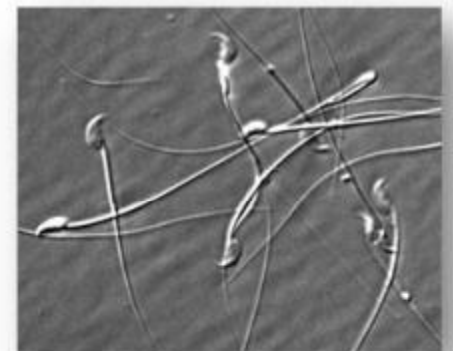


# Cryopreservation: a Tool for Colony Management & Disaster Planning

Brian W. Soper, Ph.D.  
Technical Information Services

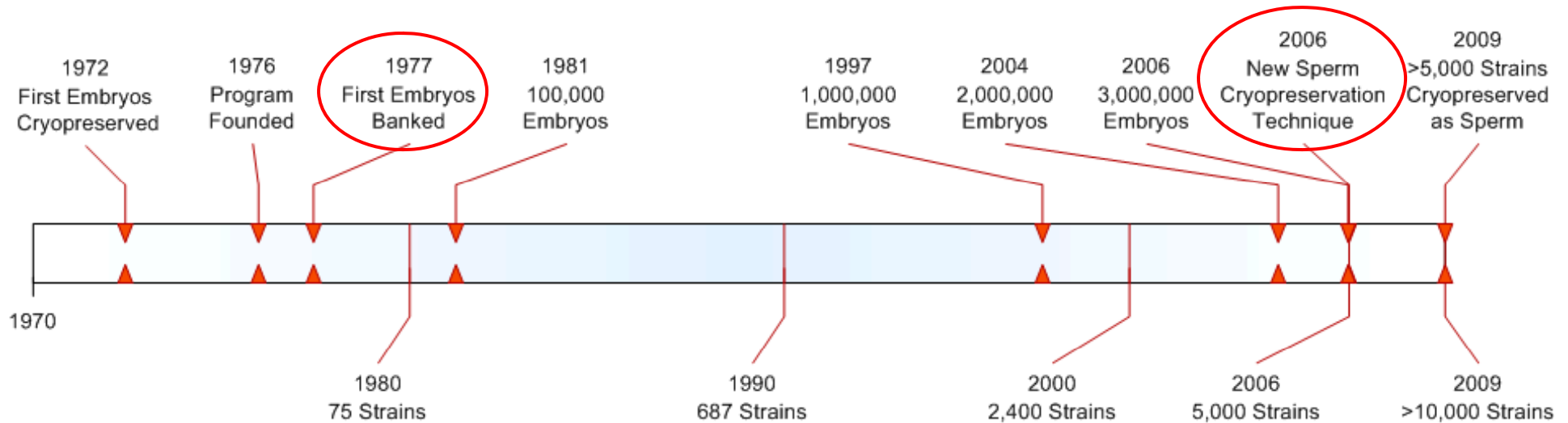


# Overview

- Why cryopreservation?
- How does it work?
- Different cryopreservation methods
  - Embryo
  - Sperm—*new* research breakthroughs
- Benefits of cryopreserving low use models
- Disaster planning



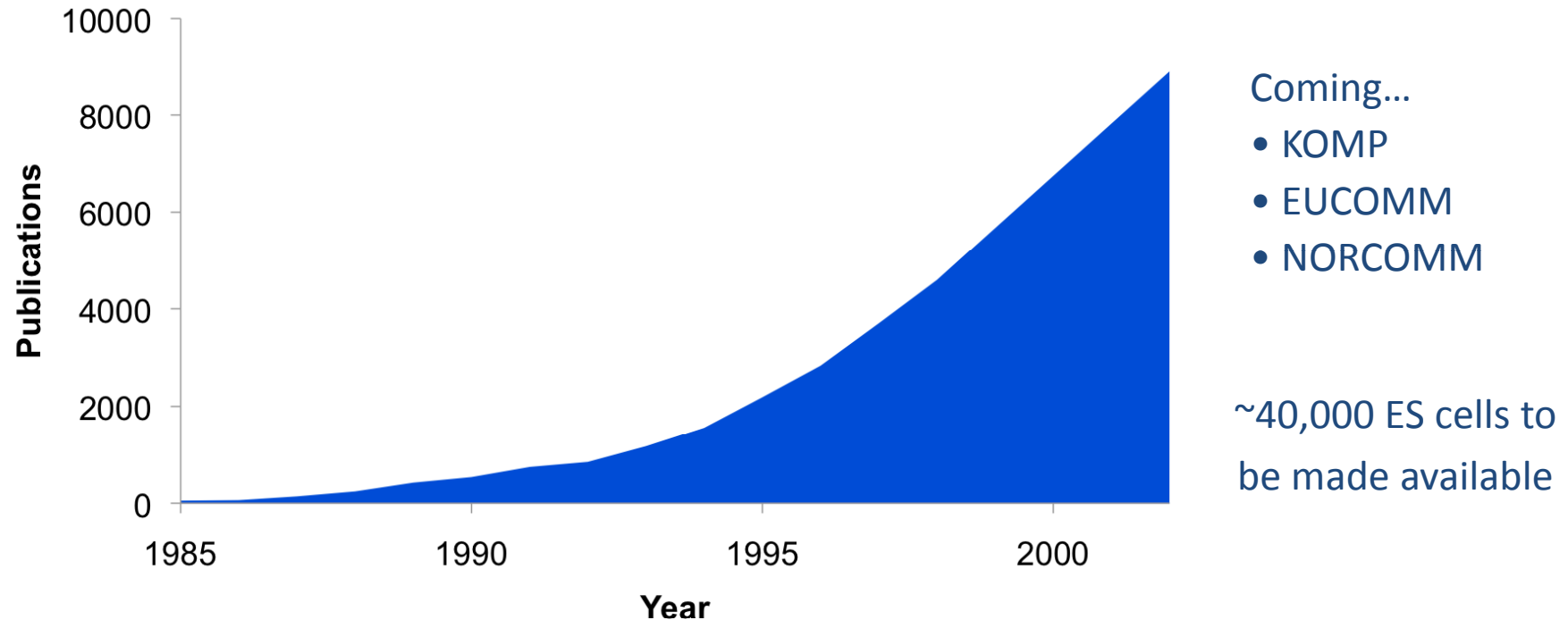
# Key Milestones - JAX Cryopreservation



To date...

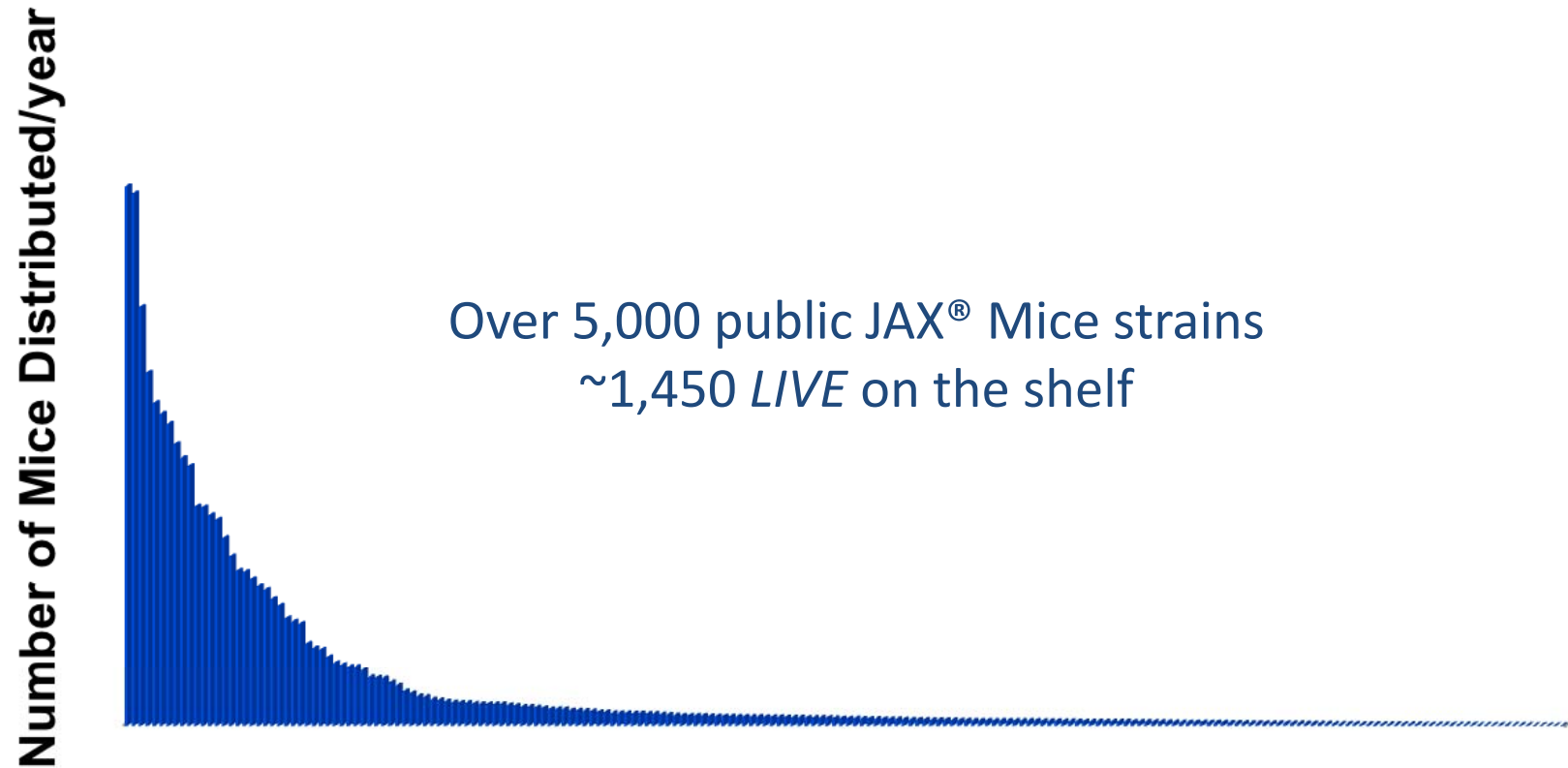
- More than 10,000 unique strains cryopreserved
- 3,500,000 embryos cryopreserved
- More than 2,500 cryorecoveries per year
- More than 12,000 embryo transfers each year

