# TALEN-based Gene Targeting Services

## Speedy Generation of Targeted Models

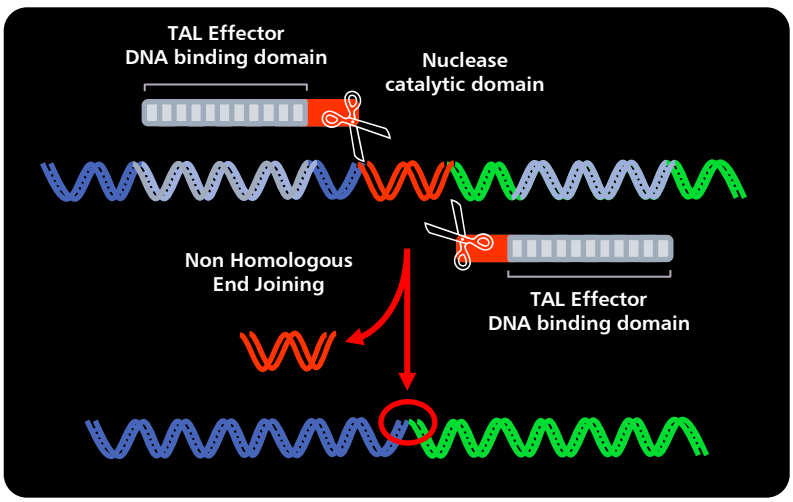
Tailor-made TALENs are used for highly site-specific gene mutations in the mammalian genome. PolyGene is offering services based on this technology.

### Introduction

TALEs (Transcription Activator-Like Effectors) are proteins that bind to DNA in a sequence-specific way. They were first discovered in the plant pathogen *Xanthomonas*, where they regulate plant genes during infection by the pathogen.

Each TALE contains a central repetitive region consisting of varying numbers of repeat units of typically 33-35 amino acids. It is this repeat domain that is responsible for specific DNA sequence recognition. Each repeat is almost identical with the exception of two variable amino acids termed the repeat-variable diresidues. The mechanism of DNA recognition is based on a code where one nucleotide of the DNA target site is recognized by the repeat-variable diresidues of one repeat.

By fusing such a TALE to a Nuclease (a "TALEN"), a highly specific DNA scissor is made. The TALEN sequences are modifiable and can be accurately matched to a target sequence by the use of a simple conversion code. The resulting TALENs have a high level of specificity, due in part to the length of their recognition site (between 15 and 20 base pairs), and also to their concerted action as a dimer. A TALEN acts as a heterodimer (2 units of a TALE DNA binding domain fused to a catalytic domain), cleaving two close sequences, and thereby increasing specificity to a complexity of 30-40 base pairs.

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## Basic Strategy

The *in silico* deduction of a functional pair of matching TALENs, then their validation on the target sequence, and finally their preparation as functional mRNAs, is completed within 8 weeks, and mutant animals are generated via intracytoplasmatic TALEN mRNA microinjection into wild-type oocytes. Within the cells, TALEN generated double strand breaks are repaired by Non Homologous End Joining, a natural repair mechanism that can be used to introduce nucleotide deletions to inactivate or knock-out a specific target gene, making it the method of choice if a constitutive knockout must be achieved in a rapid manner. A rate of approximately 10% founder offspring per injected oocyte is expected.

Another mechanism of double strand break repair uses homologous recombination, and is based on the presence of coinjected homologous DNA fragments. Iti s efficient for a majority of small targets (e.g., introduction of point mutations). However, for the insertion of large fragments (e.g. markers or cDNA's) the efficiency is not sufficient for commercial applications.

## Positioning

Generation of knockout animals via TALEN technology is particularly useful for projects that require **rapid generation of constitutive knockout animal models**. In a typical experiment, several lines are generated, 2/3 of which expected with frame-shift mutations, and thus, fully functional knockouts. Another advantage is the **strain- and species-independence**: all microinjectable mammalian oocytes can be used, in particular mouse strains that are otherwise refractory for the generation of knockout models because of their lack of available ES cell lines, as well as rats and rabbits.

References:
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