

September 9, 2021

# Designing Your *In Vivo* Studies

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Designing and successfully conducting reproducible mouse studies

Sample size planning

Communicating methods completely and accurately



Somshuvra Bhattacharya, Ph.D.

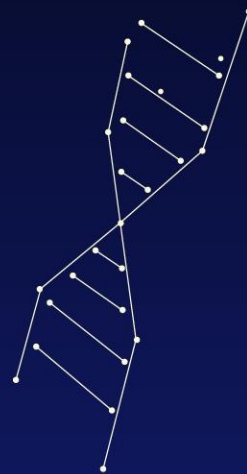
Technical Information Scientist



# JAX MISSION

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To discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.



# EMPOWERING SCIENTIFIC EXCELLENCE

Our scientific expertise is derived directly from JAX faculty and scientific researchers, who are embarking on ground-breaking research in addition to providing cutting-edge models and powerful preclinical services to researchers worldwide.

[Explore the Latest Innovations](#)



## GLOBAL EXPERIENCE. GLOBAL INFLUENCE.

JAX is a global organization hosting events and shipping mouse models to researchers worldwide, who have come to rely on the gold-standard models to answer their unique research questions.



\*white dots indicate JAX locations and shaded areas of the map are countries JAX has shipped products and services to.

# What Types of *In Vivo* Studies Are You Conducting?



- Basic science, mechanistic
  - Signaling pathway
  - Role of gene in disease
- Translational, preclinical
  - Drug, device efficacy or safety
- I am just here to learn; I am not currently conducting *in vivo* studies

# Learning Goals

- Goal One: Designing and successfully conducting reproducible *in vivo* mouse studies
  - Select appropriate *in vivo* models and controls
  - Choose quantifiable assays and meaningful readouts
- Goal Two: Sample size planning
- Goal Three: Communicating methods completely and accurately



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# Thoughtful Experimental Design Promotes the 3Rs



## REPLACE

Methods which avoid or replace the use of animals



## REDUCE

Methods which minimize the number of animals used per experiment



## REFINE

Methods which minimize suffering and hence improve animal welfare

Image Adapted From: [mdc-berlin.de/research-animal-experiments-3r/3r-principles](https://mdc-berlin.de/research-animal-experiments-3r/3r-principles)



# “The *Other* R”: Reproducibility

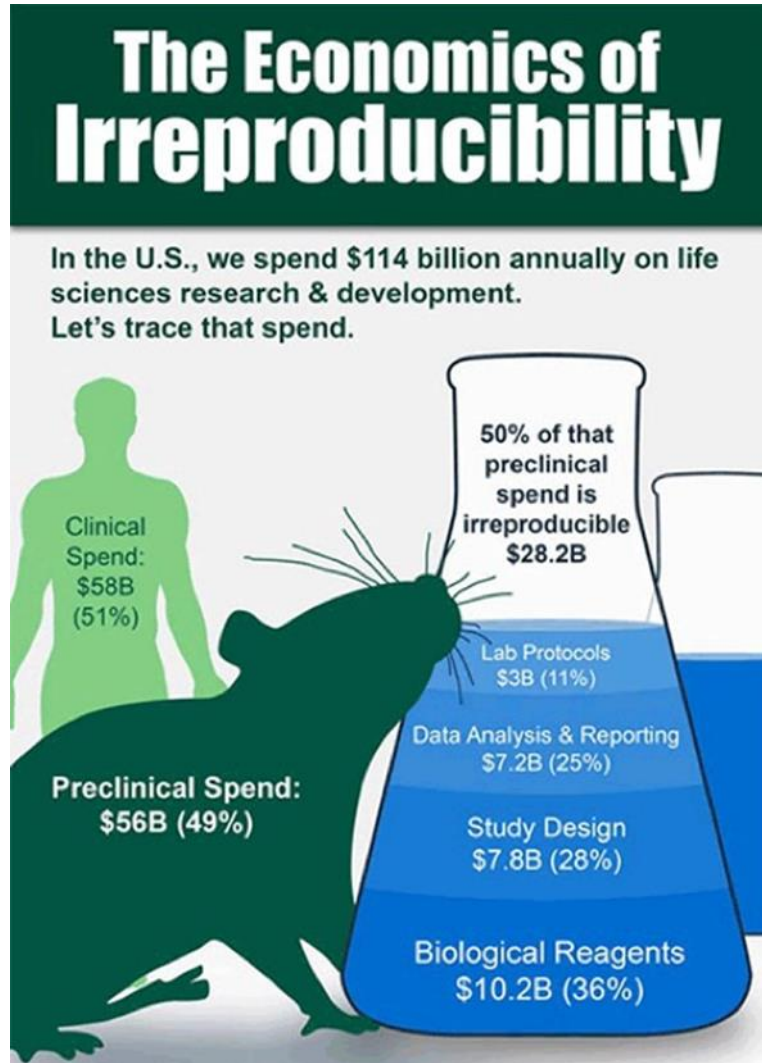


- Repeatable
  - Precision within an experiment
  - Relies on nothing changing
- Reproducible
  - Across experiments, laboratories, publications
  - Relies on parameters changing yet still supporting one robust, biological truth

Casadevall 2010 Infect Immun PMID: [20876290](https://pubmed.ncbi.nlm.nih.gov/20876290/)

Image From: [www.technologynetworks.com/informatics/articles/repeatability-vs-reproducibility-317157](http://www.technologynetworks.com/informatics/articles/repeatability-vs-reproducibility-317157)

# Relevance of Reproducibility



- High number of peer-reviewed publications involving animals are not reproducible
  - High attrition rate of novel therapeutics
- Well-designed animal studies allow for
  - Time and cost savings
  - Ethical animal use

Begley 2012 Nature PMID: [22460880](https://pubmed.ncbi.nlm.nih.gov/22460880/)  
Nature Special, Challenges in Irreproducible Research Oct 2018  
Image From: [SOURCE](#)