# Knockout & Transgenic Usage Trends



*And, the number of new strains created continues to grow!*

# JAX<sup>®</sup> Mice Repository - Strain Utilization



**Each Bar Represents One Strain**

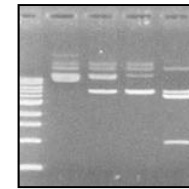
# True Cost of Low Demand Strains



Space



Time



Genotyping

*Risk of loss*

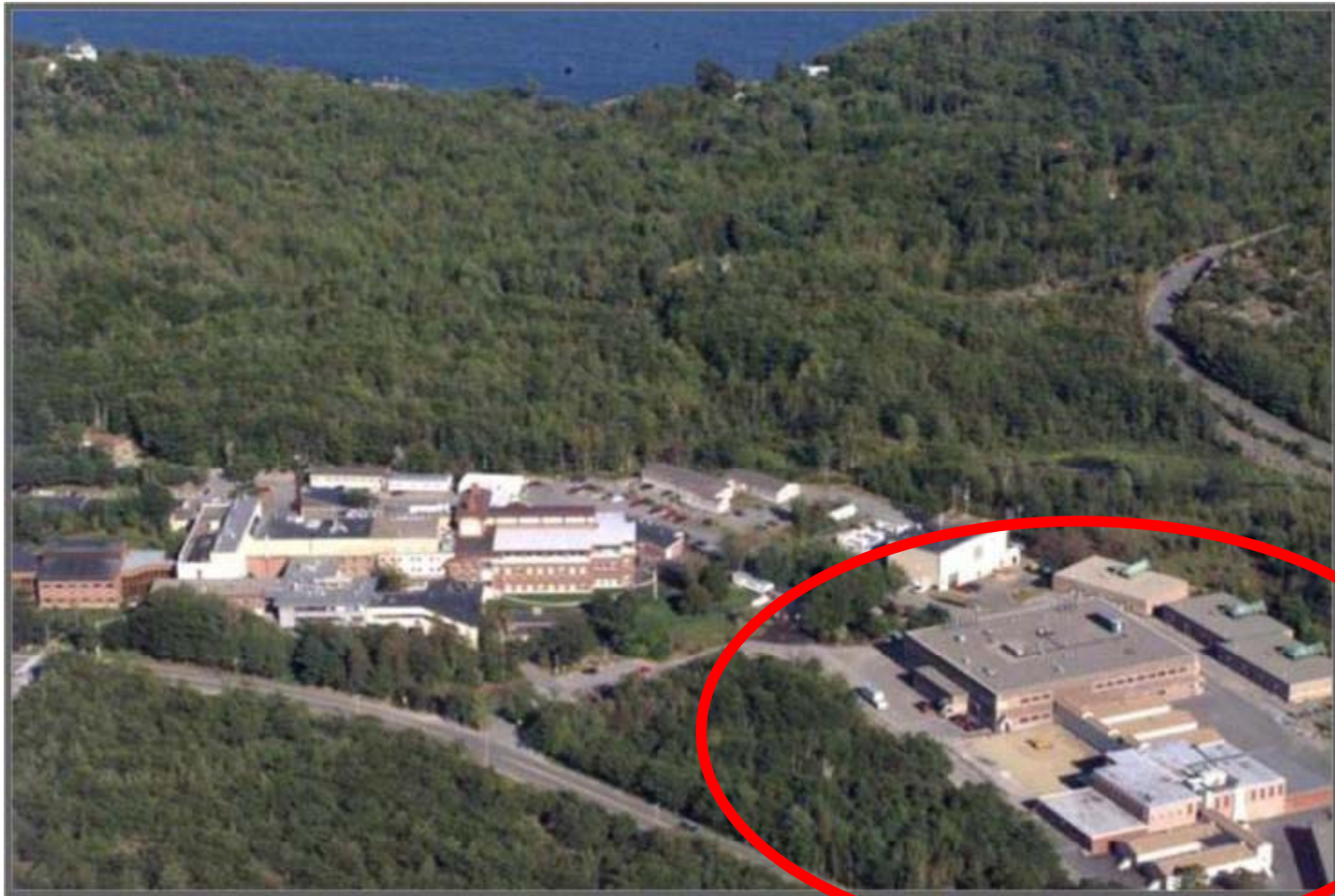


Veterinary, Technician,  
& Caretaker costs

Per Diem Charges

*Estimated average size colony maintenance:*  
~\$10,000 / year

# The Jackson Laboratory Bar Harbor Facility *over 1450 live strains*

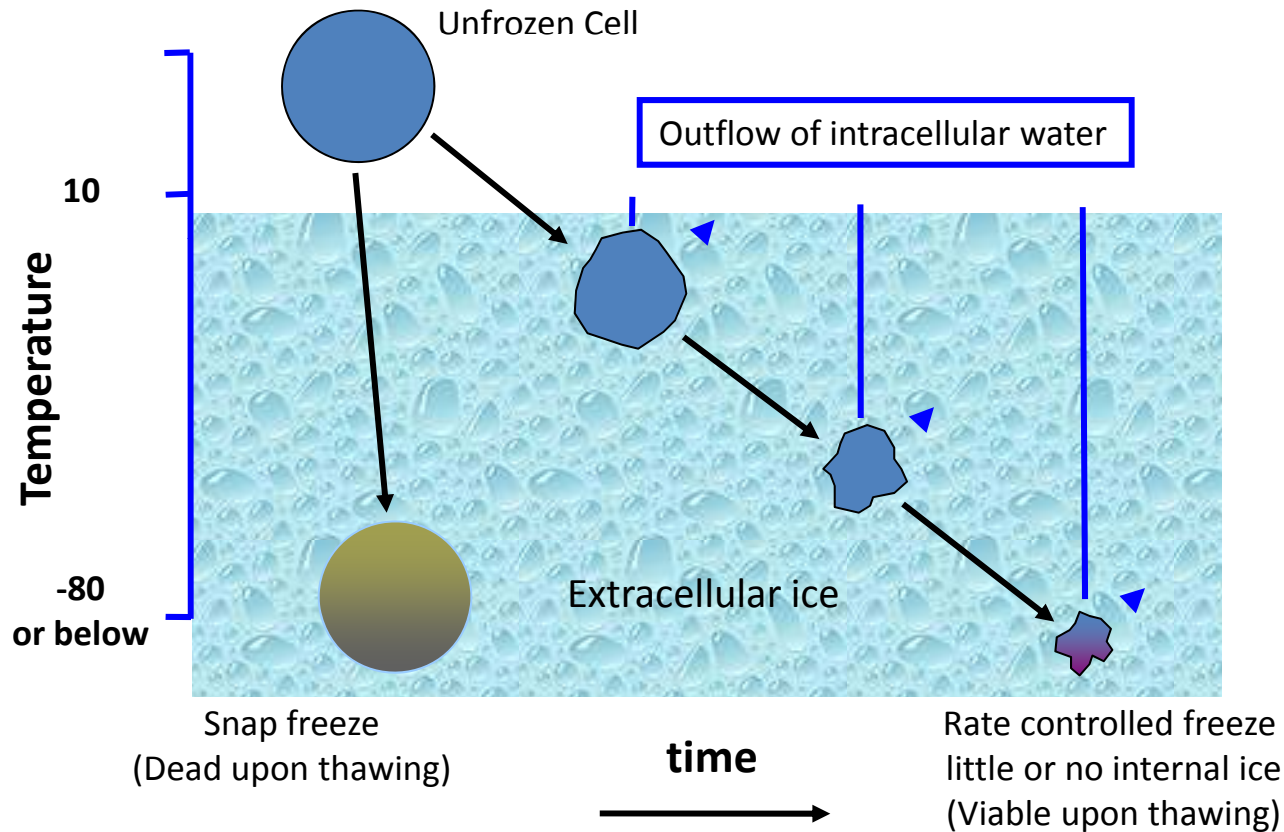


# Cryopreservation “Tank Farm”



# Cryopreservation

- + Introduction of a cryoprotectant
- + Cooling, storage below  $-80^{\circ}\text{C}$
- + *Viable & functional* after warming!



Glenister, PH and WF Rall. 1999. Cryopreservation and rederivation of embryos and gametes *in* Mouse Genetics & Transgenics: A Practical Approach, 2<sup>nd</sup> Edition, Oxford University Press, Oxford, pp27-59.

