# Existing Tools For Customized Model Generation Solutions

## Pros & Cons About The Different Methodologies

**Dr. Benedikt Wefers**
Scientific Program Manager - Custom Model Generation Solutions

**Key responsibilities:**

Develop and articulate technical and business solutions for custom model generation by partnering with our customers to understand both project and program goals

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# Custom Model Generation

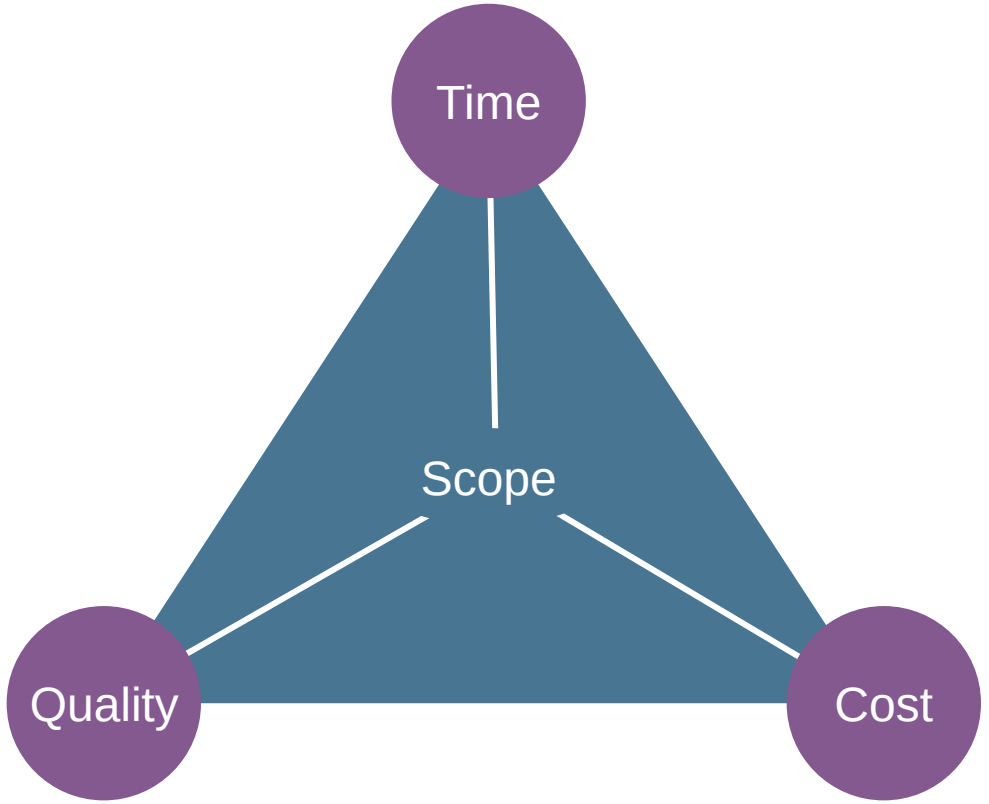
## Conceptualize, Strategize and Design

*Conceptualize*: **Why** do you need the mouse model?

- Start with the **research goal**
- Consider downstream analyses

*Strategize*: **What** is the most appropriate strategy?

- Evaluate **biological risks**
- Consider **constraints**

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# Custom Model Generation

## Conceptualize, Strategize and Design

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- Start with the **research goal**
- Consider downstream analyses

*Strategize*: **What** is the most appropriate strategy?

- Evaluate **biological risks**
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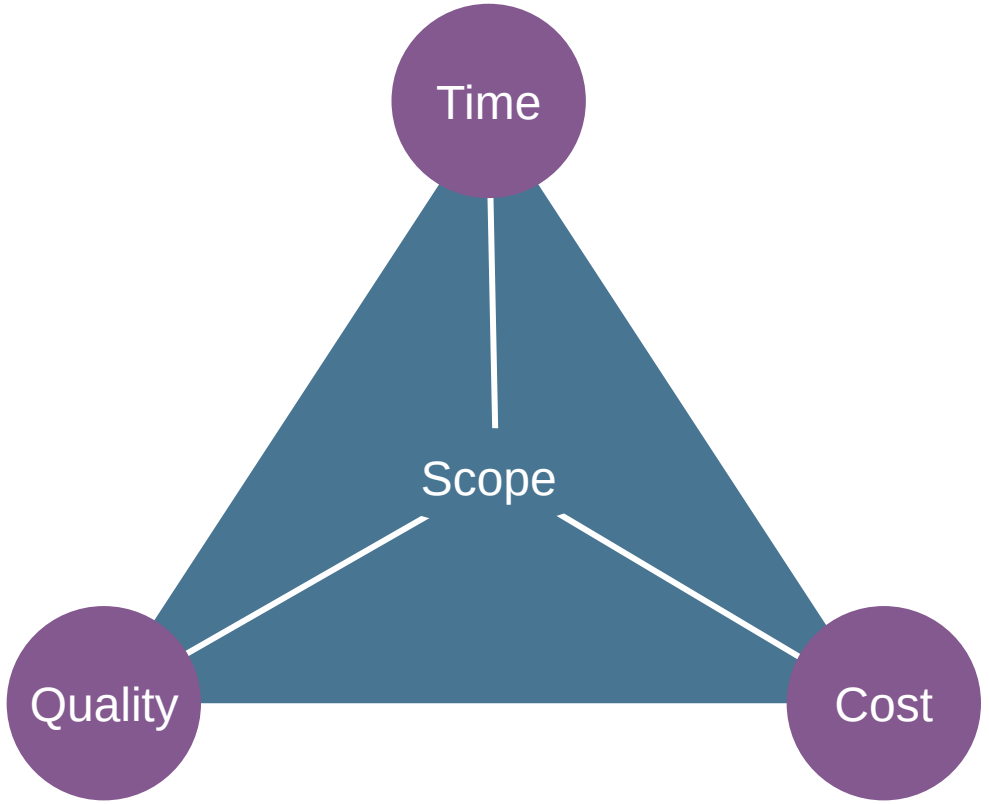
*Design*: **How** do you make the model?

