EMMA Cryopreservation Workshop

Novel concepts in mouse production and preservation

Consejo Superior de Investigaciones Cientificas

Madrid, Espagna

May 7-8, 2012

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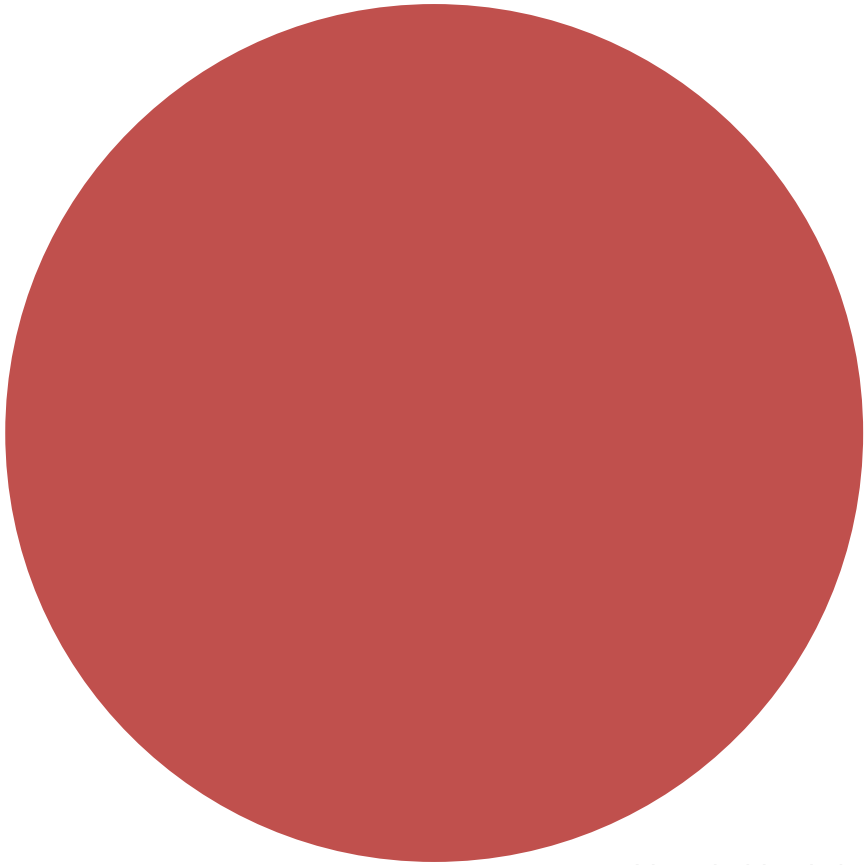
IMSR: 229,032 alleles*

