

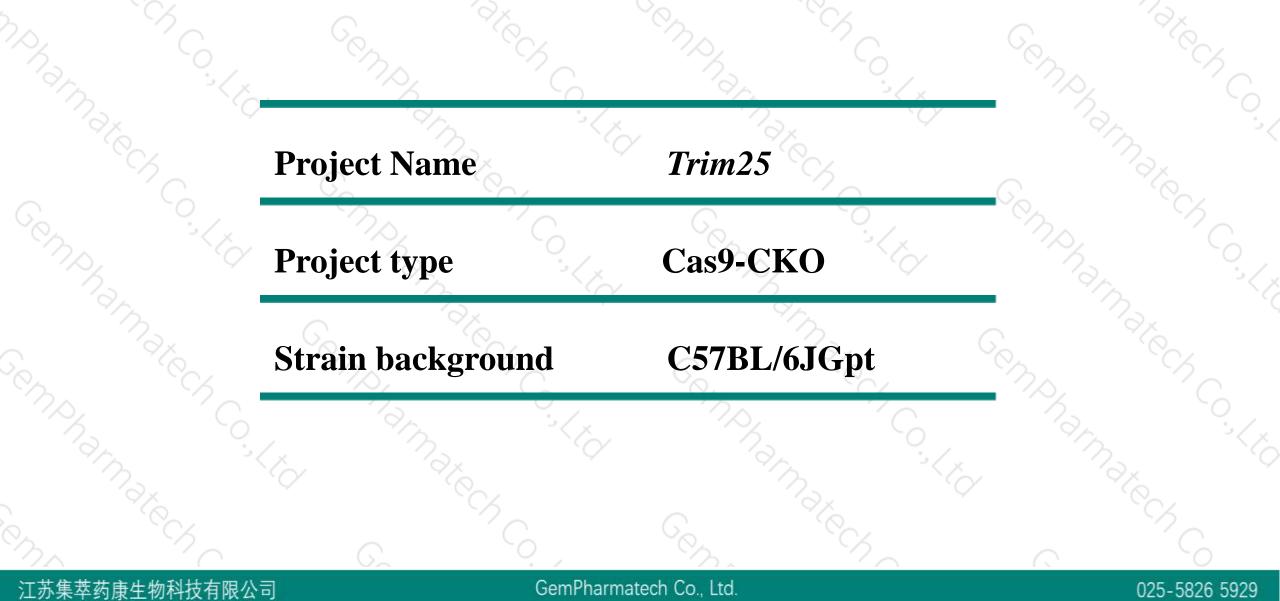
Trim25 Cas9-CKO Strategy andramater Co-te

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Project Overview

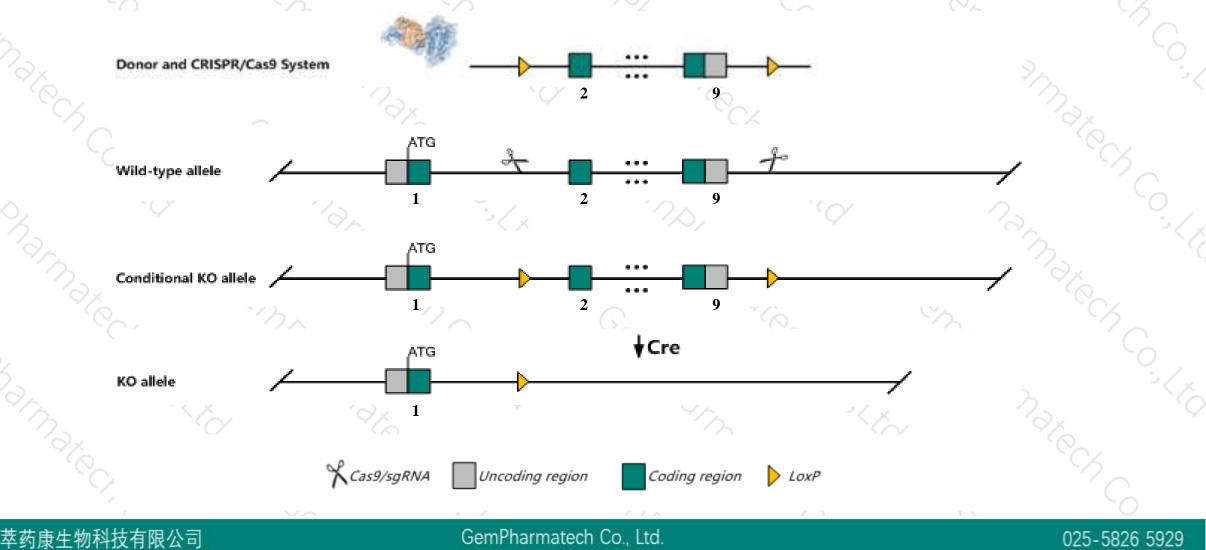




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Trim25* gene. The schematic diagram is as follows:





- The Trim25 gene has 3 transcripts. According to the structure of Trim25 gene, exon2-exon9 of Trim25-203 (ENSMUST00000107896.9) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Trim25* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice



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- According to the existing MGI data, Engineered mutations result in a compromised response to estrogen resulting in functional but small uteri.
- The Trim25 gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



