Optimize *In Vivo* Research with the Right Humanized Mouse Models

Agenda

Talk #1: Introduction to Humanized Immune System (HIS) Mouse Models

Speaker:
Ditte Olsen, PhD
Scientific Solutions Consultant
Taconic Biosciences

Talk #2: Humanized Mice Modeling Services

Speaker:
Caroline Mignard, PhD
Senior Study Director
Oncodesign Services

Talk #3: Methods to Generate a Humanized Mouse

Speaker:
Ditte Olsen, PhD
Scientific Solutions Consultant
Taconic Biosciences

Talk #4: Breeding and Handling of Humanized Mice

Speaker:
Julie Torvund-Jensen, PhD
Associate Director
Taconic Biosciences

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Existing Tools for Customized Model Generation Solutions

Pros & Cons About the Different Methodologies

Methods to Generate a Humanized Mouse

Dr. Ditte Olsen
Scientific Solutions Consultant

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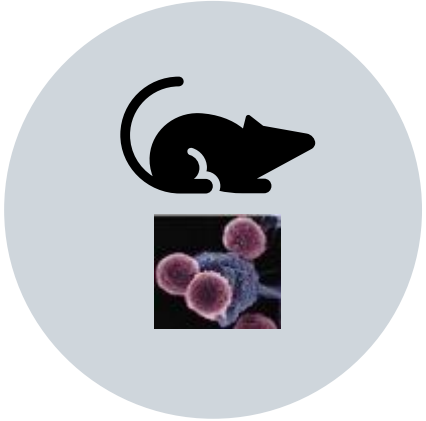
Humanized Mouse Models

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**
- **Human minigene knock-ins**
- **Partial human gene knock-ins**
- **Full genomic replacements**

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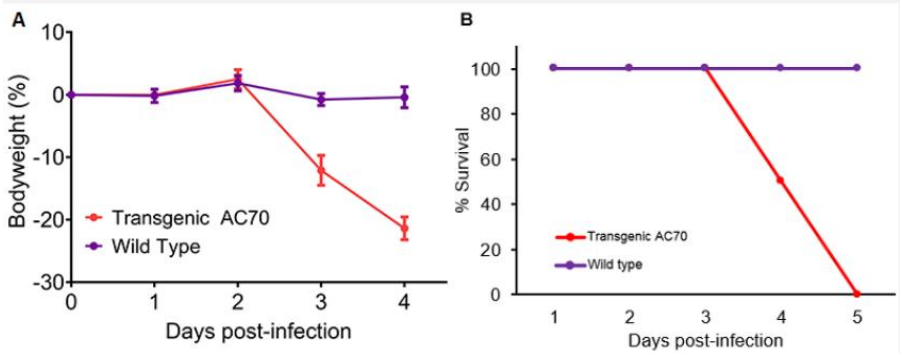
Applications of Humanized Mouse Models

- In vivo drug efficacy testing by expressing the human target in the mouse
- ADMET (absorption, distribution, metabolism, excretion, toxicology) by accurately modeling drug metabolism
- In vivo testing of complex therapeutic approaches (i.e. in vivo genome editing, enzymatic complementation, etc.) by mimicking human diseases
- Target discovery and validation by modeling human physiology and pathology
- Study of infectious disease by humanizing specific pathogen receptor



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Personal communication, Kent Tseng, UTMB

Considerations for Genetic Humanization

Human gene

- Size
- Isoforms

Murine orthologue

- Present or knock out
- Potential phenotype due to loss of function

Strategy

- Full or partial humanization
- cDNA or DNA
- Regulatory elements
- Strain

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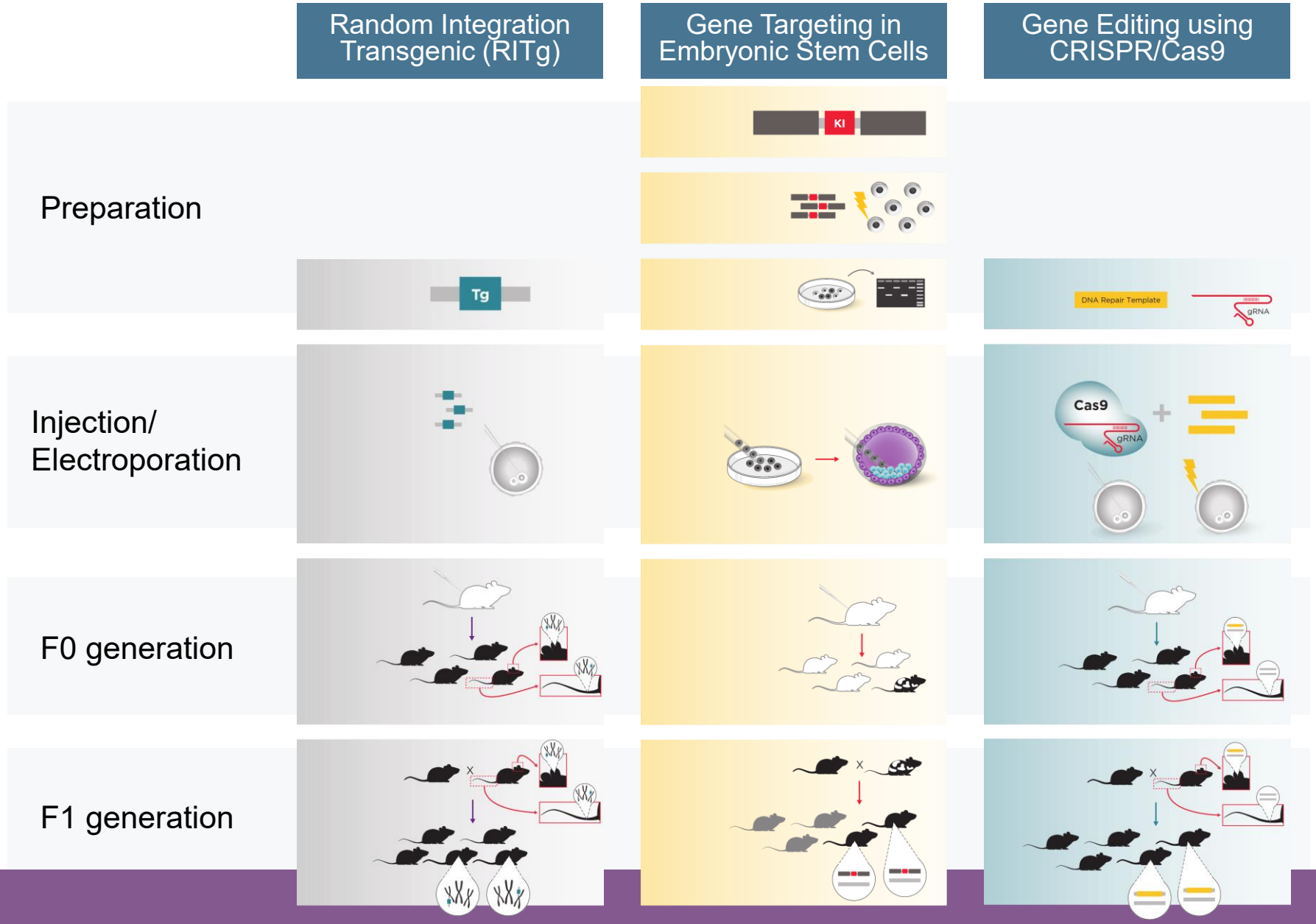
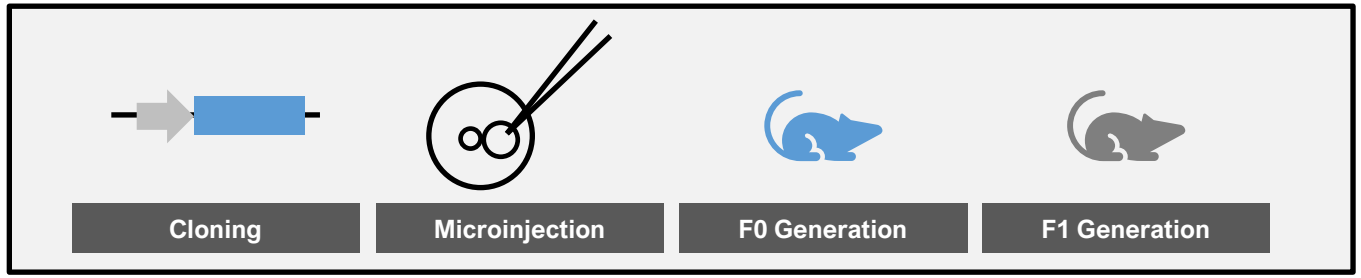
Three Major Methods for Modifying the Genome