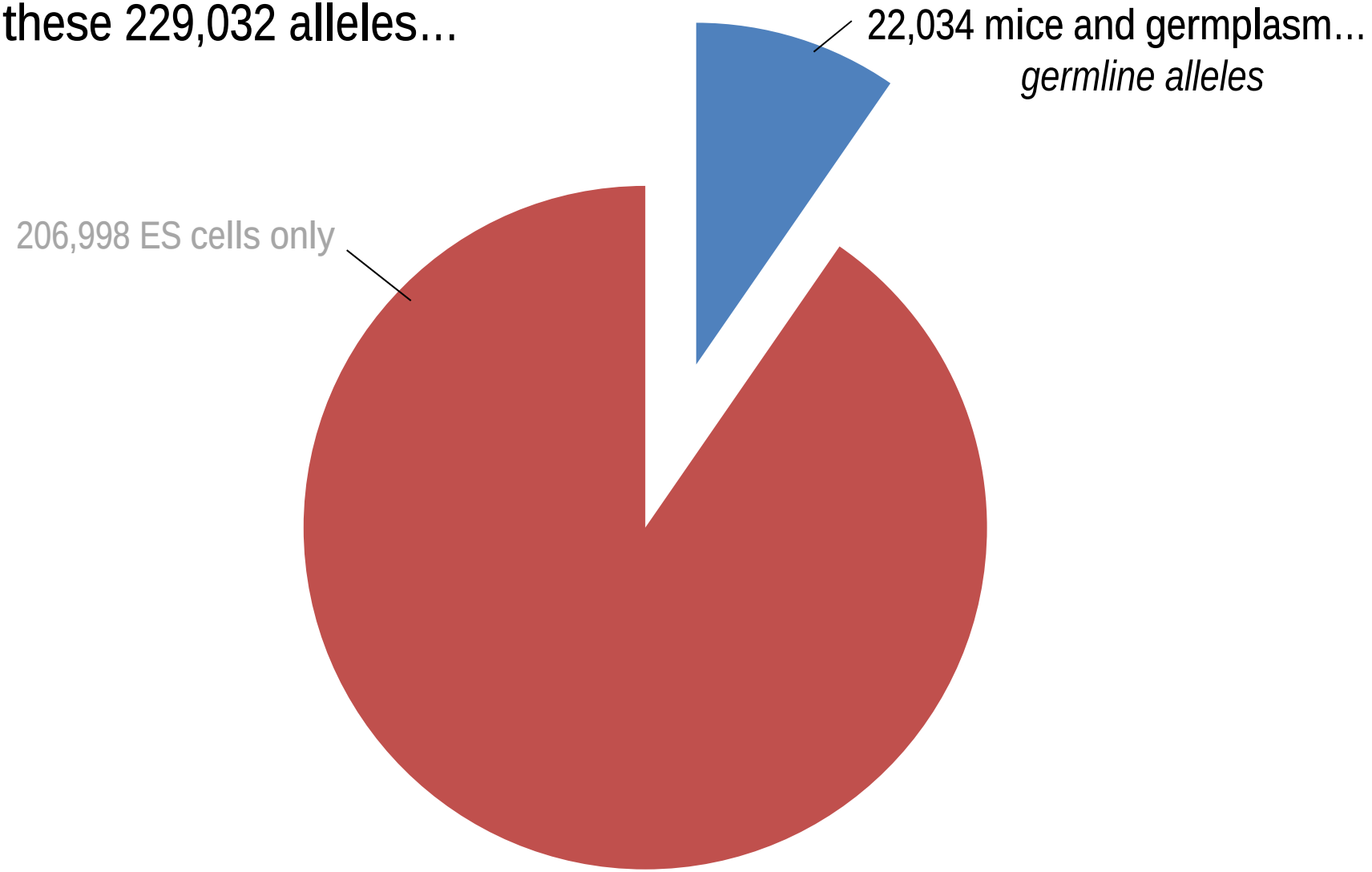
>16,000 genes (75% of genome)



- Lines held only in private labs not included
- Also includes non-genomic alleles (Cre, Flp, lacZ, etc)
- Academic, not-for-profit only (no commercial, for-profits)