# Basic Cryo Considerations

- Is the strain a unique resource?
- Cost/funding available
- Strain type and genetic background
- Likelihood of use in the future
- Quantity of resources needed to reliably recover
- What are the risks of not cryopreserving?
- What type of cryo is best suited for each strain?

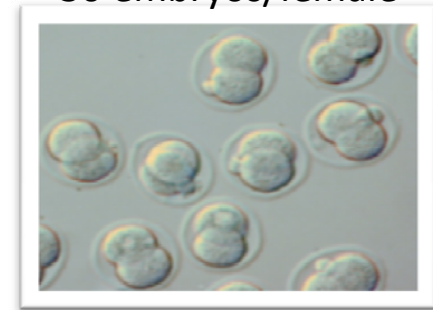


# Cryopreservation Options

## Embryos

- What you freeze *is* what you get back
- Homozygous, multiple unlinked mutations, unique genetic backgrounds

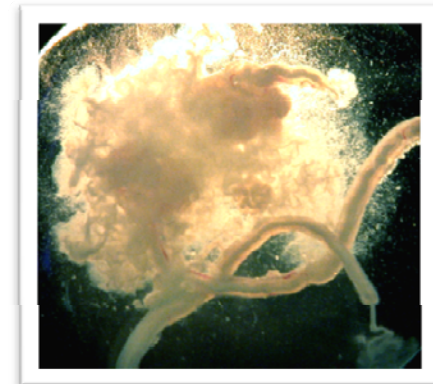
~30 embryos/female



## Sperm

- Progeny produced are heterozygotes
- Single mutations on a common inbred background: most transgenics and knockouts

~ $30 \times 10^6$  sperm/male



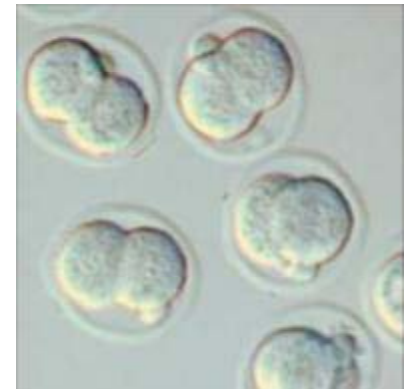
# Embryo Cryopreservation

## Advantages:

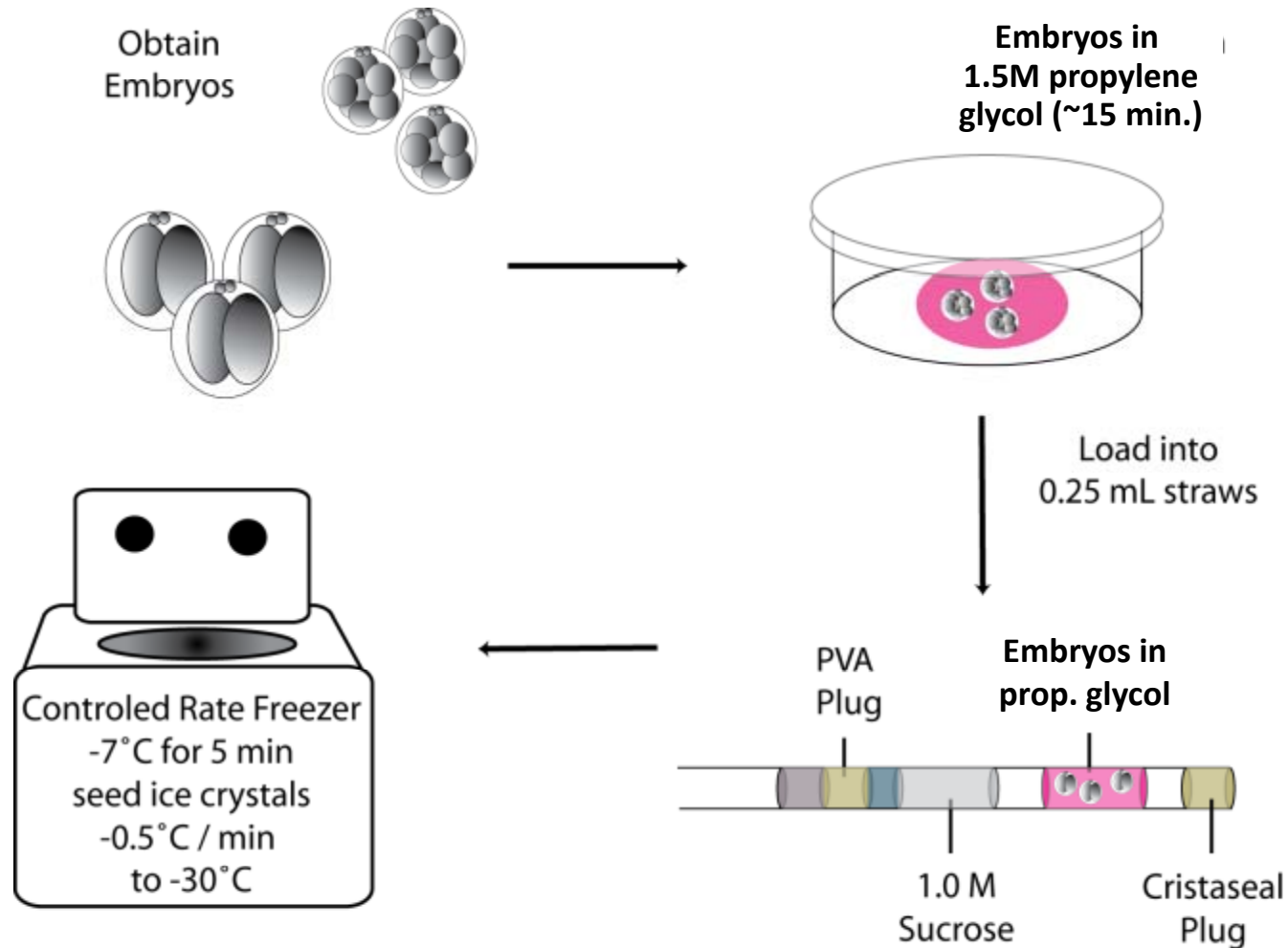
- Reliable
- Donor genotype preserved
- Embryo transfers are inexpensive and simple
  - Thaw embryos, transfer into pseudopregnant females
- Time to freeze and recover is short
- Biosafety