- Evaluate **feasibility and efficiency**
- Consider **technical risks**
- Consider flexibility
- Consider tolerances to budget
- Consider tolerances to timeline

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# Outline

- Major Methods for Genome Modification
  - Random Integration Transgenics (RITg)
  - Gene Targeting in Embryonic Stem Cells (ESC)
  - Gene Editing in Zygotes using CRISPR/Cas9
- Advancing Model Generation

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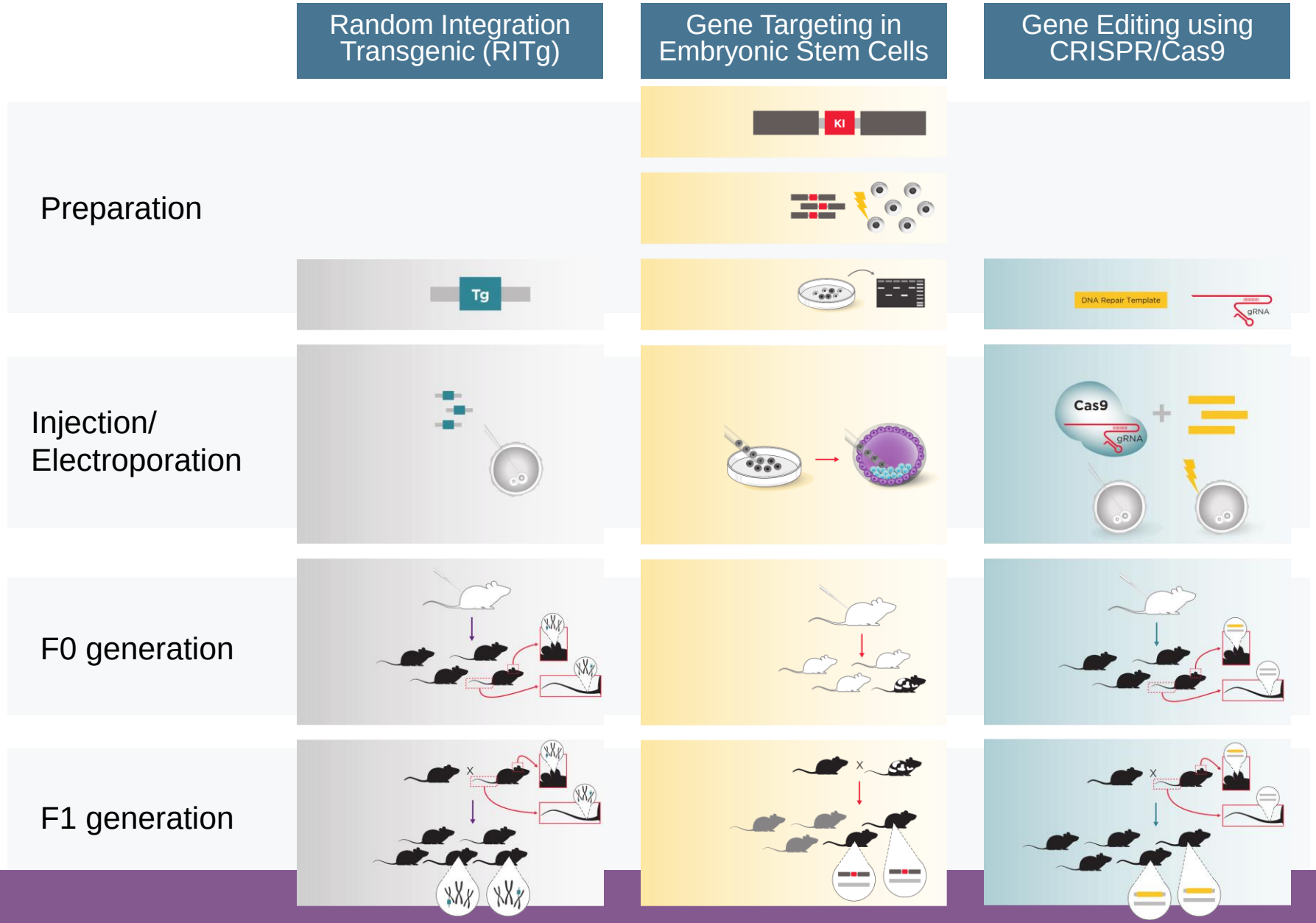
# Three Major Methods for Modifying the Genome



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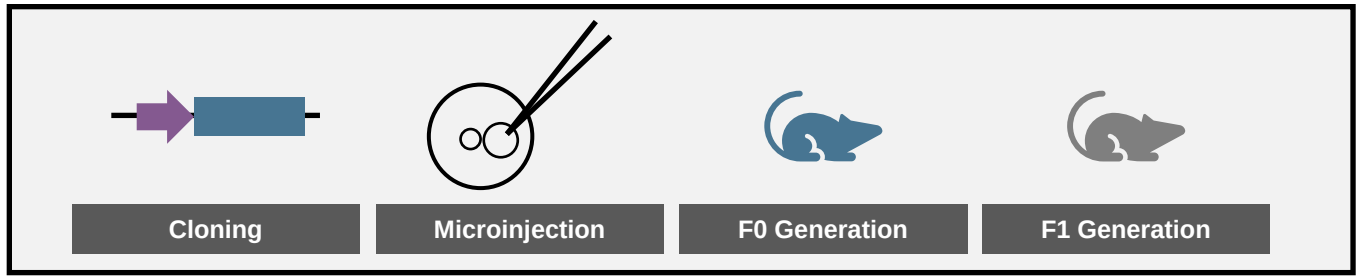
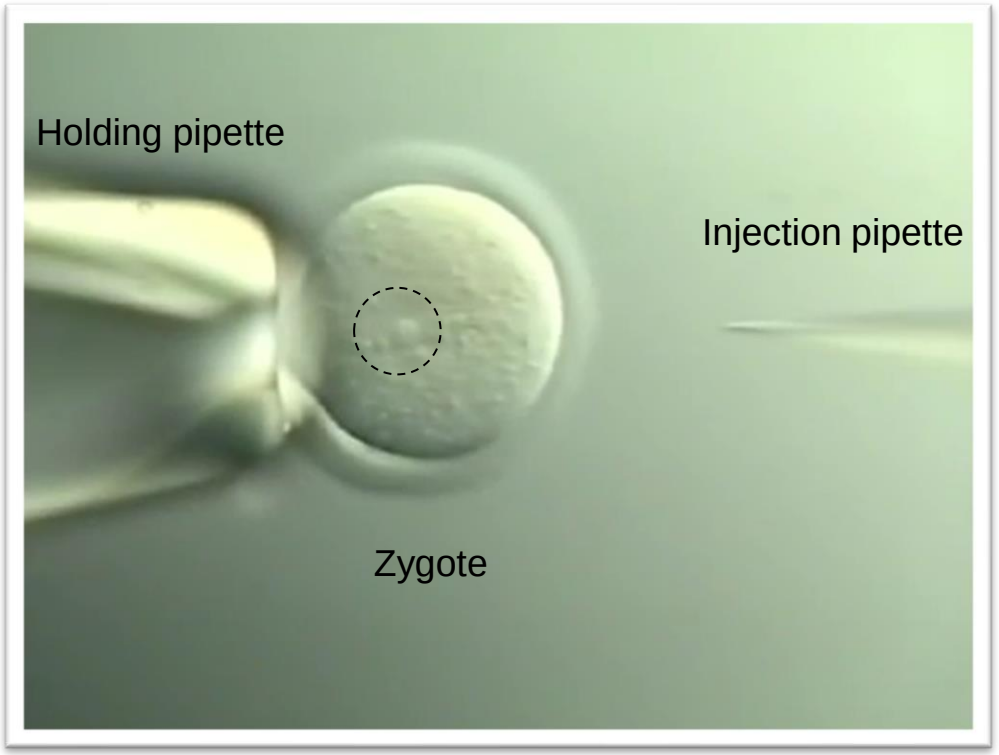
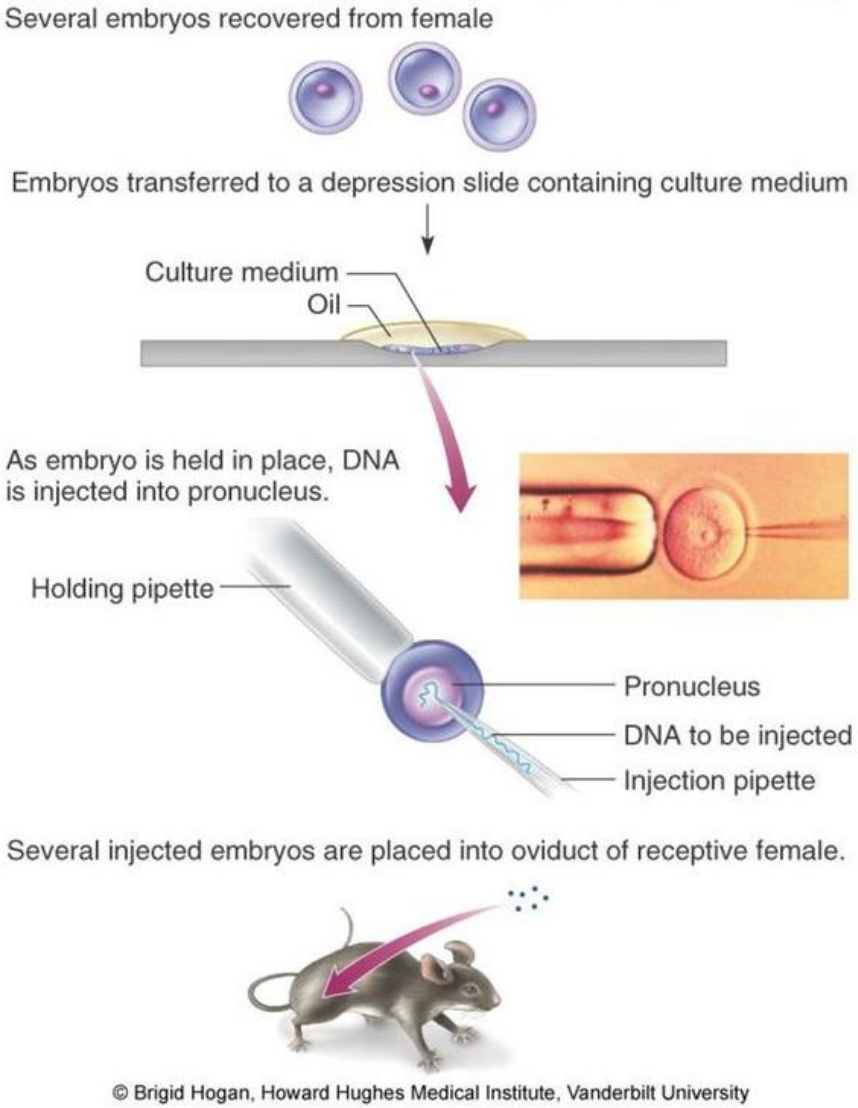
# Random Integration Transgenic Mice (RITg)

