Optimized Colony Management Solutions with Respect to the 3Rs:

Breeding and Handling of Humanized Mice

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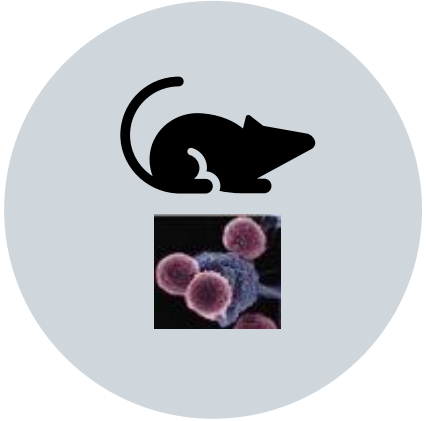
Humanized Mouse Models

Humanized rodents bridge some of the translational gaps across the species barrier

- Human tissue grafts
- Purified human immune cell populations
- Mixed human immune cells
- Human stem cells



Human cells/tissue **engrafted** into the mouse

Human genes **inserted** into the mouse genome

- Human transgenes
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- Full genomic replacements

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Breeding of Genetically Engineered Models (GEMs)

General Considerations for Breeding of GEMs

- The mouse genome can be modified using genetic engineering techniques to improve engraftment of human cells or tissues
- Humanized mouse models are genetically manipulated to integrate human genes into the animal genome
- The average number of pups produced by genetically engineered strains is determined by the genetic background

Genetic background	GEMs Litter average at birth	GEMs Litter average at weaning	Taconic inbred strains Litter average at birth
C57BL/6 congenic	6.7 ± 2.0	6.0 ± 2.1	6-8 pups/♀
BALB congenic	6.3 ± 0.9	5.9 ± 0.9	5-7 pups/♀
FVB congenic	9.7 ± 1.9	9.1 ± 1.9	8-10 pups/♀
129 Congenic	6.9 ± 3.2	6.4 ± 3.4	6-8 pups/♀
NTac:SD	10.5 ± 1.3	10.1 ± 1.2	10-12 pups/♀
STOCK (mixed)	7.3 ± 2.3	6.7 ± 2.2	

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The importance of proper upfront planning

Well-defined cohort goals are crucial for designing optimized breeding plans

- Questions to be asked:
  - Is an adverse phenotype expected?
  - Can you use study animals of both sexes?
  - Which age-range should the study animals have?
  - Do you need wildtype littermate controls?
  - How frequently do you need cohorts?

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Breeding Strategies

Definition of terms

Timed-Pregnant Setup:

Breeding time: ~24-72 hours.

Goal: Produce pups at specific embryonic/postnatal age

Peak Breeding:

Breeding time: 1-3 weeks.

Goal: Produce 1 drop of litters with limited age-range.

Synchronous Breeding:

Breeding time: 3-9 weeks.

Goal: Produce 1-3 drops of litters.

Continuous Breeding:

Breeding time: Unlimited

Goal: Continuous production of offspring.