Random Integration Transgenics (RITg)

Random Integration Transgenic (RITg)

Model Generation Process



Transgene Injection (PNI)

- Plasmid based cloning
- BAC based cloning
- YAC based cloning

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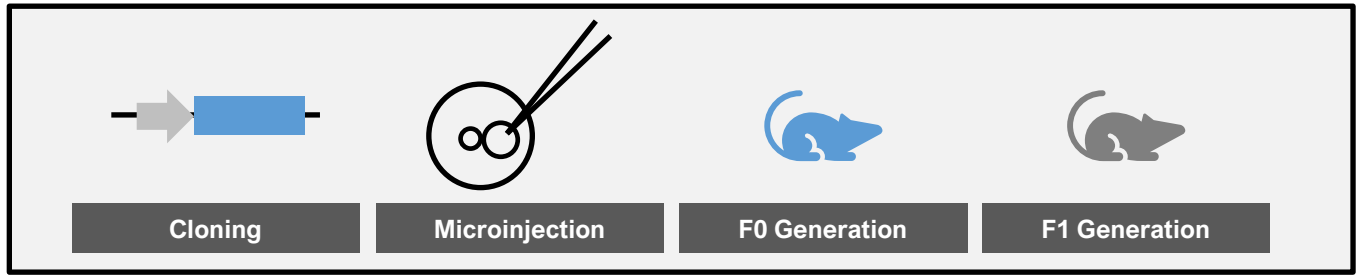
Model Generation Process



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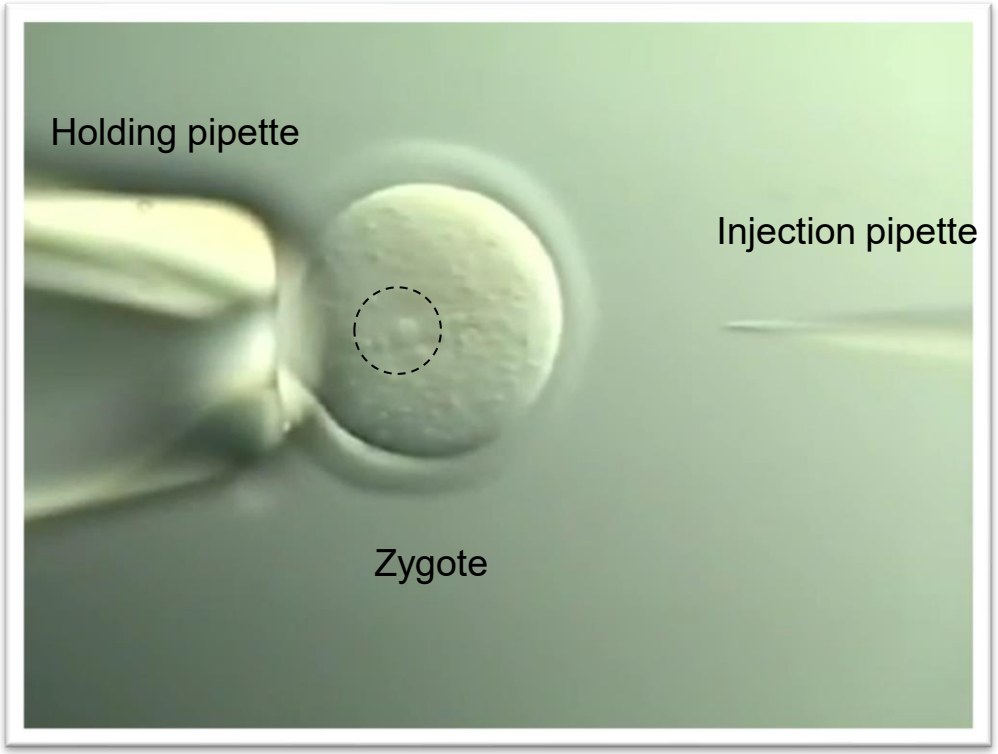

