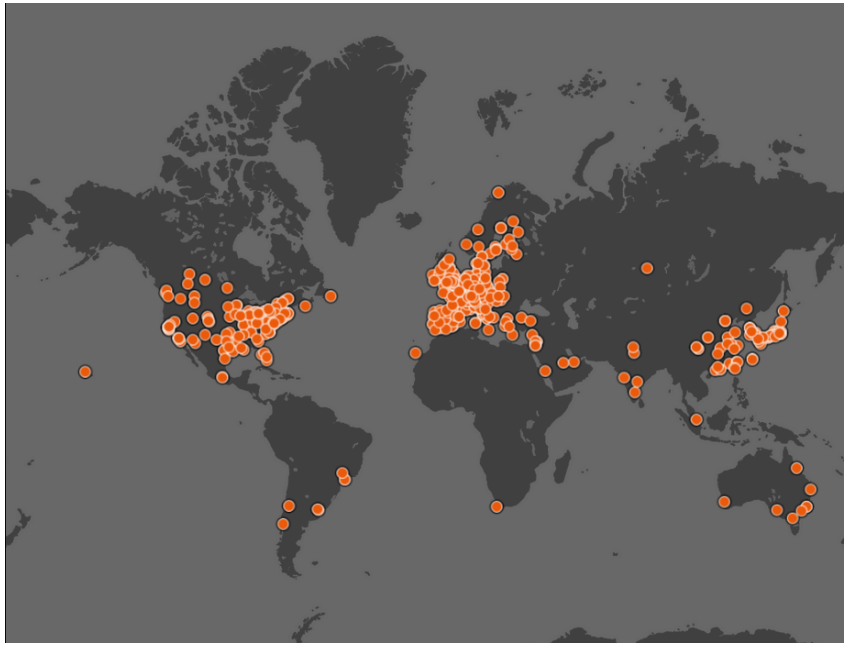
Sharing mutations: Biobanks are still required in the post-CRISPR/Cas9 era

Martin D. Fray

MRC Harwell Institute, UK

European Mouse Mutant archive



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What is a biobank?

- Banks make a return on investments

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

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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

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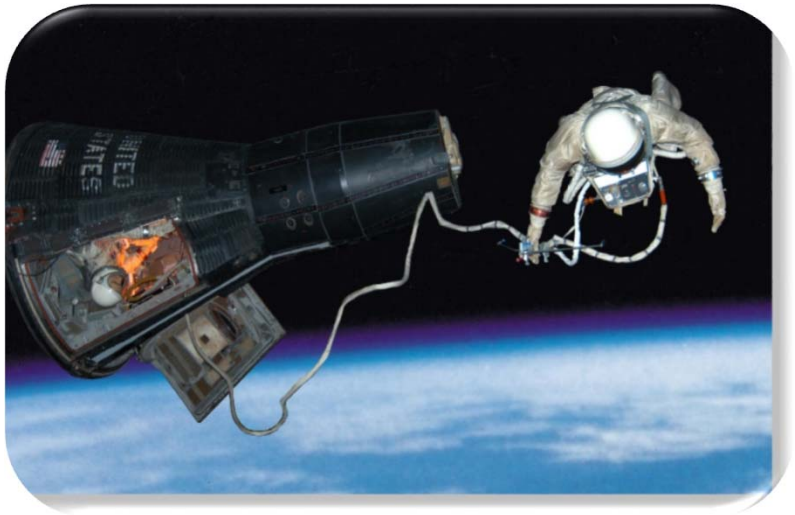
Know thy mice!

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

Genetic anchor points

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



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Reproducibility – vital to progress

PERSPECTIVE

The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman[1]*, Iain M. Cockburn[2], Timothy S. Simcoe[2,3]

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

- 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

Arguments still apply post CRISPR

- Need to educate new users:

  C57BL/6NTac-Gnb4$^{em2(IMPC)/}$H

  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

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Obligations on biobanks

www.findmice.org

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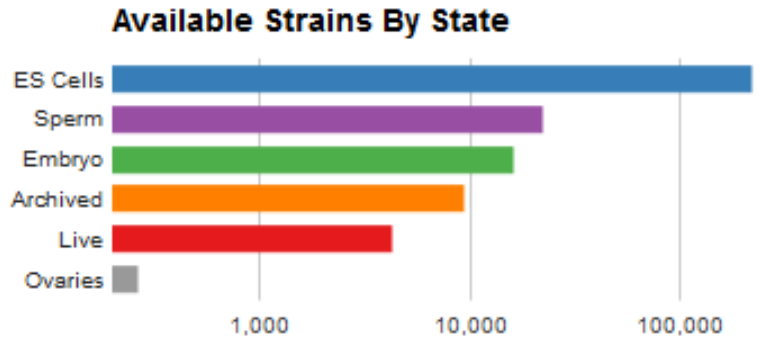
What is possible?

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



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

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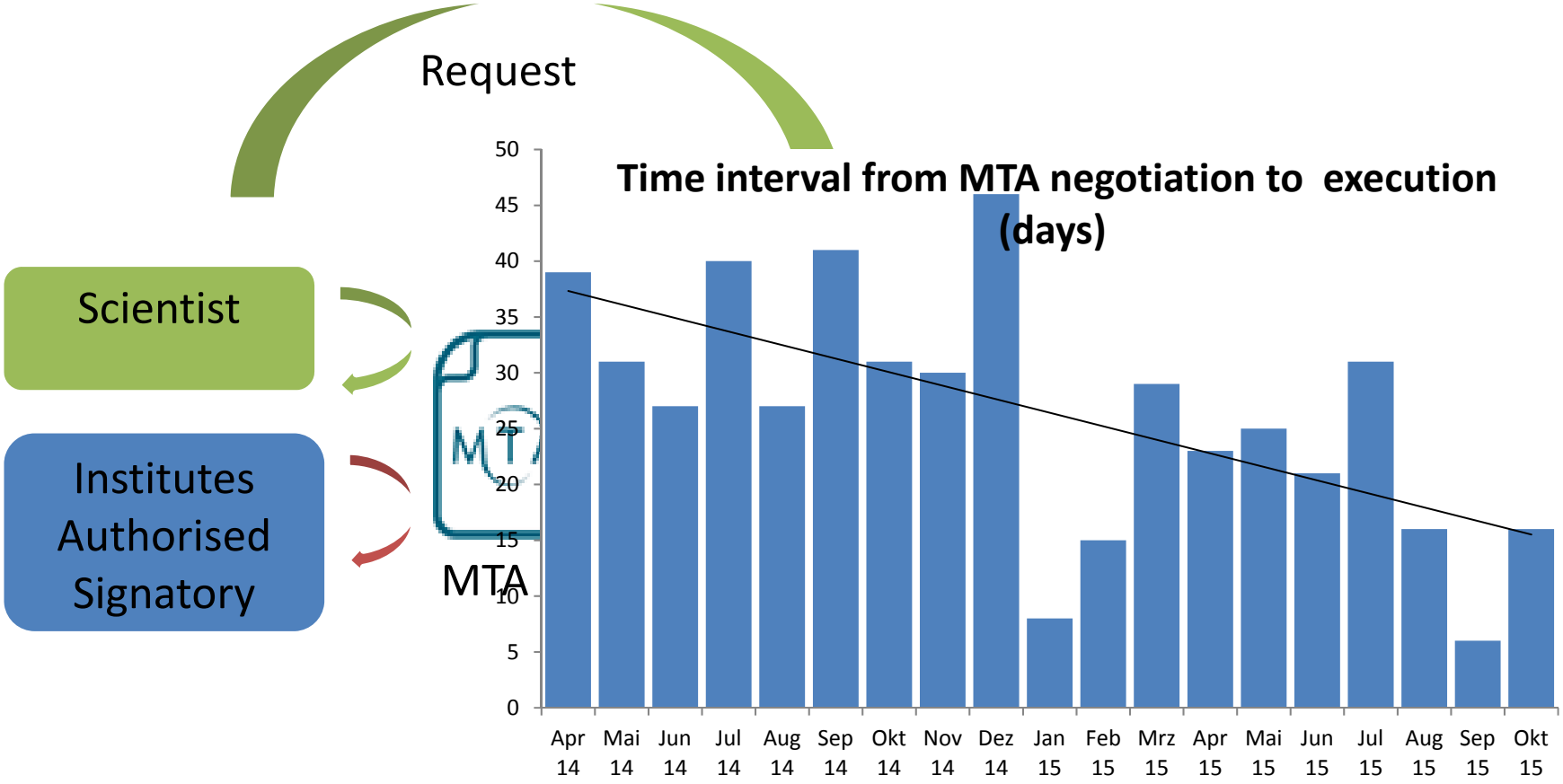
Document processing