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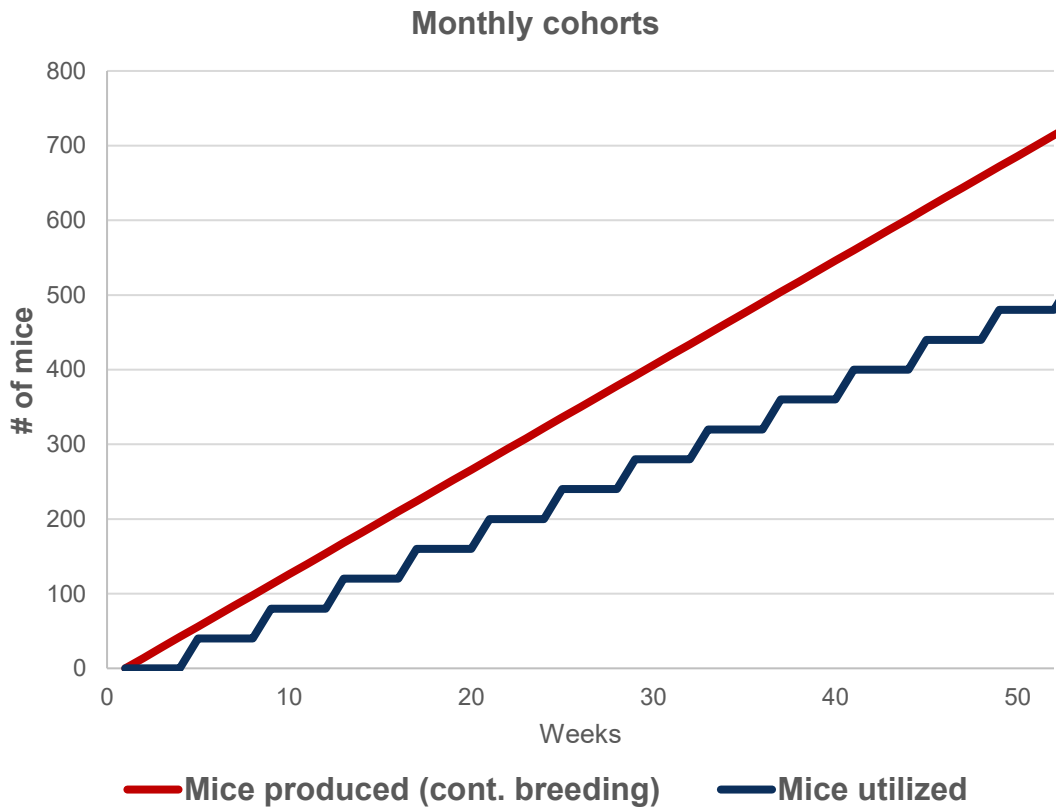
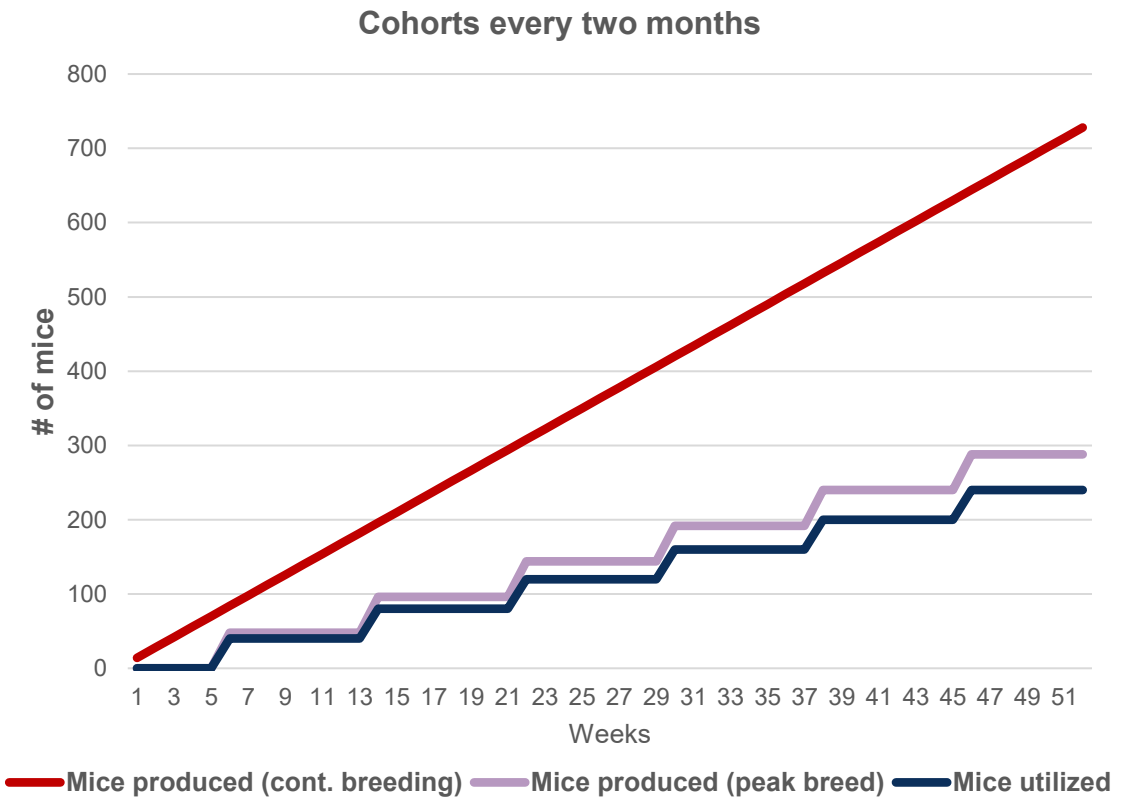
The importance of proper upfront planning

How frequently do you need cohorts?





