Dual Promoter Models

Reproducible Transgenesis via ROSA26/CAG Promoters

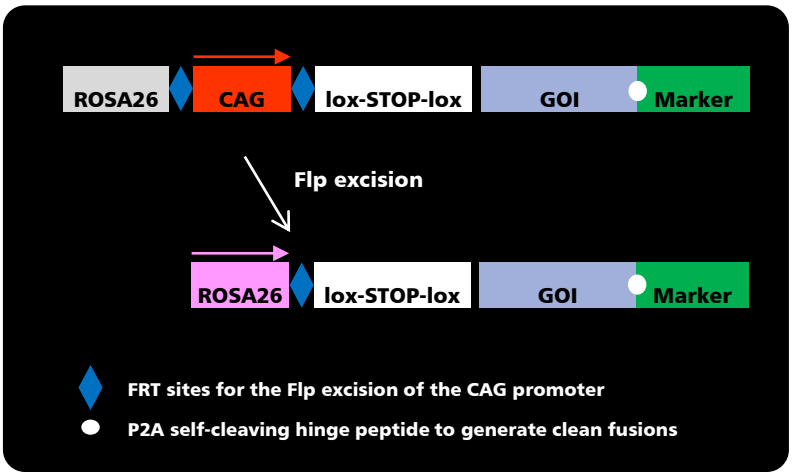
Conditional transgene expression in two versions (high/low), and co-expressed with marker proteins. Tightness, conditional activation and marker expression are pretested and guaranteed.

Rationale

The obvious downside of random transgenesis is its randomness: although proper design will reasonably grant success, several mouse lines are generated and must be bred and analysed, and an appropriate mouse line chosen. Reproducible model designs are desirable to limit excessive animal experimentation as well as manpower.

The ROSA26 locus is an excellent target for the placement of a transgene. This gene was identified as a ubiquitously expressed mouse gene with unknown function, which does not show haploinsufficiency phenotypes (morbidity upon loss of one allele), and in fact, not even knocking out the gene on both alleles causes a phenotype. It is hence well-suited for knocking in genes that require ubiquitous (though limited) expression, or, because of its property as an open locus, allows to knock in a promoter-cDNA cassette.

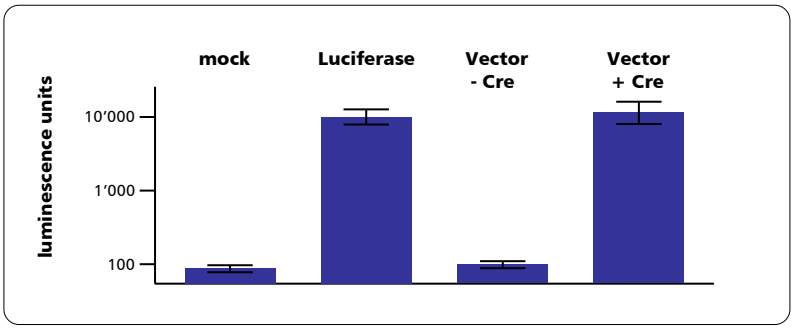
PolyGene takes this a step further: the standard targeting yields two versions (low ubiquitous or high ubiquitous expression, both conditional). As a free option, a marker can be added for tracking gene expression, and, because all vectors are pre-established, this duplicate model is offered at competitive pricing.



A cDNA (Gene of Interest, "GOI") is inserted either simply, or as a gene fusion (5' or 3' optional, with a choice of expression marker genes such as fluorescence or luminiscence marker genes). Targeting is efficient at the ROSA26 locus, and resulting chimeric mice are immediately bred with Flp deleters. As a result, the first generation of mice includes both, ROSA26-driven and CAG-driven transgenics.

Performance

The mouse ROSA26 gene is ubiquitously expressed, and knock-ins have been widely used for expression of foreign DNA. The classic CAG promoter is composed of the chicken beta-actin promoter and a CMV-derived enhancer, and is amongst the most common drivers for ubiquitous transgene expression. *In vivo* studies show about 100-fold enhancement of expression with marker genes when compared to ROSA26-based markers. We take advantage of both, while carefully avoiding interference between them. A tight stop-cassette allows for Cre/loxP activated conditional expression.



The STOP cassette is tight: before/after Cre/loxP recombination of a client vector. Cell culture baseline ("mock") corresponds to native vector, the recombined vector reaches maximum luminescence levels of a constitutive luciferase marker.
CAG-based expression is shown.

Price and Time Lines

Service	Duration	Deliverables	Price
Complete model based on your cDNA clone	5-7 months	founder animals for Rosa26-cDNA AND CAG-cDNA	28'000 €
Use of additional markers not listed	1-2 months	Full non-exclusive FTO if rights are available	please inquire
Accessory services (breeding, expression testing etc.)	1-2 months	tbd	please inquire