## Model Generation Process

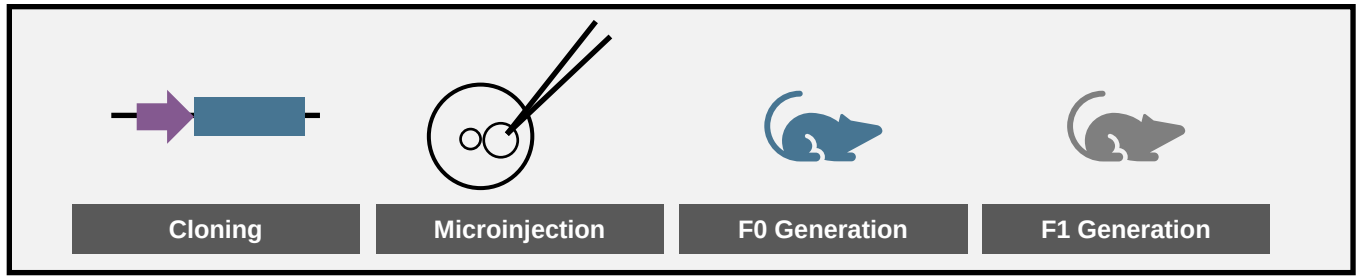


# Random Integration Transgenic Mice (RITg)

## Model Generation Process




**Major Advantages**

- Wide range of transgene designs possible (e.g., promoter choice, tags or reporters)
- Feasible for both mouse and rat models
- 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
- Possibility for multiple integrations

**Suitability**

- Over(expression) of plasmid- and BAC-based transgenes

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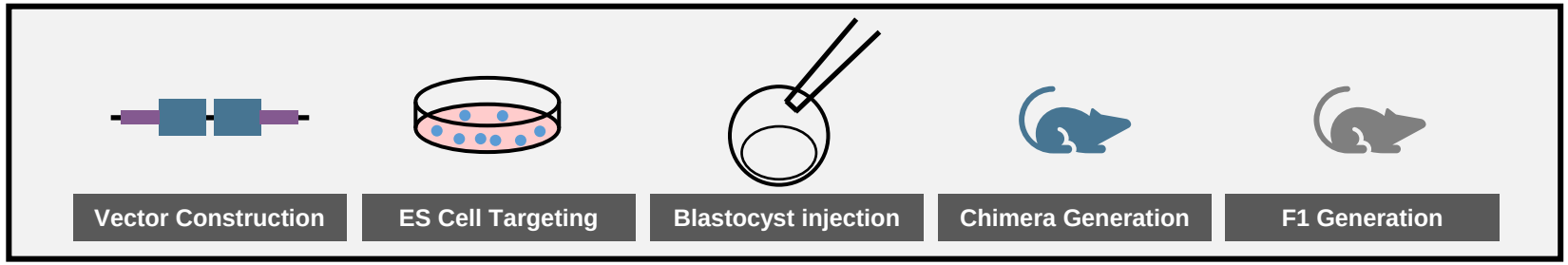
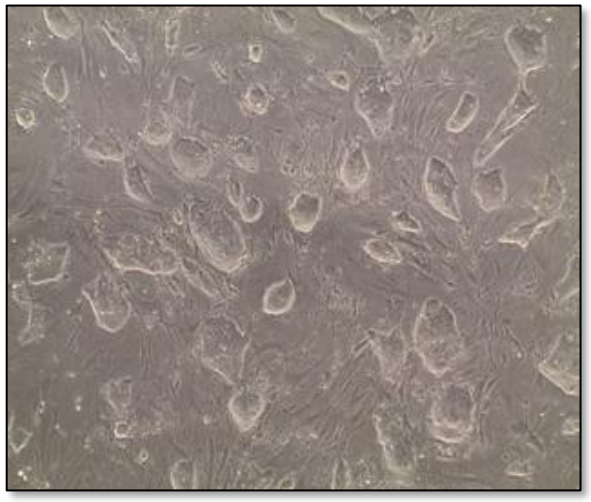
# Gene Targeting in Embryonic Stem Cells (ESCs)