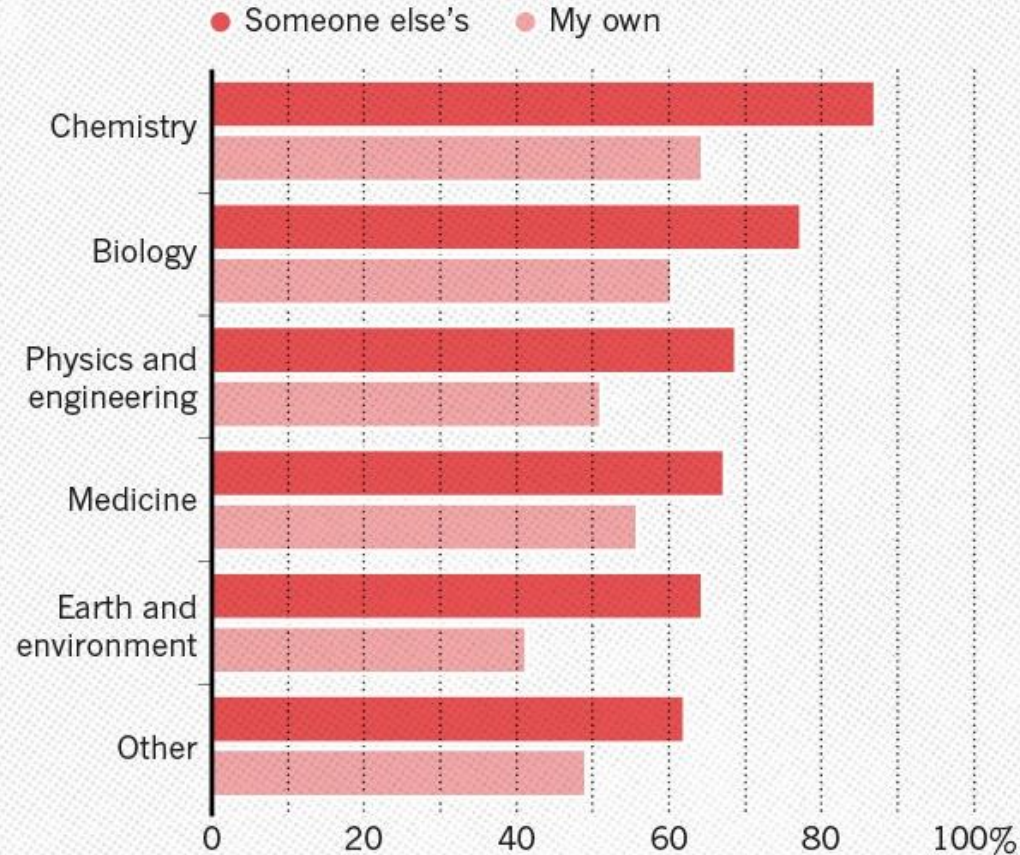
# Have You Ever Failed to Reproduce Experimental Results?

- A. I have personally failed to reproduce experimental results.
- B. I know of someone who has failed to reproduce experimental results.
- C. I have never tried to reproduce experimental results.
- D. I have always succeeded in reproducing experimental results.

# You Are Not Alone!

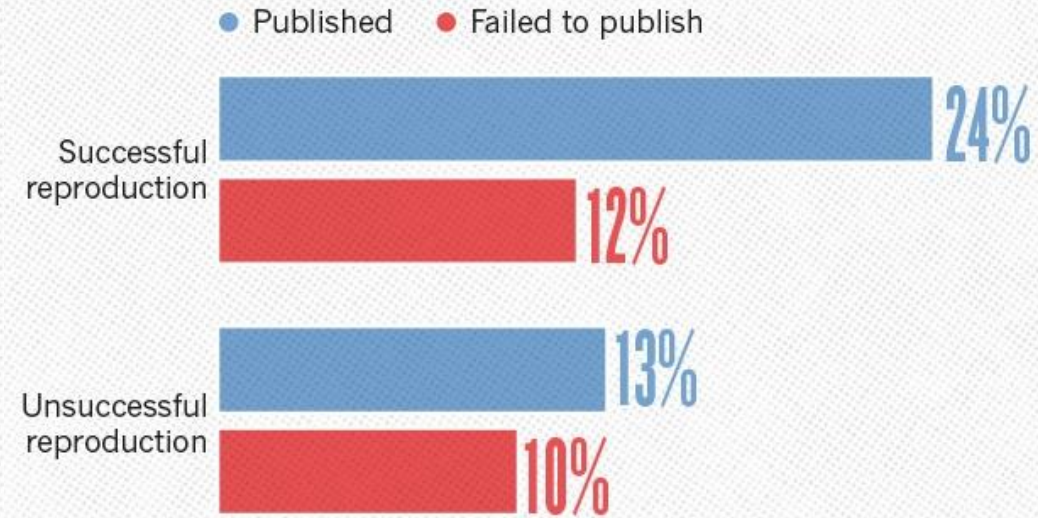
## HAVE YOU FAILED TO REPRODUCE AN EXPERIMENT?

Most scientists have experienced failure to reproduce results.



## HAVE YOU EVER TRIED TO PUBLISH A REPRODUCTION ATTEMPT?

Although only a small proportion of respondents tried to publish replication attempts, many had their papers accepted.



Number of respondents from each discipline:  
Biology **703**, Chemistry **106**, Earth and environmental **95**,  
Medicine **203**, Physics and engineering **236**, Other **233**

©nature

Baker M. 1,500 scientists lift the lid on reproducibility. Nature. 2016 May 26;533(7604):452-4. doi: 10.1038/533452a. PMID: 27225100.

# Gaps in Knowledge Can Result in Irreproducibility

- Two laboratories made a knockout of gene X and both models have been studied extensively for 10 years
- Found to differ in response to diet-induced obesity and hepatic steatosis
- What could account for differences?

	Laboratory A = <i>Fabp1</i> <sup>tm1Ddsn</sup>	Laboratory B = <i>Fabp1</i> <sup>tm1Bin</sup>
<b>Backcross method</b>	C57BL/6J from JAX 7 times using marker assistance	6 times to C57BL/6NCr (original paper); 6 times to C57BL/6J from JAX (done by collaborator)
<b>Strain maintenance</b>	Hom x Hom	Hom x Hom
<b>Refresh</b>	2 times to C57BL/6J (2007, 2012)	Not mentioned
<b>Other</b>	Generation (N and F) used is listed in figure legends	
	Controls were C57BL/6J purchased directly from JAX for each experiment	
	<b>*Substrain screen identified the KO mice being 40% C57BL/6N</b>	

Newberry 2015 Am J Physiol Gastrointest Liver Physiol PMID: [26251469](#)

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# Resources for *In vivo* Model Selection

The Science, Primary Literature



[pubmed.ncbi.nlm.nih.gov/](http://pubmed.ncbi.nlm.nih.gov/)



[informatics.jax.org/](http://informatics.jax.org/)



[phenome.jax.org](http://phenome.jax.org)

Mouse Repositories



<https://mice.jax.org/>



Mutant Mouse Resource & Research Centers supported by NIH



Create a Model *de novo*



[www.jax.org/jax-mice-and-services/custom-model-generation](http://www.jax.org/jax-mice-and-services/custom-model-generation)

ASK US!

[micetech@jax.org](mailto:micetech@jax.org)

1.800.422.6423 (US) |

1.207.288.5845 (International)

# Disease Mode of Action

- Mice are models
  - Mimic human disease symptoms but may not follow the same mode of action
  - Single model may only partially reconstruct a human disease
- ***Consider testing multiple disease models and determine whether they are relevant to your hypothesis, therapy***

## ***Common models for Human Type II Diabetes***



*Lep<sup>db/db</sup>* [000642](#)



*Lep<sup>ob/ob</sup>* [000632](#)