## Considerations:

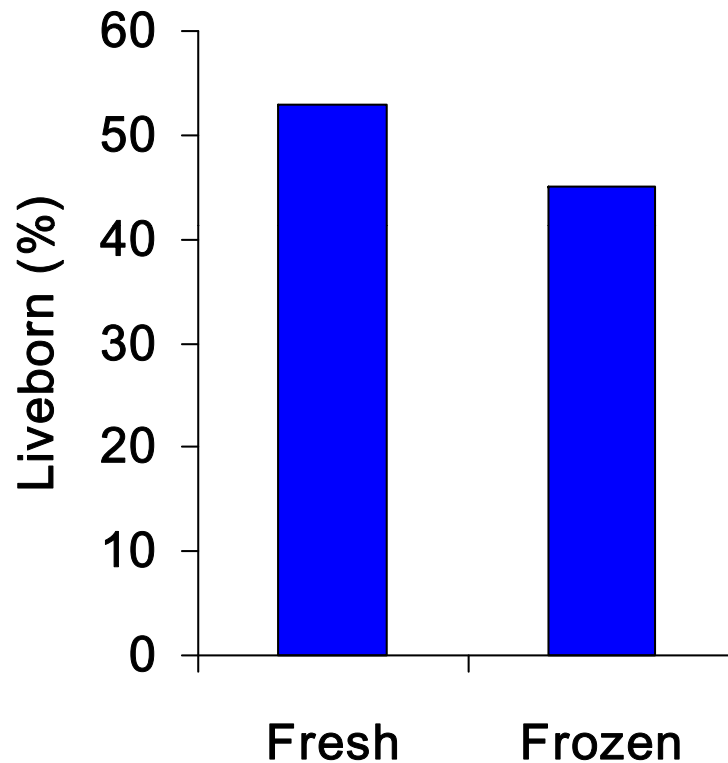
- More upfront cost (to freeze)
- Yield / animal moderate



# Embryo Cryopreservation Protocol



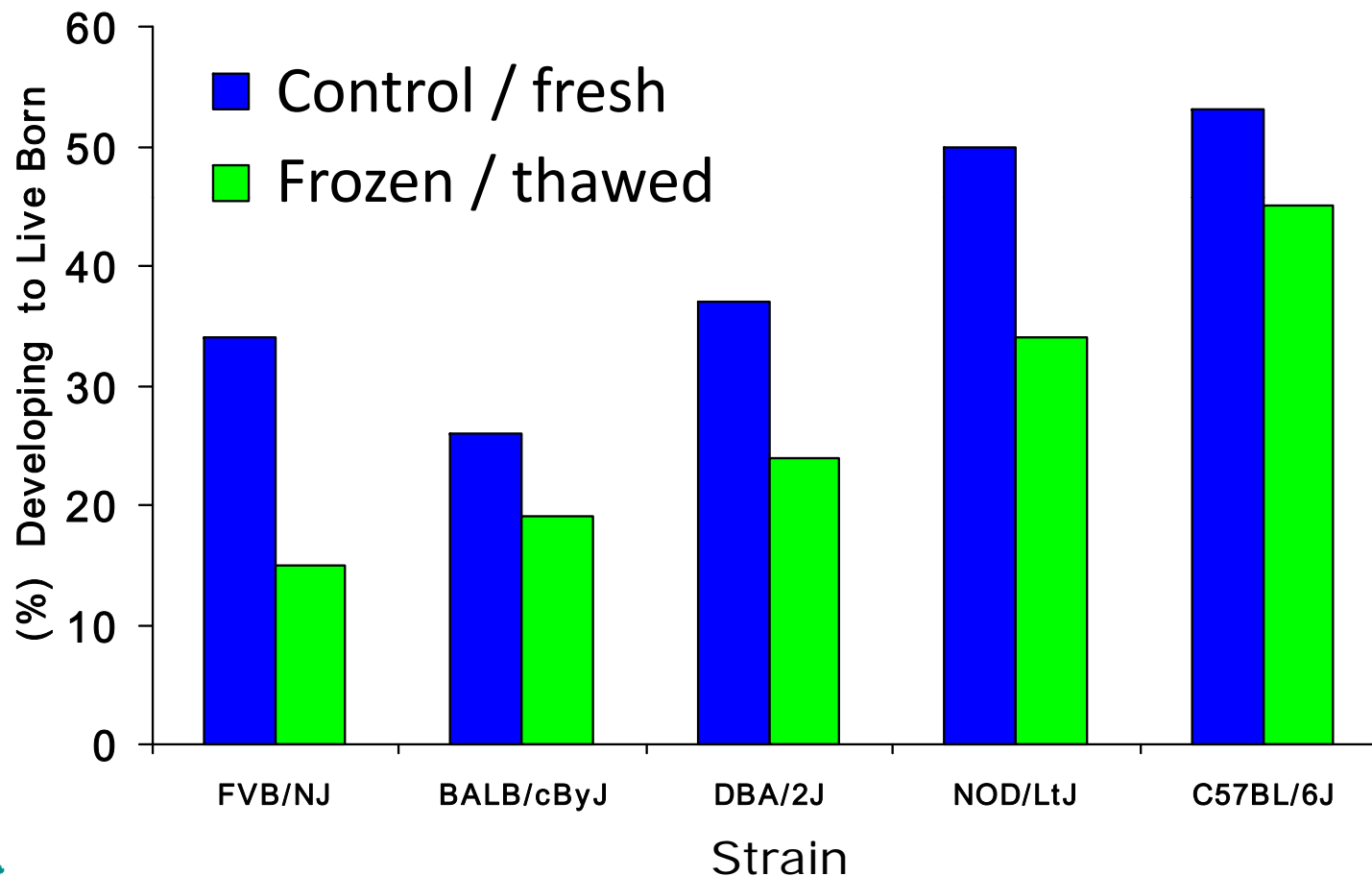
# Embryo Cryopreservation is Robust



Used by JAX since early 70's

- ~5000 strains preserved
- Easy to recover
- Excellent biosafety

# Influence of Genetic Background on Embryo Cryopreservation Success



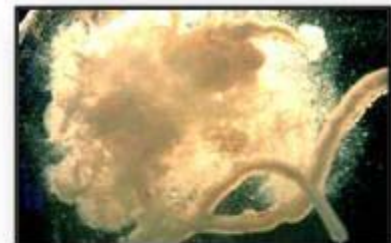
# Sperm Cryopreservation

## Advantages:

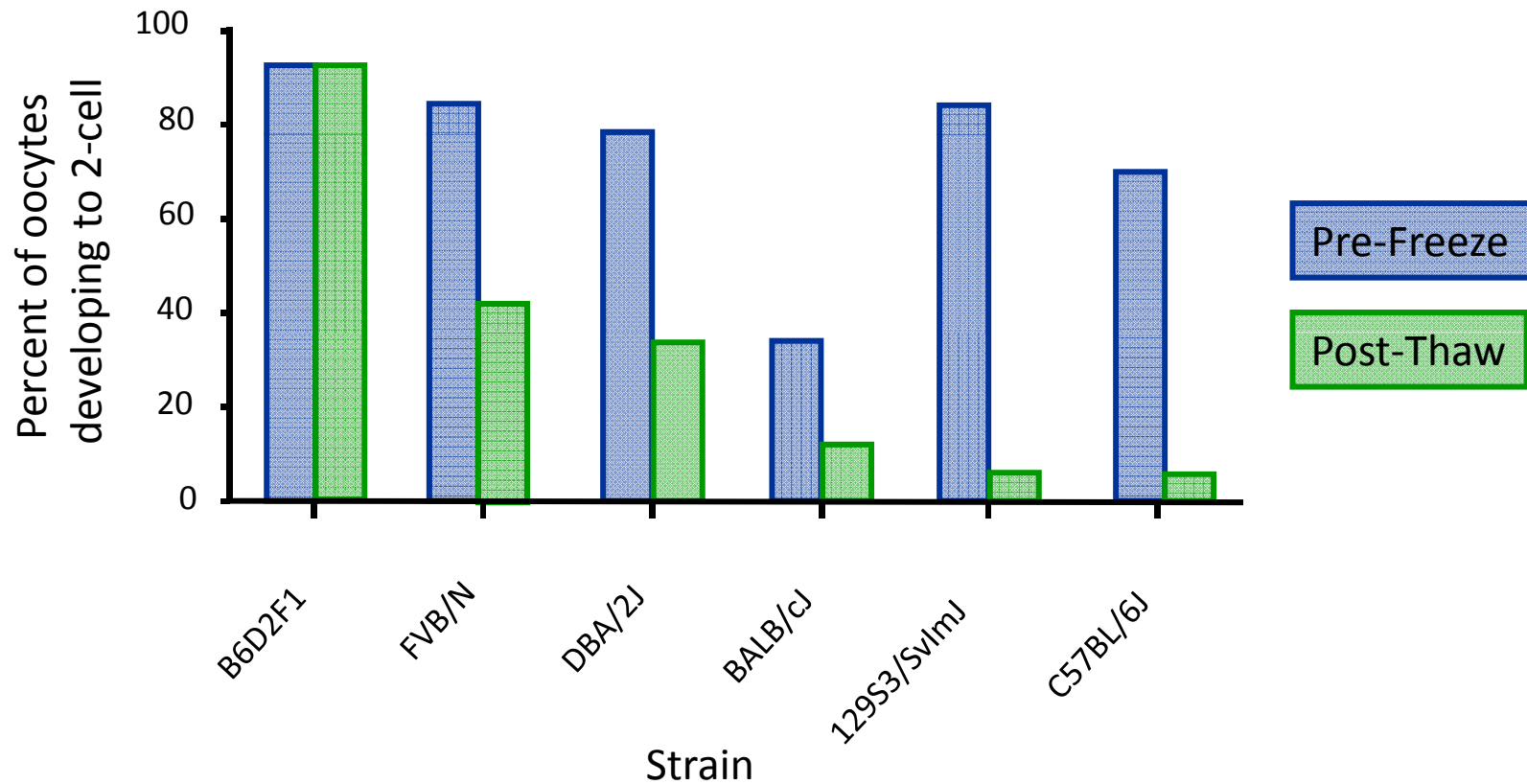
- Abundant material (30 million sperm / male)
- Simple (and now efficient)
- Low upfront cost
- Single mutations on a common inbred background

