TACONIC
Models For Life.™

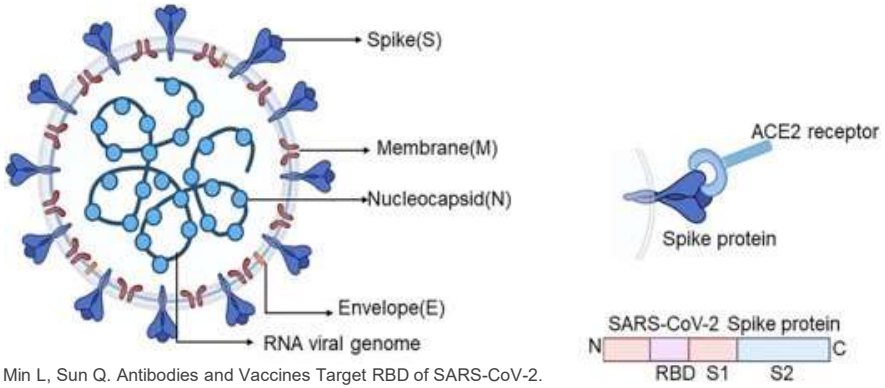
CRISPR/Cas9 as ONE tool to improve animal models

Mark Johnson, PhD
Associate Director, Product Development
Scientific Services
Taconic Biosciences

January 9, 2024

Applications for Genetically Engineered Animal Models in Infectious Disease and Vaccine Research

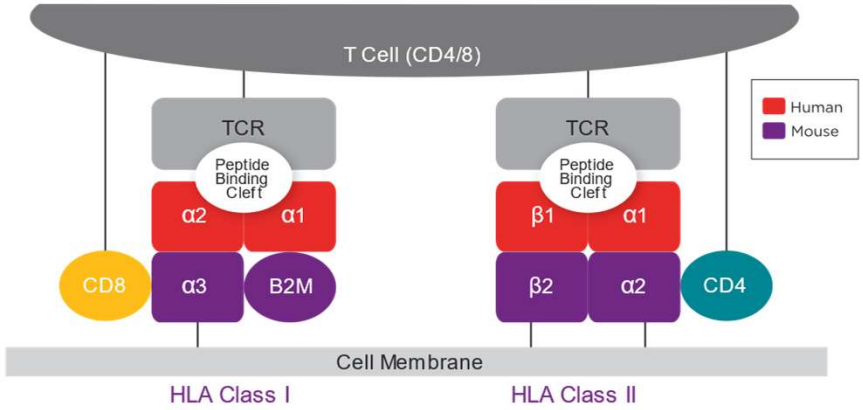
Conferring Infectivity / Susceptibility



Min L, Sun Q. Antibodies and Vaccines Target RBD of SARS-CoV-2. Front Mol Biosci. 2021 Apr 22;8:671633. doi: 10.3389/fmolb.2021.671633. Licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

- Humanization of surface proteins necessary for viral entry
- Introduction of human genetic elements that facilitate or enhance infection by pathogens
- Models used for infection studies and measuring efficacy of vaccine therapeutics

Humanized Antigen Presentation

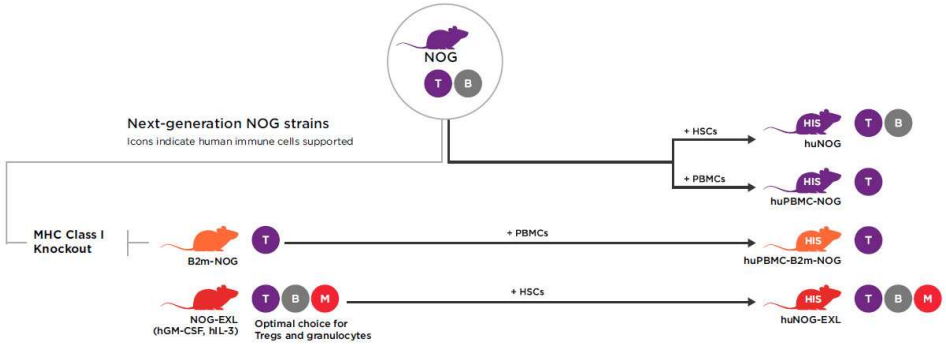


- Mice expressing human MHCs (HLAs)
- Transgenes confer human-like presentation of antigens
- Mouse/Human chimeric HLAs facilitate T-cell responses
- Widely used for vaccine development