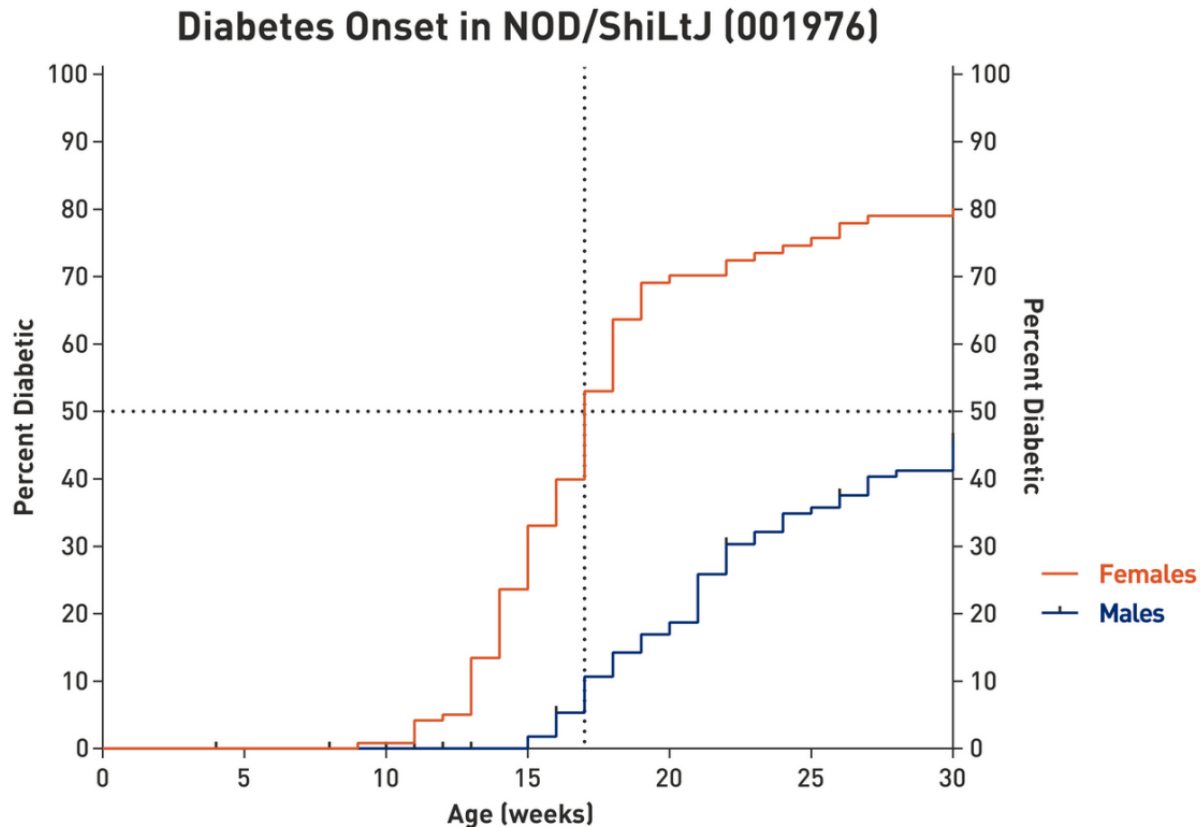
Wang 2015 Curr Diabetes Rev PMID: [24809394](#)



# Model Selection May Impact Experimental Design



NOD/ShiLtJ: Type I Diabetes  
[001976](#)




- Mice vary in disease onset
- Not all mice become diabetic
- Females and males differ in disease susceptibility
- Hence, it is a good idea to make sure sample size is appropriate

# Model Sex May Impact Experimental Design

- Disease prevalence, severity may differ
  - Human populations vs mouse models
    - Lupus BXSB/MpJ ([000740](#))
    - Rett Syndrome ([Rett Syndrome Mouse Model Resource](#))
  - Baseline characteristics in male vs female mice may differ
- Published literature may be sex-skewed for historical or logistical reasons rather than biological reasons
  - 5.5x more male animals are used in neuroscience
    - Beery 2011 Neurosci Biobehav Rev PMID: [20620164](#)
  - Females are no more variable than males
    - Prendergast 2014 Neurosci Biobehav Rev PMID: [24456941](#)

# Model Sex May Impact Experimental Design

-  National Institutes of Health  
Office of Research on Women's Health
  - Expectation that sex will be factored into research designs, analyses, and reporting in vertebrate animal and human studies
  - Strong justification required to propose to study only one sex
- **Consider using both sexes**
  - May lead to better understanding of sex differences, if applicable
  - Both sexes may be needed to more accurately model human disease
  - Potential to reduce breeding costs, animal use numbers



# Considering Phenotype for Experimental Design

- “Genetic model” does not equate to “disease uniformity”
  - Age
  - Sex
  - Genetic background
  - Environmental factors (stress, health status)
- ***Adjust experimental design to accommodate known model characteristics***

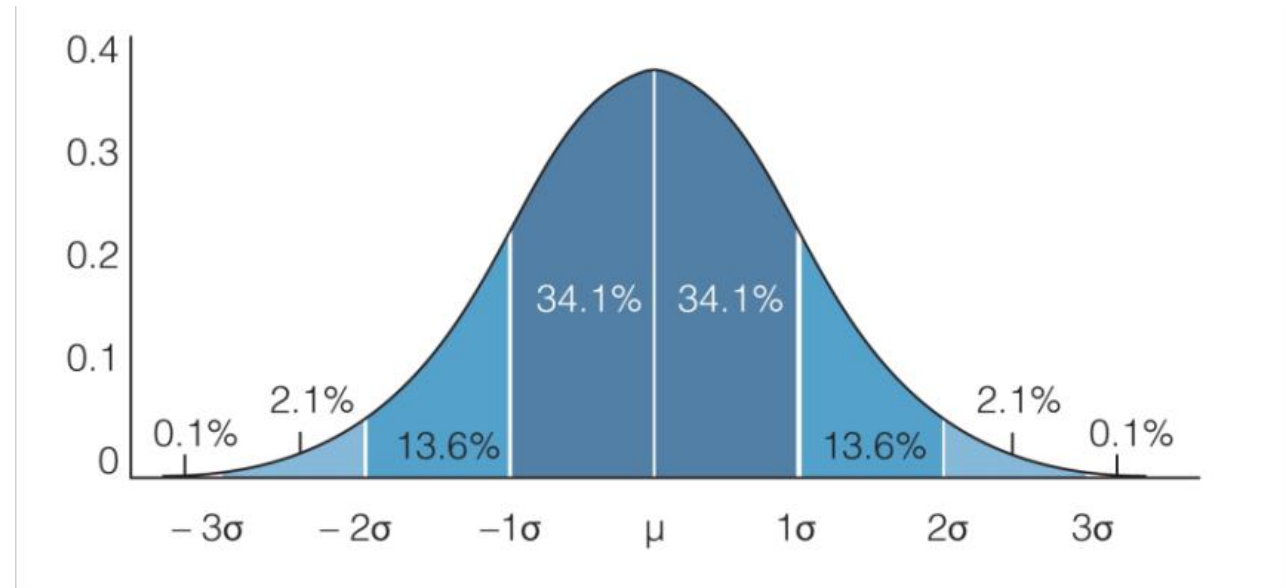
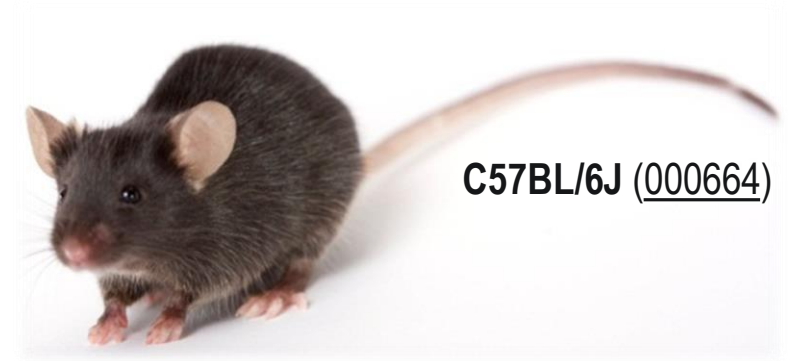