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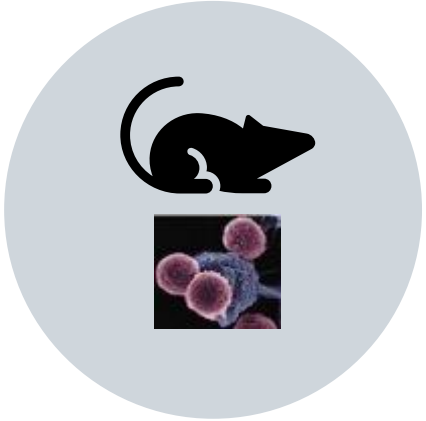

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Humanized Mice Generated by RITg

Considerations for Breeding Models Generated by Random Integration Transgenics (RITg)

- Human genes are randomly inserted into the mouse genome
- Risk of deleterious mutations at integration site
- Breeding to homozygosity not recommended unless transgene insertion has been mapped
- Each founder animal is unique and mosaic
  - Requires extensive transgene expression analysis to identify most suitable founder line
  - Recommended testing for:
    - Partial integration
    - Germline transmission
    - Copy number analysis
    - Transgene mapping
  - Recommended to establish colony from a single F1 carrier
- Cryopreservation recommended at an early generation number as transgenic expression may be epigenetically silenced over time

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Humanized Mice Generated by Gene Targeting in ESCs

Considerations for Breeding Models Generated by Gene Targeting in Embryonic Stem Cells

- Commonly used to replace a mouse gene with a human gene
- Human proteins may not compensate for loss of mouse protein → functional knock-out
- Founder animals are chimeric
  - Assess germline transmission
- Assess production performance as the breeding colony is being established
- Heterozygous (HET) x HET breeding
  - Litter size
  - Genotype split
- Homozygous (HOM) x HOM breeding
  - Litter size
  - Burden assessment
- Cryopreservation recommended at an early generation number

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Humanized Mice Generated by CRISPR/Cas9 Gene Editing

Considerations for Breeding Models Generated by Gene Editing in Zygotes using CRISPR/Cas9

- Commonly used to introduce small and less-complex mutation in the mouse genome
- Risk of introducing unwanted mutations
- Founder animals may carry more than one modified allele at the targeted site → recommended to sequence F1 HET offspring at the gene of interest
- Establishing breeding colony from a single F1 HET animal reduces risk of propagating mosaicism and fixation of off-target mutations
- Thorough on- and off-target characterization reduces risk if establishing breeding colony from multiple F1 animals
- Cryopreservation recommended at an early generation number