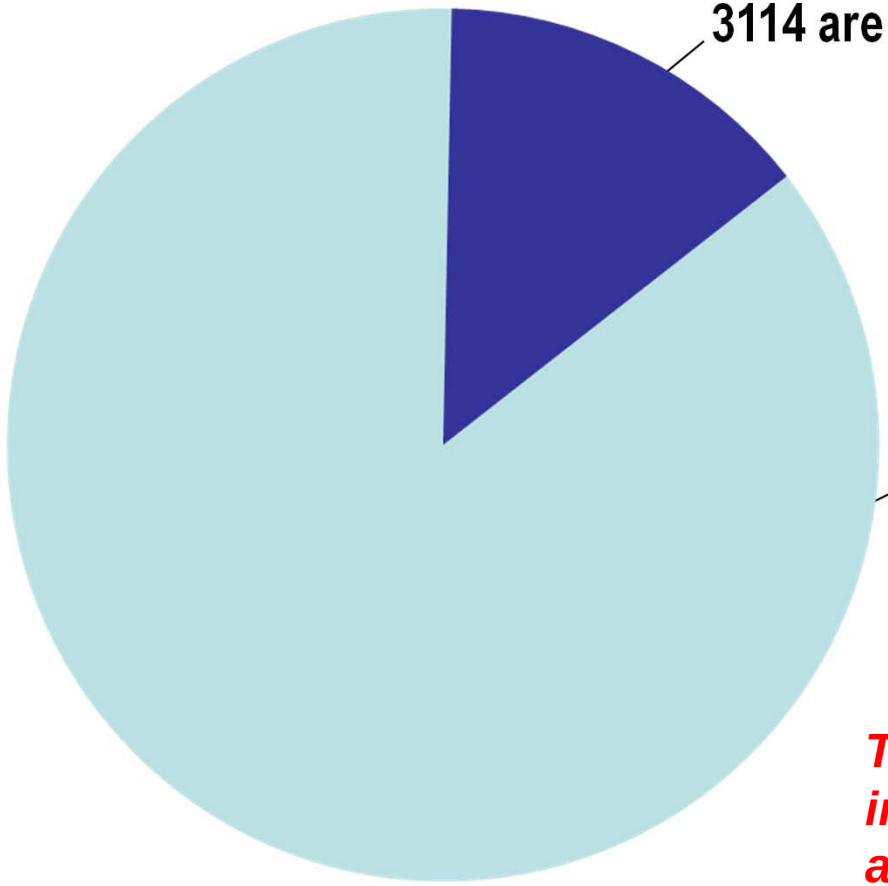
Induced mutant strain resource, October 2011

Of these 229,032 alleles…



90% of alleles are *NOT* germline

Of these 22,034 germline alleles existing as mice and germplasm…

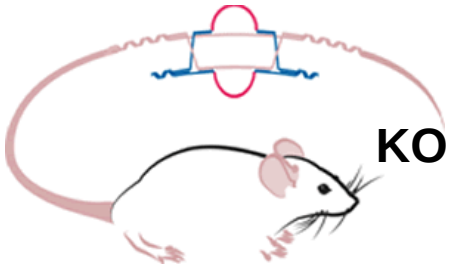


3114 are live mice…most also germplasm

18,920 (~86%) are only germplasm
…includes embryos and/or sperm

Therefore, most germline alleles in repositories are in suspended animation, requiring recovery to live mice for use.

IKMC (INTERNATIONAL KNOCKOUT MOUSE CONSORTIUM)



EUCOMM

NorCOMM

TIGM

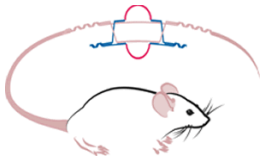
17,000 genes

- 8,500 genes (KOMP)
- 8,000 genes (EUCOMM)
- 500 genes (NorCOMM)