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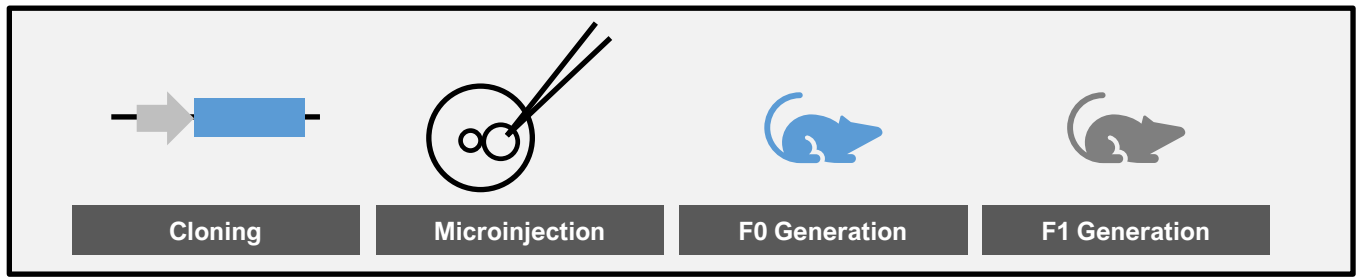

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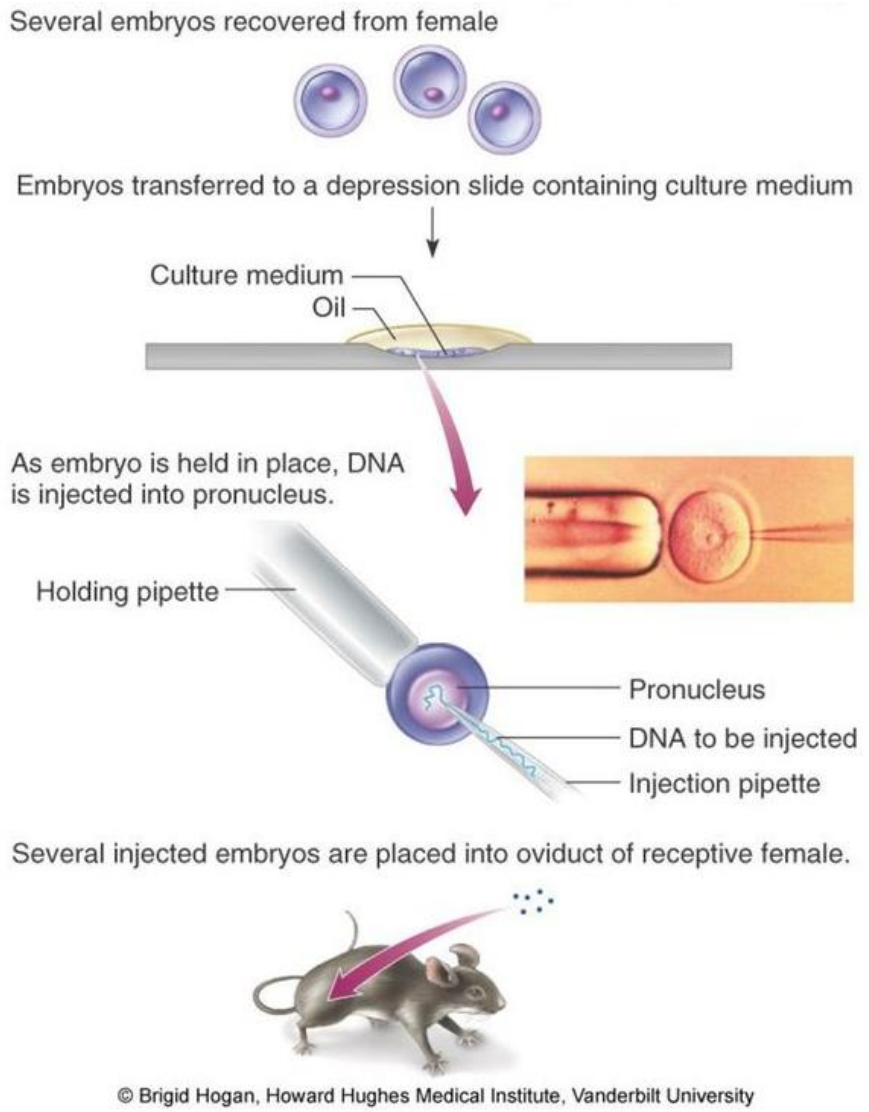

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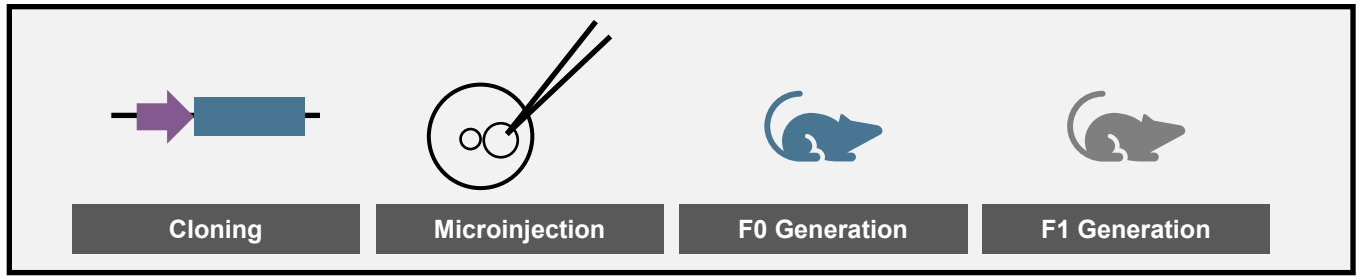

Suitable for:

- Overexpression Studies
- Multiple Copy Integration Desired
- Reporter or Marker Genes

Major Advantages

- Wide range of transgene designs possible (e.g., promoter choice, tags or reporters)
- Feasible for both mouse and rat models with a wide range of genetic background
- Relatively fast production of founder (F0) animals
- Dynamic range in transgene expression (e.g. F0s with varied expression patterns or levels)

Major Disadvantages

- Integration(s) can cause deleterious mutations
- BACs: Passenger genes may provide complication
- Each integration will vary in location, copy number, transgene structure, and expression profile
- Founder animals are likely to be mosaic

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hTNFα

Random transgenic integration model for rheumatoid arthritis studies

- Tumor necrosis factor α (TNFα) is a cytokine involved in inflammation and immune responses
- Implicated in the pathogenesis of human rheumatoid arthritis
- C57BL/6 mouse expressing the human TNF-α transgene,
  - Generated by random integration
    - Expressing constitutive levels of hTNFa, which activates mouse TNFR1 but not TNFR2
    - Human TNFα is not significantly inducible with LSP, while mouse TNFα is.