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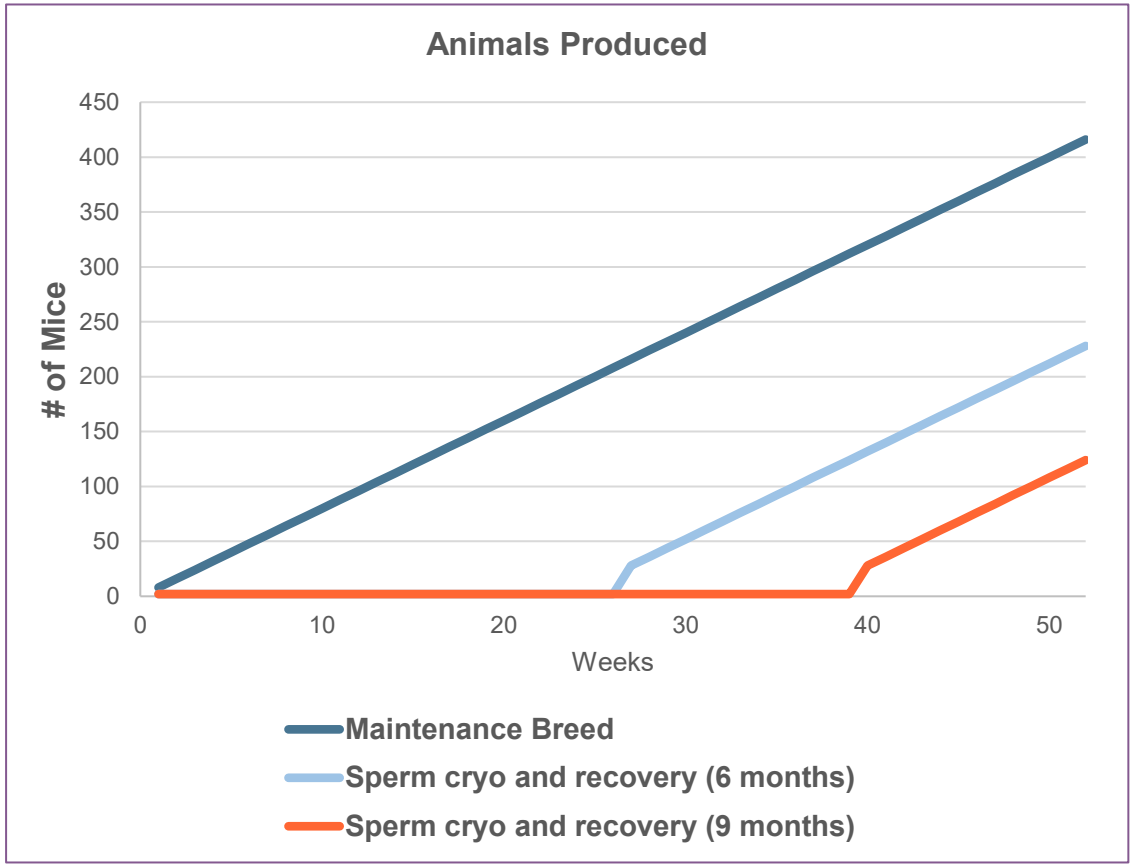
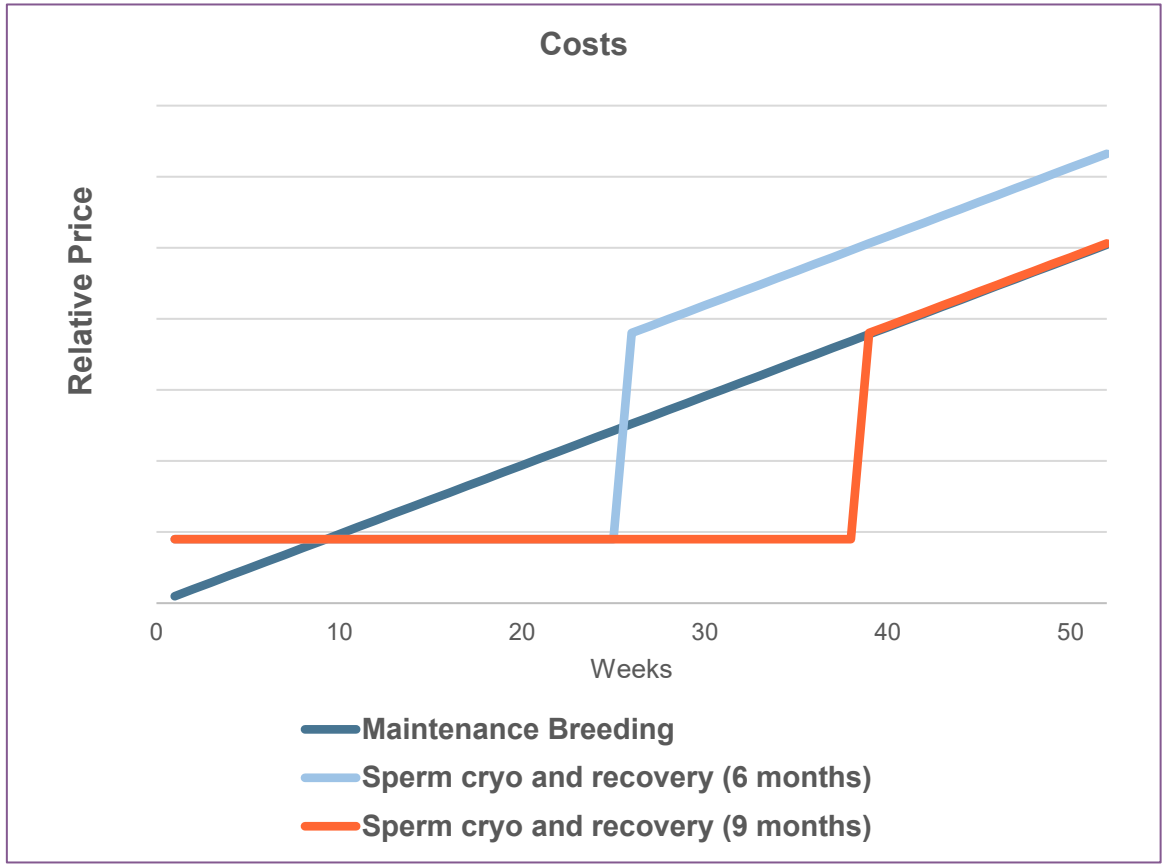
Cryopreservation