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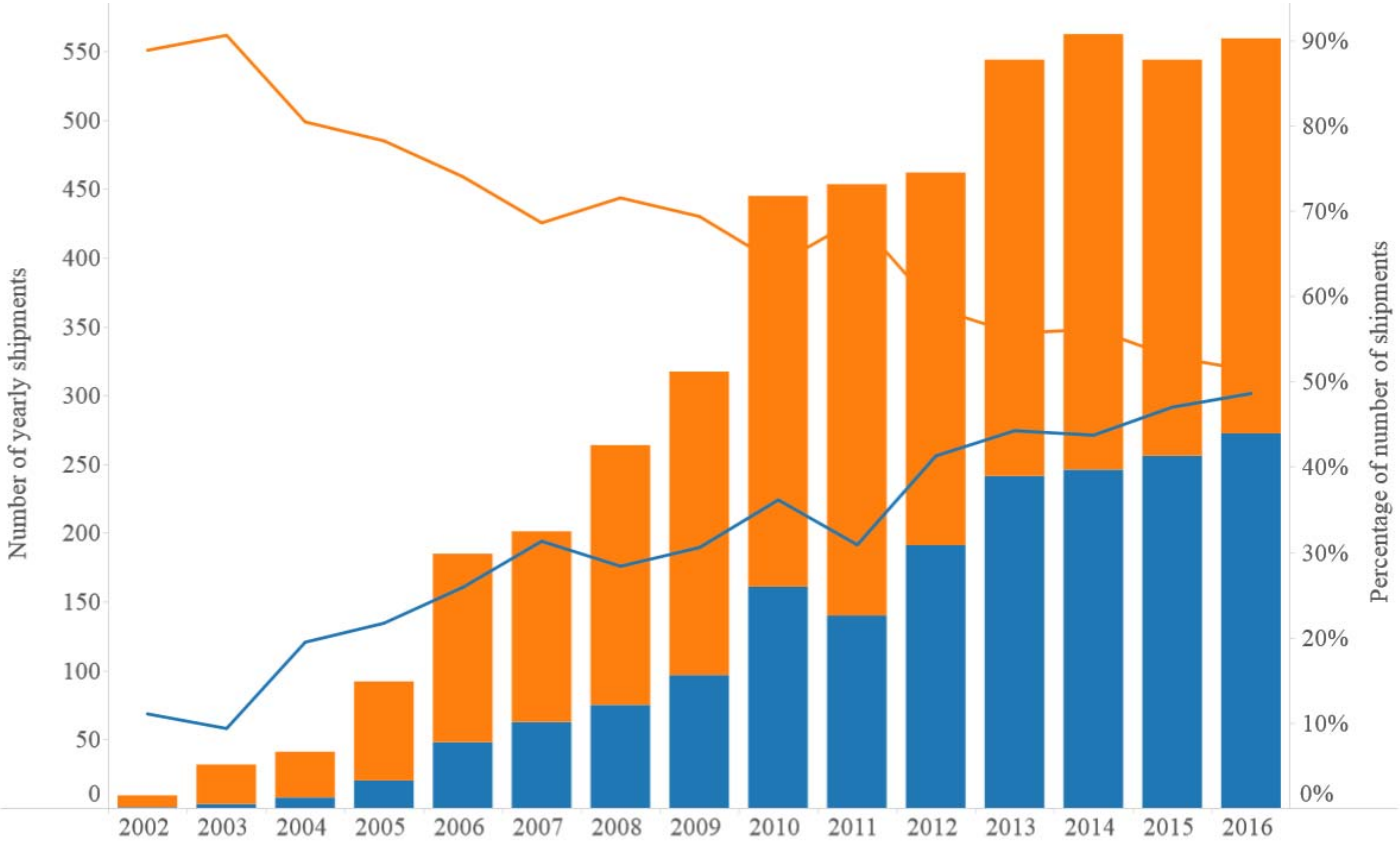
Animal care: live or frozen