## Model Generation Process



Mouse ES cells

Chimeras

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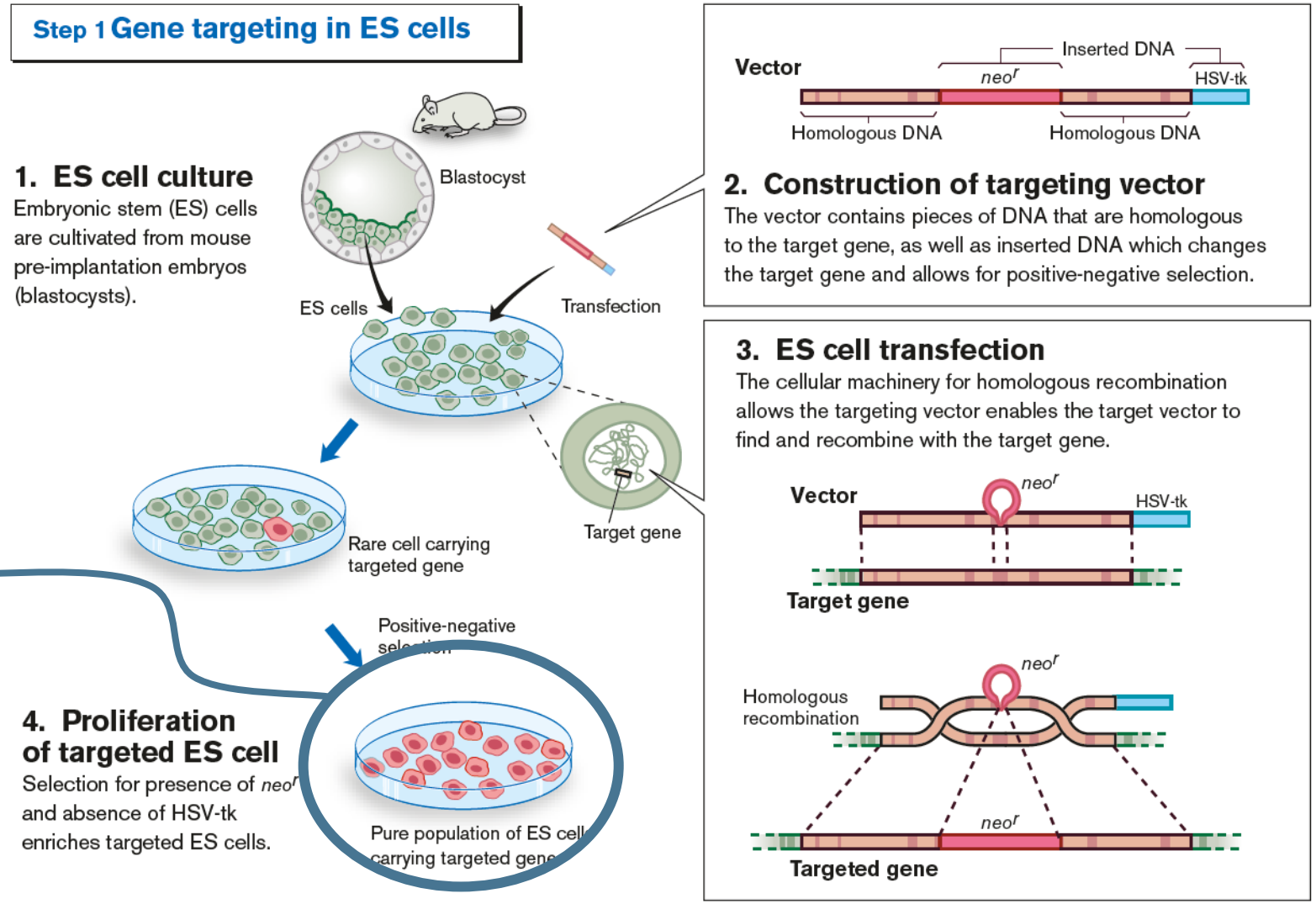
# Gene Targeting in Embryonic Stem Cells (ESCs)