A tool to reduce animal wastage, costs, genetic drift, and risk of catastrophic loss





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Breeding and Handling of Immunodeficient and Humanized Immune System Mice

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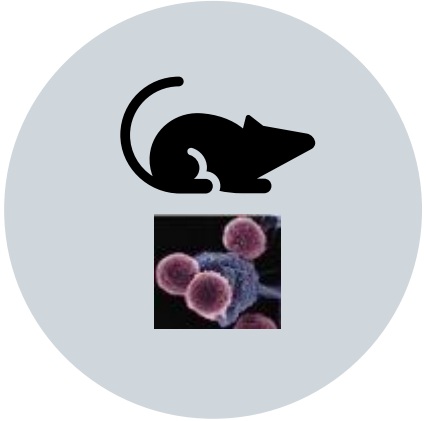

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Breeding of Immunodeficient Mice

Special Considerations for NOG Breeding

- Mouse models in the NOG portfolio are genetically modified
- Minimize handling due to stress sensitivity
- Maintain at Opportunist-Free (OF) health standard, autoclaved food/water, weekly bedding change
- Monitor for poor nursing or cannibalism; replace breeders as needed
- Pre-wean mortality: <5%
- Adverse Phenotype classified as “Not Harmful”

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Breeding of Immunodeficient Mice

Example: Breeding of FcResolv® NOG-EXL

- Genetically complex model
- Nomenclature: NOD.Cg-*Fcgr2b*^{tm1Ttk} *Fcer1g*^{tm1Rav} *Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac
- Available genotypes: ko/ko;ko/ko;sp/sp;ko/(ko/y);tg/wt
- Breeding formats:
  - ko/ko;ko/ko;sp/sp;ko/ko;**wt/wt** ♀ x ko/ko;ko/ko;sp/sp;ko/y;**tg/wt** ♂
  - ko/ko;ko/ko;sp/sp;ko/ko;**tg/wt** ♀ x ko/ko;ko/ko;sp/sp;ko/y;**wt/wt** ♂

Genetics

Summary

Species: Mouse
Strain Type: Congenic
Allele Type: Constitutive Knockout,Spontaneous Mutant,Random Transgenic,Genetically Humanized
Coat Color: Albino
Genetic Background: NOD

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Handling of Immunodeficient and Humanized Immune System Mice

Special Considerations for NOG and HIS Handling

- Generally non-aggressive and can be group housed
- Need a long acclimatization period due to stress sensitivity
  - ≥7 days for NOG mice
  - ≥10 days HIS mice
- Acclimation requires attention to diet and hydration
- Require high-level biosecurity
- Plan for reasonable overage as attrition may occur
- Taconic offers free consultation prior to shipping first HIS order

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Appearance of Humanized Immune System Mice