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Promoting frozen shipments

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

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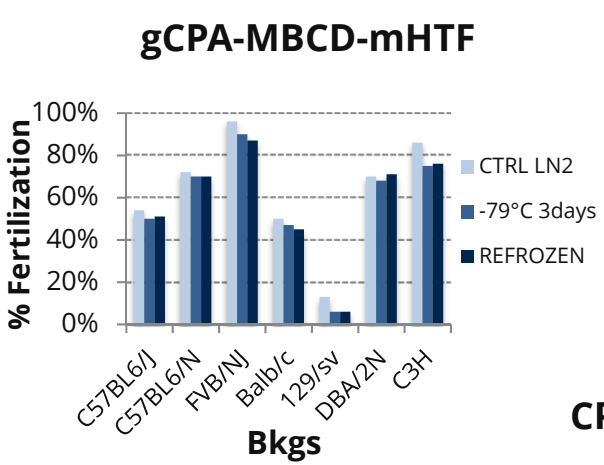
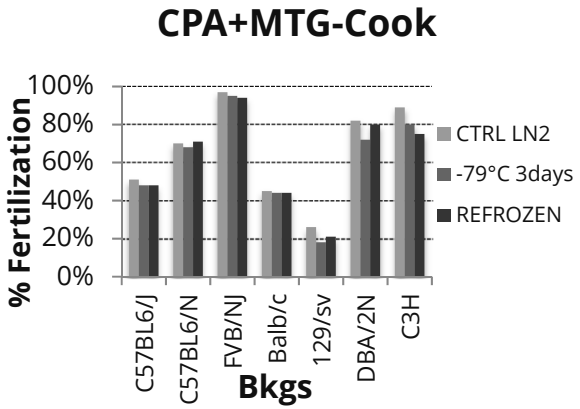
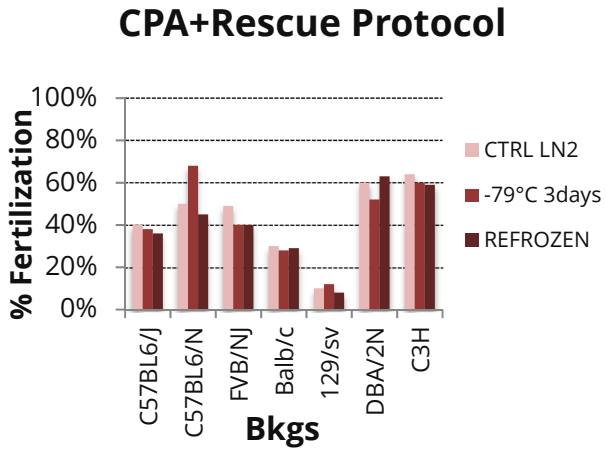
Dry-ice: Robust & cheap







- Safe >2 years
- Can be returned to LN_2
- Raspa et al (2018) Therio, 107, 41-49

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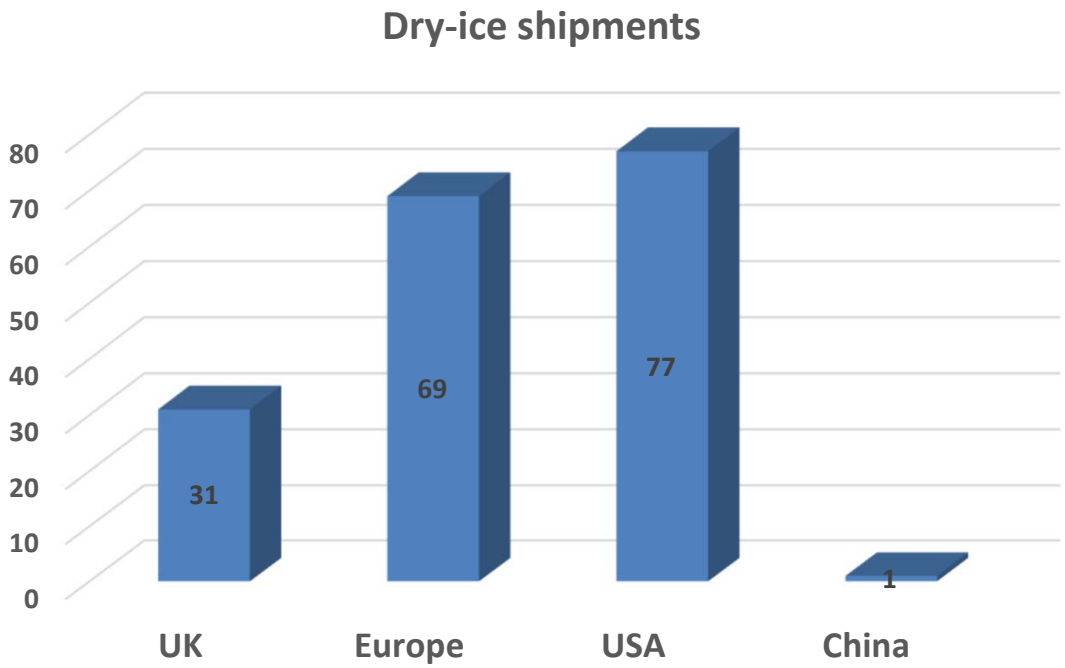
Sperm shipments on dry-ice