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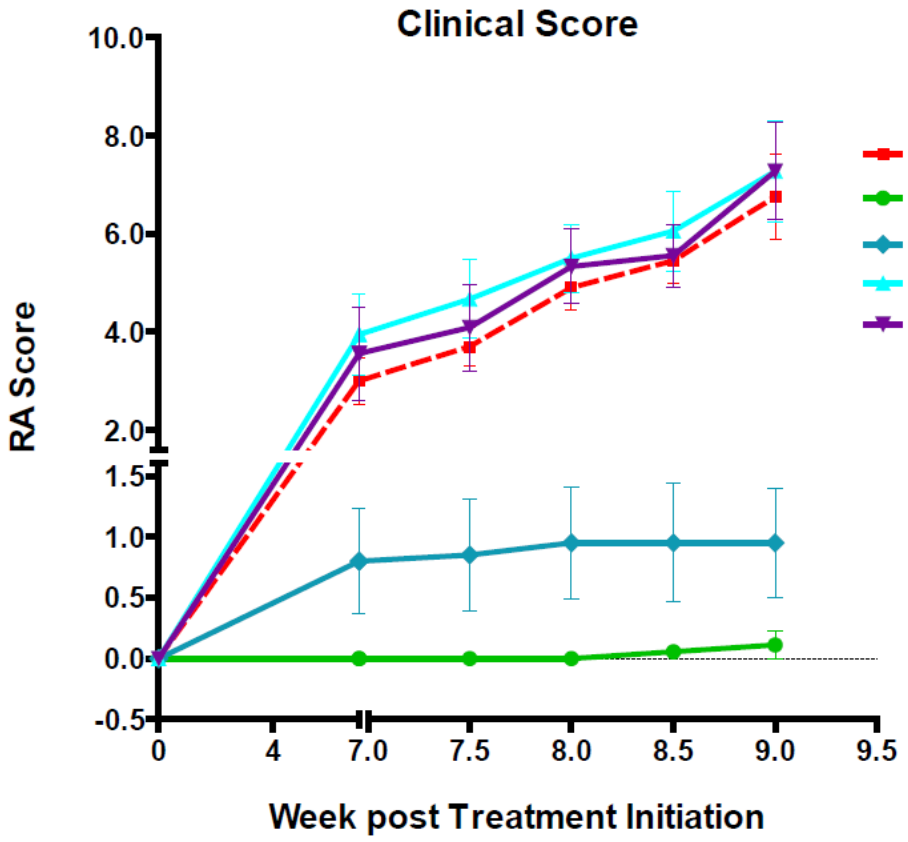
hTNFα

Random transgenic integration model for rheumatoid arthritis studies



Arthritis Clinical Scores
Two-Way ANOVA

Time	P<0.0001
Time/Treatment	P<0.0001
Treatment	P<0.0001

Post-hoc LSD Test

G1 vs G2	P<0.0001
G1 vs G3	P<0.0001
G1 vs G4	P=0.559
G1 vs G5	P=0.709

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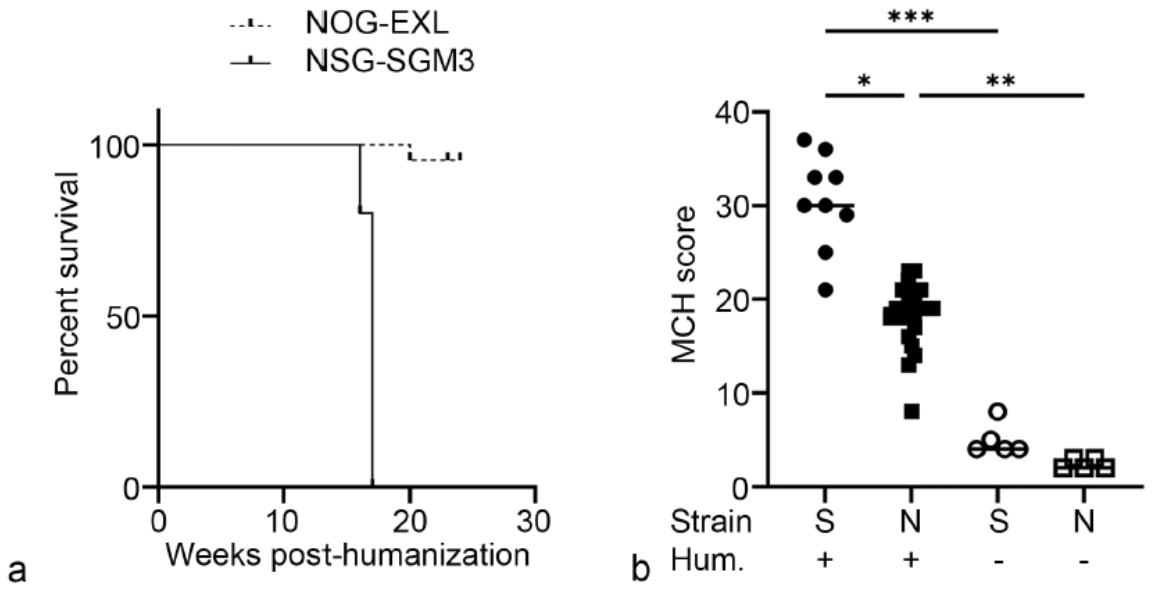
Graph from Taconic's webinar by Heildelberg Pharma:
Human TNF-α Transgenic Mouse Model of Spontaneous Arthritis

NOG-EXL Expressing Human Cytokines

Random transgenic integration causes different expression levels which may cause big differences between models

- NOG-EXL: NOG: NOD.Cg*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(SV40/HTLV-IL3,CSF2)10-7Jic/JicTac
  - Genetically humanized using random transgenic integration of genes encoding human GM-CSF and human IL-3 to support myeloid cell populations
- NSG-SGM3: NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV IL3,CSF2, KITLG)1Eav/MloySzJ



	NSG-SGM3 (16 WPE)	NOG-EXL (22 WPE)
Histiocytosis with activated macrophages	+++	++
Hemophagocytosis	++	++
Anemia	+++	+++
Mast cell hyperplasia	+++	-
Increased eosinophilopoiesis	++	+
Meningeal involvement	+	-
Skin involvement	+	-
Leukocytosis	+	-
Longer survival	-	+++

Gene Targeting in Embryonic Stem Cells (ESC)