- Mild scruffy coat appearance, slightly hunched, and pale
- Animals should still appear bright, alert, and active
- Body Conditioning Score on the lower end of BCS 3 (2+)
- Slender appearance with no excessive adipose storage and score of 2+ is considered healthy
- If animals appear slow, lethargic, and severely hunched and/or pale, veterinary staff should be contacted to evaluate the animals

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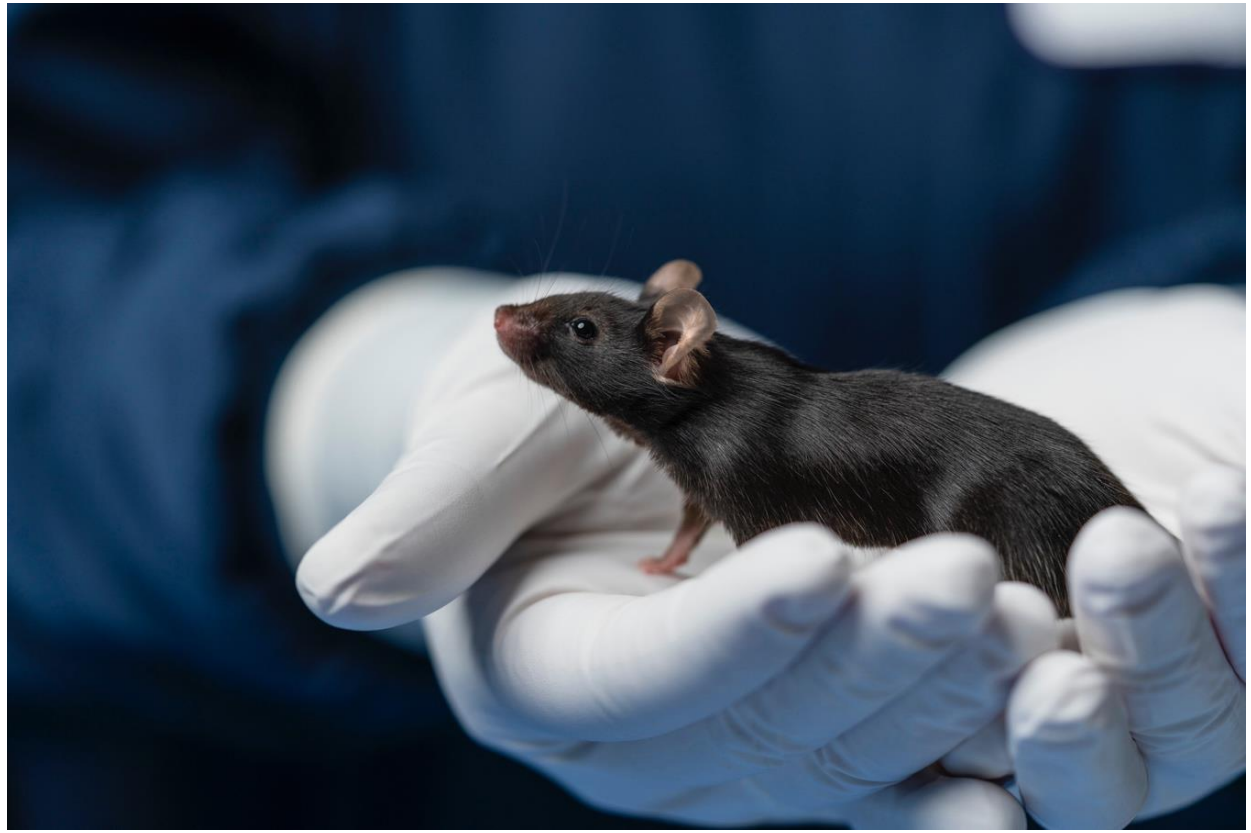
Breeding and Handling of Humanized Mice

Summary and Closing Remarks



- Breeding of any mouse model requires careful planning
- The method used to generate a genetically engineered model is important for downstream breeding
- Minimize handling and ensure high biosecurity when breeding of immunodeficient mouse models
- Humanized immune system models are sensitive and require special attention

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Thank You!