3R

Extensive molecular characterization of multiple clones

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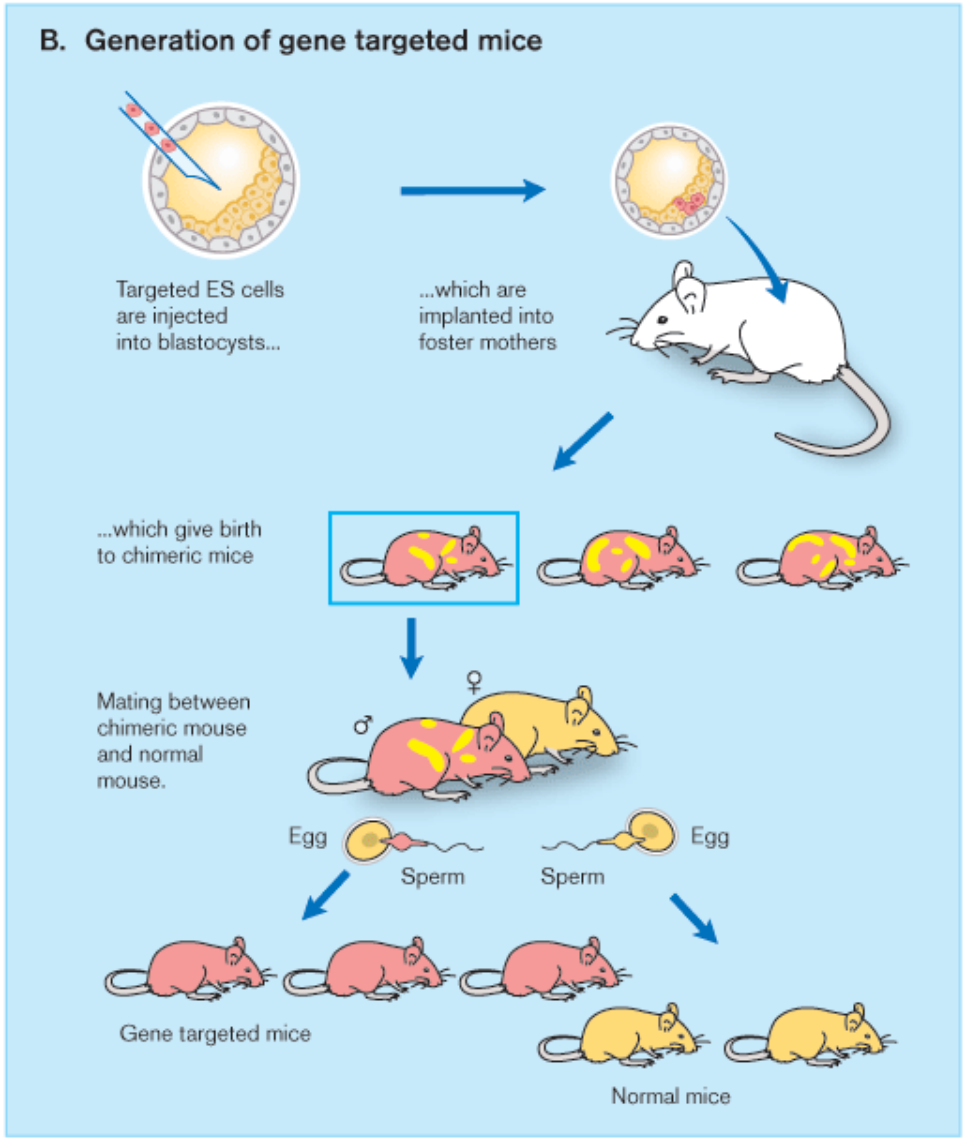
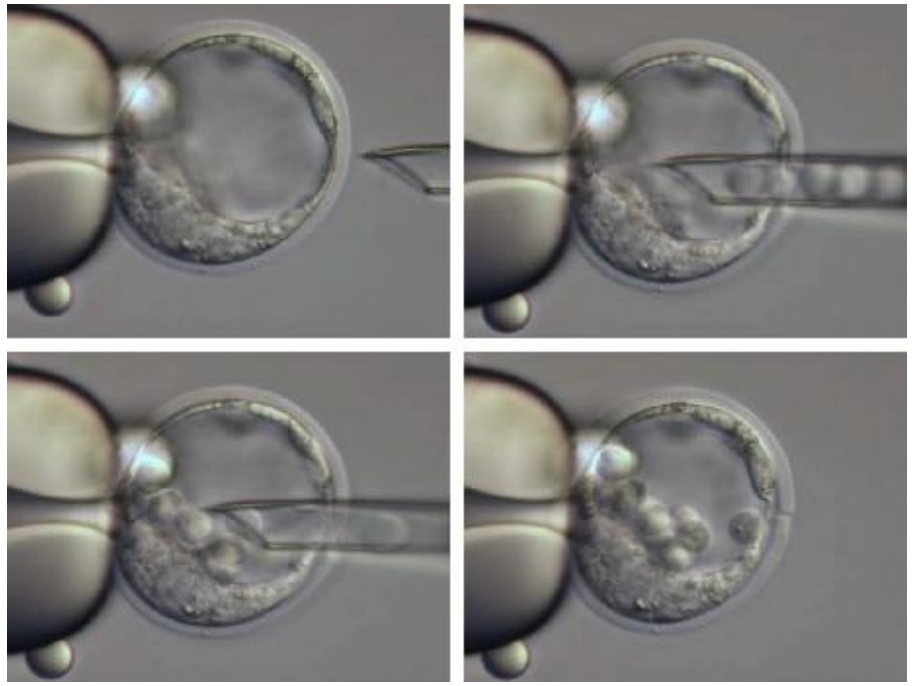
Press release. NobelPrize.org. Nobel Prize Outreach AB 2023. Sat. 2 Dec 2023. <https://www.nobelprize.org/prizes/medicine/2007/press-release/>

# Gene Targeting in Embryonic Stem Cells (ESCs)



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Advanced information. NobelPrize.org. Nobel Prize Outreach AB 2023. Sat. 2 Dec 2023.
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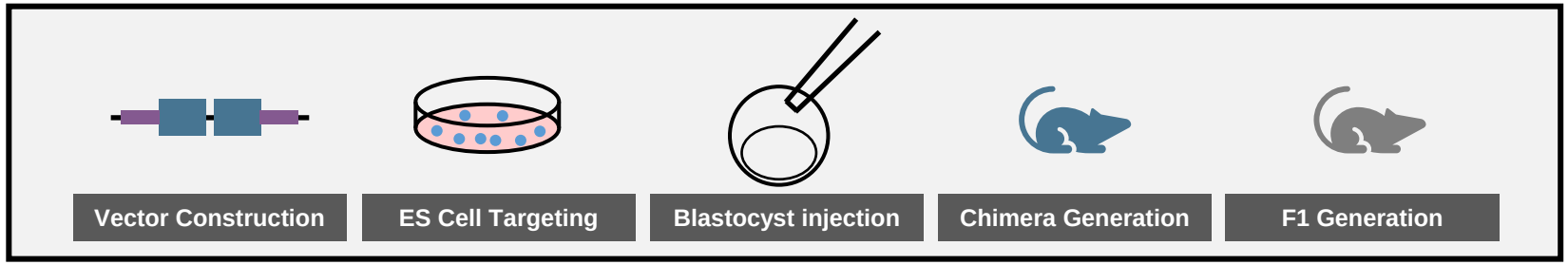
# Gene Targeting in Embryonic Stem Cells (ESCs)

## Model Generation Process




**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**

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

**Suitability**

- Large and complex modifications (e.g., targeted humanizations >150 kb)