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Unfrozen materials

Refrigerated transport boxes

- ✓ Frozen/thawed embryos from archive

- ✓ Epididymides (sperm) in Lifor

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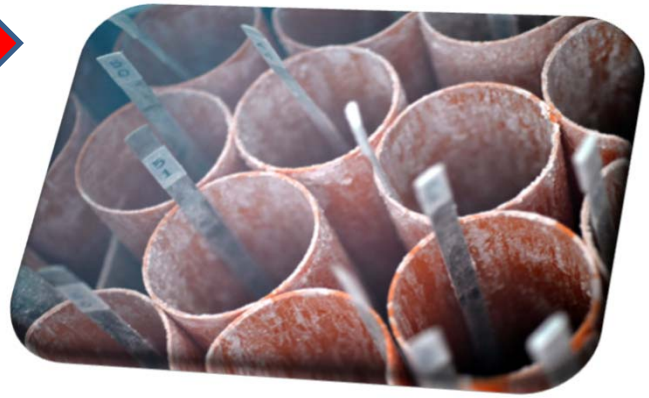
Animal free banking/exchange

Unfrozen epididymides
Unfrozen embryos



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

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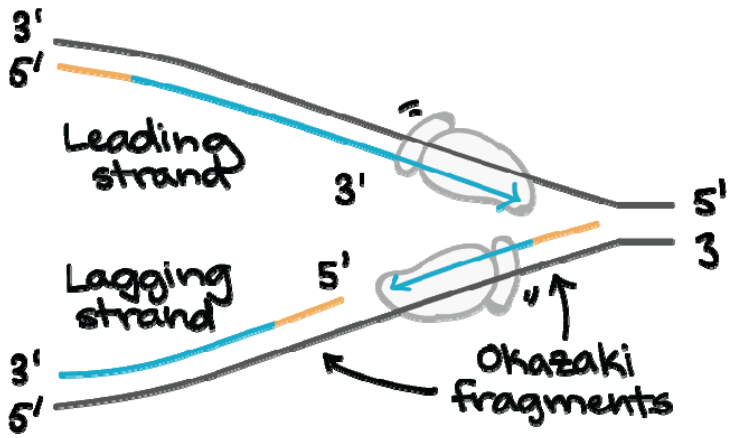
Folie 17

MF4 Martin Fray; 15.11.2017

Biobanks still have currency

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



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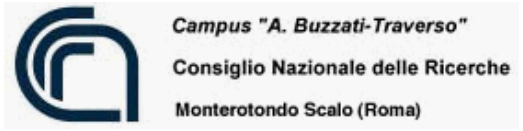
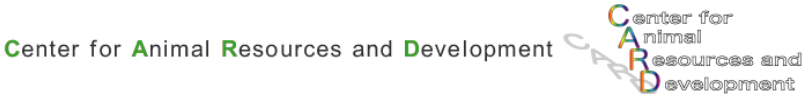
Acknowledgements

- Marcello Raspa – CNR
- Ferdinando Scavizzi - CNR
- Lluis 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



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Unfrozen epididymides

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

Turn-around-time: 2016

- 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?

