

Gas5 Cas9-CKO Strategy

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Project Overview



Project Name

Gas5

Project type

Cas9-CKO

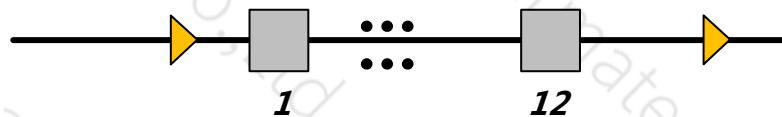
Strain background

C57BL/6JGpt

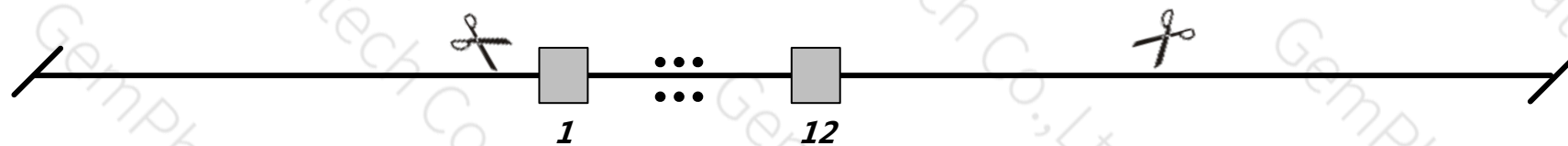
Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gas5* gene. The schematic diagram is as follows:

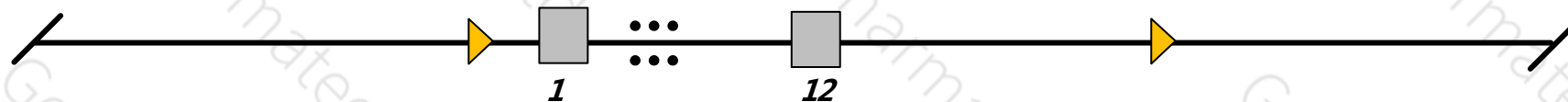
Donor and CRISPR/Cas9 System



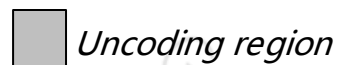
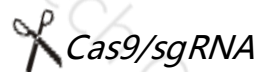
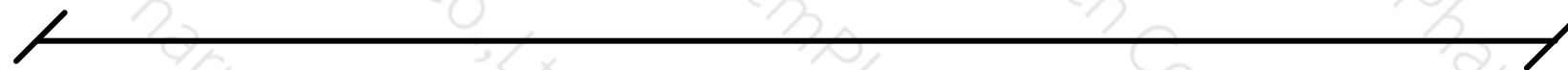
Wild-type allele



Conditional KO allele



KO allele



Technical routes

- The *Gas5* gene has 25 transcripts. According to the structure of *Gas5* gene, exon1-exon12 of *Gas5*-224 (ENSMUST00000162558.7) transcript is recommended as the knockout region. The region contains all of the sequence. Knock out the region will result in disruption of gene function.
- In this project we use CRISPR/Cas9 technology to modify *Gas5* 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 or cell types.

- Knocking out this gene will also knock out 15 genes including *Snord80*, *Gm25789*, *Snord47*, *Gm26224*, *Gm22489*, *Gm22357*, *Gm23212*, *Snord78*, *Gm50452*, *LOC115487643*, *LOC115487678*, *LOC115487638*, *LOC115487644*, *LOC115487645*, *LOC115487640*, with unknown effects.
- The *Gas5* gene is located on the Chr1. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Gas5 growth arrest specific 5 [*Mus musculus* (house mouse)]

Gene ID: 14455, updated on 18-Nov-2019

Summary

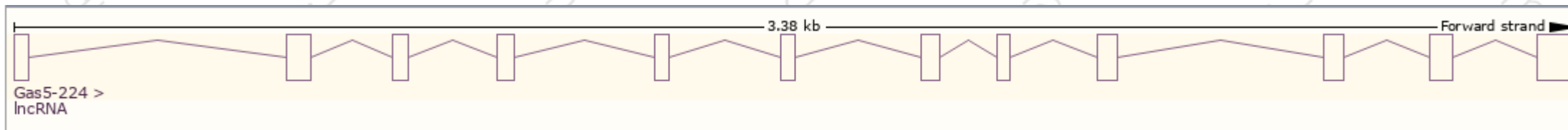
Official Symbol	Gas5 provided by MGI
Official Full Name	growth arrest specific 5 provided by MGI
Primary source	MGI:MGI:95659
See related	Ensembl:ENSMUSG00000053332
Gene type	ncRNA
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gas-5; Snhg2; Mir5117
Expression	Broad expression in liver E14 (RPKM 95.8), CNS E11.5 (RPKM 88.1) and 16 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

The gene has 25 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	Flags
Gas5-218	ENSMUST00000161229.7	2556	No protein	Retained intron	-	TSL1
Gas5-222	ENSMUST00000162163.7	1614	No protein	Retained intron	-	TSL1
Gas5-211	ENSMUST00000158890.7	1066	No protein	Retained intron	-	TSL5
Gas5-223	ENSMUST00000162289.7	904	No protein	Retained intron	-	TSL5
Gas5-204	ENSMUST00000158161.7	872	No protein	Retained intron	-	TSL5
Gas5-220	ENSMUST00000161461.7	793	No protein	Retained intron	-	TSL1
Gas5-208	ENSMUST00000158438.7	779	No protein	Retained intron	-	TSL5
Gas5-213	ENSMUST00000160429.7	759	No protein	Retained intron	-	TSL3
Gas5-205	ENSMUST00000158157.7	723	No protein	Retained intron	-	TSL3
Gas5-212	ENSMUST00000160152.7	712	No protein	Retained intron	-	TSL3
Gas5-202	ENSMUST00000158037.1	636	No protein	Retained intron	-	TSL2
Gas5-214	ENSMUST00000160497.1	629	No protein	Retained intron	-	TSL2
Gas5-219	ENSMUST00000161380.7	600	No protein	Retained intron	-	TSL5
Gas5-215	ENSMUST00000160516.1	572	No protein	Retained intron	-	TSL2
Gas5-216	ENSMUST00000160661.1	549	No protein	Retained intron	-	TSL3
Gas5-207	ENSMUST00000158404.7	539	No protein	Retained intron	-	TSL5
Gas5-201	ENSMUST00000065709.11	530	No protein	Retained intron	-	TSL2
Gas5-206	ENSMUST00000158399.7	422	No protein	Retained intron	-	TSL2
Gas5-221	ENSMUST00000161623.7	403	No protein	Retained intron	-	TSL2
Gas5-225	ENSMUST00000163001.1	370	No protein	Retained intron	-	TSL2
Gas5-203	ENSMUST00000158119.7	665	No protein	lncRNA	-	TSL5 GENCODE basic
Gas5-210	ENSMUST00000158706.7	557	No protein	lncRNA	-	TSL2 GENCODE basic
Gas5-224	ENSMUST00000162568.7	502	No protein	lncRNA	-	TSL1 GENCODE basic
Gas5-217	ENSMUST00000161095.7	430	No protein	lncRNA	-	TSL5 GENCODE basic
Gas5-209	ENSMUST00000158661.7	346	No protein	lncRNA	-	TSL3 GENCODE basic

The strategy is based on the design of *Gas5-224* transcript, The transcription is shown below



Genomic location distribution



If you have any questions, you are welcome to inquire.
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