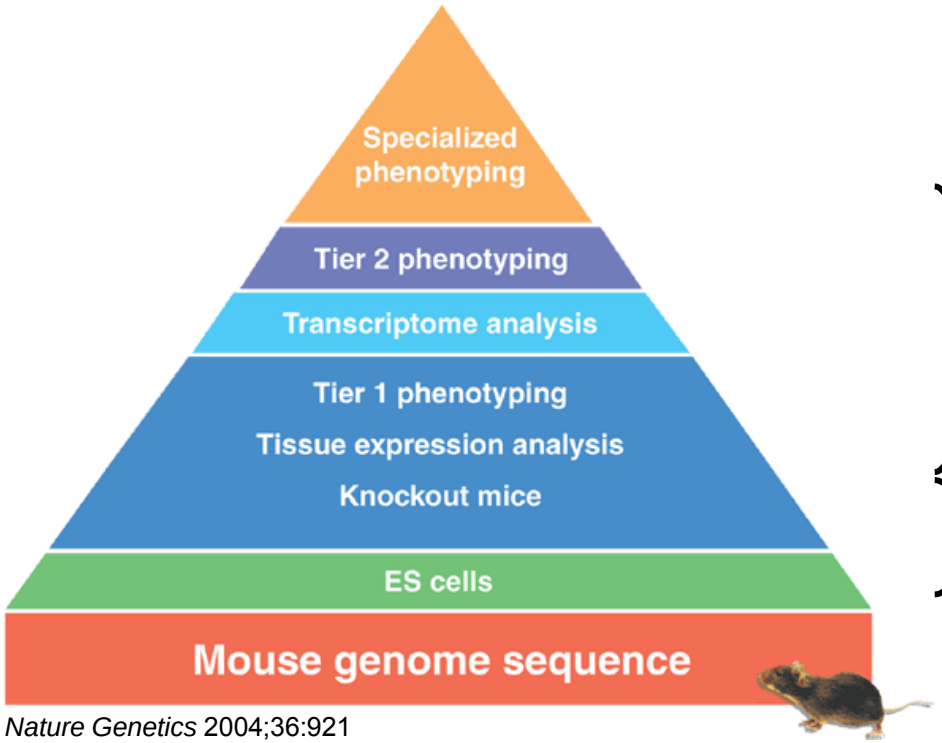
- -C57BL6/N
- -*lacZ*-tagged
- -K/O alleles:
  - conditional null
  - targeted trap
  - deletion

Mutant ES cell resource + **Vector resource**

250-500 mutant mice/yr



KOMP:
Knockout Mouse Project



Nature Genetics 2004;36:921

KOMP2

2011-2021
~US$275million
8500 mouse lines
with phenotype

KOMP1

2006-2011
~US$55million
8500 ES cell lines

In 2003, a meeting at the Banbury Center, Cold Spring Harbor developed a proposal for high throughput gene knockouts and phenotyping for every gene in the mouse genome…***KOMP***

IMPC International Mouse Phenotyping Consortium

Welcome to the International Mouse Phenotyping Consortium

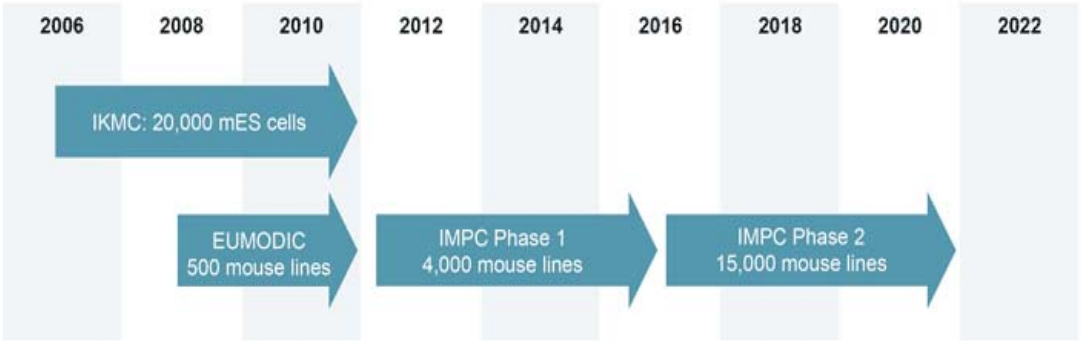
The International Mouse Phenotyping Consortium (IMPC) comprises a group of major mouse genetics research institutions along with national funding organisations formed to address the challenge of developing an encyclopedia of mammalian gene function.

The IMPC envisages a ten year programme to undertake a broad-based, systematic genome-wide phenotyping project of knockout mice generated from the embryonic stem cell mutant resources developed by the International Knock-out Mouse Consortium (IKMC). Each mutant line will undergo a broad suite of high-throughput tests to identify developmental, anatomical, physiological, behavioural and pathological phenotypes.

