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Novel Technologies for Optimizing Speed and Quality of Custom Transgenic Mouse Models

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Abstract

Three innovative technologies were developed to enable the generation of better translational, transgenic mouse models in the shortest possible time, without the use of CRISPR, shortening the time to initial experiments by 3 to 4 months. Data will be presented describing goGermline 2.0, OzBIG, and the Double Flex Switch System in recently established models.

goGermline 2.0 is a method aimed at improving efficiency of generating transgenic chimeras in which all the chimeras are: 1) male, and 2) can transmit the genome of the injected, targeted ES cell, but not that of the host embryo. This method ensures production of germline-transmitted F1 hets in the first litter for every model. To achieve this increased efficiency, two strains were engineered and crossed to produce host embryos in which male embryos are spermatogenesis-deficient and can be visually distinguished from females. The overall impact is the reduction of the time to first experiments by an average of 4 months, and a significant 3Rs improvement based on a 95% reduction in the number of mice required to generate a new model.

OzBIG is a method for gene-targeted genomic replacement of DNA fragments at least an order of magnitude greater in size than fragments in conventional gene targeting constructs. OzBIG is based on developing gene targeting constructs with very large insertion fragments by engineering low-copy, bacterial artificial chromosomes rather than conventional high-copy plasmid clones. Optimization of design, construction, selection, and screening of gene-targeted ES cells has enabled the humanization of mouse genes by full genomic replacement of these genes with fragments well over 200 kb. Based on gene size distribution, OzBIG increases the number of genes that can be humanized with a single targeting event from 10% to 90%.

Double Flex Switches are allele designs based on the use of two DNA recombinase-mediated "flip and excise" (FLEx) elements to provide leakproof control of recombinase-activated, conditional knockout or knock-in alleles. The system was established to resolve the problem of leakiness using the common StopFLox in conditional transgenic alleles. Head-to-head comparison of Double FLEx and StopFLox elements in gene-targeted models shows the Double FLEx system completely blocks leaky expression, whereas StopFLox does not. We now use Flex or Double Flex switches in all conditional allele mouse model designs.

References

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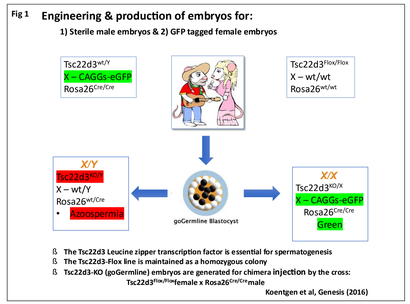
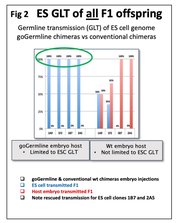
For more information:
https://www.ozgene.com/

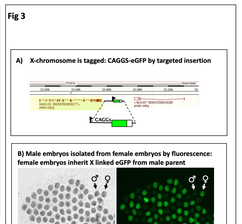
goGermline 2.0

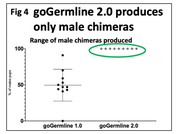
Problem: embryo host competition



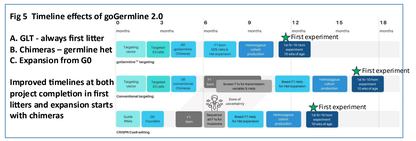
Strategy: engineer male embryo hosts





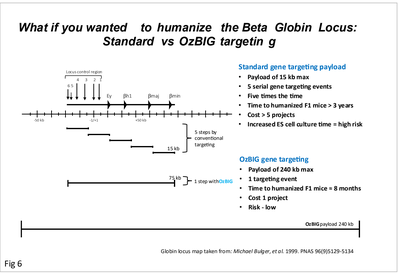


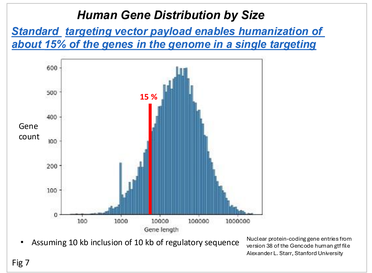
Result: fastest possible time to 1st exp