Image from: [SOURCE](#)

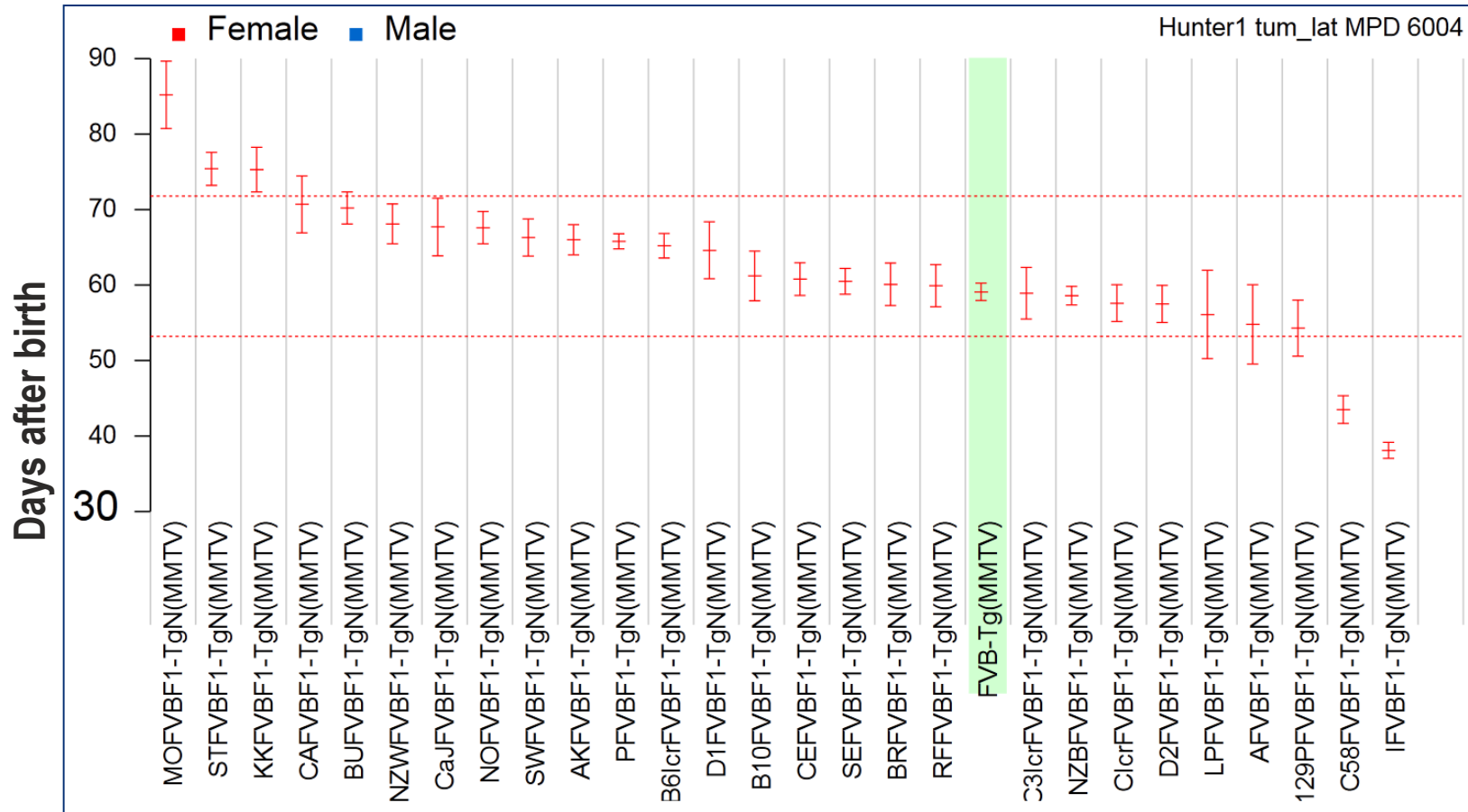
# Selecting Controls

- Ctrl/Scrambled vs experimental shRNA, siRNA
- No surgery, sham surgery vs experimental surgery
- Contralateral vs ipsilateral
- Vehicle vs experimental treatment
- **Wild Type vs Genetic Mutant**
  - Genetic Background
  - Substrain differences



# Genetic Background Influences Phenotype

Mammary Tumor Latency



# Genetic Drift: Substrain Divergence

**Substrains:** Branch of an inbred strain known or suspected to be genetically different from the parent colony.

**Colonies are considered substrains when. . .**

- 1) Separated from the parent colony for 20+ breeding generations
- 2) Phenotypic differences with the parent colony are discovered