TACONIC
Models For Life.™

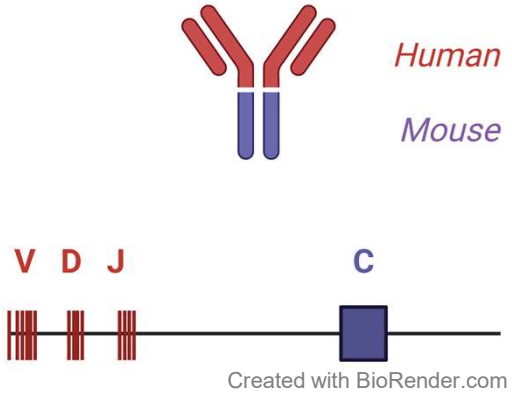
Applications for Genetically Engineered Animal Models in Infectious Disease and Vaccine Research

Humanized Immune System Platforms



- Genetic inactivation of mouse immune system
- Humanization of cytokines and immune factors supporting development of specific hematopoietic lineages
- Engraftment of human immune progenitor cells
- Examples: NSG mice from Jackson Labs, NOG mice from Taconic

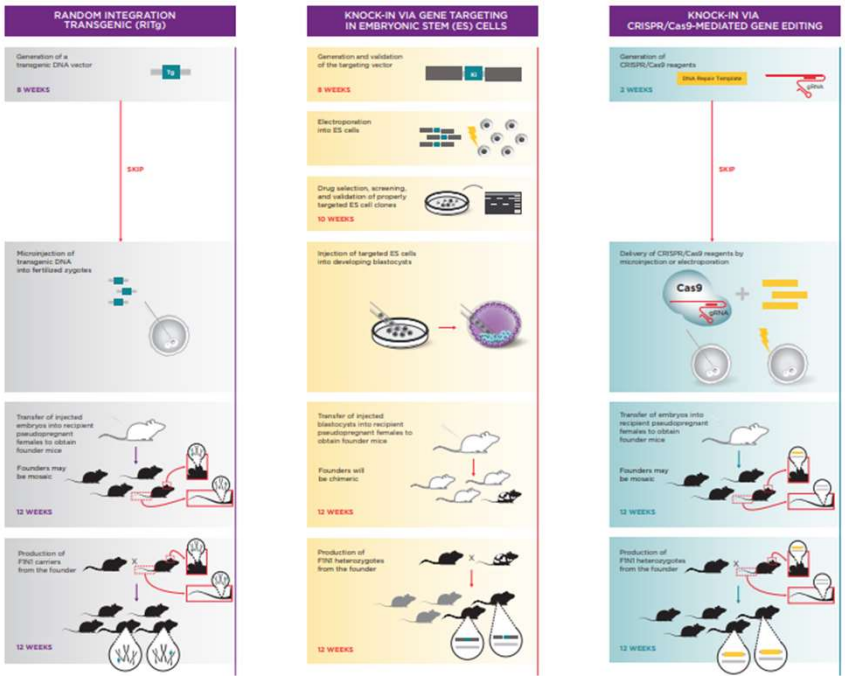
Humanized Antibody Platforms



- Introduction of engineered transgenes encoding human immunoglobulin repertoires, V(D)J and/or C segments.
- Some platforms retain mouse constant domains and generate human/mouse chimeric antibodies
- Examples: Trianni Mouse, OmniMouse, AlivaMab Mouse

TACONIC
Models For Life.™

Genome Engineering Technologies for Model Generation in Rodents



	Random Integration Transgenesis	Gene Targeting in ES Cells	CRISPR/Cas9 Gene Editing
Targeting Method	Zygote Pronuclear Injection	ES Cell Electroporation	Zygote Pronuclear Injection or Electroporation
Type of Integration	Random	Targeted	Targeted
Species	Any[1]	Mouse	Any[1, 2]
Mouse Genetic Backgrounds	Any[1]	Only for strains with established ES cells	Any[1, 2]
Level of Precision	Low	High	Medium, Increasing
Maximum Size of Insertion	≤ 250 kb (BAC)	≤ 180 kb	≤ 5 kb, Increasing
Model Generation Time	≥ 9 months	≥ 12 months	≥ 6 months
Time to First Cohort	≥ 15 months	≥ 18 months	≥ 12 months

[1] any from which zygotes can be harvested and manipulated
[2] any where genome sequencing data is available

