Research

Animal Gene Editing Laboratory (AGEL)

Genetically modified animals are powerful tools for studying physiological function of genes *in vivo* in the whole animal context. The primary goal of AGEL is to provide services in creating genetically modified animal models and to conduct research on developing new gene editing technologies and novel animal models for human diseases. AGEL provides a wide spectrum of gene editing services in animals, including creation of transgenic rodents by pronuclear injection and gene knockin/knockout in mice via ES cell targeting. AGEL employs a number of gene editing methods including the recently developed TALEN and CRISPR/Cas9 nuclease-based techniques. We provide a comprehensive range of services that cover all of the steps involved in generating genetically modified animals including design of gene targeting strategy, synthesis and preparation of reagents and production of F1 heterozygotes etc.

Services Provided by AGEL

1) Gene targeting via ES cells

- Design and construction of gene targeting vector
- Generation of gene targeted ES cell lines
- Creation of knockout/knockin chimeras by blastocyst injection
- Production of F1 heterozygotes (germline transmission)

For floxed conditional knockout mice, we strongly advise researchers to search the IMPC database (https://www.mousephenotype.org/) to find out if the consortium has already generated targeted ES cell lines or knockout animals for the gene of interest. Researchers may directly order targeted ES cells from the consortium, and AGEL can generate the knockout animals by blastocyst injection.

2) Rodent transgenesis

- Construction of tissue specific promoter driving transgene expression cassette
- BAC engineering: insertion of reporter gene, exon deletion or single/multiple nucleotide mutations
- Stable transfection and screening for ES cells containing the genetically modified BAC
- Creation of transgenic animals by pronuclear injection or blastocyst injection (BAC transgenesis)

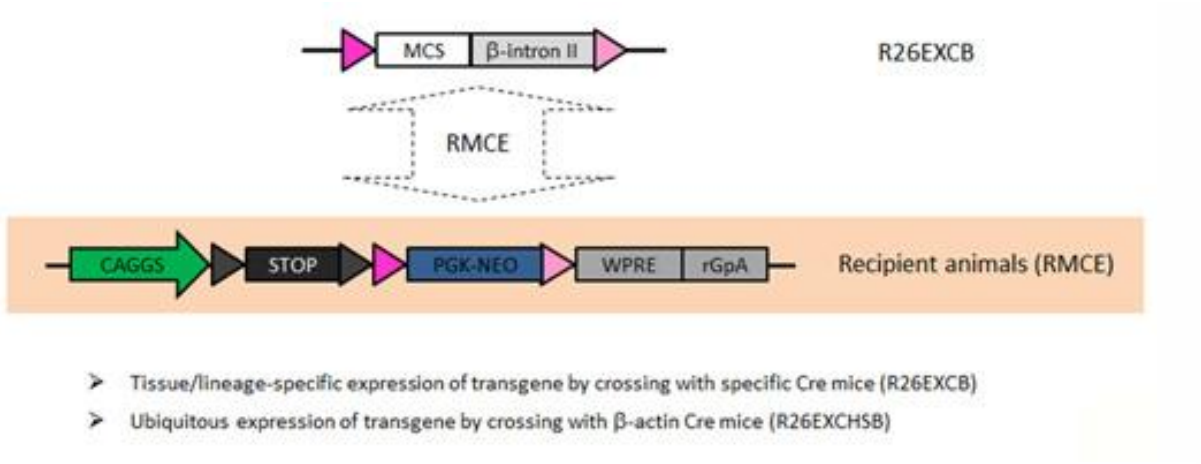
Pronuclear injection of transgene construct is the traditional way to overexpress a protein coding gene or to express a reporter gene under a tissue-specific promoter in animals. However, the expression pattern of the transgene in animals generated by this method can be highly variable from line to line, and from generation to generation, due to random

integration (positional and copy number) of the transgene. These issues occur less frequently (but are not completely eliminated) with BAC transgenesis.