It is anticipated that this landmark programme will produce a paradigm shift in our understanding of basic molecular, cellular and systems biology, as well as feed the biopharmaceutical pipeline by enhancing our understanding of the genetic bases for disease.

Importantly, this internationally co-ordinated programme will build upon the major mouse genetics programmes and infrastructures around the world enabling access to high quality, freely accessible phenotype data covering all major organ systems, as well as access to mouse knockout strains of lasting biological and medical value.

An IMPC Steering Committee (SC) was formed to develop an operational plan and harness the major world-wide mouse research programmes and infrastructures in a strategic and coordinated effort.



Events Calendar

« November »

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

Latest News

Library of gene function will speed up disease research

Dr. Mark Moore appointed Executive Director for IMPC

Industry Work Group Report is ready to download

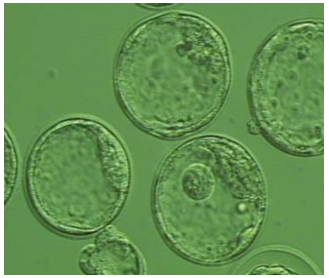
Phenotyping Work Group Report is ready to download and comment upon

Industry Liaison Workshop, London, April 7th, 2011

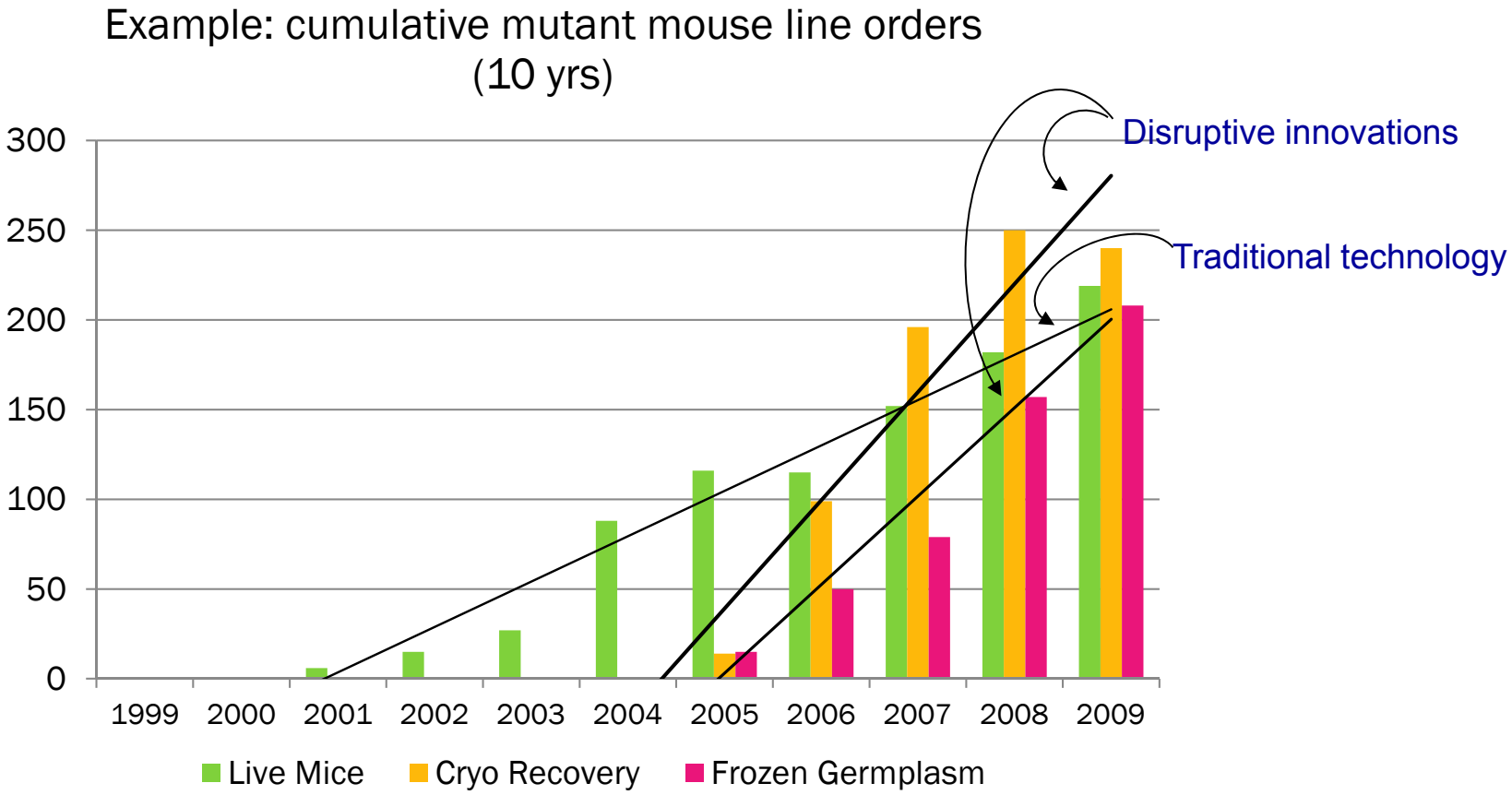
Phenotype Work Group Report