## Considerations:

- Preserves only one-half the genome
- IVF needed for recovery
- Historically, poor success for strains on certain inbred backgrounds



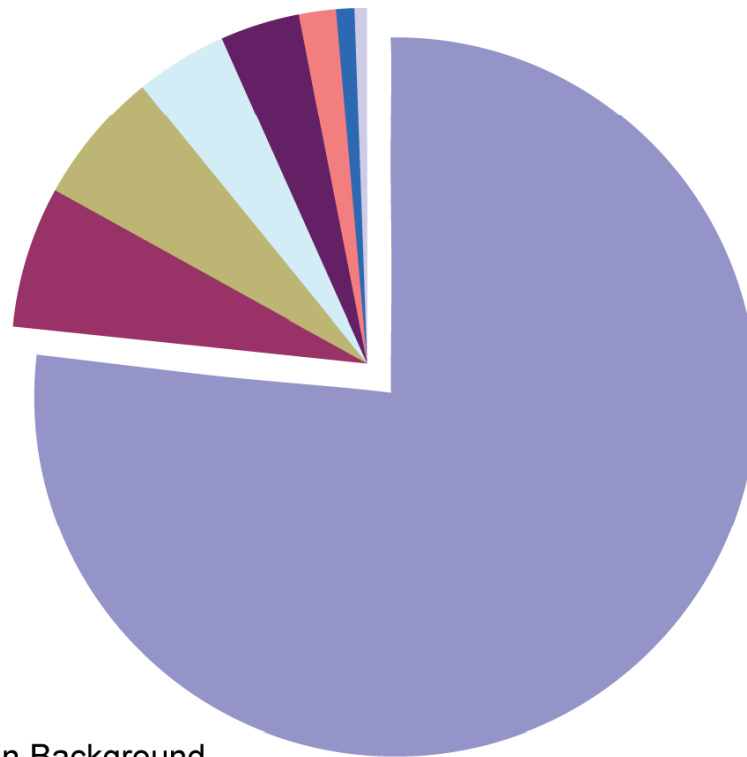
# Influence of Genetic Background on *Traditional* Sperm Cryopreservation Success



Can a reproducible and cost effective  
method be developed for  
cryopreserving and recovering mouse  
sperm?

# Background Usage - Frequency

Jan. '04 to Jan. '06  
670 mouse strains donated to  
JAX<sup>®</sup> Mice Repository



## Inbred Strain Background




# Traditional Sperm Cryo Protocol



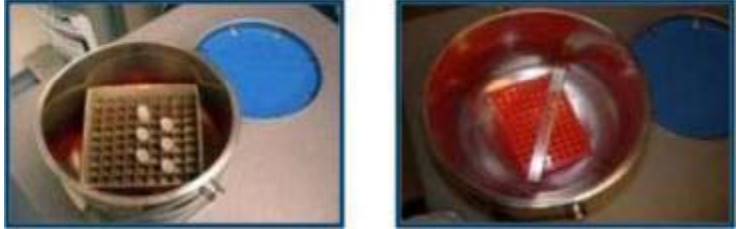
sperm collected into cryoprotective medium

sperm dispensed into pre-labeled containers



vials  
ampules  
straws


static vapor freezing




storage  
-196°C

thawing

quality control




motility



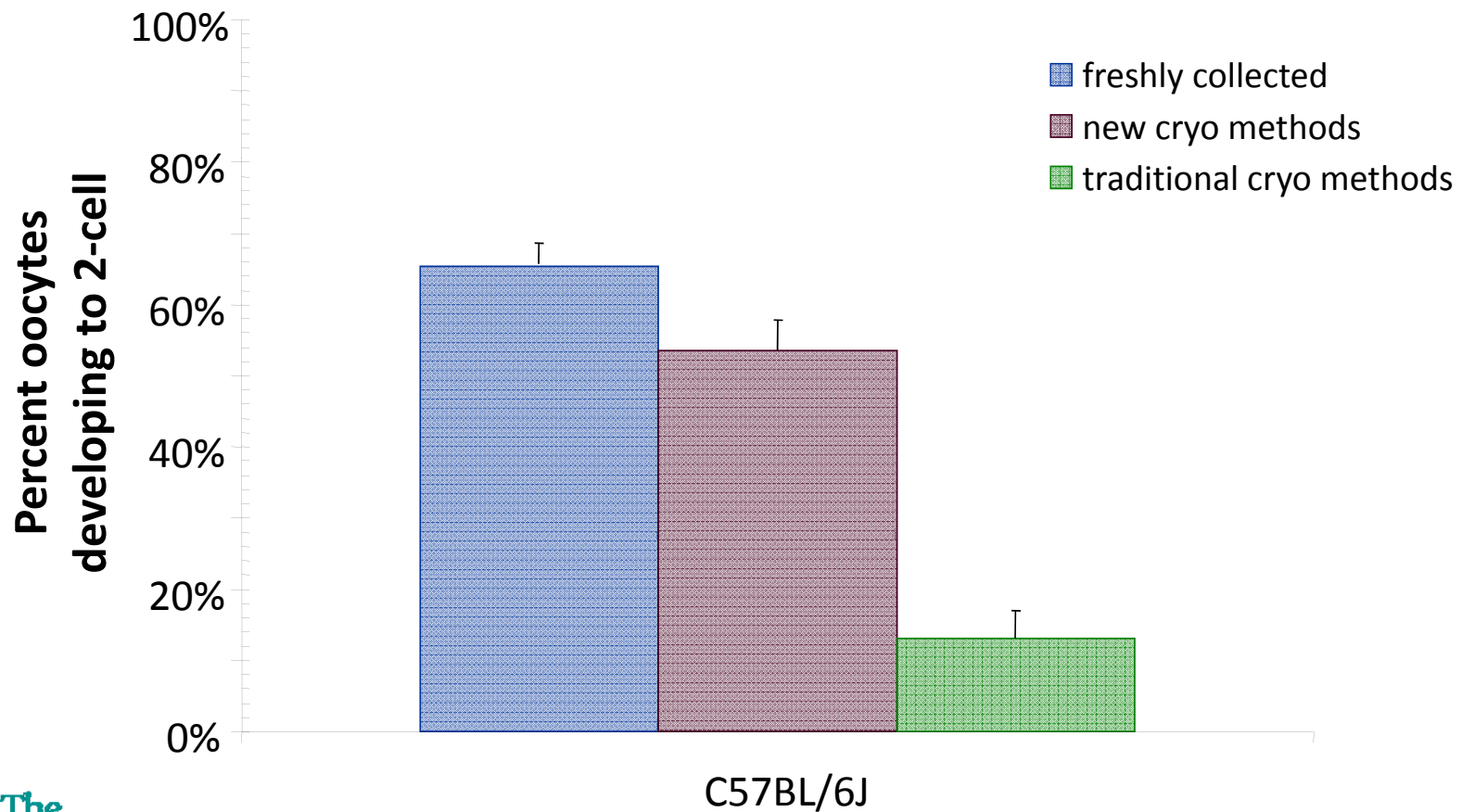
% intact membranes

*in vitro* fertilization



% to 2-cell

# New Sperm Cryopreservation Methods



# New Sperm Cryopreservation Methods

C57BL/6J Live Born

- Freshly collected sperm - 50% of embryos to live born
- *New* cryo methods - 44%
- *Traditional* cryo methods - 17%