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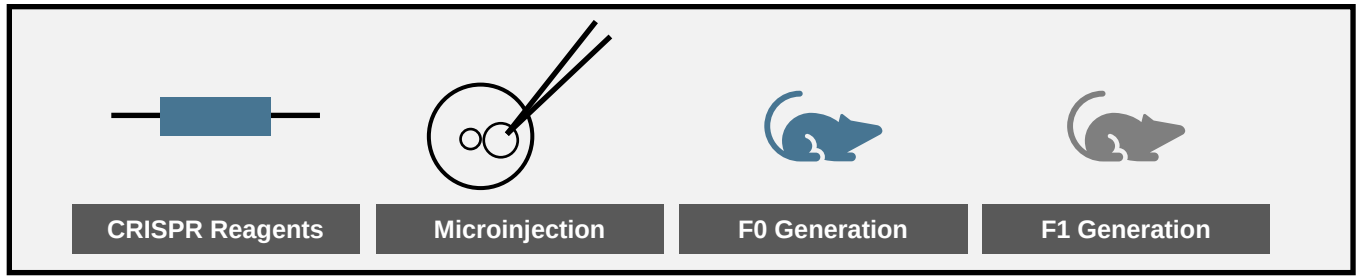
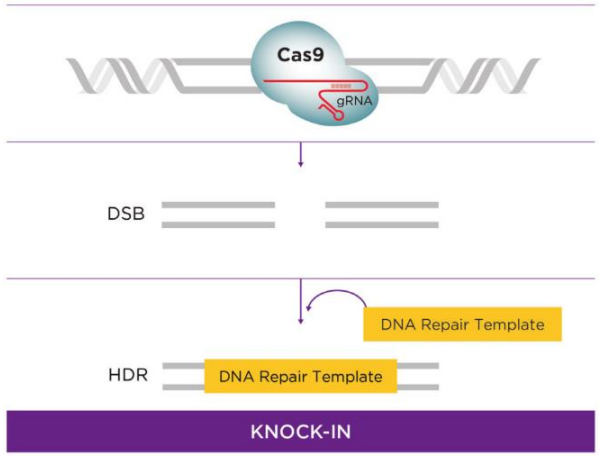
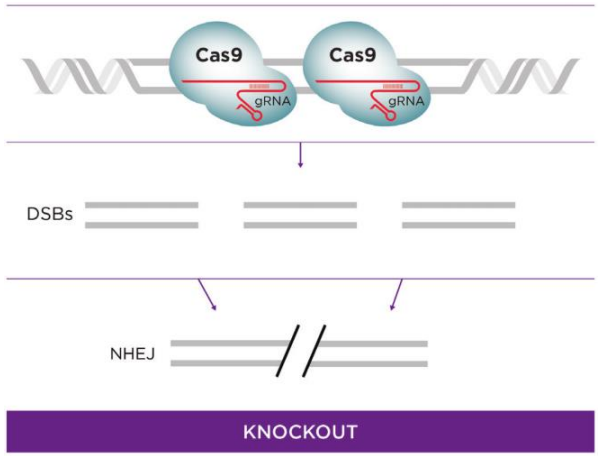
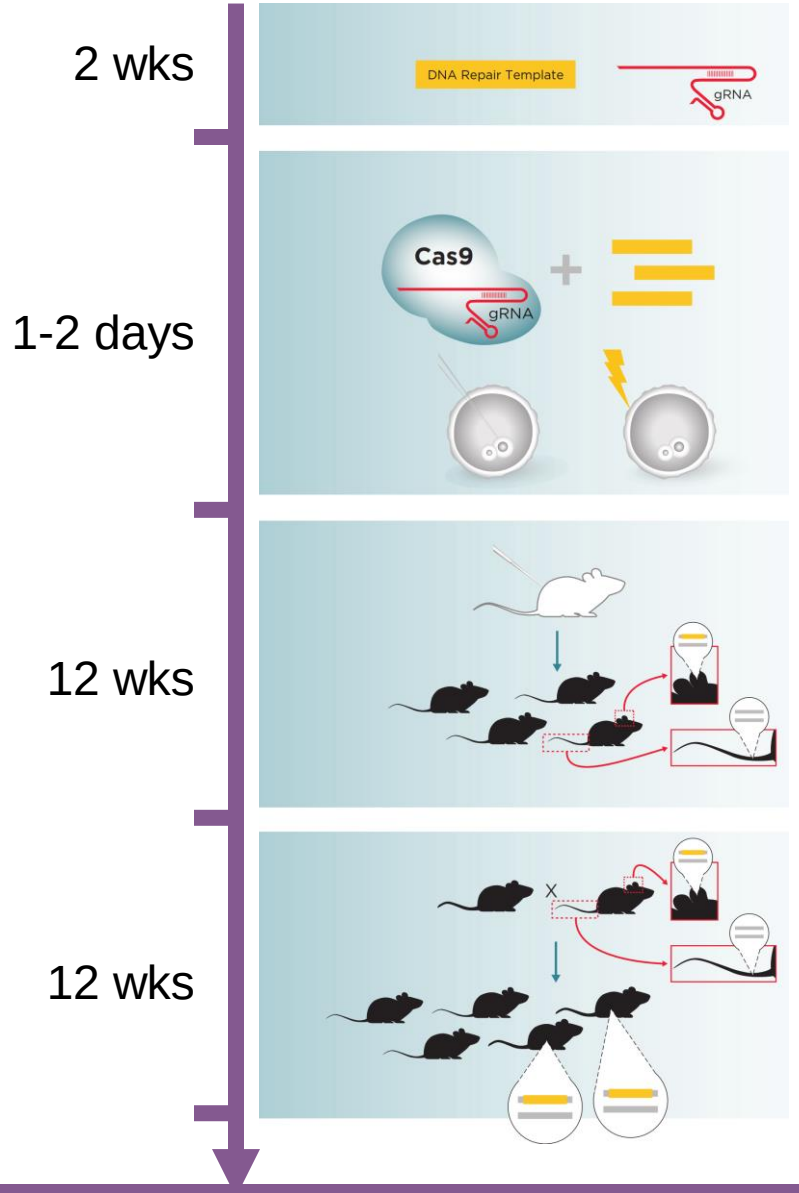
# Gene Editing in Zygotes Using CRISPR/Cas9

## Model Generation Process



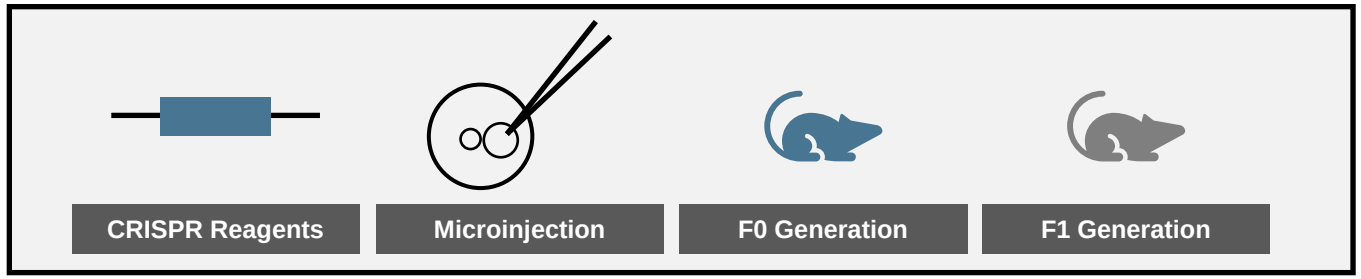



# Gene Editing in Zygotes Using CRISPR/Cas9

## Model Generation Process




**Major Advantages**

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

**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

**Suitability**

- Small and less-complex modifications (e.g., KOs, knock-in point mutations, fluorescent tags, knock-ins <10 kb)

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# Choosing the Right Tool for every Model

Targeting efficiency of CRISPR *in vivo* projects vs. ESC-based targeting

| | ES Cells | CRISPR |
|---|---|---|
| Constitutive Knockout | ●● | ●●●● |
| Point Mutation Knock-in | ●● | ●●●● |
| Short Tag Knock-in | ●● | ●●● |
| Cre Recombinase Knock-in | ●●● | ●●● |
| Reporter Knock-in | ●●● | ●●● |
| cDNA Knock-in | ●●● | ●●● |
| Conditional Knockout | ●●●● | ●● |
| Genomic Replacement | ●●●● | ● |