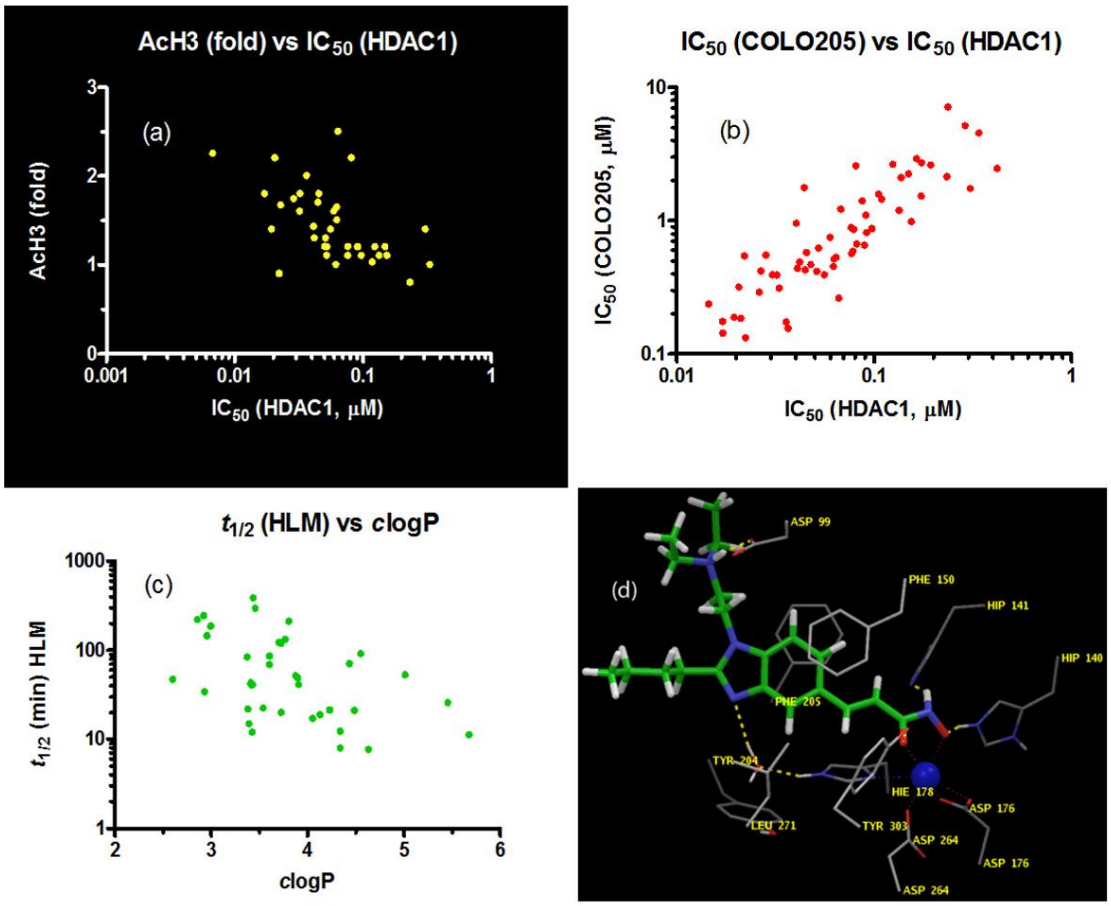
3) Transgene expression at Rosa26 locus

- Cre-mediated tissue/lineage-specific transgene expression at *Rosa26*
- Tetracycline inducible transgene (protein-coding gene or shRNA) expression at *Rosa26* with Cre-mediated tissue/lineage specificity

As a more precise alternative to pronuclear injection of expression constructs, we have developed a transgene expression platform that allows for a transgene cassette to be integrated as a single copy specifically at the *Rosa26* locus. The expression of the transgene is under the control of a ubiquitous CAGGS promoter with a preceding floxed transcriptional stop signal inserted between the promoter and the transgene coding sequence. Tissue/lineage specificity of transgene expression is achieved by Cre-mediated deletion of the floxed “STOP”. Thus, by crossing with lines expressing Cre under the control of a range of tissue-specific promoters one can achieve different patterns of transgene expression with same animal model, ─ one model for multiple studies. Transgene expression can also be controlled temporally by tetracycline induction. The highly controllable (spatial and temporal) and predictable (single copy at predetermined genomic locus) nature of this system makes it ideal for studying the pathogenesis of gene mutations that may cause predisposition to human disease, because individual transgenic lines with different mutations can be directly compared for phenotypic differences. In addition to protein-coding genes, this system also supports shRNA expression under a well characterized miRNA expression cassette. This facilitates acute knockdown of gene expression at any developmental stage (tetracycline-inducible) and in any tissue (with a specific Cre line of choice). Integration of individual transgenes at the docking site in ES cells is mediated by a highly efficient recombinase-mediated cassette change (RMCE) process, which allows for rapid generation of multiple transgenic lines simultaneously.