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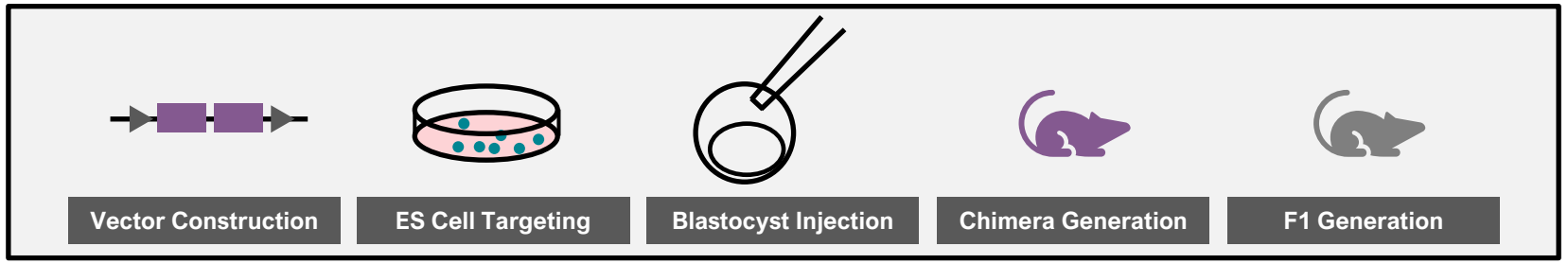
Model Generation Process



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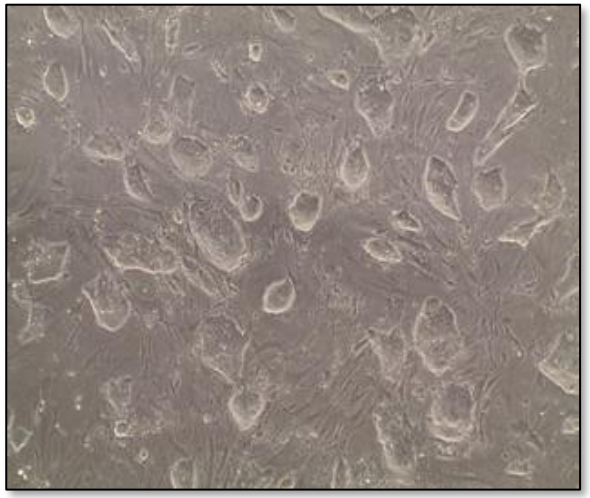
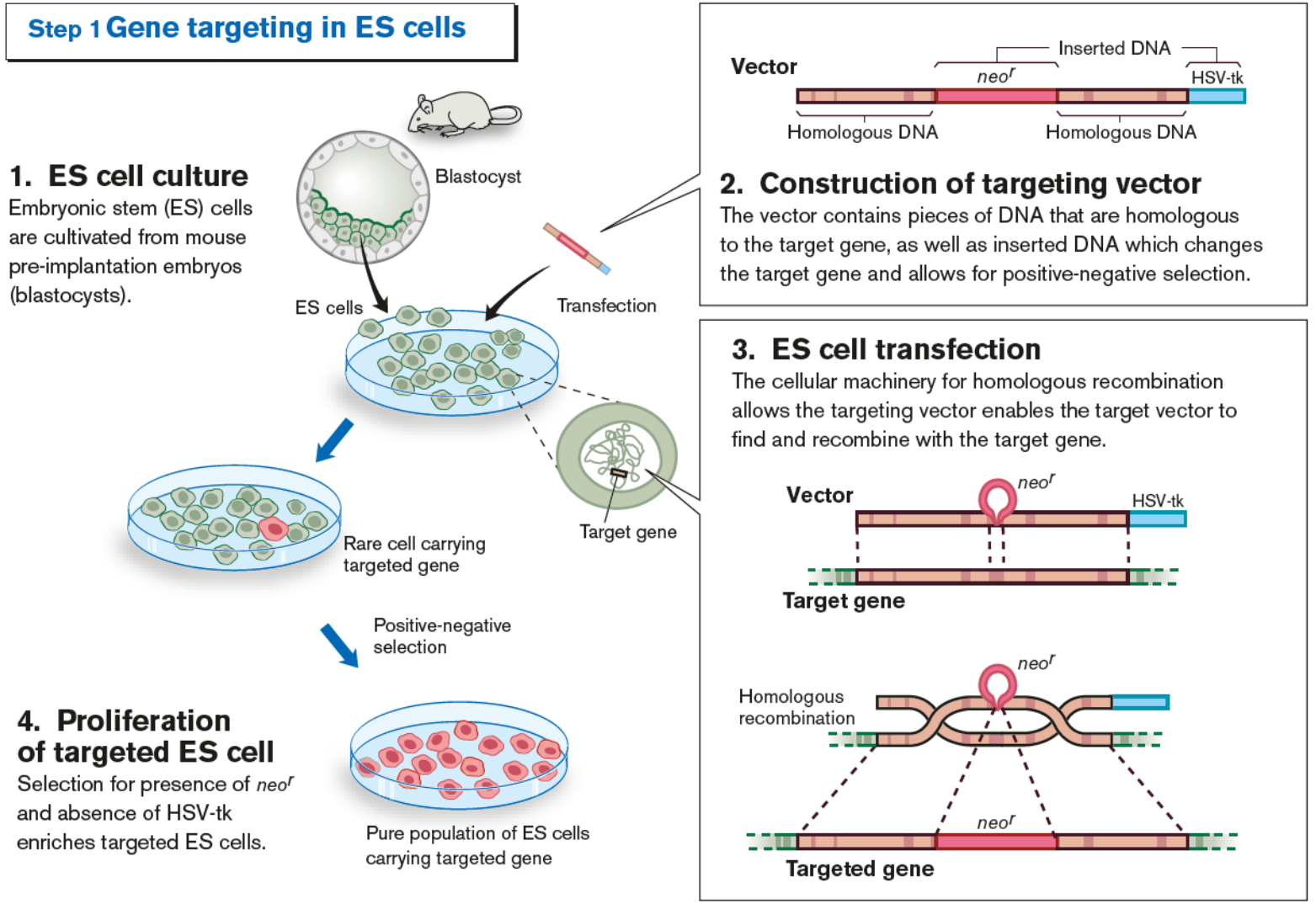