●●●● Excellent ●●● Good ●● OK ● Poor

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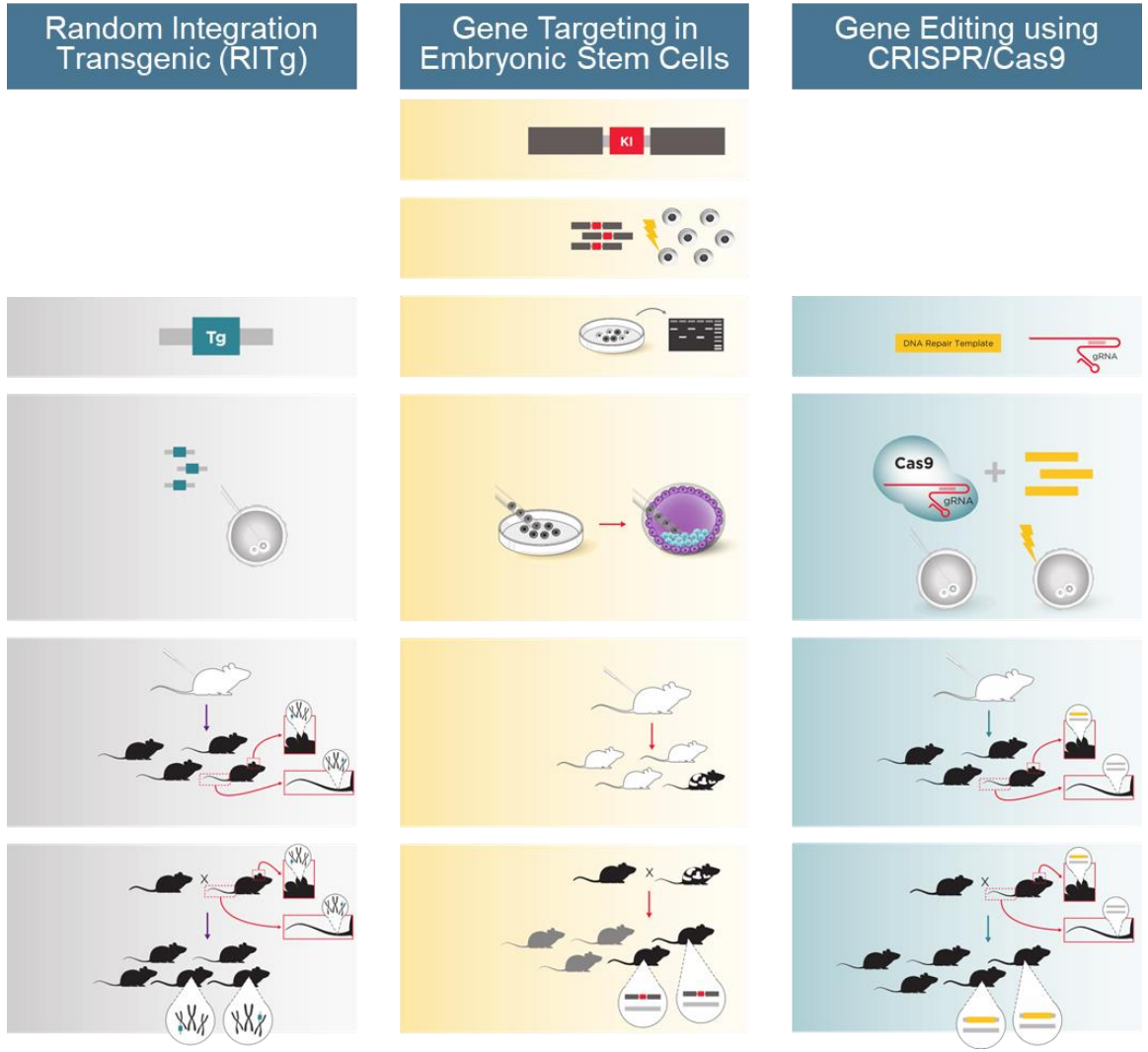
# Advancing Model Generation

## The founder challenge

Which founder should I use to establish the line?

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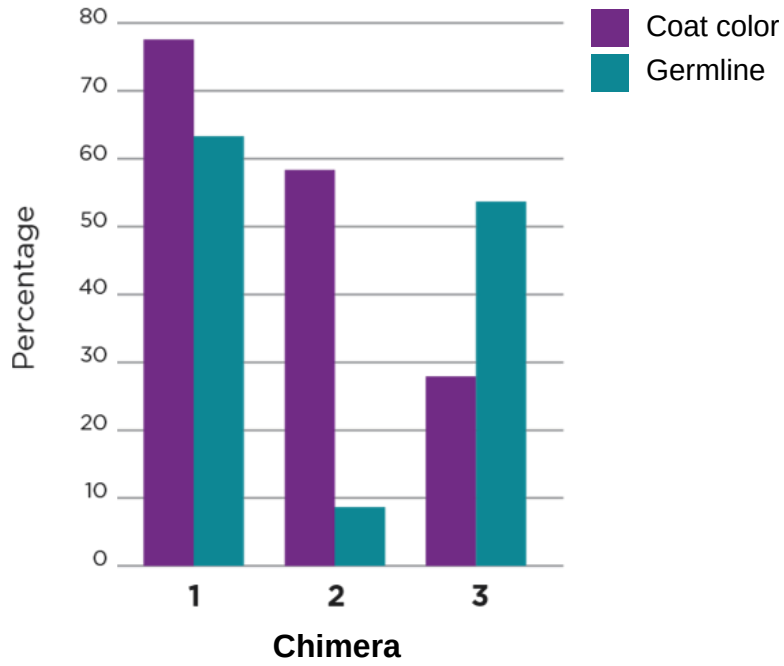
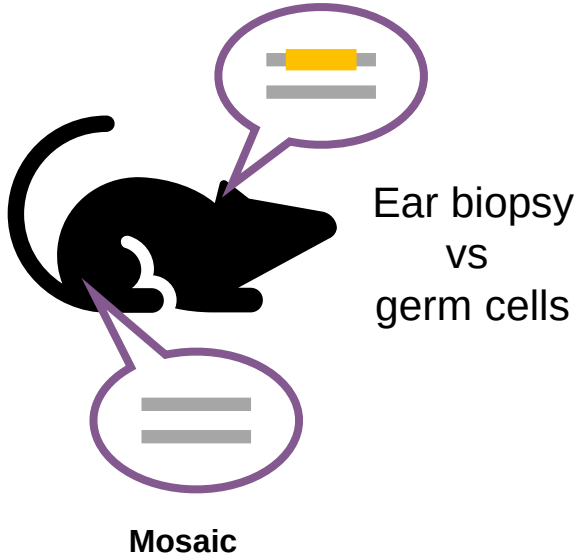
# Advancing Model Generation

## The founder challenge

- Founders are most likely mosaic or chimeric
  - Allele frequency in germline unknown