- Tissue/lineage-specific expression of transgene by crossing with specific Cre mice (R26EXCB)
- Ubiquitous expression of transgene by crossing with β-actin Cre mice (R26EXCHSB)

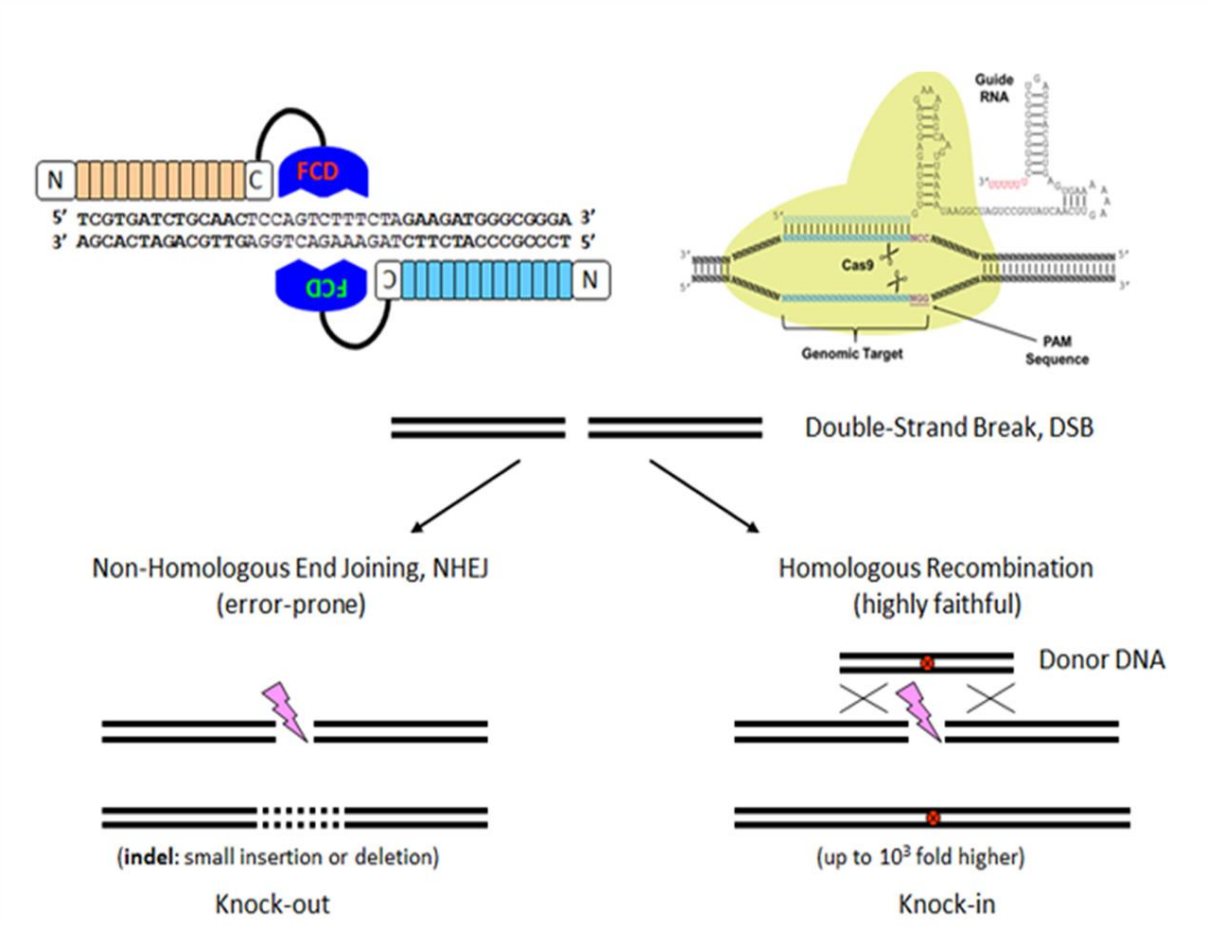


4) TALEN & CRISPR/Cas9 mediated gene editing in mice and rats

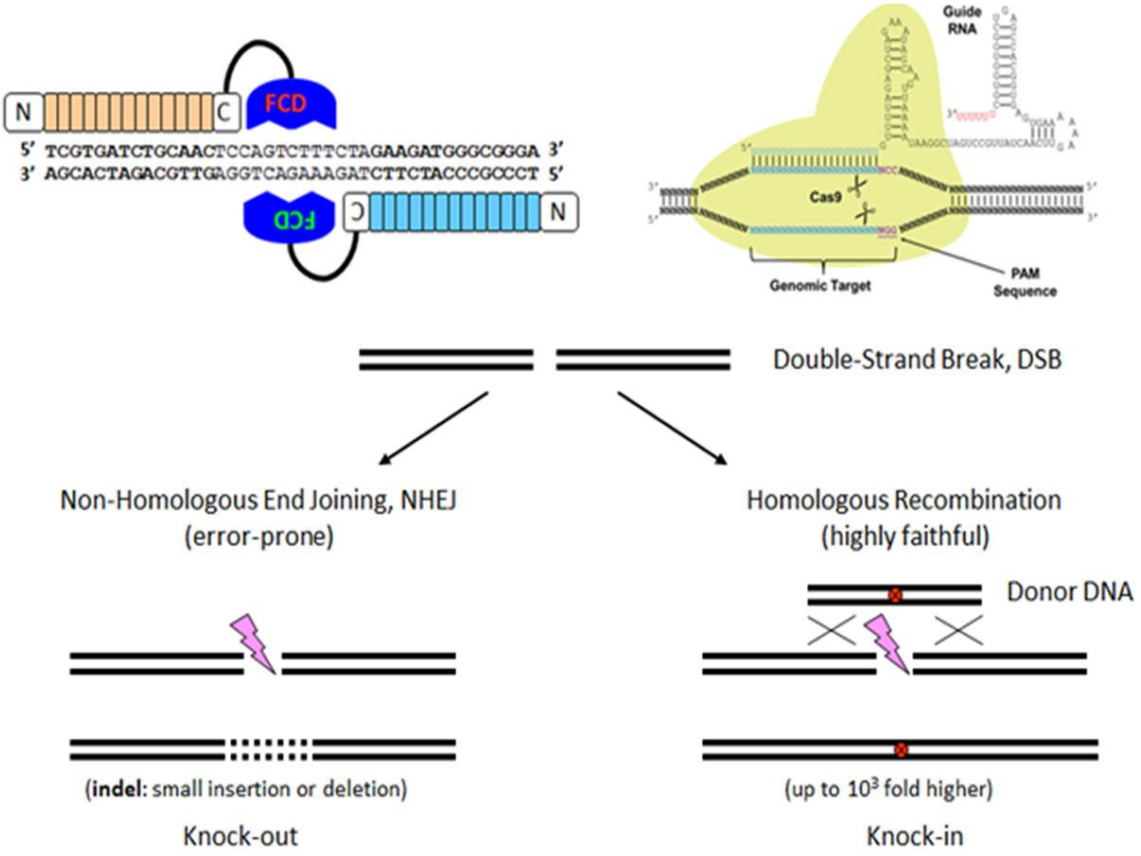
- Individual or large scale TALEN plasmid assembly and activity assay
- TALEN or CRISPR/Cas9 mediated reading frame shift (*indel*) or single/multiple nucleotide mutagenesis in mice and rats

AGEL provides full support for the creation of genetically modified rodents using TALEN or CRISPR/Cas9 technology, including assembly and construction of specific TALEN plasmids, activity testing of TALENs and gRNA (see RESOURCES), reading frame shift mutagenesis in cells and animals (mice and rats), oligonucleotide-mediated single/multiple nucleotide

mutagenesis in mice and rats etc. AGEL's services are comprehensive, from design of the targeting strategy, synthesis and preparation of the reagents, microinjection of oocytes, to analysis of chimeric founders by deep sequencing and breeding of mutation segregated F1 heterozygotes.



5) Ad hoc services: In addition to the services listed above, AGEL provides advice and technical supports for custom's projects relating to genetically modified animals and all enquiries are welcome. Please contact us AGEL-BRC@brc.a-star.edu.sg and we will get back to you as quickly as possible.


N
C
FCD
5′ TCGTGATCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGGA 3′
3′ AGCACTAGACGTTGAGGTCAGAAAGATCTTCTACCCGCCCT 5′
FCD
C
N
Guide RNA
Cas9
Genomic Target
PAM Sequence
Double-Strand Break, DSB
Non-Homologous End Joining, NHEJ
(error-prone)
Homologous Recombination
(highly faithful)
Donor DNA
(indel: small insertion or deletion)
Knock-out
(up to 10³ fold higher)
Knock-in