Introducing OzBIG: A robust process for generating mouse models with 20 – 240 kb targeted replacement

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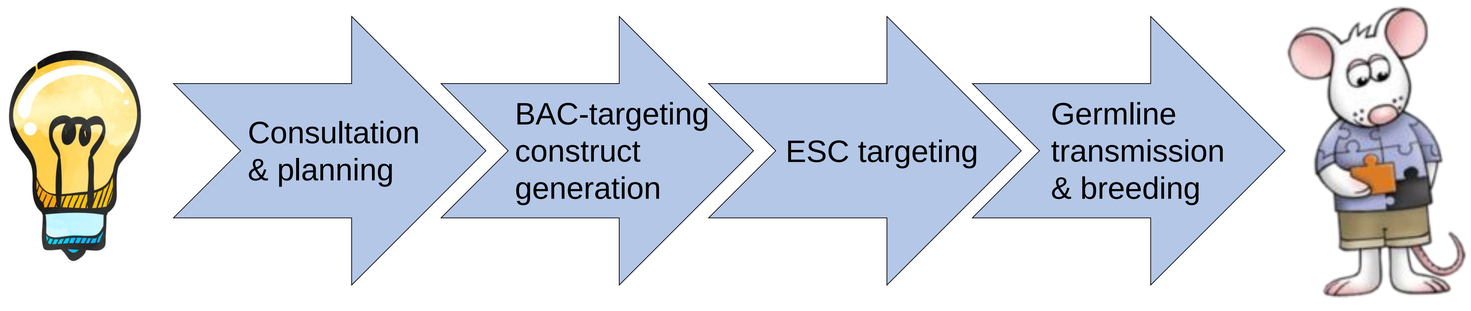
Abstract Demand for large, genomic humanized mouse models inspired our development of a system, called OzBIG, for robust creation of large, gene-targeted replacement models. This system enables our routine generation of mouse models with very large (20 to 240 kb) genomic replacements. The process for developing OzBIG models is the result of a collaborative effort combining the technological strengths of Ozgene and Gen-H. Large, BAC-based targeting vectors engineered by Gen-H are used to efficiently generate targeted ES cells using conventional gene targeting coupled with a screening platform developed at Ozgene. OzBIG targeted ES cells are then used to develop germline heterozygous chimeras via goGermline™ embryo injection. The targeting method is independent of exogenous nucleases, like CRISPR/Cas9, thereby eliminating the need to analyze for off-target effects. The ability to routinely create large modifications using OzBIG vastly expands the gene set accessible to genomic humanization. Traditional gene targeting using plasmids to deliver the integrative payload has a technical upper size limit of approximately 20 kb, which limits single vector genomic humanization to approximately 10% of all mouse genes. BAC-based OzBIG targeting vectors, while technically more difficult to assemble, can carry up to 240 kb or more of integrative payload. The payload improvement achieved by using our BAC-based targeting vectors increases the gene set amenable to humanization to approximately 90% of all mouse genes. In addition to these large genomic humanizations, we are using OzBIG to build large, non-humanized transgenics, such as complex reporters and synthetic expression systems. As an example, we present development of a conditional Pax7 allele for Cre-inducible expression of the pathognomonic *Pax7-Foxo1* chimeric oncogene and the expression marker eYFP. The *Pax7-Foxo1* fusion protein drives the childhood muscle cancer alveolar rhabdomyosarcoma and this model will be used to explore the biology of several key aspects of this sarcoma.

The successful partnership between Ozgene and Gen-H offers full-package generation of transgenic mouse lines with industry-leading timelines and a proven track record.

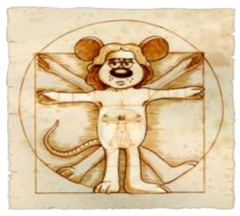
1.5 years of co-operation yielding >10 ESC-confirmed transgenes with a 0% failure rate.

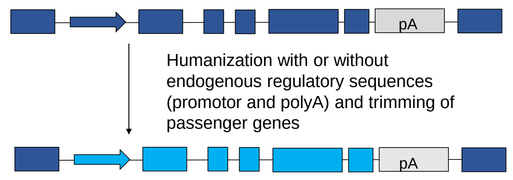
- average transgene insertion: 52 kb
- average time to ESC: 5 months



