

***Rosa26-CAG-LSL-EGFP-3*HA-WPRE-polyA* Mouse Model Strategy**

-CRISPR/Cas9 technology

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Project Overview

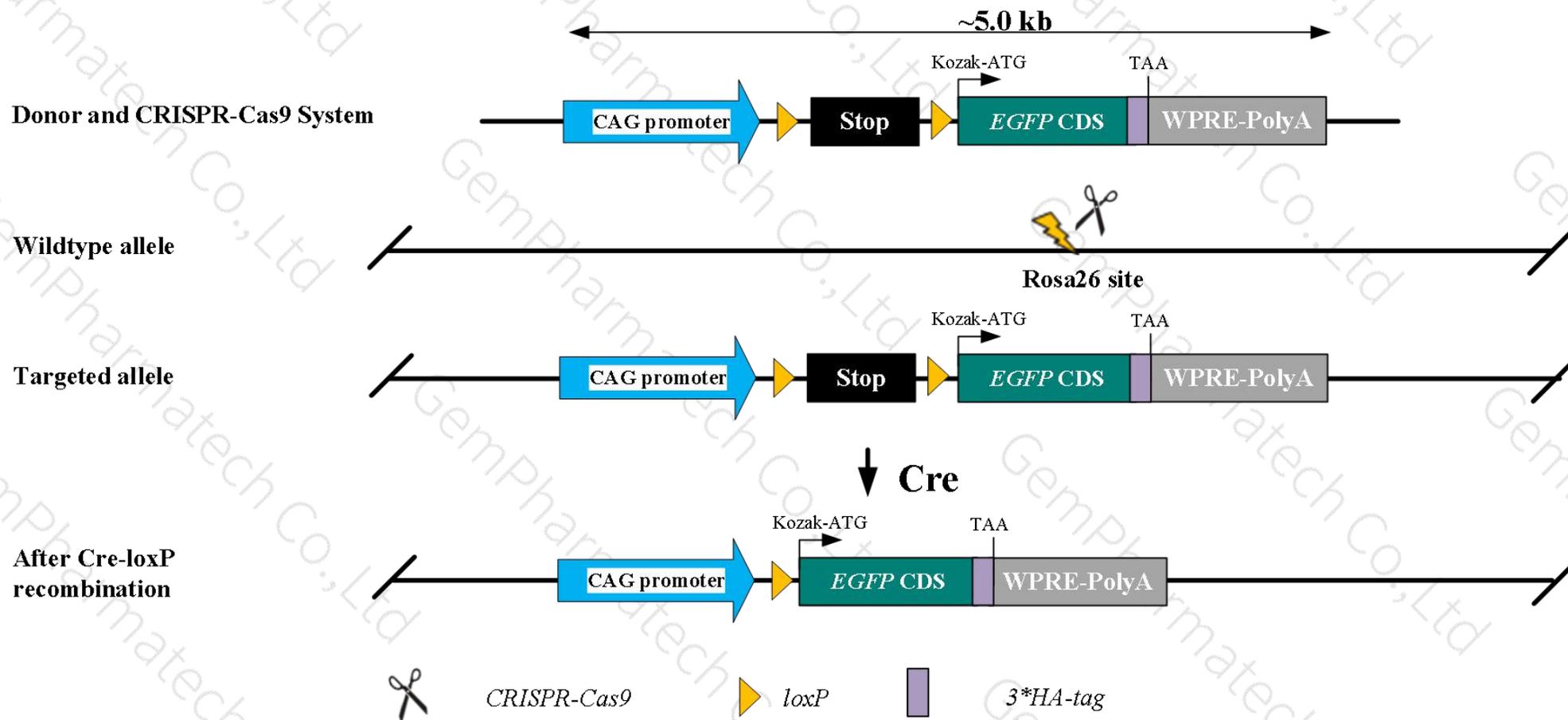
Project Name Rosa26-CAG-LSL-EGFP-3*HA-WPRE-polyA

Project Type Cas9-KI(Rosa26)

Background C57BL/6JGpt

Strategy

The *CAG-LSL-EGFP-3*HA-WPRE-polyA* fragment was inserted into *Rosa26* site of mice and the schematic diagram is as follows:



Technical Description

- *Rosa26*, located on mouse chromosome 6, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- A construct containing the loxP-floxed cassette with *EGFP-3*HA* driving by CAG promoter was inserted into the Gt(ROSA)26Sor locus via homologous recombination. The floxed Stop cassette contains a strong pA stop sequence which prevents the *EGFP-3*HA*. This entire targeting vector was inserted between exons 1 and 2 of the Gt(ROSA)26Sor locus.
- In this project, the *CAG-LSL-EGFP-3*HA-WPRE-polyA* fragment was inserted into *Rosa26* site of mice by CRISPR/Cas9 technology. The brief process is as follows: CRISPR/Cas9 system and donor were microinjected into the fertilized eggs of C57BL/6JGpt mice, and obtained positive F0 generation mice. The F0 positive mice were mated with C57BL/6JGpt mice, The pups will be genotyped by PCR, followed by sequence analysis.

- *Rosa26* is located on Chr6. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., Cre) strains, if the other gene is also on Chr6, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

如您有任何疑问，欢迎垂询。

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