Mouse ES cells



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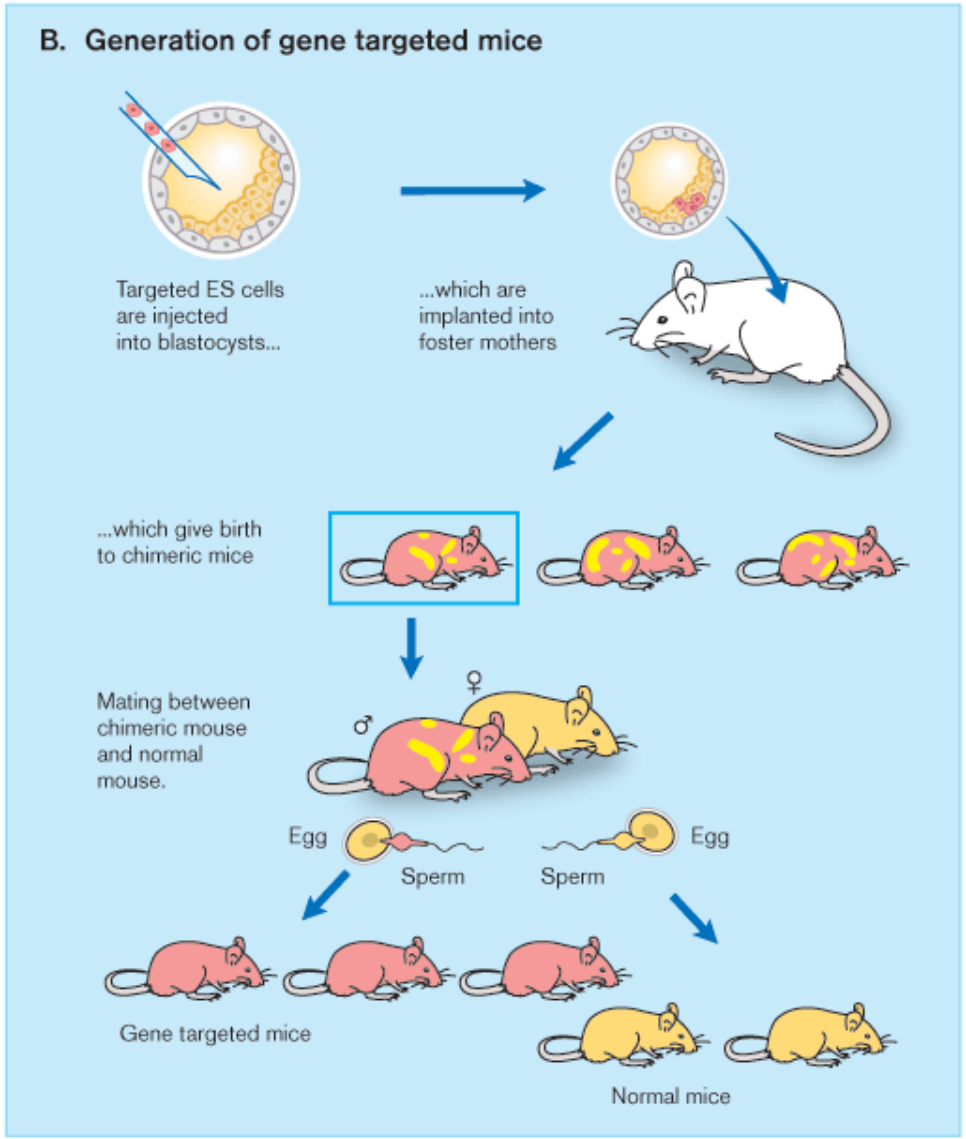

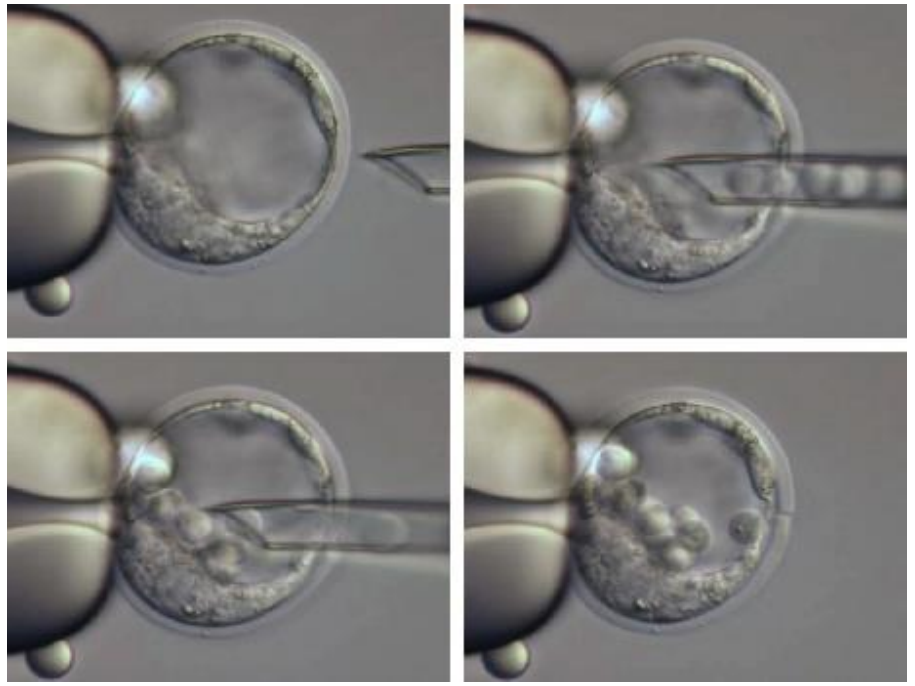

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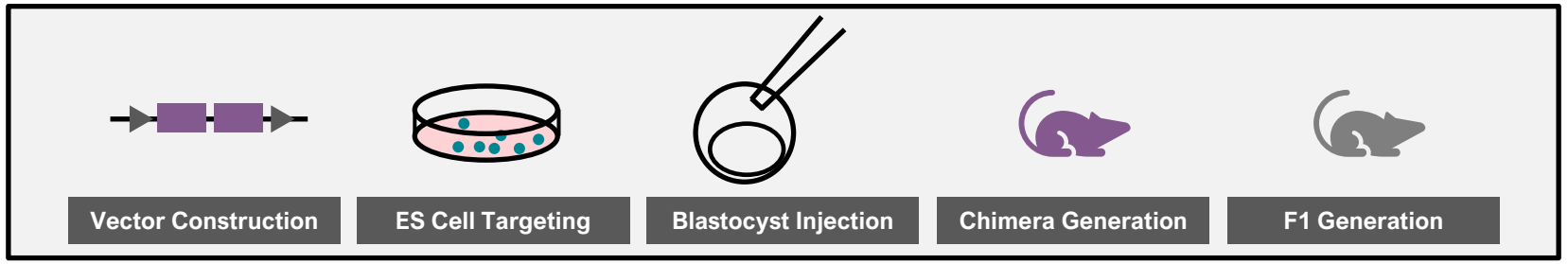
Gene Targeting in Embryonic Stem Cells (ESCs)