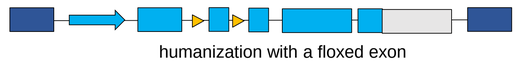
Enabling complex transgene structures

- Humanizations





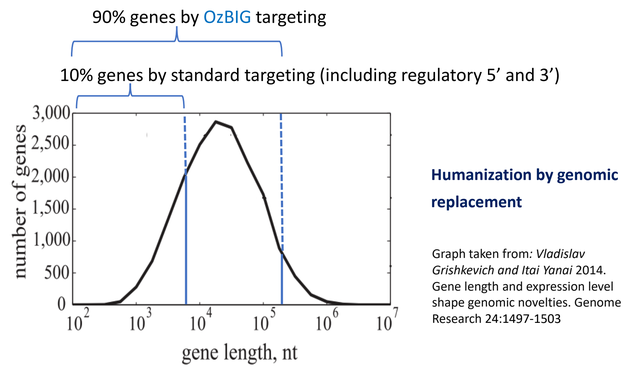
- Humanizations with a conditional knock-out



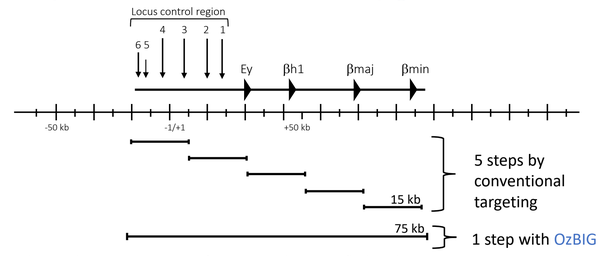
- Humanizations with a conditional mutation knock-in

- Synthetic reporter constructs

OzBIG Large Gene-Targeted Replacements & Insertions



Example: what if you wanted to humanize the entire β-globin locus?



Globin locus map taken from: Bulger, *et al.,* 1999. Conservation of sequence and structure flanking the mouse and human β-globin loci: The β-globin genes are embedded within an array of odorant receptor genes. PNAS 96(9)5129-5134

Conventional gene targeting
- Targeting vector payload of **15 kb max**
- **5** serial gene targeting events
- Time to humanized F1 mice **> 3 years**
- Cost > 5 projects
- **High risk** - due to ES cell culture time

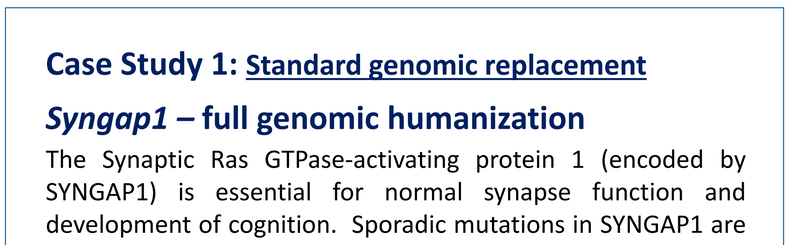
OzBIG gene targeting
- Targeting vector payload of **240 kb max**
- **1** targeting event
- Time to humanized F1 mice **~ 7 months**
- Cost = 1 project
- **Low risk**

Case Study 1: Standard genomic replacement

Syngap1 – full genomic humanization

The Synaptic Ras GTPase-activating protein 1 (encoded by SYNGAP1) is essential for normal synapse function and development of cognition. Sporadic mutations in SYNGAP1 are responsible for rare, dominant disorders of intellectual disability, epilepsy autism and sensory processing. The SynGAP Research Fund (https://www.syngapresearchfund.org/) sponsored the development of a humanized model as the first step towards an animal model enabling the study and therapeutic development for these rare diseases.

Transgene design
A Floxed genomic fragment of 37.5 kb carrying the entire human SYNGAP1 gene, including 2 kb of promoter sequence, and the ZBTB9 gene to the Stop codon replaced the mouse 34.7 kb orthologous genomic region.



Progress:
Initiation → targeting construct: 3 months
targeting construct → confirmed ESC: 3 months
ESC → GLT: 3 months

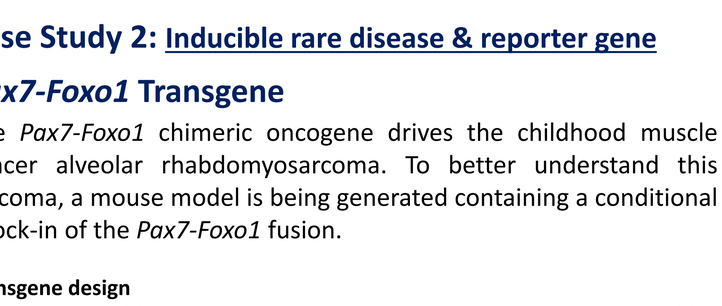
Case Study 2: Inducible rare disease & reporter gene

Pax7-Foxo1 Transgene

The *Pax7-Foxo1* chimeric oncogene drives the childhood muscle cancer alveolar rhabdomyosarcoma. To better understand this sarcoma, a mouse model is being generated containing a conditional knock-in of the *Pax7-Foxo1* fusion.