New frontiers in suspended animation

- Since 1972, embryo cryopreservation in liquid nitrogen has been the gold standard for preserving mutant mouse lines.
- Sperm cryopreservation receiving more attention
- Enhancements in techniques made to promote stability during storage, ensure viability upon recovery, reduce cost, increase speed, control quality, make reliable



Technological improvements that anticipate future needs



RATIONALE:

1-Live mice in most demand

2-Frozen formats are innovative, leading edge technology

3-Use of frozen formats mice reduces maintenance and distribution costs

Projects in progress…

-efficient derivation of iPS cells from mutant lines

-enhanced intracytoplasmic nuclear injection (ICNI)

-targeting in mutant iPS cells

-restoring viability (e.g., motility) to dried sperm

-mercury-free mechanical zona drilling (RosDrill^{c})

-molecular vectors as reliable (recoverable) genetic storage formats

-warp-speed congenics

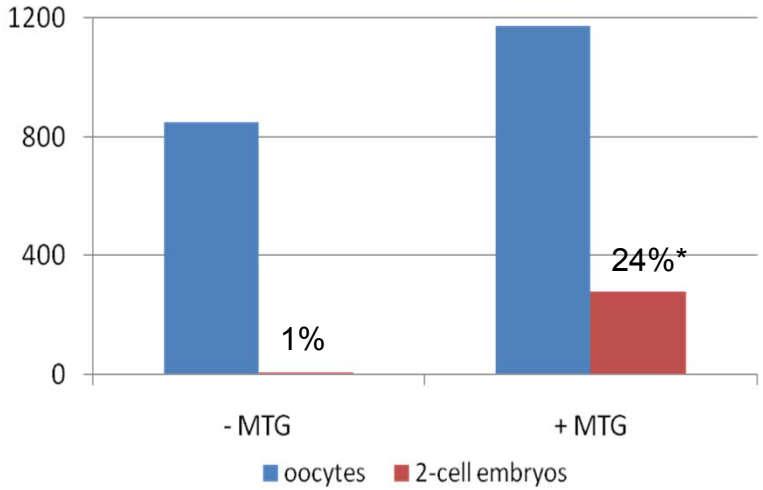
		Type of User		
Procedure	Format	Individual Lab	Transgenic facility	Repository
		self	intramural	extramural distribution
Preserve	Embryos	√	√	√
	Sperm		√	√
	ES cells	√		√
Maintain	Embryos	√	√	√
	Sperm		√	√
	ES cells	√		√
Recover	Embryos	√	√	√
	Sperm		√	√
	ES cells			√