Model Generation Process



Suitable for:

- Small and less-complex modifications (e.g., KOs, knock-in point mutations, fluorescent tags, knock-ins <10 kb)
- Large and complex modifications for e.g., targeted humanization > 150 kb

Major Advantages

- Targeted insertion of a well-defined modification (e.g., single copy, intact structure)
- Very large and complex modifications possible
- Extensive and thorough validation (e.g., Southern blots with internal and external probes) prior to generating mice

Major Disadvantages

- Requires an appropriate ES cell line
- Limited to mouse model generation
- Time consuming

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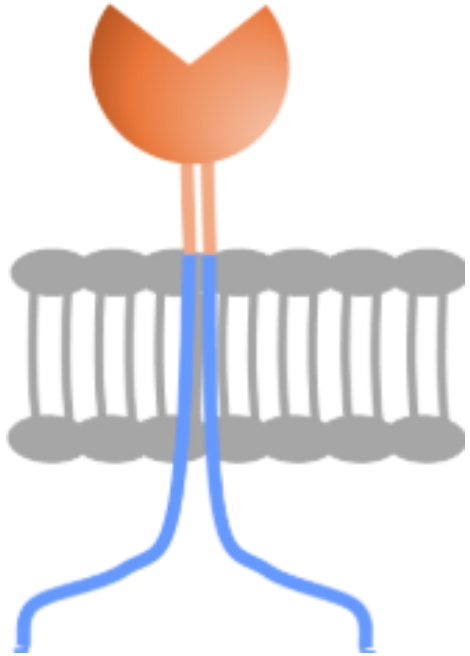
New hTFRC model

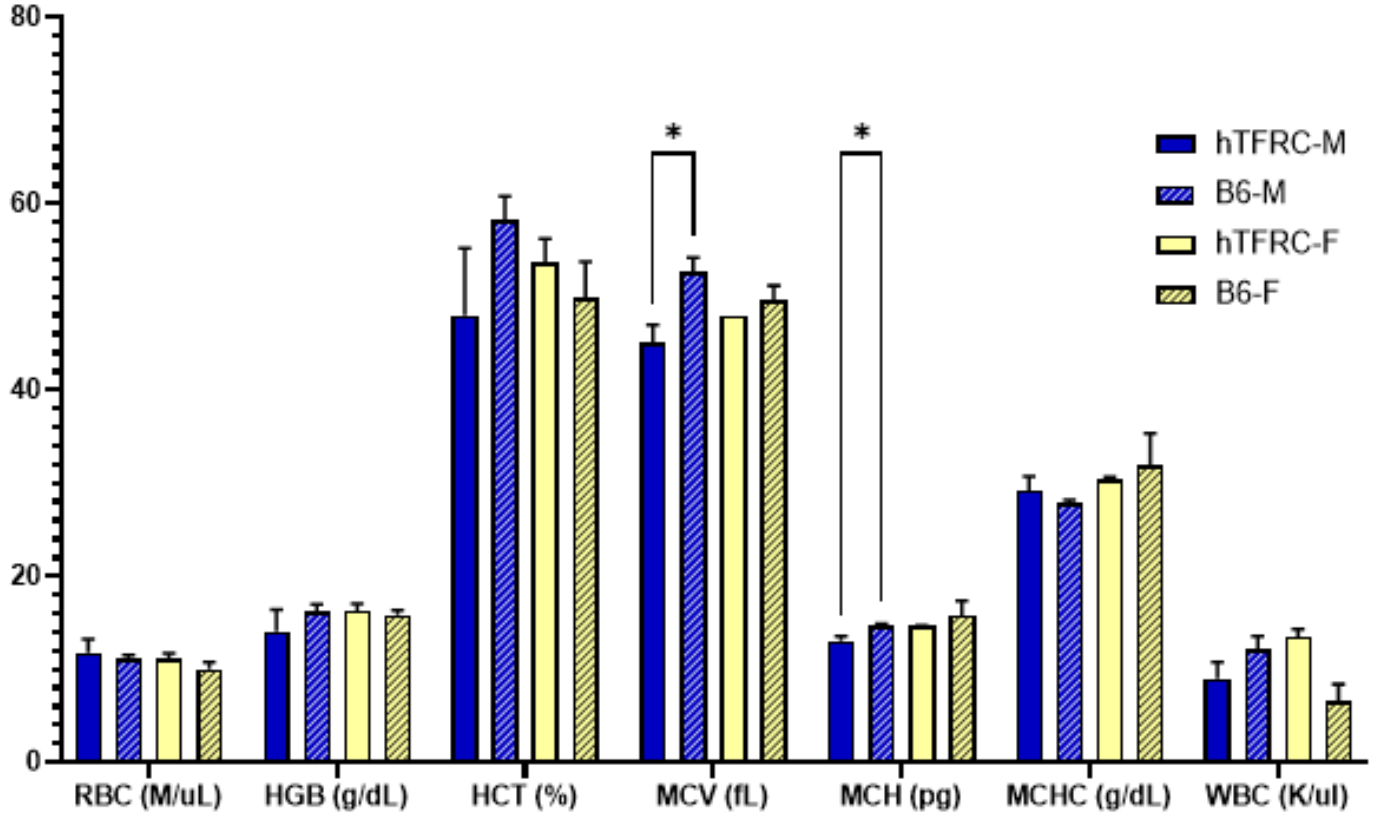
Partial humanization generated using gene targeting in ES cells

- *TFRC* encodes transferrin receptor-1 (TfR1),
  - cell-surface receptor required for import of iron from transferrin into cells by receptor-mediated endocytosis
- Useful for studying TfR1 transcytosis-mediated delivery of therapeutic monoclonal antibodies across the blood-brain barrier (BBB)
- Model is partly humanized: extracellular domain is humanized while the transmembrane and intracellular domains remain murine



hTFRC: Orange indicates the region of humanization (aa 89-760, exons 4-19). The intracellular and transmembrane domains remain the native murine sequence (exons 1-3: blue).