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Trim25 tripartite motif-containing 25 [Mus musculus (house mouse)]

Gene ID: 217069, updated on 3-Feb-2019

Summary

Official Symbol	Trim25 provided by MGI
Official Full Name	tripartite motif-containing 25 provided by <u>MGI</u>
Primary source	MGI:MGI:102749
See related	Ensembl:ENSMUSG0000000275
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA960166, AL022677, EFP, Zfp147
Expression	Ubiquitous expression in placenta adult (RPKM 10.1), adrenal adult (RPKM 9.4) and 28 other tissuesSee more
Orthologs	human all

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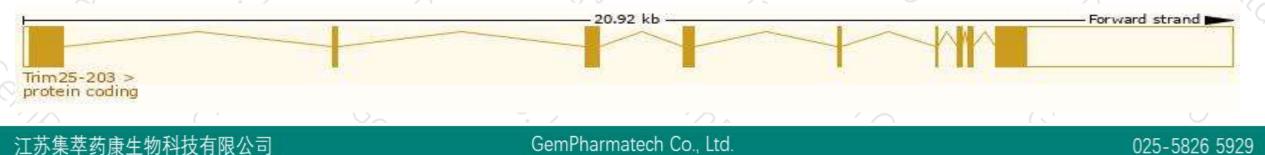
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trim25-203	ENSMUST00000107896.9	5590	<u>634aa</u>	Protein coding	CCDS36278	<u>Q61510</u>	TSL:1 GENCODE basic APPRIS P2
Trim25-201	ENSMUST0000000284.6	5566	<u>626aa</u>	Protein coding	-	Q5SU71	TSL:1 GENCODE basic APPRIS ALT2
Trim25-202	ENSMUST00000100627.8	1433	<u>387aa</u>	Protein coding	-	Q5SU72	TSL:5 GENCODE basic

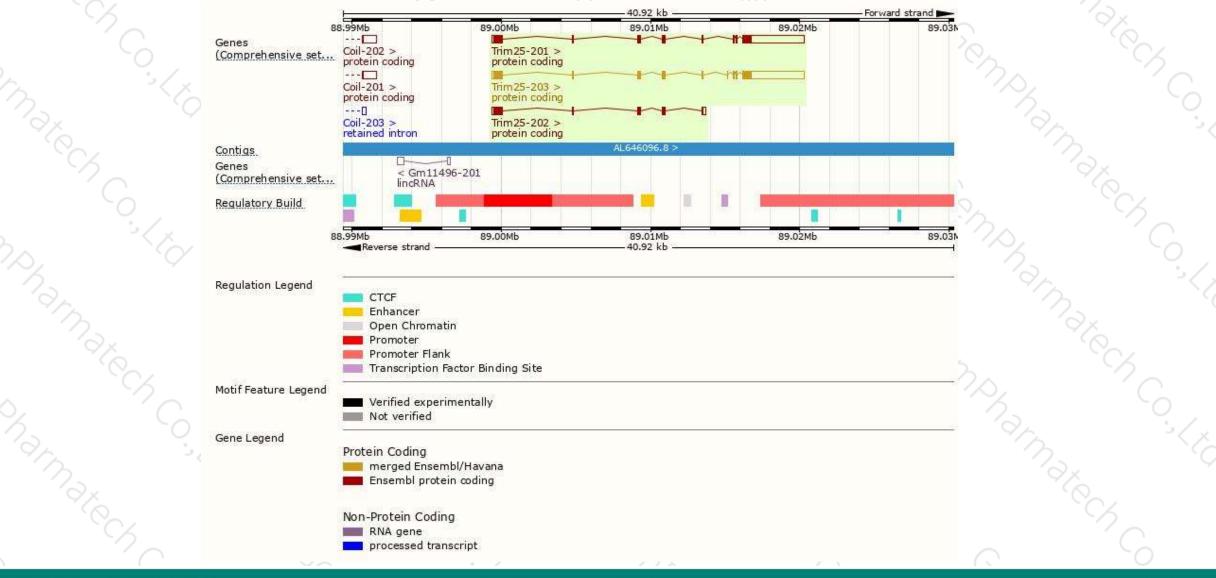
The strategy is based on the design of *Trim25-203* transcript, The transcription is shown below



Genomic location distribution



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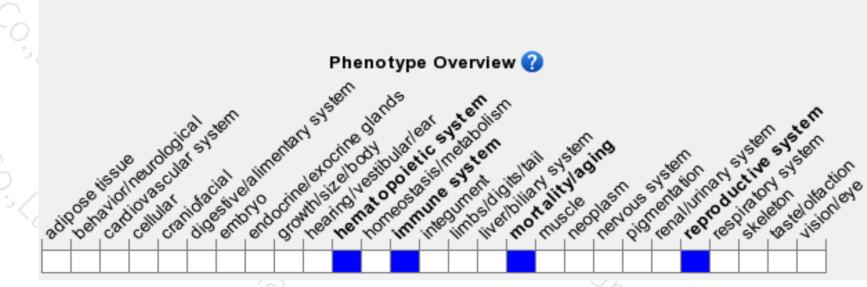
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Engineered mutations result in a compromised response to estrogen resulting in functional but small uteri.





If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



