

GENE TARGETING SERVICES

Precise Genome Editing Tools

PolyGene provides a complete range of gene targeting services in mice and other rodents. Constitutive and conditional knockouts and knock-ins are offered using homologous recombination in ES cells, or via nuclease-based engineering technologies such as TALENs or CRISPR/Cas9. PolyGene is highly specialized in difficult-to-achieve genomic alterations such as the manipulation of repetitive regions, large targets, or highly sophisticated configurations.

Gene Targeting

A knockout mouse is a biological model for the disruption of a gene of interest (GOI). The loss of expression often causes a change in phenotype and enables *in vivo* studies of gene function. Likewise, a gain of function is achieved by targeting a gene with the wild-type version of a foreign gene or marker, or with a mutant gene copy ("knock-in").

PolyGene offers a complete gene targeting service, or optionally, the execution of partial procedures. Careful design and free-of-charge consultancy are viewed as an inseparable part of an optimal design, as are unlimited and meticulous post-project care.

Constitutive Knockout



The gene of interest or the relevant exon (GOI) is deleted constitutively.

Conditional Knockout



Flanking the GOI by recombination sites (e.g. loxP) allows its deletion in a tissue-specific or inducible manner.

Constitutive Knock-in



The GOI is replaced by a mutant version, or by a marker cDNA.

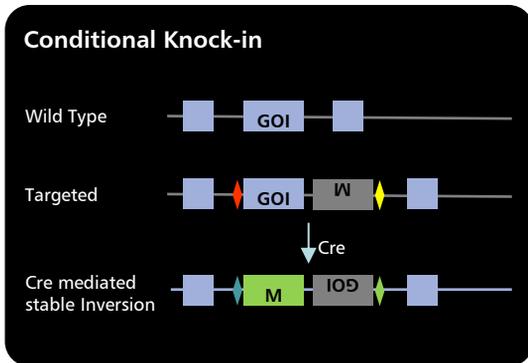
What Method to Choose?

Strategy	Scope	Method of choice	Timelines
Knockout	All	Nucleases	3-4 months
Conditional Knockout	Flanking 0.5 - 3kb	ES cell technology	8-10 months
Conditional Knockout	Flanking > 3kb	Double targeting, Nucleases	12-15 months
Knock-in	Point mutation	ES cell technology, Nucleases	8-10 months
Knock-in	cDNA, Marker	ES cell technology	8-10 months

Specialized Gene Targeting

While gene targeting is always tailor-made, there are certain strategies that require specialized tools aimed at enabling unusual goals (conditional knock-ins are an example); or just new techniques (e.g., nuclease-based targeting technologies).

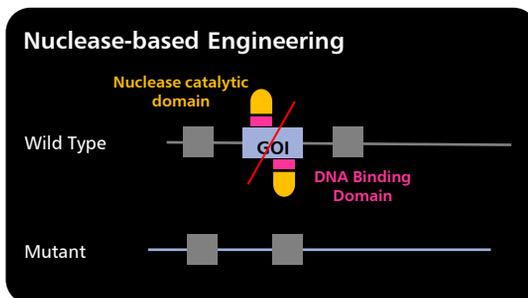
For conditional knock-ins, where a gene is replaced with a mutant only under certain conditions or in certain tissues (nicely mimicking what could be found in a human disease case), we offer a selection of strategies.



Cre/Flp mediated recombination opens up many avenues to mimic human disease within an animal model. A silent mutant allele (M) is inserted into the genome, until activated by Cre mediated inversion. Depending on the transcriptional regulation of Cre, the mutant allele can be activated spatially or upon induction.

The example shows stable, irreversible inversion by the use of mutant lox sites. Stable reversion to wild type can also be achieved when using additional Cre or Flp mutants; or minigene approaches allowing for conditional activation of a foreign cDNA. For all strategies, tested base vectors are available at PolyGene.

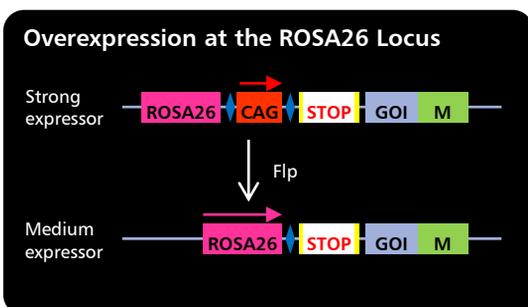
Simple targeting tasks can now be accomplished by using a variety of nuclease based techniques, thereby circumventing ES cells unavailable for certain species or strains:



Nuclease-based technology is particularly useful for projects that require rapid generation of constitutive knockout animal models.

Here, a TALEN acts as a heterodimer, thereby increasing specificity to a complexity of 30–40 base pairs. In a CRISPR/Cas9 system, the sequence specific RNAs are designed similarly, and can either be used as a monomer using nucleases, or as a dimer with nickases to reduce off-site effects.

Ready-to-go transgenesis: rapid and convenient overexpression at the ROSA26 locus:



Knocking the cDNA into a well characterized locus such as ROSA26 is a means to control insertion site and thus reproducibility.

PolyGene offers a promoter switch technology, which allows tissue-specific or inducible GOI expression either from the ROSA26 promoter or the ~100-fold stronger CAG promoter. The pretested model can accommodate expression marker coexpression, and is Cre-inducible.

For more information on each topic, please find the corresponding product sheet or visit us at www.polygene.ch.