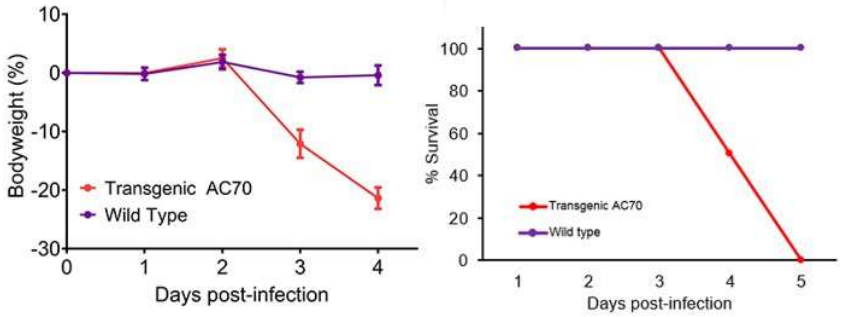
TACONIC
Models For Life.™

Example of CRISPR/Cas9 Advantages for Model Development

Precision, Speed, Accessibility

Random Integration Transgenic Humanized ACE2 Model

Expressing full length human *ACE2* driven by exogenous promoter



- Infection causes complete lethality
- Unknown location of transgene
- Unknown transgene copy number
- Unpredictable tissue distribution
- Relied on chance of a suitable allele
- Required backcrossing

CRISPR/Cas9 Point-Mutation Humanized ACE2 Model

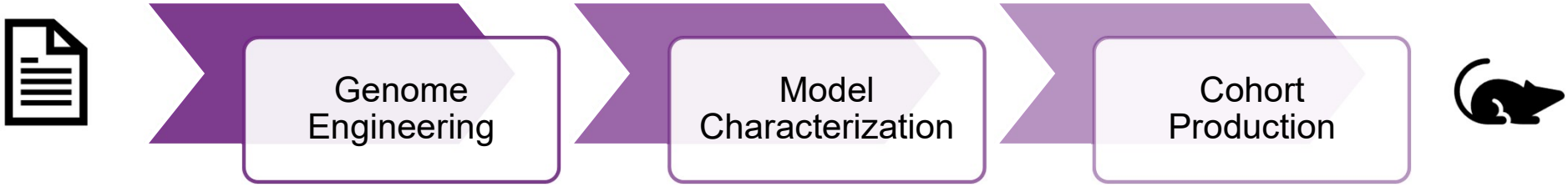
Expressing mouse *Ace2* from endogenous locus with humanization of only amino acid H353

- H353K confers SARS-CoV-2 infectivity in mice
- Receptor stoichiometry is maintained
- Receptor retains endogenous signaling
- Receptor is expressed where expected
- No backcrossing necessary
- Precision editing, but 6 months faster than ES cell approach
- Translatable to other strains and species

TACONIC
Models For Life.™

Preparedness: Tactics for Rapid & Efficient Model Development

Create generalized work plans for model development suited for specific classes of pathogens



Apply state-of-the art technologies and industry tricks

TACONIC
Models For Life.™

Preparedness: Community-Driven Model Repositories

Reproductive Lifecycle is FIXED. Even with CRISPR/Cas9, the shortest amount of time it takes to generate a *de novo* mouse model and obtain a first experimental cohort is still **~ 1 YEAR**

Anticipate need →

Generate animal models **NOW** and **cryobank** genetic lines

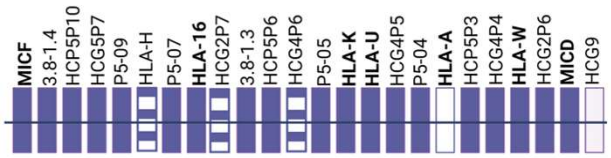
Public Health Organizations & Disease Foundations

Model Generation Cores & Commercial Model Providers

Cryopreserved Models

Expand Repertoire of HLA-expressing Models

- Create transgenic models for the remaining human HLAs
- Map and characterize HLA transgenes and/or
- Create knock-in versions targeted to safe-harbor loci
- Create engineered transgene(s) encoding full set of HLA



An HLA gene region of Human Chr. 6
Created with BioRender.com

Create Model Collections for Pathogen Classes

- Humanize common receptors for viral classes
- Introduce susceptibility factors for classes of pathogens or related to human populations
- Create new models on genetic backgrounds permissive to studying infectious diseases

Clear the path for rapid recovery and distribution

- Create cryorecovery and colony expansion plans
- Anticipate and clear regulatory hurdles to international shipment and global distribution
- Design legal terms to be permissive to sharing with research community

TACONIC
Models For Life.™

TACONIC
Models For Life.™

Thank you for your attention.

Mark Johnson, PhD
Mark.Johnson@taconic.com