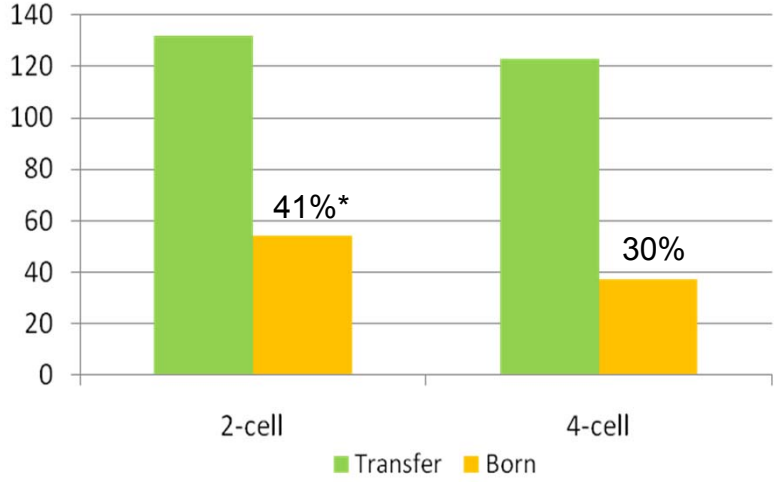
Eg. Lab may want to preserve only (natural) embryos, not sperm
Eg. Facility may want to preserve (mostly) embryos (IVF) and sperm (few…slow, sure)
Eg. Repository may want to preserve ESC & (mostly) sperm (many…fast, cheap, versatile)

Technology enhancement:

Protection against cryo-induced oxidation damage of sperm

B6.Cg-*Mecp2*$^{tm1.1Jae}$/Mmcd:





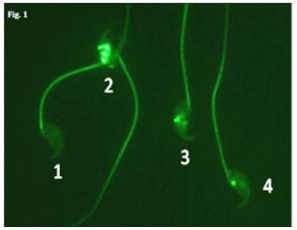
Other mutant strains:

Mutant strain background	Freezing Media	No. mutant strains tested	No. 2-cell embryos (%)
C57BL/6J	CPM	20	604 (6%)
	CPM + MTG	13	627 (15%)*
FVB/N-CD1	CPM	41	3990 (31%)
	CPM + MTG	30	5278 (52%)*

*Reproduction 2012

Technology replacement:

Unconventional preservation of mouse sperm*



In vitro development of embryos from ICSI using freeze-dried sperm:

Genetic background	Treatment	No. eggs survived	No. 2-cell embryos (%)	No. blastocysts (%)
B6D2F1/J	Fresh	75	70 (93%)	55 (73%)
	Freeze-dried	77	68 (85%)	44 (57%)
C57BL/6J	Fresh	59	51 (86%)	41 (69%)
	Freeze-dried	52	47 (90%)	28 (54%)

Genetic screening of 3 generations of mice from ICSI using freeze-dried sperm:

Genetic background	Treatment	P	F1	F2	F3	No. MS alleles tested	No. MS mutations detected
B6D2F1/J	Natural mating	8	35	26	28	1424	0
	Fresh ICSI	8	41	29	29	1584	0
	Freeze-dried ICSI	6	24	22	20	1056	0
C57BL/6J	Natural mating	8	24	24	20	2176	0
	Fresh ICSI	10	29	35	26	2880	0
	Freeze-dried ICSI	8	20	19	18	1824	0

**Zygote, 2009*

Technology improvement:

Simpler, faster, cheaper sperm preservation by evaporative drying*

B6.129P2-APOE^{tm1Unc}:	Fresh Sperm ICSI Group	ED Sperm ICSI Group
No. oocytes injected	380	364
No. oocytes survived ICSI	149	128
ICSI survival rate (%)	39.2%	35.2%
No. 2-cell embryos obtained	137	114
Fertilization rate (%)	91.9%	89.1%
No. pups born	52	20
Birth rate (%)	38.0%	17.5%
Sex ratio (M/F)	25/27	12/8