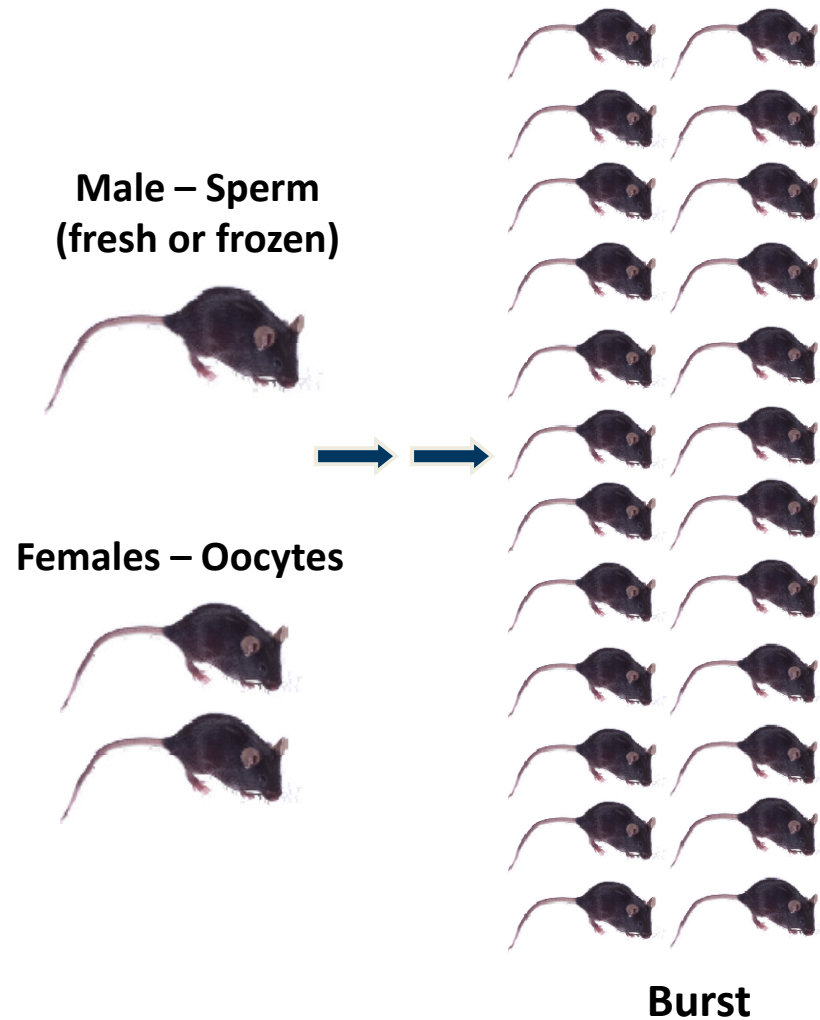
Starting with 100 oocytes...

- *Traditional* methods yield ~10 2-cell embryos and 0-1 live born
- *New* methods yield ~50 embryos and ~22 live born

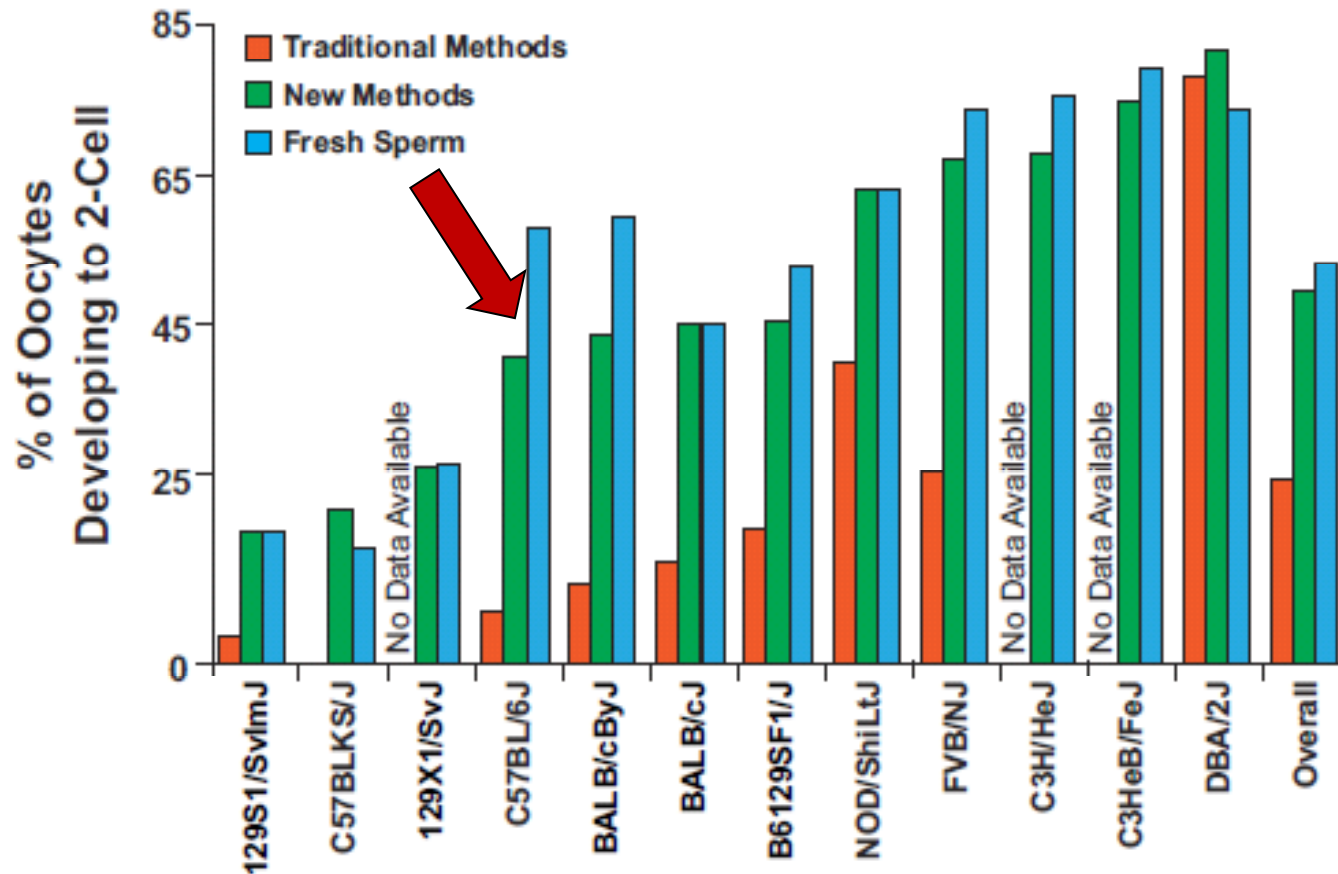
*Highly Reproducible!*

# Easy Speed Expansion with Sperm Cryo

Sperm Doses	2-cell embryos (using 2♀♀s / IVF)	Pups Born
1	20	7
2	40	14
3	60	21
4	80	28
5	100	35
6	120	42
7	140	49
8	160	56
9	180	63
10	200	70