- 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$^{em2(IMPC)/H}$
- Bystander mutations
- Sperm vs ovary freezing

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What to keep and how to keep it?

- Target & bystander mutations
- Sperm vs embryo freezing
- Avoid confusion over allele names
- C57BL/6NTac-Gnb4$^{em2(IMPC)/}$H
- Cite repository numbers

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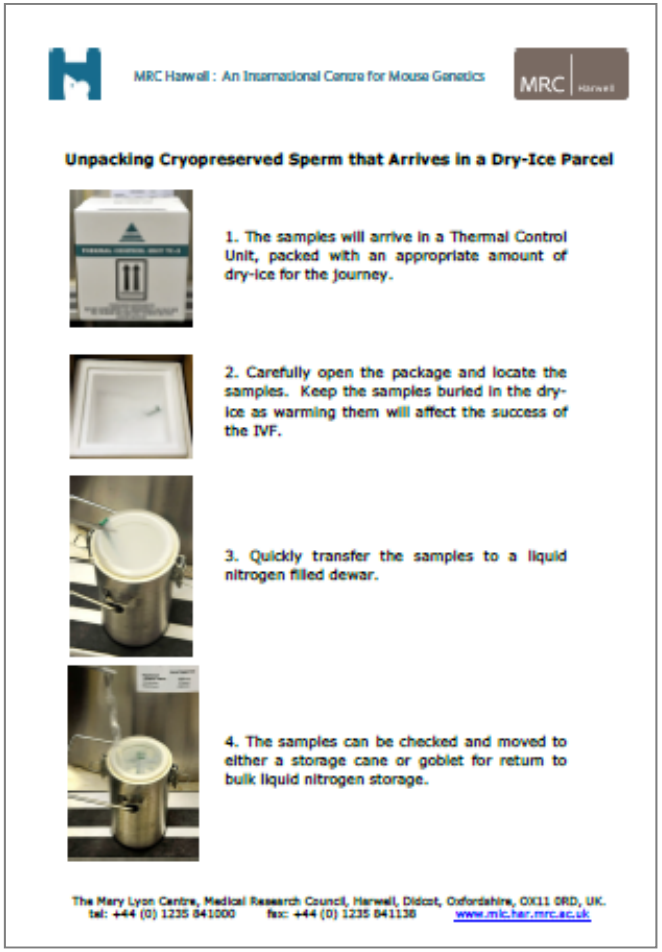
Dry-ice shipments: info sheets



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Unpacking Cryopreserved Sperm that Arrives in a Dry-Ice Parcel

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

2. 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.

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

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

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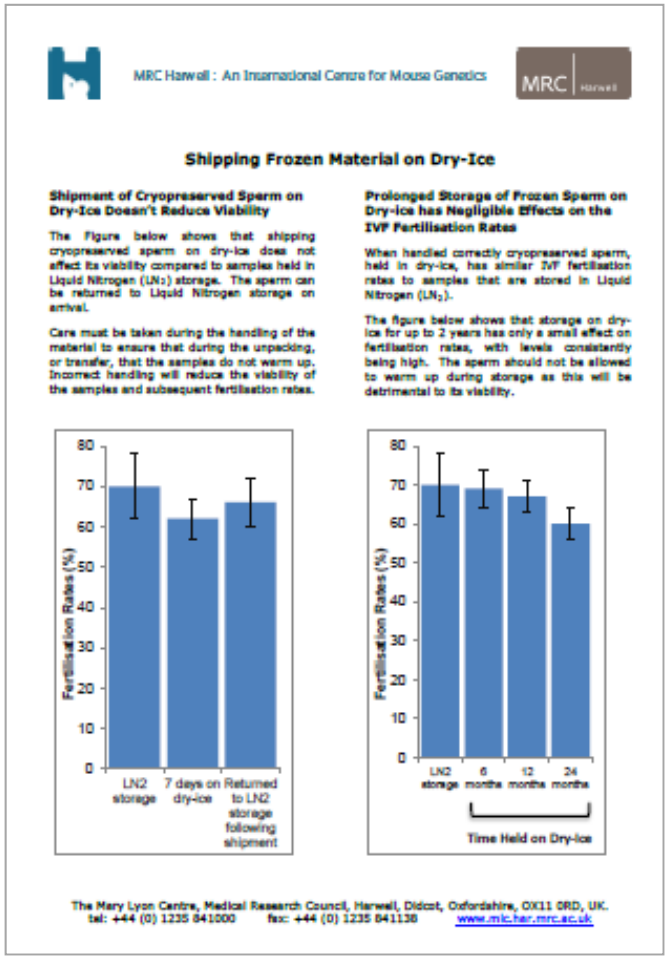
tel: +44 (0) 1235 841000 fax: +44 (0) 1235 841138 www.mlc.har.mrc.ac.uk