New hTFRC model

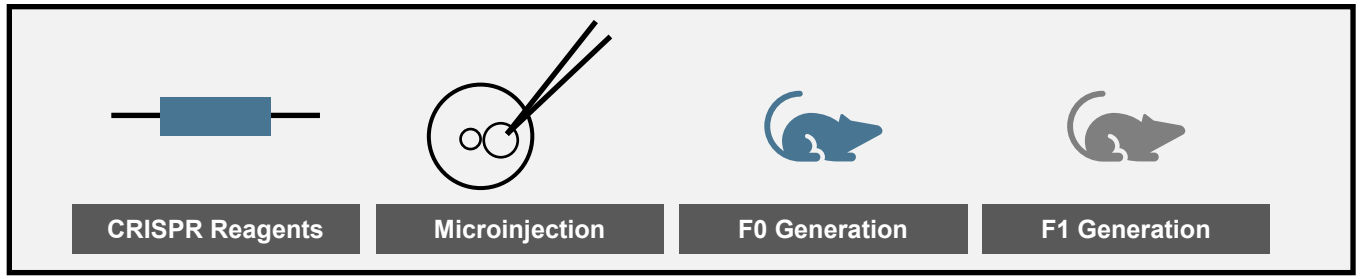


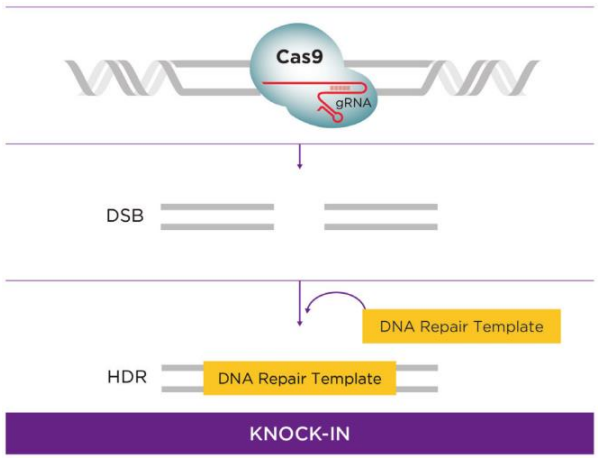
Taconic's hTFRC model express minimal subclinical microcytic anemia, as evidenced by decreased MCV but normal RBC and hemoglobin when compared to standard B6 mice. Complete blood count (CBC) analysis included count of red blood cells (RBC), hemoglobin concentration (HGB), percent hematocrit (HCT), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC)

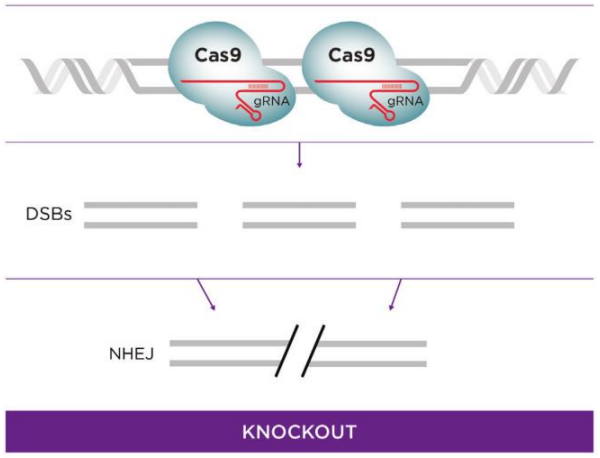
Gene Editing in Zygotes using CRISPR/Cas9

Gene Editing in Zygotes Using CRISPR/Cas9

Model Generation Process

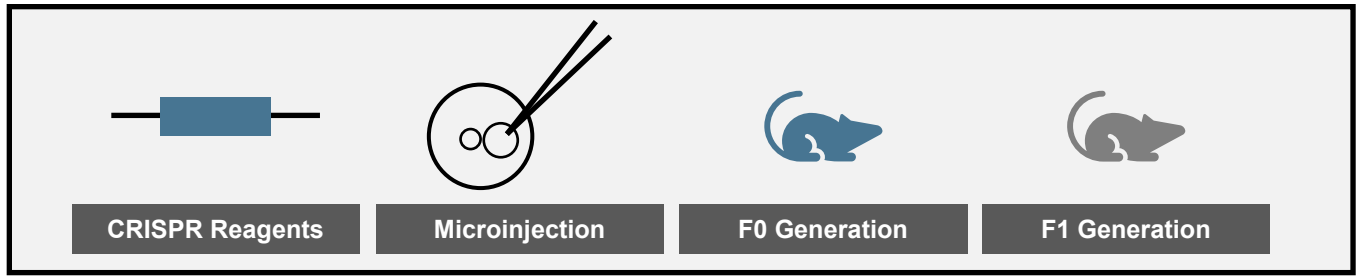






Gene Editing in Zygotes Using CRISPR/Cas9

Model Generation Process



Suitable for:

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Major Advantages

- Feasible for both mouse and rat models
- Flexibility (e.g., choice of genetic background), ease of design and reagent preparation
- Usually, fastest timeline to F1 animals

Major Disadvantages

- Risk for off-target mutations
- Risk for secondary on-target mutations
- Different quality control or genetic validation procedures *in vivo* (vs. gene targeting in ES cells)
- Size of modifications is limited
- Founder animals are likely to be mosaic

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ACE1 R1279Q

Point mutation introduced into mouse gene using CRISPR/Cas9

- ACE1 is increased in postmortem Alzheimer's disease (AD) brain tissue
- ACE1 R1279Q has been identified in several AD families
- The R1284Q mutation was introduced into exon 25 and two additional silent mutations were inserted into exon 25 to generate a restriction site (Mfe I) for analytical purposes.
- **Results:**
  - ACE1 was increased in neurons, but not microglia or astrocytes, of KI brains, which became elevated further with age.
  - Angiotensin II (angII) and angII receptor AT1R signaling were also increased in KI brains.
  - Autosomal dominant neurodegeneration and neuroinflammation occurred with aging in KI hippocampus, which were absent in the cortex and cerebellum.
  - Hippocampal neurodegeneration was completely rescued by treatment with brain-penetrant drugs that inhibit ACE1 and AT1R.

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L. K. Cuddy, et al. Aβ-accelerated neurodegeneration caused by Alzheimer's-associated *ACE* variant R1279Q is rescued by angiotensin system inhibition in mice. *Sci. Transl. Med.* **12**, eaaz2541 (2020).

Custom Model Generation

Summary and Closing Remarks

- The project goal determines the strategy and the methodology
- There are several methods to generate a transgenic humanized mouse model
  - RITg, ESC, CRISPR/Cas9
- The different methods has their own strengths and weaknesses

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Thank You

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