# New Sperm Cryo Methods Improve Recovery



*Across all major inbred strains!*

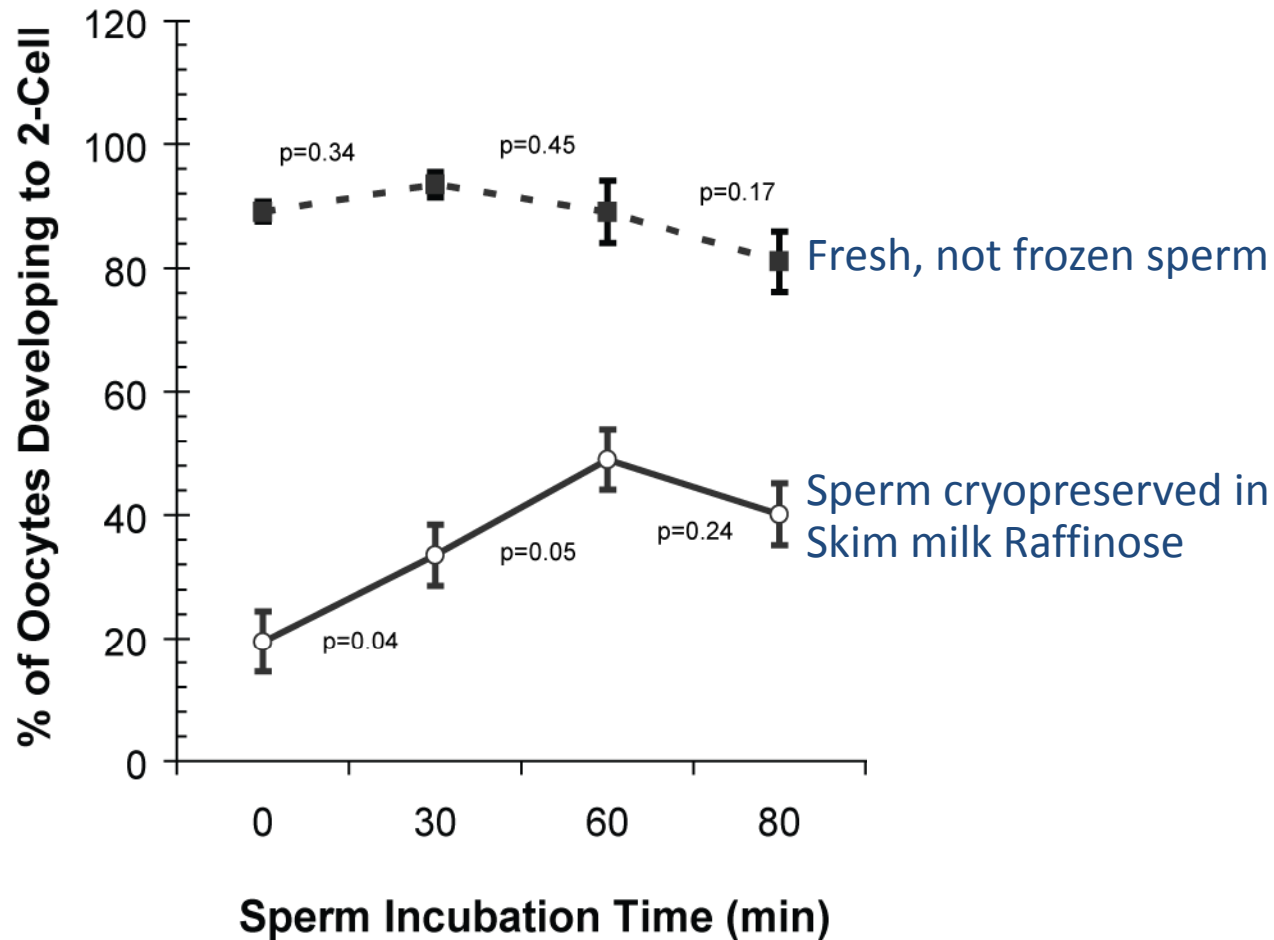
# What's the Secret?

## *Optimize, Optimize, Optimize*

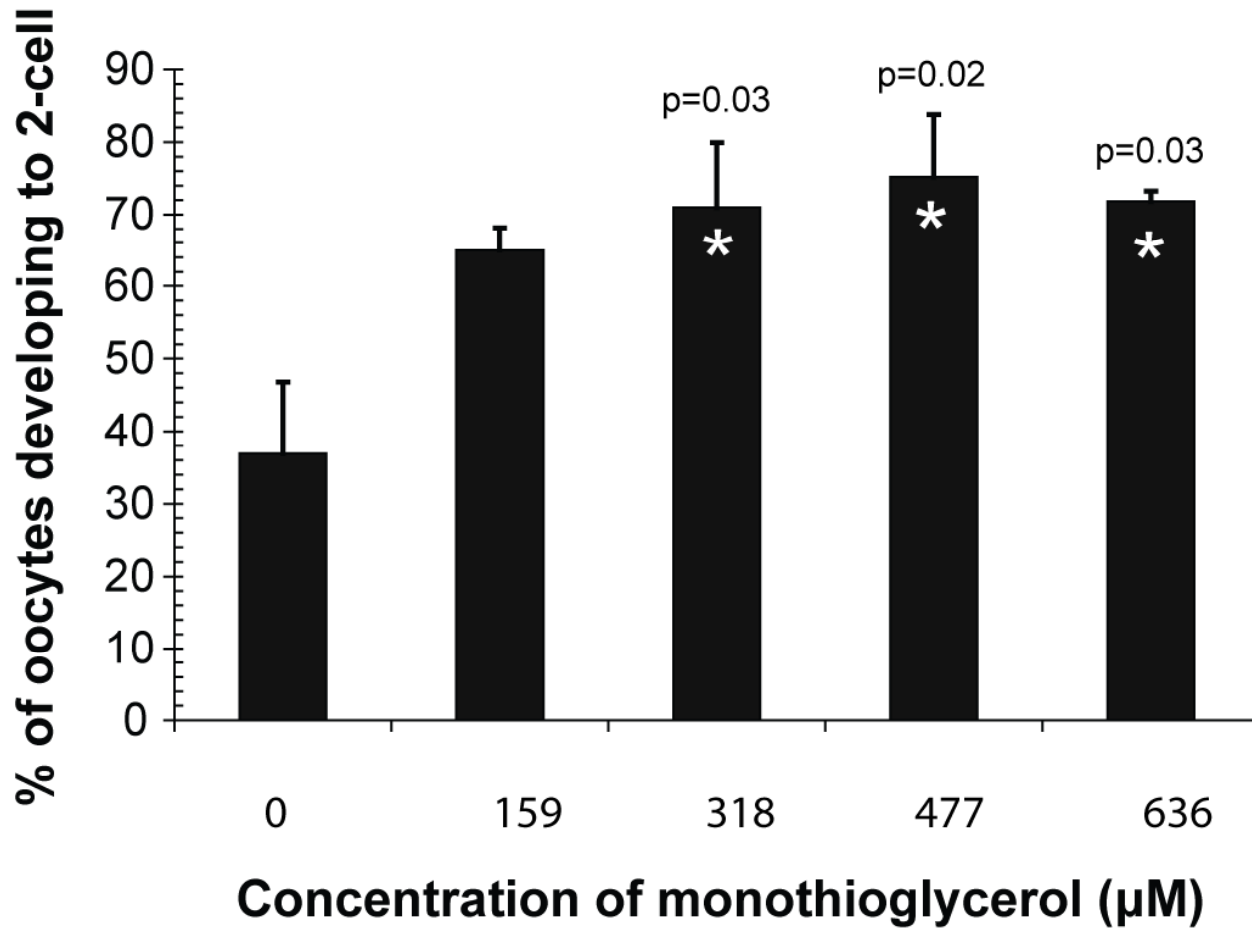
- Use straws for freezing instead of vials
- Slow cooling rates
- Optimized thawing temperature
- Incubate thawed sperm prior to IVF
- Optimized cryoprotectant