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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_2) 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_2).

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.

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Now and into the future

- 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

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Generated 6500 lines

500 using gene editing techniques

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43K strains in MGI: only 50% are public

www.findmice.org

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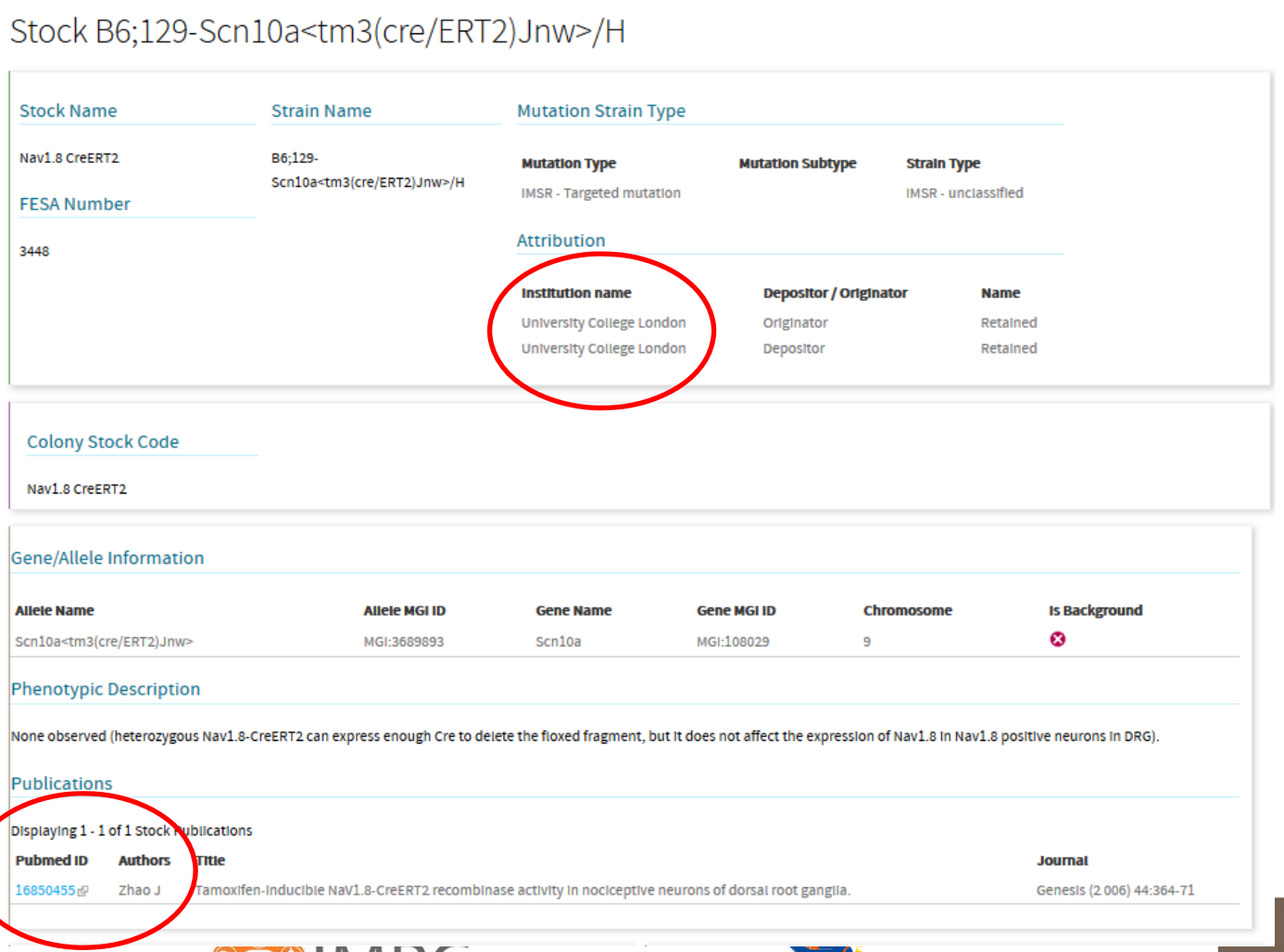
MouseBook – acknowledgements



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

Stock Name

Nav1.8 CreERT2

FESA Number

3448

Strain Name

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

Mutation Strain Type

Mutation Type	Mutation Subtype	Strain Type
IMSR - Targeted mutation		IMSR - unclassified

Attribution

Institution name	Depositor / Originator	Name
University College London	Originator	Retained
University College London	Depositor	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 (2 006) 44:364-71

Promoting awareness

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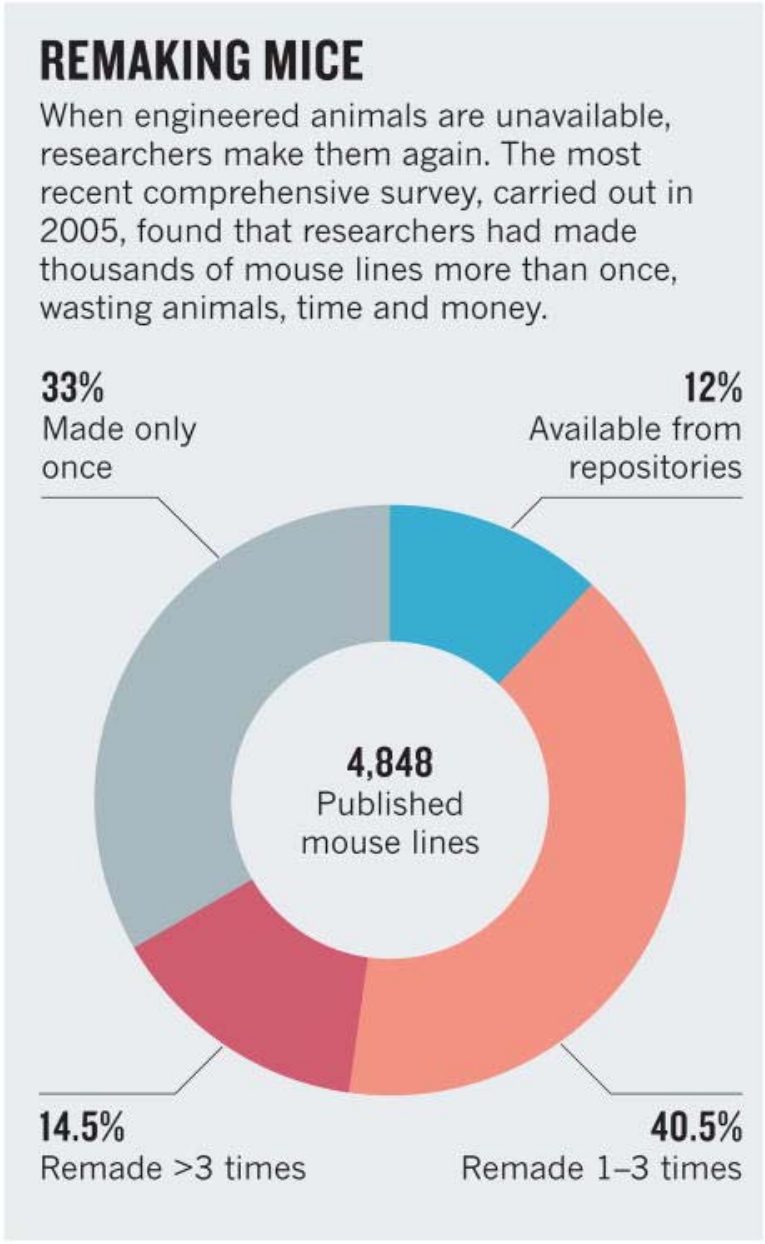

Lloyd et al (2015) Nature, 522, 151

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