	Fresh Sperm ICSI Group	ED Sperm ICSI Group
No. natural mating pairs	5	6
No. of litters born	5	5
Litter rate	100%	83.3%
No. pups born	30	28
Sex ratio (M/F)	13/17	17/11

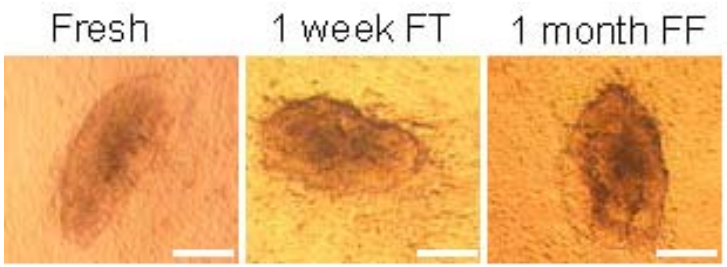
*Reproduction 2012

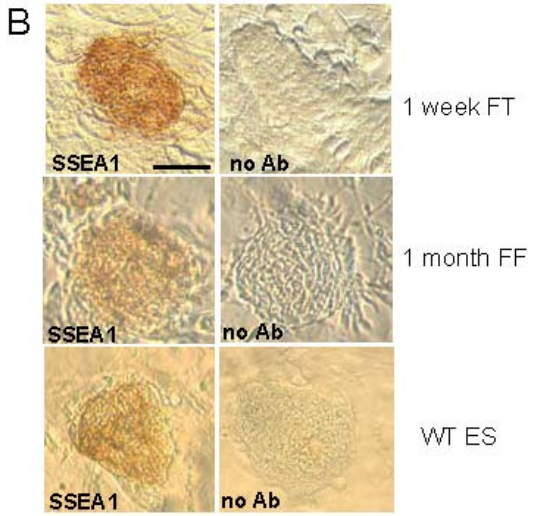
Disruptive technology:

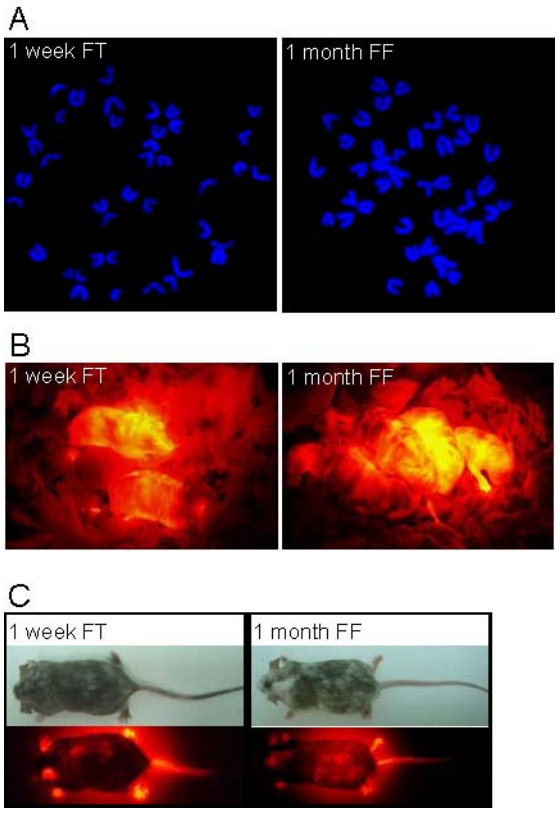
Freezing & storage of somatic tissues/cells for rederivation*

(Oct3/4, Sox2 and Klf4)

iPS cells from…	No. iPS clones	No. AP & SEA1 positive (%)
Frozen tail (1 week)	38	31 (71%)
Frozen fibroblasts (1 month)	15	11 (73%)







*Trans Tech, 2011

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