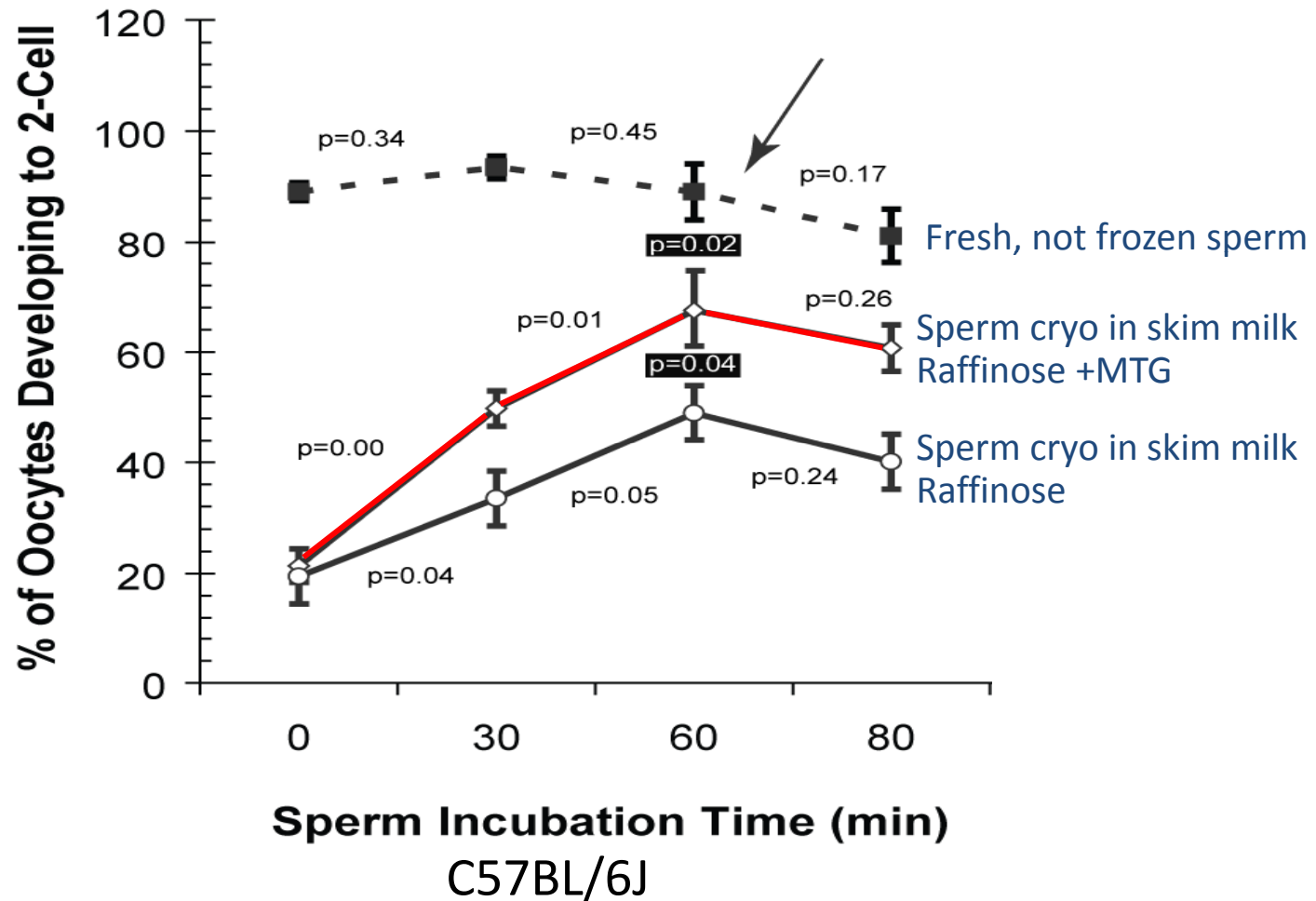
# Traditional Sperm Cryo Media



# Monothioglycerol (MTG) Improves Sperm Cryo



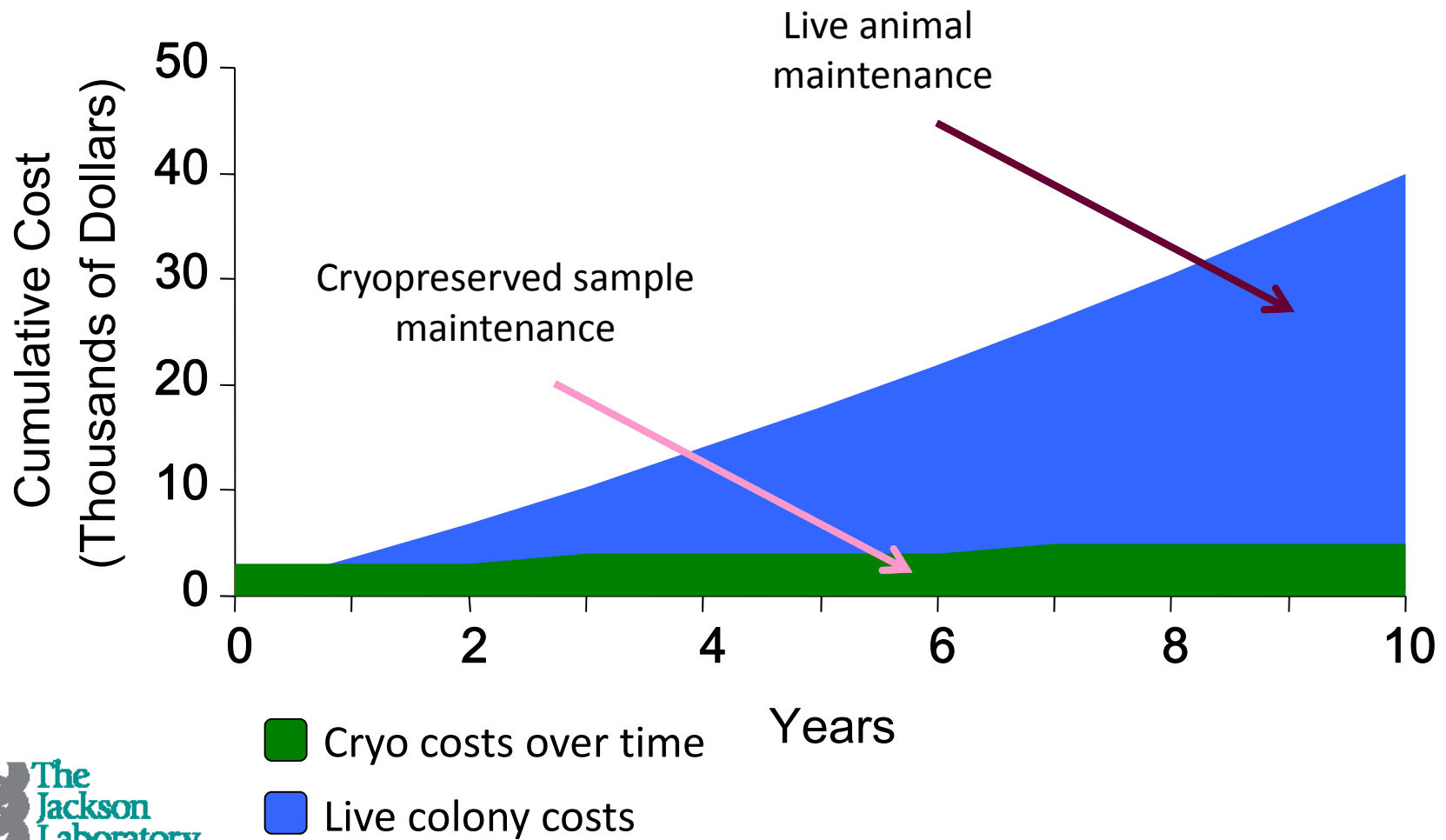
# Monothioglycerol (MTG) Effect



# Cryopreservation Options Compared

	Embryos	Sperm
Pros	Reliable Entire genome preserved Simple recovery Great biosafety	Now Reliable! Simple cryo Low upfront resources- (2 males) Great biosafety
Cons	Higher upfront resource (females=\$\$)	Half genome

# Benefits of Cryopreserving Low Use Strains



# How Will You Protect Your Mice?



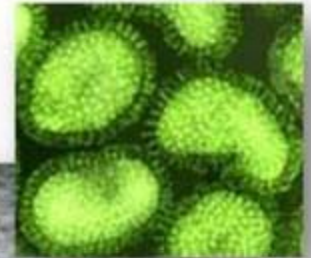
# Benefits of Cryo – Disaster Recovery

- University of Texas – 2001  
Tropical Storm Allison
- Louisiana State & Tulane–2005  
Hurricane Katrina
- Hundreds of researchers affected & millions  
of research dollars lost
- Years of delay for project recovery
- Permanent loss of valuable mouse resources



# Disaster Comes In Many Forms

- Disease outbreaks – not a matter of *if* but *when*
- Breeding errors or breeding cessation
- Facility equipment failures
- Contamination
- Genetic drift



# Develop Your Own Insurance Plan

## *Prevention*

- Maximize safety of your facility and resources
- Watch out for silent disasters

## *Action Plan*

- What will you do if...?

## *Recovery*

- How will you get your strains back?



# Cryopreservation Options

## *Do It Yourself!*

- JAX Cryopreservation Course
  - <http://courses.jax.org>
- Sperm Cryo Kit



## **JAX<sup>®</sup> Alliance Program for Transgenic Cores**

- Sperm cryo kit discounts
- Includes IVF, QC & storage

## *Donate Your Strain*

- JAX Genetic Resources
  - [www.jax.org/grc/index](http://www.jax.org/grc/index)
- Sponsored Strain Donation

### **Donate a Mutant Strain to The Jackson Laboratory Repository**

Strains are evaluated once a month by the Genetic Resource Committee. To ensure that your strain is evaluated at the next meeting, please submit your strain no later than the 3<sup>rd</sup> Wednesday of the month.

Please enter information for no more than one strain at a time. The information you submit is used to evaluate your strain - please be as detailed as possible.

A confirmation email is sent to the email address provided upon our receipt of your submission. If you do not receive the confirmation email please contact [Deborah Boswell](#).

#### **Contact Information**

Your name:  **REQUIRED**

Institution:  **REQUIRED**

Street:

City:  St:  Zip:

## *Freeze Team*

- Cryopreservation of a large number of strains *facility-wide*
- ***We come to you*** and can cryopreserve 120 strains or more/week



# Thank you!

- Breeding & colony management
- Revolutionary cryopreservation & recovery
- Phenotyping & efficacy testing
- Genetic research services
- Surgical & preconditioning services
- Flexibility to develop customized approaches
- Use of innovative technologies and state-of-the-art equipment



## JAX<sup>®</sup> Mice & Services

[jaxservices@jax.org](mailto:jaxservices@jax.org) • 1-800-422-6423 • 1-207-288-5845 • [www.jax.org/jaxmice](http://www.jax.org/jaxmice)