**Nomenclature:** Strain name “/” Laboratory code(s)

e.g. CBA/CaGnLeJ

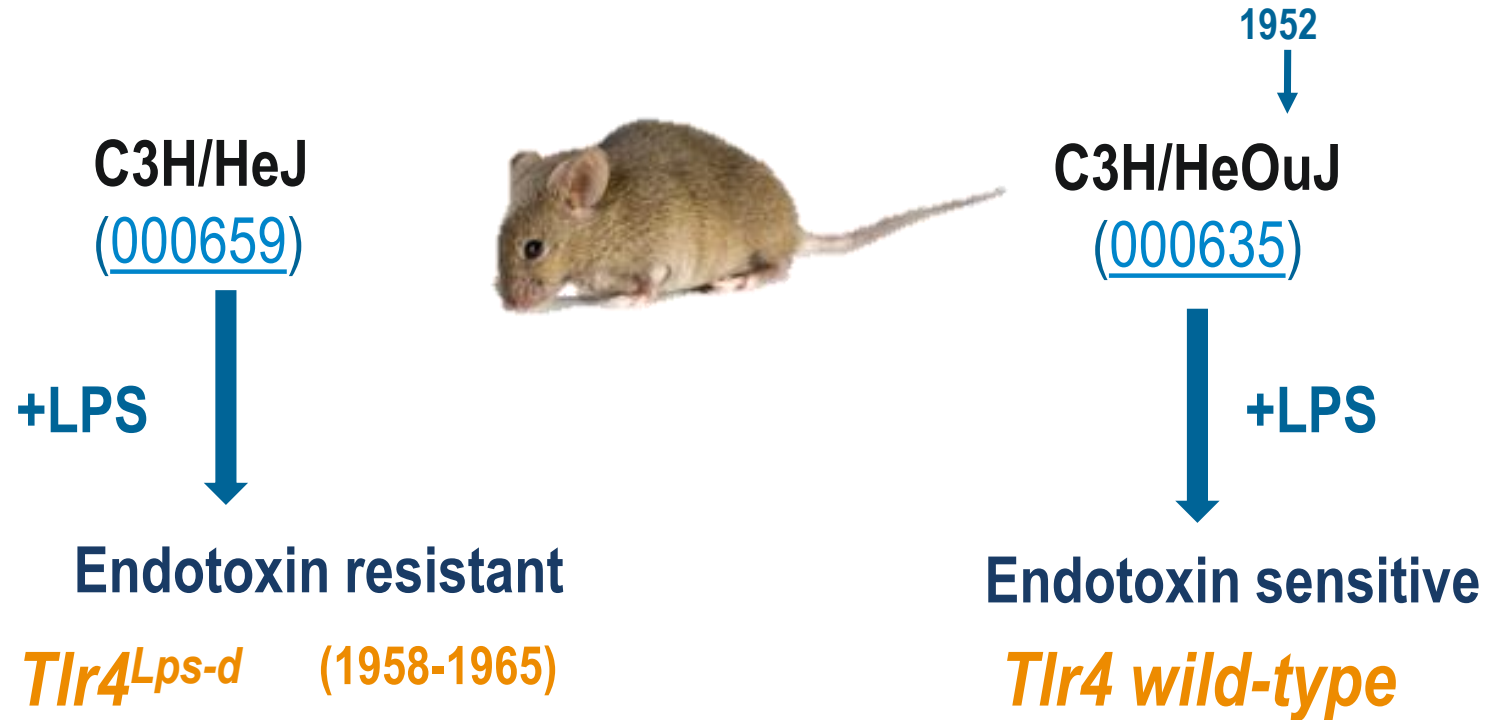
Parent strain

Substrain designations (cumulative)

Laboratory maintaining strain

LAB CODE	ORGANIZATION
CrI	Charles River Laboratories
Hsd	Envigo (formerly Harlan Laboratories)
J	The Jackson Laboratory
N	National Institutes of Health
Rj	Centre D'Elevage R. Janvier
Tac	Taconic Farms, Inc.

# Genetic Drift Contributes to Data Variability

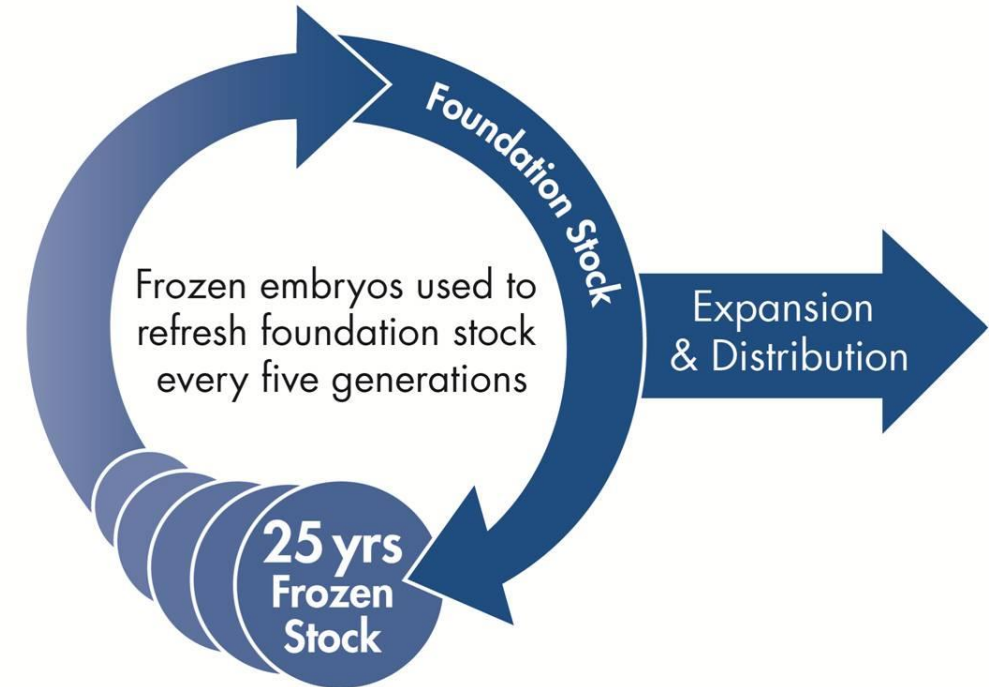


Sultzer BM. 1968. *Nature* PMID: [4877918](#)  
Watson J et al. 1978. *J Immunol* PMID: [202651](#)  
Poltarak A et al. 1998. *Blood Cells Mol Dis* PMID: [10087992](#)  
Poltarak A et al. 1998. *Science* PMID: [9851930](#)



# Avoid Genetic Drift Through Genetic Stability

- ***Know the history, genetic background, and substrain of your mouse***
  - Mouse repositories maintain genetic quality. Purchasing cohorts, new breeder pairs, or cryorecovering mice is a relatively small investment.
  - Ask collaborators for known genealogy
  - Verify genetic background with tools like Genome Scanning or GigaMUGA



US patents 7592501, 8110721

Lloyd 2015 Nature PMID: [26062496](https://pubmed.ncbi.nlm.nih.gov/26062496/)

<https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/why-jax-mice/patented-genetic-stability-program>

# JAX Genetic Quality Control Program

- Highly skilled animal caretakers
  - Intensive training program
  - Lab animal science and genetics courses
- Rigorous colony management protocols
- Systematic screens for variant genotypes and phenotypes
  - Quality control SNP and unwanted alleles genotyping panels
  - Allele-specific genotyping assays
  - Coat color changes, strain specific phenotype validation



<https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/why-jax-mice/genetic-quality-control-program>

# Use Colony Management Practices That Minimize Impact on Phenotype

## *Avoid accidentally selecting for mild or strong phenotypes*

- Maintain a defined breeding rotation and mating scheme
- Keep pedigrees and excellent records, phenotype may appear after breeding
- Refresh genetic background regularly
- [Cryopreserve](#) unique strains
- Purchase cohorts from a trusted vendor that minimizes genetic drift



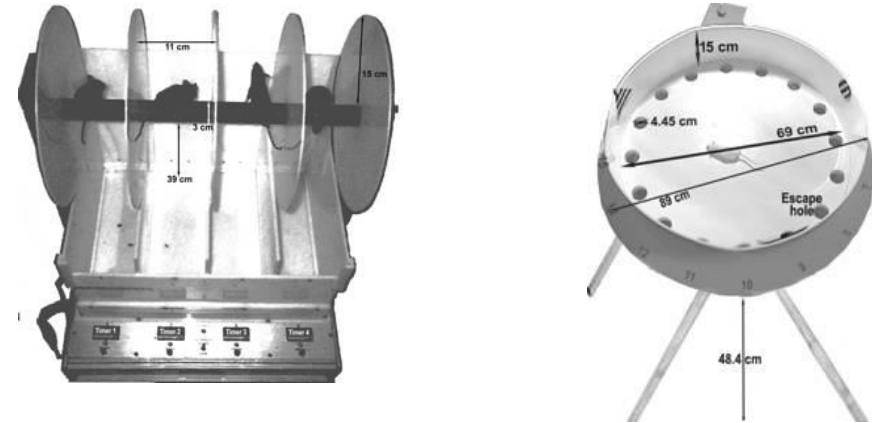
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# Assay Selection Depends on *In Vivo* Model

- Inherent strain characteristics may make them unsuitable test subjects
  - Hearing loss and startle responses (Zheng 1999 Hear Res PMID: [10320101](#))
- Repeated measures on test subjects may lose sensitivity, experience high variability
  - Locomotor assays
- **Select many complementary assays, adjust study design**