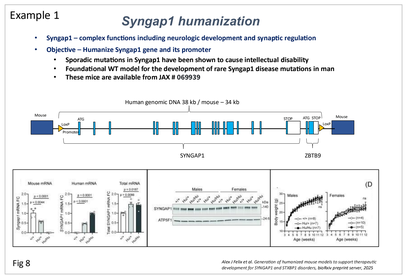
OzBIG

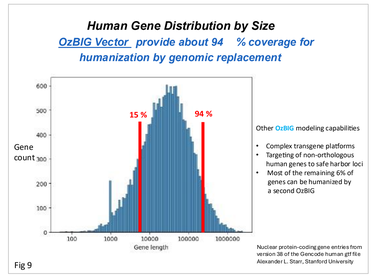
Problem: genomic replacement limited to 15 kb with standard vector



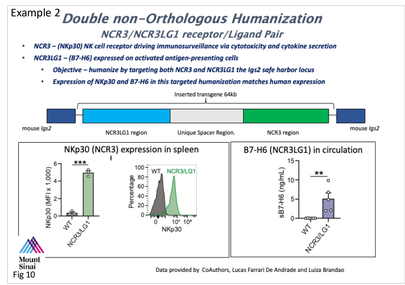


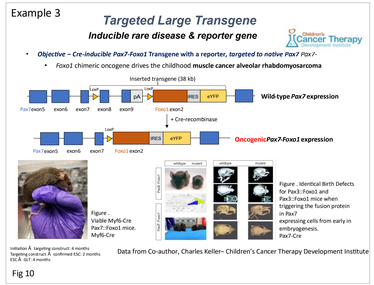
Strategy: Overcome payload limitations with BAC targeting constructs





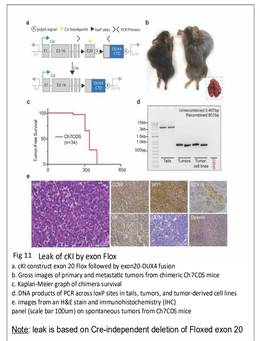
Result: Genomic replacement of any gene of any size is now possible

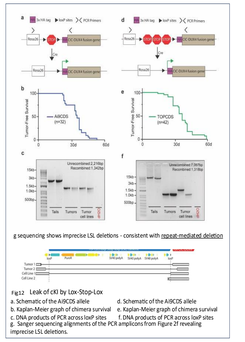


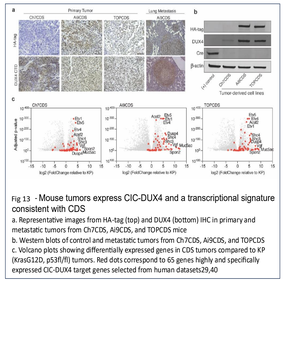


Double FLEx

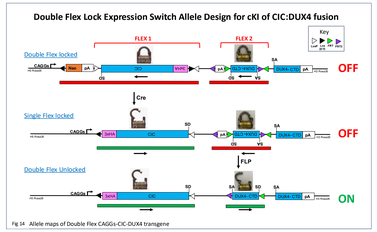
Problem: LoxPStopLoxP switches leak

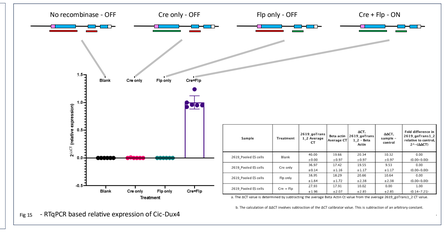






Strategy: use two FLEx switches, test in vitro and then in vivo





Result: Double FLEx enables otherwise impossible Onco & Immuno models