipment ~37.78 days
- Live (n=112)
  - Order to MTA sign-off ~44.8 days
  - MTA to shipment ~108.3 days



# What to keep and how to keep it?

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- Avoid confusion: use a specific naming system
- G0 = PRO/2972.1e
- G1 = Pcdh20-Del-Cas-Line1-B6N
- G2+ = Pcdh20-Del4-EM1-B6N
- C57BL/6NTac-Gnb4<sup>em2(IMPC)/H</sup>
- Bystander mutations
- Sperm vs ovary freezing






# What to keep and how to keep it?

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- Target & bystander mutations
- Sperm vs embryo freezing
- Avoid confusion over allele names
- C57BL/6NTac-Gnb4<sup>em2(IMPC)/H</sup>
- Cite repository numbers




# Dry-ice shipments: info sheets





MRC Harwell : An International Centre for Mouse Genetics




### Unpacking Cryopreserved Sperm that Arrives in a Dry-Ice Parcel

- 


The samples will arrive in a Thermal Control Unit, packed with an appropriate amount of dry-ice for the journey.
- 

Carefully open the package and locate the samples. Keep the samples buried in the dry-ice as warming them will affect the success of the IVF.
- 


Quickly transfer the samples to a liquid nitrogen filled dewar.
- 

The samples can be checked and moved to either a storage can or goblet for return to bulk liquid nitrogen storage.

The Mary Lyon Centre, Medical Research Council, Harwell, Didcot, Oxfordshire, OX11 0RD, UK.  
 tel: +44 (0) 1235 841000 fax: +44 (0) 1235 841138 [www.mlc.har.mrc.ac.uk](http://www.mlc.har.mrc.ac.uk)



MRC Harwell : An International Centre for Mouse Genetics



### Shipping Frozen Material on Dry-Ice

#### Shipment of Cryopreserved Sperm on Dry-Ice Doesn't Reduce Viability

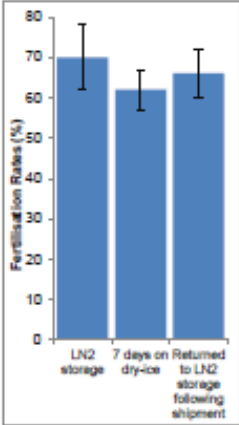
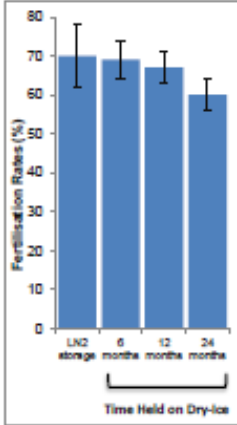
The Figure below shows that shipping cryopreserved sperm on dry-ice does not affect its viability compared to samples held in Liquid Nitrogen (LN<sub>2</sub>) storage. The sperm can be returned to Liquid Nitrogen storage on arrival.

Care must be taken during the handling of the material to ensure that during the unpacking, or transfer, that the samples do not warm up. Incorrect handling will reduce the viability of the samples and subsequent fertilisation rates.

#### Prolonged Storage of Frozen Sperm on Dry-ice has Negligible Effects on the IVF Fertilisation Rates

When handled correctly cryopreserved sperm, held in dry-ice, has similar IVF fertilisation rates to samples that are stored in Liquid Nitrogen (LN<sub>2</sub>).

The figure below shows that storage on dry-ice for up to 2 years has only a small effect on fertilisation rates, with levels consistently being high. The sperm should not be allowed to warm up during storage as this will be detrimental to its viability.

The Mary Lyon Centre, Medical Research Council, Harwell, Didcot, Oxfordshire, OX11 0RD, UK.  
 tel: +44 (0) 1235 841000 fax: +44 (0) 1235 841138 [www.mlc.har.mrc.ac.uk](http://www.mlc.har.mrc.ac.uk)



# Now and into the future

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- Robust sperm freezing/IVF protocols
- Ultra-superovulation
- *In vitro* recombination
- Oocyte vitrification in straws
- Shipment of unfrozen embryos/gametes
- Sperm shipments on dry ice
- Universal non-surgical transfer





# IMPC

International Mouse Phenotyping Consortium



Generated 6500 lines  
500 using gene editing techniques



# 43K strains in MGI: only 50% are public



## 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



State	Count (approx.)
ES Cell	25,000
Embryo	22,000
Live	18,000
Ovaries	15,000
Sperm	12,000

The relative proportion of the "state" in which mouse resources are maintained across all repositories (not a measure of unique strains).

Search for:  [Search](#) [Reset](#) [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
- ARC (Animal Resources Centre) Australia
- CARD (Center for Animal Resources and Development) Japan
- CMMR (Canadian Mouse Mutant Repository) Canada
- EMMA (European Mouse Mutant Archive) Germany
- EMS (Dr. Elizabeth M. Simpson, Ph.D.) Canada
- HAR (MRC Harwell) UK
- JAX (JAX Mice and Services) 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.