Images from: [SOURCE](#)

# Assay Selection May Depend on Human Relevance

- Assay may not fully model a process
  - *Select several complementary assays to test a hypothesis and understand limitations*
- A mouse assay may not match what is measured in patients
  - *Develop new assays which may better recapitulate clinical measures or give robust quantifiable data*

# Handling Stress May Affect Experimental Readout

## *Implement mindful handling practices*

- Plan for consistency in experimenter, keep notes
- Reduce handling to a minimum
- Remember that researchers and animal care staff have common goals: promoting scientific discovery and maintaining animal welfare



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# Sample Size Considerations

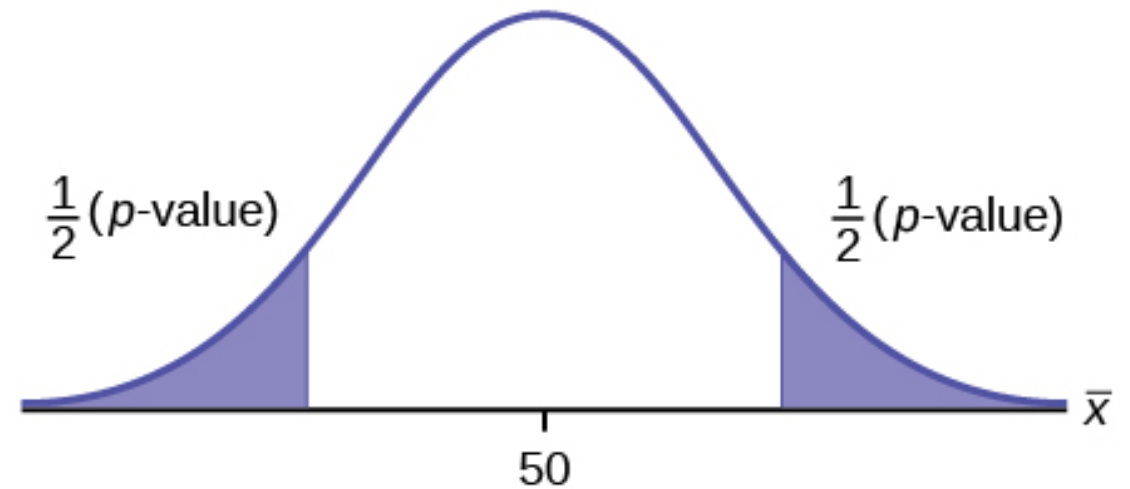
- Mice are biological “reagents”
  - Natural variation in phenotype onset
  - Observations *in vitro* may not match observations *in vivo*
- Human donor cells contribute variability
- Based on your assay, how many mice do you need to see a statistically *and* biologically significant difference?
  - Too few – experiment cannot provide reliable answers
  - Too many – waste of resources for minimal gain



[theguardian.com/science/2019/may/31/sexist-research-means-drugs-more-tailored-to-men-says-scientist](https://www.theguardian.com/science/2019/may/31/sexist-research-means-drugs-more-tailored-to-men-says-scientist)

# p-Value

- **Probability of measuring a false positive**
- /s there a difference between 2 groups?
- Does not provide indication of how large the difference is
  - A statistically significant  $p < 0.05$  can be obtained in a variety of ways
    - Small sample size with large effect size
    - Large sample size with small effect size
- Does not provide a probability that a finding can be reproduced

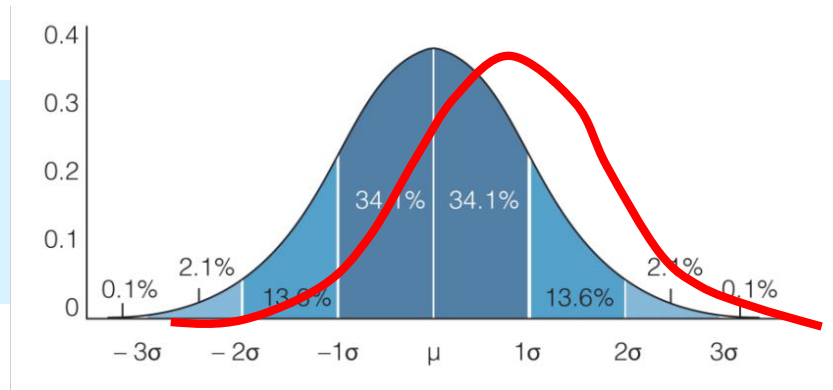


Images from: [SOURCE](#)  
Halsey 2015 Nat Meth PMID: [25719825](#)  
Sullivan 2012 J Grad Med Edu PMID: [23997866](#)

# Effect Size

- *How big* is the difference between 2 groups? *How well* does the intervention work?

$$\text{Effect size} = \frac{\text{Mean}_{\text{Expt}} - \text{Mean}_{\text{Ctrl}}}{\text{St Dev}}$$



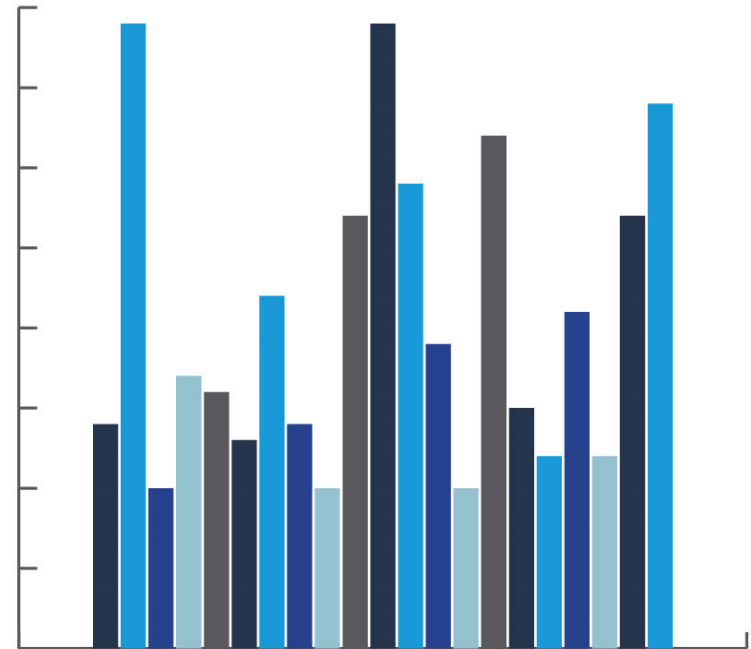
- “Acceptable” effect sizes vary by discipline
  - If effect size is 0.8, then the mean of the experimental group is **0.8 SD above the mean of the control group**.
  - When reported with a confidence interval, provides a probability that a finding can be reproduced

# Pilot Studies and Power Analysis

- Power - probability of finding a true difference between groups
  - “Acceptable” power is determined by discipline
- Shares a strong relationship with p-value, effect size, and sample size
- Pilot experiment
  - Start with a small n; choose an assay and collect data; calculate a p-value, effect size, and power.
  - If the pilot is underpowered, increase power by
    - Increasing sample size
    - Choosing new assays that show stronger effects

# Experiment Planning and Statistical Analysis

- Enlist the help of an experienced biostatistician when designing your experiments
- Perform pilot studies to calculate power and estimate appropriate sample size
- Randomize mice into study groups; blind the study, if possible
- Report p-value and effect size in statistical analyses



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# Reporting Your Research

## Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals

Carol Kilkenney , Nick Parsons, Ed Kadyszewski, Michael F. W. Festing, Innes C. Cuthill, Derek Fry, Jane Hutton, Douglas G. Altman

Published: November 30, 2009 • <https://doi.org/10.1371/journal.pone.0007824>

- Survey of 271 animal studies, 72 in mice
  - Key methods and results had omissions/uncertainty
    - Strain, sex, age, weight
    - Randomization, blinding
    - Sample size, statistical methods (identification of, appropriate usage of)

Kilkenny 2009 PLoS One PMID: [19956596](https://pubmed.ncbi.nlm.nih.gov/19956596/)

# Reporting Your Research

- Reproducibility relies on accurate and complete reporting of methods, results, statistical analysis
- NIH encourages greater scientific rigor and transparency in experimental details
- Reporting of *In Vivo* Experiments (ARRIVE) Guidelines as a tool to improve communication and reproducibility

[ARRIVE guidelines](#)

[NIH Rigor and Reproducibility](#)

<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html>



# How Can You Use the ARRIVE Guidelines?

## Title

1. Accurate & concise description

## Abstract

2. Background, objectives, methods, key findings and conclusions

## Introduction

3. Background
4. Objectives

## Methods

5. Ethical statement
6. Study design (blinding/randomisation)
7. Experimental procedures (How? When? Where? Why?)
8. Experimental animals (species, sex, weight)
9. Housing and husbandry
10. Sample size
11. Allocation experimental groups
12. Experimental outcomes
13. Statistical methods

## Results

14. Baseline Data
15. Numbers Analysed
16. Outcomes & estimation
17. Adverse events

## Discussion

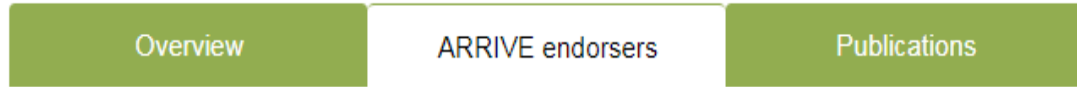
18. Interpretation & implications
19. Generalisability and translation
20. Funding

# The ARRIVE Essential 10: Author Checklist

- Study design
- Sample size
- Inclusion and exclusion criteria
- Randomization
- Blinding
- Outcome measures
- Statistical methods
- Experimental animals
- Experimental procedures
- Results

Percie du Sert N 2020 PLoS One PMID: [32663221](https://pubmed.ncbi.nlm.nih.gov/32663221/)

# ARRIVE: Animal Research Reporting In vivo Experiments



- Journals
- Funders
- Universities
- Learned societies
- Organisations

## Journals

Journals are invited to endorse the ARRIVE guidelines to improve the standard of reporting of animal research in the scientific literature. Journals can endorse the ARRIVE guidelines by incorporating the guidelines into their Instructions for Authors or Editorial Policies to ensure authors are aware of the guidelines when reporting animal research.

To improve the impact of the ARRIVE guidelines we encourage journals to actively implement the guidelines. To assist in this process, an [ARRIVE checklist](#) is available to download. The checklist should be completed by the author and can be used during the review process to ensure published research is of the highest standard.

- *Nano Communication Networks*
- *Nano Energy*
- *Nano Today*
- *NanoWorld Journal*
- *Nature*
- *Nature Biotechnology*
- *Nature Cell Biology*
- *Nature Chemical Biology*
- *Nature Chemistry*
- *Nature Communications*
- *Nature Genetics*
- *Nature Immunology*
- *Nature Materials*
- *Nature Medicine*
- *Nature Methods*
- *Nature Nanotechnology*
- *Nature Neuroscience*
- *Nature Protocols*
- *Nature Structural and Molecular Biology*
- *Neural Development*
- *Neurobiology of Disease*
- *Neurobiology of Learning and Memory*
- *Neurobiology of Pain*

Image From: [SOURCE](#)

## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a | Confirmed

- The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided  
*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*

### Bioethics policy

#### Studies involving animals and human research participants

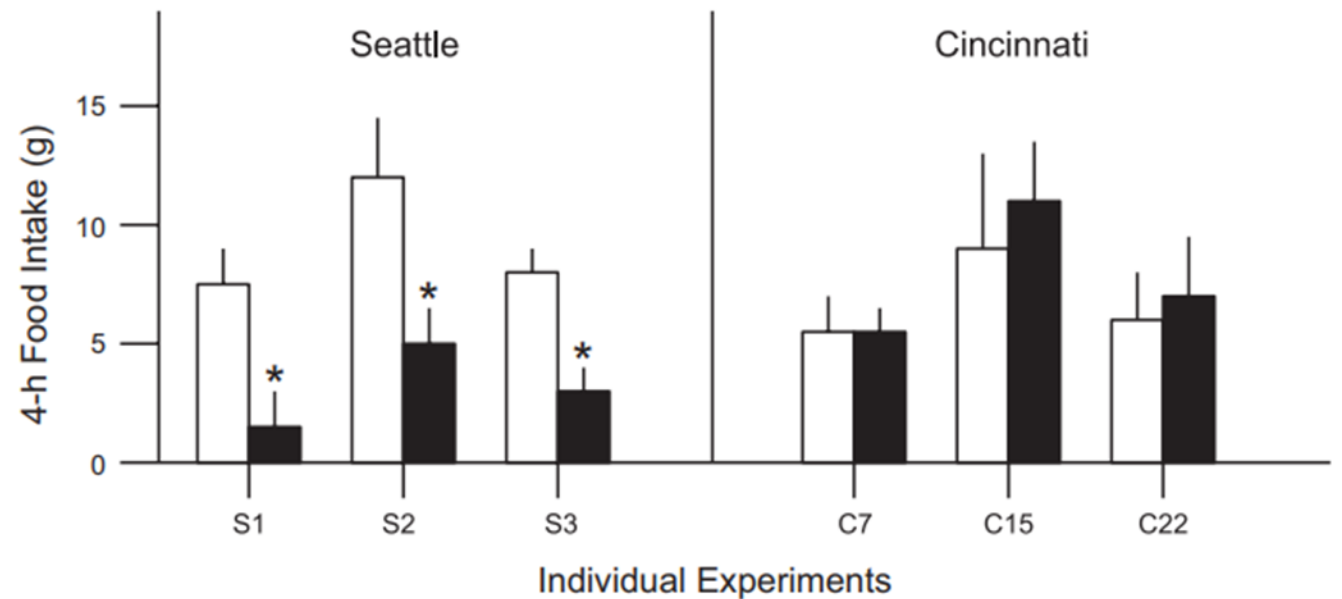
All authors of life sciences manuscripts complete an [editorial policy checklist](#) to verify their compliance with the Nature Research journals' editorial policies.

For primary research manuscripts in the Nature Research journals (Articles, Letters, Brief Communications, Technical Reports) reporting experiments on live vertebrates and/or higher invertebrates, the corresponding author must confirm that all experiments were performed in accordance with relevant guidelines and regulations. The manuscript must include a statement identifying the institutional and/or licensing committee approving the experiments, including any relevant details. Sex and other characteristics of animals that may influence results must be described. Details of housing and husbandry must be included where they are likely to influence experimental results. We recommend following the ARRIVE reporting guidelines when documenting animal studies ([PLoS Bio 8\(6\), e1000412, 2010](#)).

Image From: [Source](#)  
Image From: [Source](#)

# And What If You Still Can't Reproduce Experimental Results?

- Consider any other factors that might play a role in your findings
- Consider the possibility that the original findings are not reproducible
- Publish negative or alternate findings



Woods, S. C., & Langhans, W. 2012. Am J Physiol Endocrinol Metab PMID: [23074241](#)

# Summary

- Reproducible *in vivo* research studies depend on a thorough understanding and characterization of your selected mouse model(s)
- Biological factors, colony management practices, and experimenter behavior can influence consistency
- Well-powered and well-reported *in vivo* studies build strong foundations for future discoveries

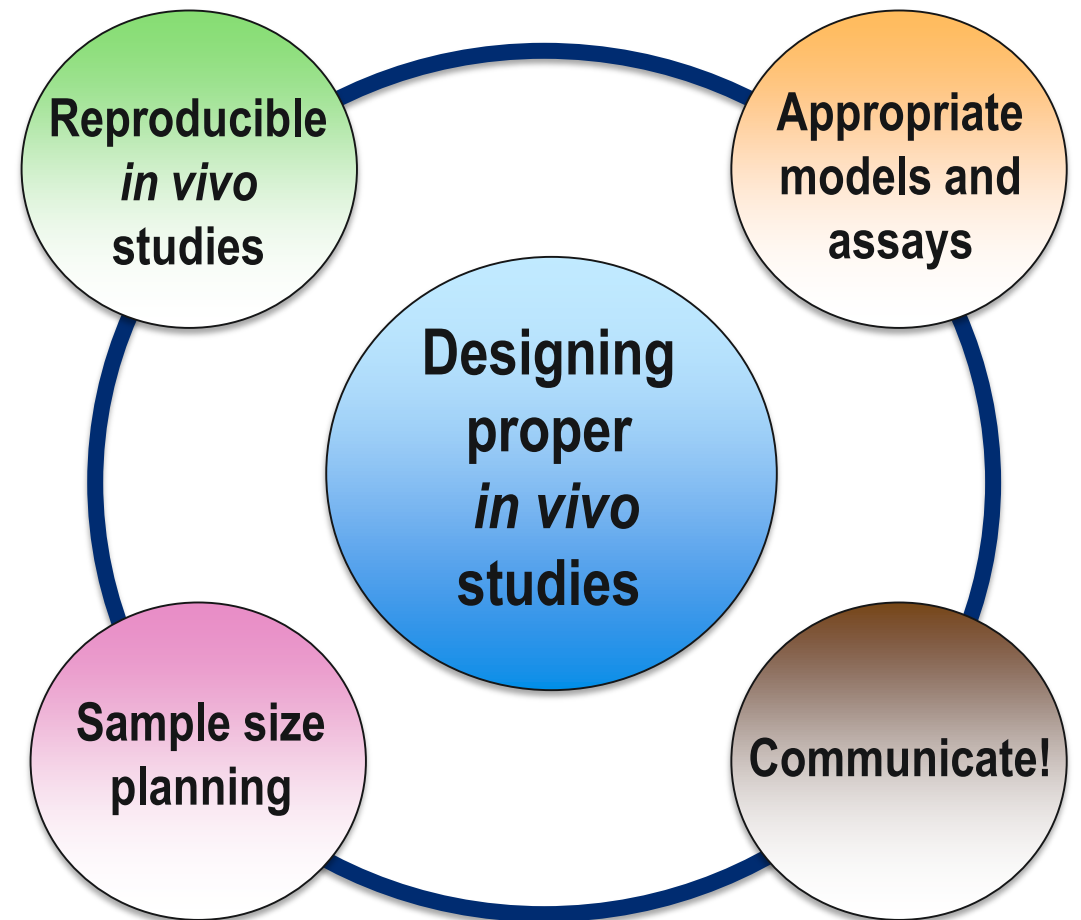


Image From: [SOURCE](#)

# JAX TECHNICAL INFORMATION SCIENTIST (TIS)

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1.207.288.5845 (International)



Be sure to check out our live and on-demand webinars.

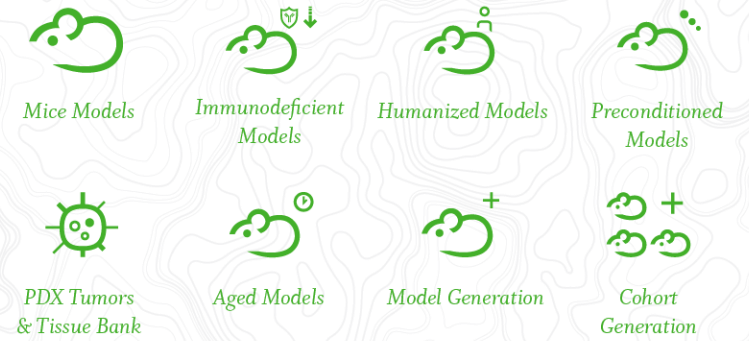


# JAX MICE AND SERVICES

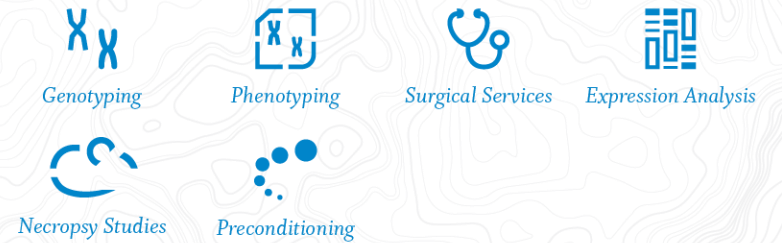
Precise models, powerful services, and experience experts



## MODEL ACCESS



## TARGET VALIDATION/ TAILORED CHARACTERIZATION



## PRECLINICAL SOLUTIONS





# Upcoming JAX Virtual Events

Subscribe to JAX Events announcements email list: <https://subscribe.jax.org/>

- JAX Tech Talk, Episode 43: Let's Talk Assessing Efficacy of GvHD Therapeutics
  - Sep 14, 2021, 1:00 pm USA Eastern Time (New York)
- Humanized Mice Journal Club: Human KIT+ Myeloid Cells Facilitate Visceral Metastasis by Melanomas
  - Sep 21, 2021, 10:30 am USA Eastern Time (New York) / 4:30 pm CEST (Berlin)
- Antibody Drug Development Summit, Fall 2021 Event, Humanized In Vivo Resources for the Preclinical Development Pipeline
  - Sep 22-23, 2021, 11:00 am USA Eastern Time (New York) / 5:00 pm CEST (Berlin)



# THANK YOU FOR THE ADVENTURE

At JAX, we enjoy the journey as much as reaching the destination, and we're so happy you joined us.

Have more questions?  
Want to discuss a project?



[micetech@jax.org](mailto:micetech@jax.org)



1.800.422.6423 (USA)

1.207.288.5845 (International)