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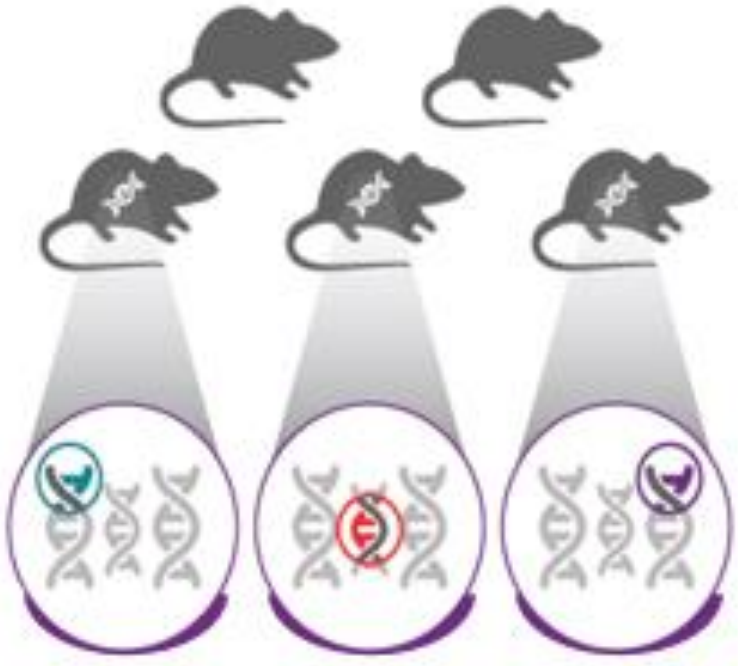
# Advancing Model Generation

## The founder challenge

- Founders are most likely mosaic or chimeric
  - Allele frequency in germline unknown
  - Fertility of founder potentially compromised
  - RITg: need to breed and analyze multiple sublines



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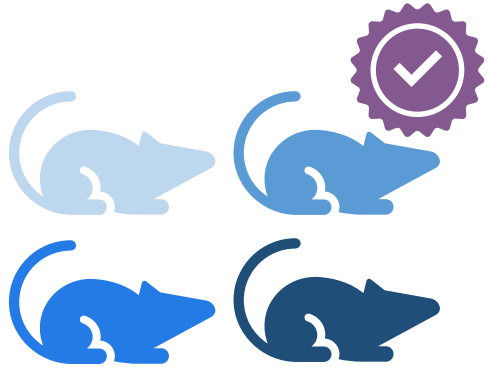
# Advancing Model Generation

Reducing timelines, risks, and surplus animals without compromising quality

- Concept: Data-informed founder selection

| | Allele frequency | Expression analysis | Fertility |
|---|---|---|---|
| **RITg** | Mosaicism (qPCR) | Tissue-specific expression levels (qRT-PCR) | *In vitro* fertilization |
| **ES Cells** | Chimerism (qPCR) | - | *In vitro* fertilization |
| **CRISPR** | Mosaicism (NGS)<br>OT analysis (NGS) | - | *In vitro* fertilization |



Identify the best founder

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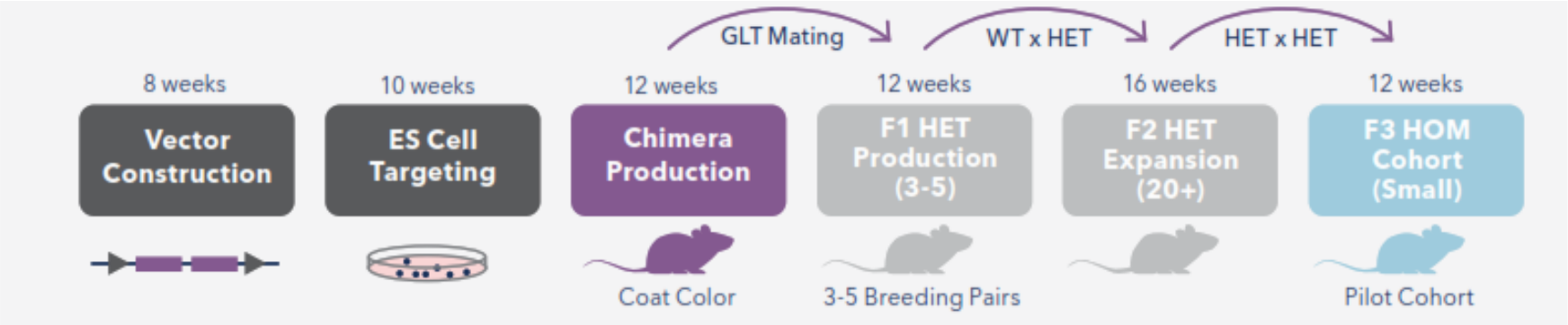
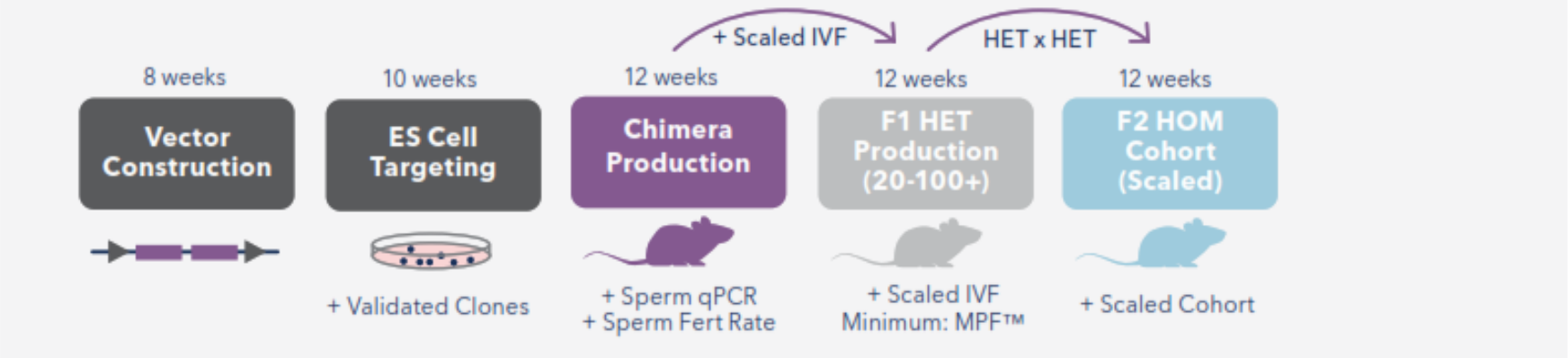
# Advancing Model Generation

Reducing timelines, risks, and surplus animals without compromising quality

- Concept: Data-informed founder selection & Faster and scalable generation of F1 animals




3R

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# Custom Model Generation

## Summary and Closing Remarks

- The project goal determines the strategy and the methodology.
- There are different methods with their own strengths and weaknesses.
- There is not THE ULTIMATE method for custom model generation.
- Careful evaluation of the optimal strategy, methodology and project design is not only important for the research goals, but also for the 3Rs.

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# Thank You

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