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



# MouseBook – acknowledgements

Stock B6;129-Scn10a<tm3(cre/ERT2)Jnw>/H

Stock Name	Strain Name	Mutation Strain Type		
Nav1.8 CreERT2	B6;129-Scn10a<tm3(cre/ERT2)Jnw>/H	<b>Mutation Type</b> IMSR - Targeted mutation	<b>Mutation Subtype</b>	<b>Strain Type</b> IMSR - unclassified
<b>FESA Number</b> 3448	<b>Attribution</b>			
	<b>Institution name</b> University College London University College London	<b>Depositor / Originator</b> Originator Depositor	<b>Name</b> Retained Retained	

Colony Stock Code
Nav1.8 CreERT2

Gene/Allele Information					
Allele Name	Allele MGI ID	Gene Name	Gene MGI ID	Chromosome	Is Background
Scn10a<tm3(cre/ERT2)Jnw>	MGI:3689893	Scn10a	MGI:108029	9	✖

Phenotypic Description	
None observed (heterozygous Nav1.8-CreERT2 can express enough Cre to delete the floxed fragment, but it does not affect the expression of Nav1.8 in Nav1.8 positive neurons in DRG).	

Publications			
Displaying 1 - 1 of 1 Stock Publications			
Pubmed ID	Authors	Title	Journal
16850455	Zhao J	Tamoxifen-Inducible Nav1.8-CreERT2 recombinase activity in nociceptive neurons of dorsal root ganglia.	Genesis (2006) 44:364-71





# Promoting awareness

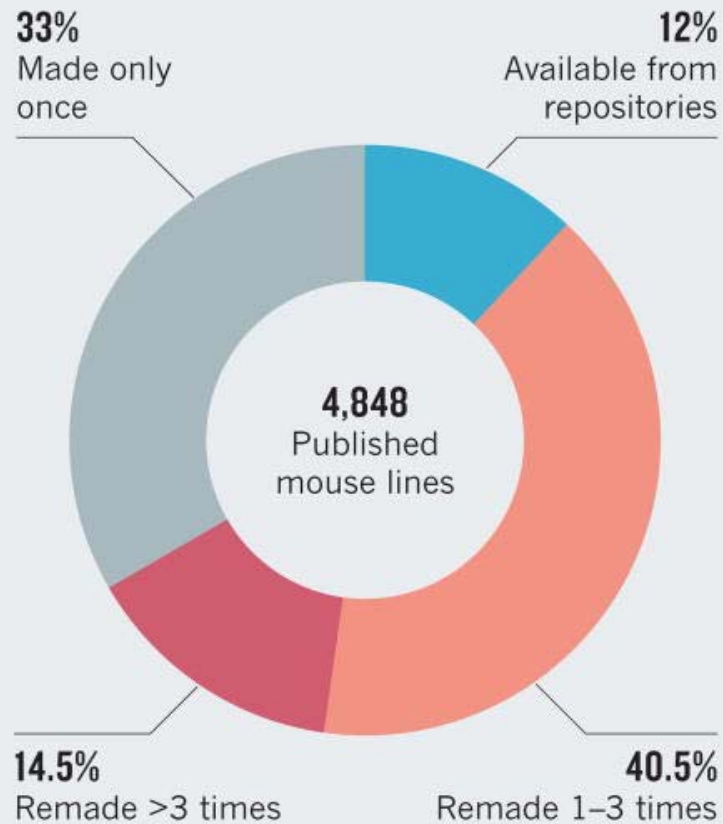
The collage features several key resources:

- Archiving**: A section titled "Safe guard your mouse lines against loss and ensure a co-operative future for mouse research" with a sub-header "Archiving Mouse Lines - Why Archive a Line? How Much Does it Cost? How do I Submit a Line for Archiving?". It includes an introduction and a list of links for further information.
- MouseBook**: A resource for mouse lines, featuring a calendar and a list of links.
- MTA**: A resource for mouse lines, featuring a list of links.
- AMRC**: The Association for Mouse Research, featuring a search bar and a list of links.
- Ximbio**: A platform for searching, sourcing, and sharing reagents, featuring a search bar and a list of links.
- IMPC**: The International Mouse Phenotyping Consortium, featuring a logo and a list of links.
- Infrafrontier**: A platform for mouse disease models, featuring a logo and a list of links.
- MRC Harwell**: A platform for mouse research, featuring a logo and a list of links.



## REMAKING MICE

When engineered animals are unavailable, researchers make them again. The most recent comprehensive survey, carried out in 2005, found that researchers had made thousands of mouse lines more than once, wasting animals, time and money.



Lloyd et al (2015) Nature, 522, 151