Transgene design
To create a conditional *Pax7-Foxo1* fusion, the mouse *Pax7* gene was modified such that exons 8 and 9 were flanked with loxP sites, and a downstream cassette containing exon2 of *Foxo1* followed by an IRES and eYFP coding sequence was inserted.

In the resulting transgenic mouse, wild-type *Pax7* will be expressed by default. Induction of Cre-recombinase will cause genomic rearrangement to yield a model of the oncogenic genotype with a fluorescent reporter of expression. Cre expression triggers excision of the 3' terminal *Pax7* sequence, resulting in fusion with the downstream *Foxo1* exon. The IRES-eYFP sequence allows expression of the oncogene to be inferred via expression of the fluorescent marker.



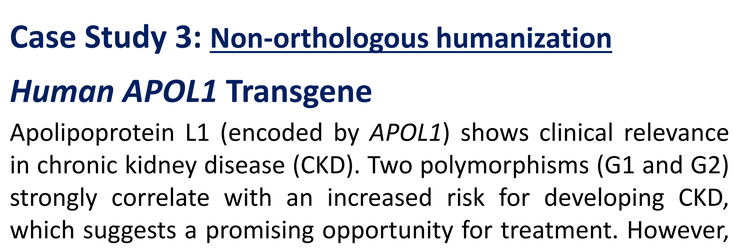
Progress:
Initiation → targeting construct: 4 months (higher complexity)
targeting construct → confirmed ESC: 2 months
ESC → GLT: underway

Case Study 3: Non-orthologous humanization

Human APOL1 Transgene

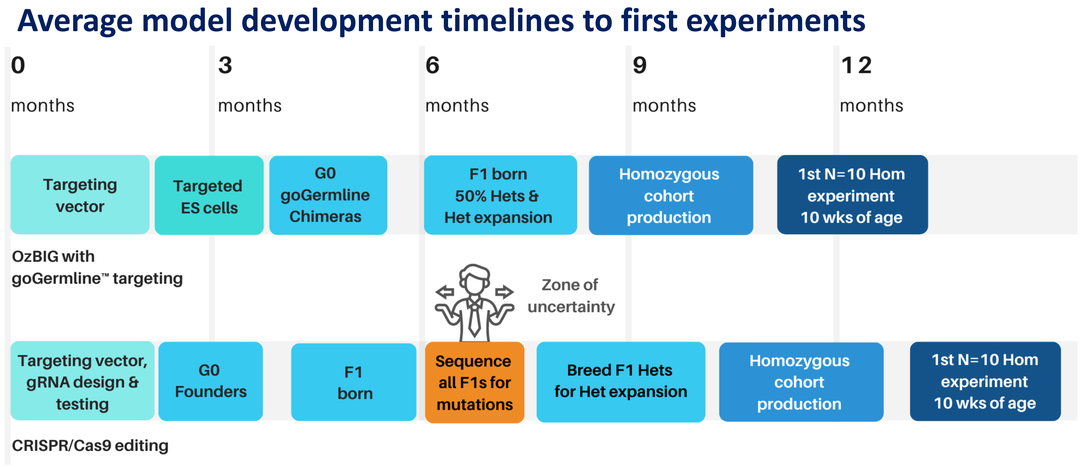
Apolipoprotein L1 (encoded by *APOL1*) shows clinical relevance in chronic kidney disease (CKD). Two polymorphisms (G1 and G2) strongly correlate with an increased risk for developing CKD, which suggests a promising opportunity for treatment. However, *APOL1* is lacking in rodents, which greatly hinders drug discovery efforts. We have generated a mouse model for expression of human *APOL1* to support these studies.

Transgene design
A targeting construct was created to insert the human gene *APOL1* into the murine safe harbor locus, *Igs2*. To optimize for expression in relevant tissues and at relevant levels, 10 kb of upstream sequence was included in the transgene to capture the endogenous human regulation of expression. All *APOL1* exons were sequenced to confirm the presence of the desired *APOL1* allele, G1.



Progress:
Initiation → targeting construct: 2 months
targeting construct → confirmed ESC: 2 months
ESC → GLT: 4 months

OzBIG/goGermline™
Average model development timelines to first experiments



For more information:

Ozgene: https://www.ozgene.com/
Gen-H: https://www.gen-h.de/

OzBIG press release: