GemPharmatech™

Hes7 Cas9-CKO Strategy

Designer:Xueting Zhang

Reviewer:Ruirui Zhang

Design Date: 2024-3-8

Overview

Target Gene Name

- Hes7

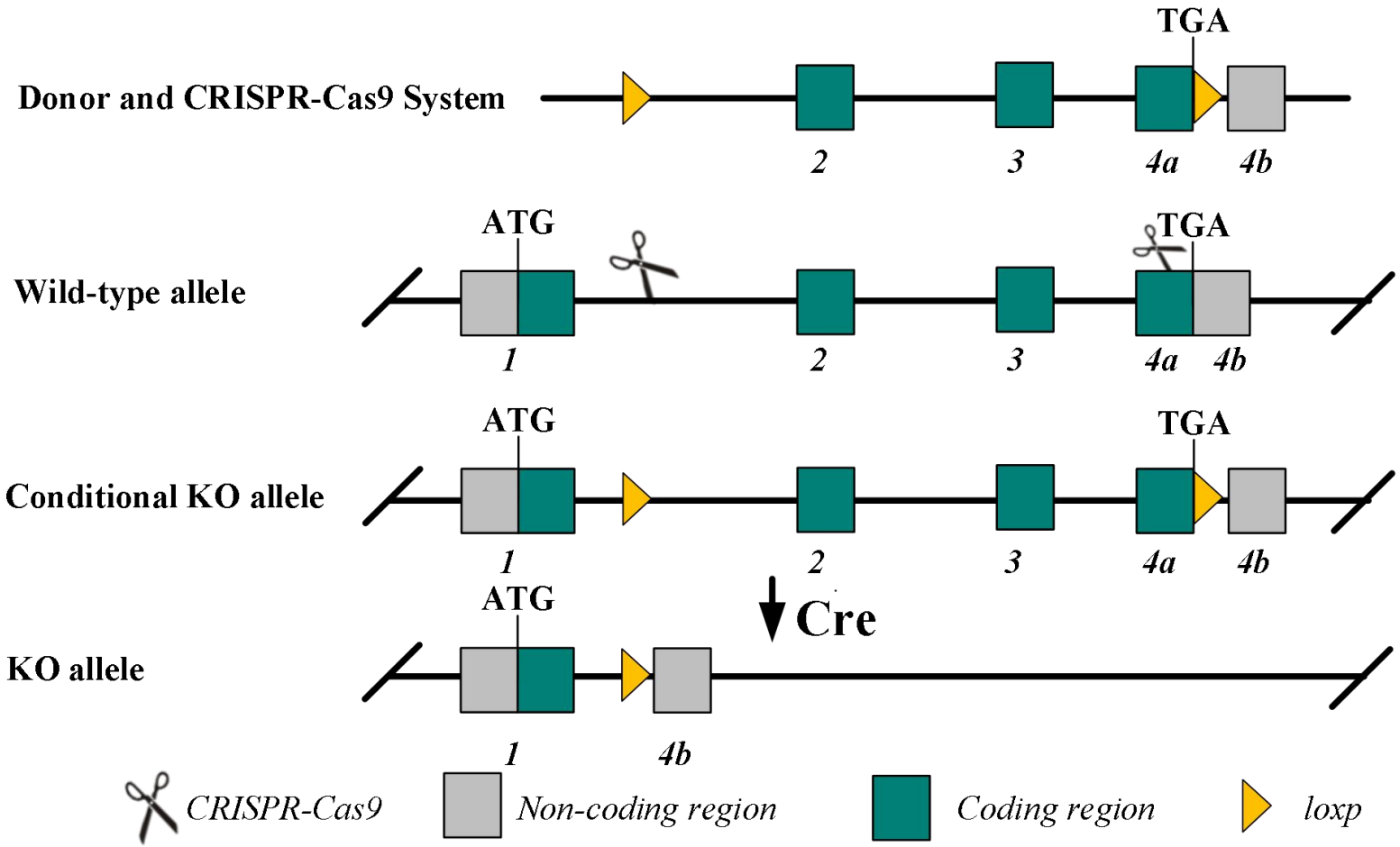
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Hes7* gene.

Technical Information

- The *Hes7* gene has 1 transcript. According to the structure of *Hes7* gene, exon2-exon4 of *Hes7*-201 (ENSMUST00000024543.3) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Hes7* gene. The brief process is as follows: CRISPR-Cas9 system 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- The flox mice will be 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.

Gene Information

Hes7 hes family bHLH transcription factor 7 [Mus musculus (house mouse)]

Gene ID: 84653, updated on 12-Apr-2023

Summary

Official Symbol	Hes7 provided by MGI
Official Full Name	hes family bHLH transcription factor 7 provided byMGI
Primary source	MGI:MGI:2135679
See related	Ensembl:ENSMUSG00000023781
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	bHLHb37
Summary	Enables RNA polymerase II transcription regulatory region sequence-specific DNA binding activity. Involved in negative regulation of transcription, DNA-templated. Acts upstream of or within several processes, including negative regulation of transcription by RNA polymerase II; post-anal tail morphogenesis; and somitogenesis. Predicted to be active in nucleus. Is expressed in several structures, including central nervous system; retina; small intestine; unsegmented mesenchyme; and urinary system. Human ortholog(s) of this gene implicated in spondylocostal dysostosis. Orthologous to human HES7 (hes family bHLH transcription factor 7). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

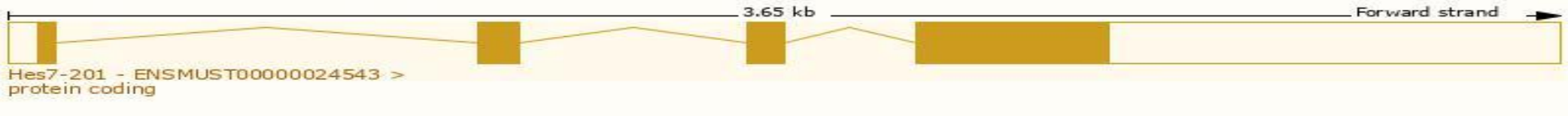
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 1 transcript,and the transcript is shown below:

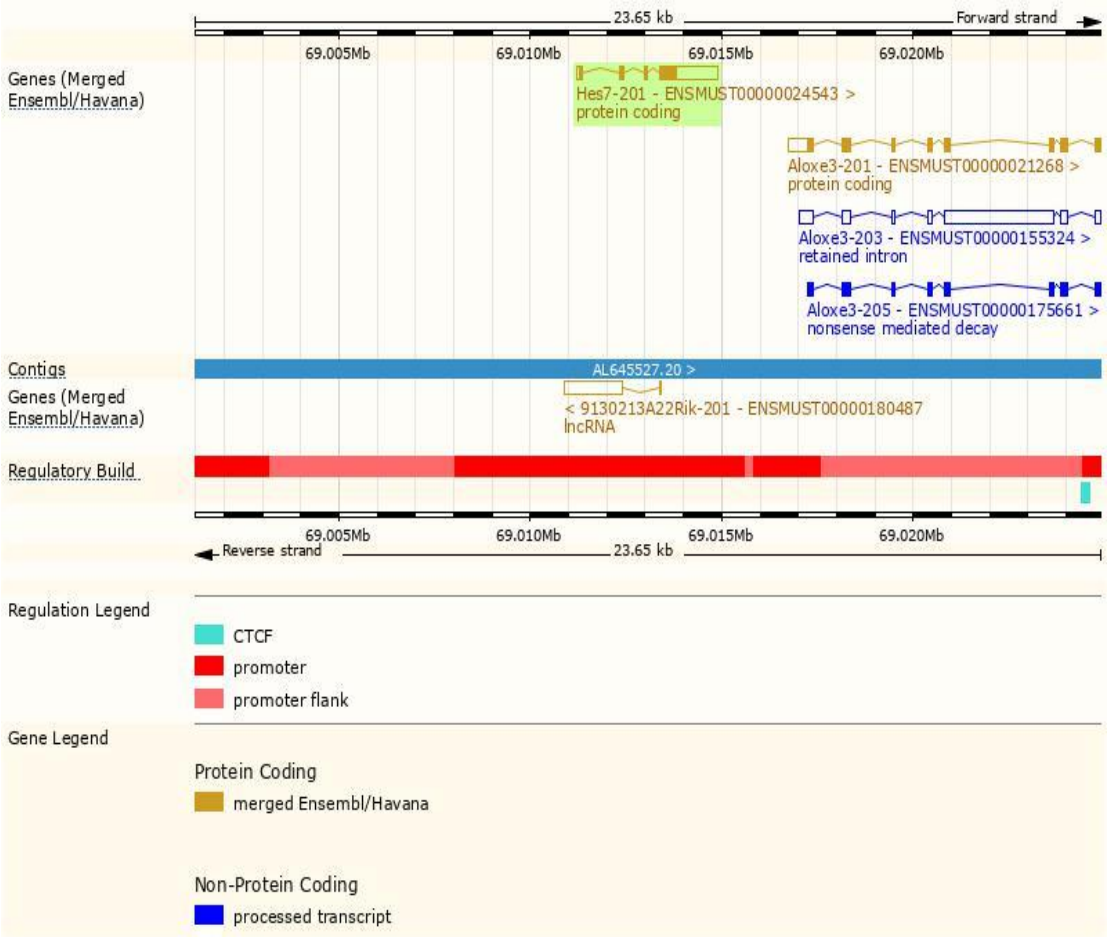
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000024543.3	Hes7-201	1809	225aa	Protein coding	CCDS24883	Q8BKT2	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1

The strategy is based on the design of *Hes7*-201 transcript, the transcription is shown below:



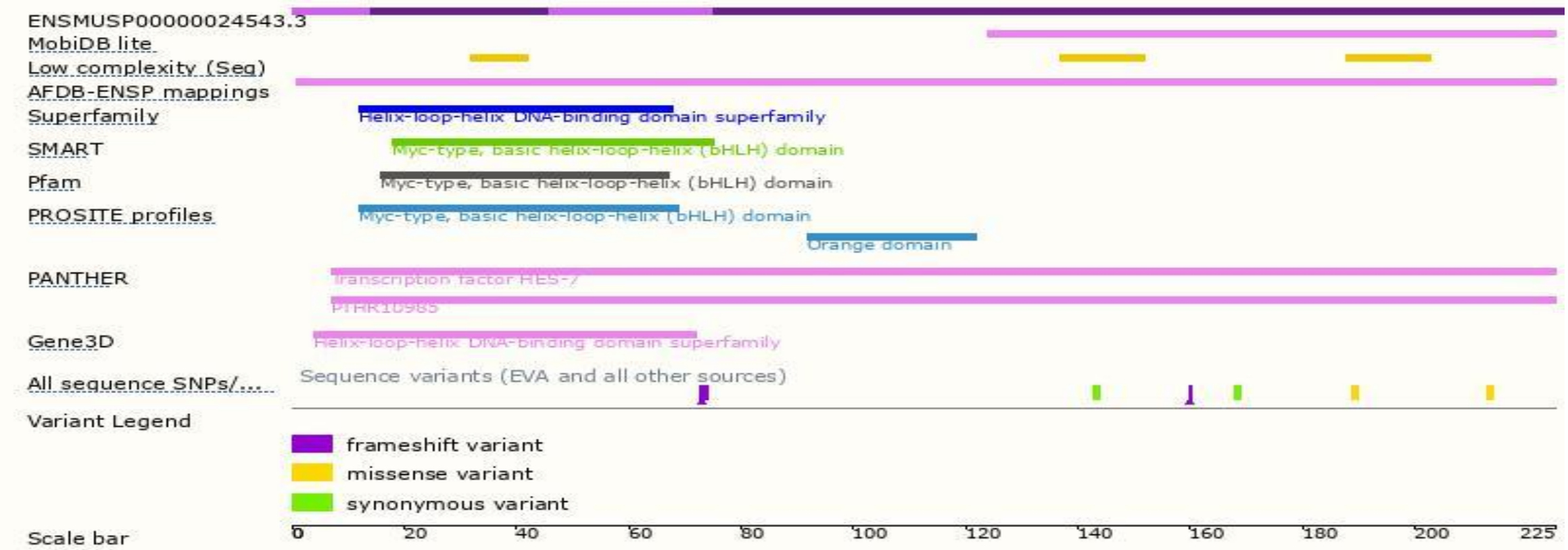
Source: https://www.ensembl.org

Genomic Information



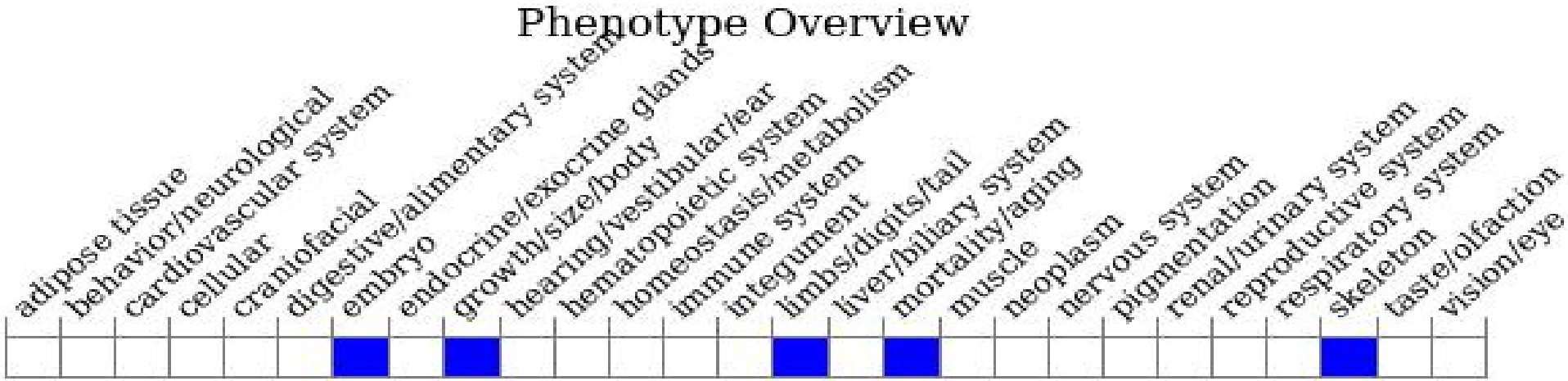
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygotes for a targeted null mutation exhibit disrupted somite formation leading to skeletal defects including short trunk and tail, reduced numbers of ribs, and deformed and fused vertebrae, and neonatal death. Some heterozygotes have tail kinks.

Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data,homozygotes for a targeted null mutation exhibit disrupted somite formation leading to skeletal defects including short trunk and tail, reduced numbers of ribs, and deformed and fused vertebrae, and neonatal death. Some heterozygotes have tail kinks.
- The floxed region is near to the N-terminal of *Aloxe3* gene,this strategy may influence the regulatory function of the N-terminal of *Aloxe3* gene.
- There will be 2-4 base mutations in 3'UTR of *Hes7* gene,and the effect is unknown.
- *9130213A22Rik* gene may be destroyed directly.
- *Hes7* is located on Chr11. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference

Bessho Y, et al., Dynamic expression and essential functions of Hes7 in somite segmentation. Genes Dev. 2001 Oct 15;15(20):2642-7

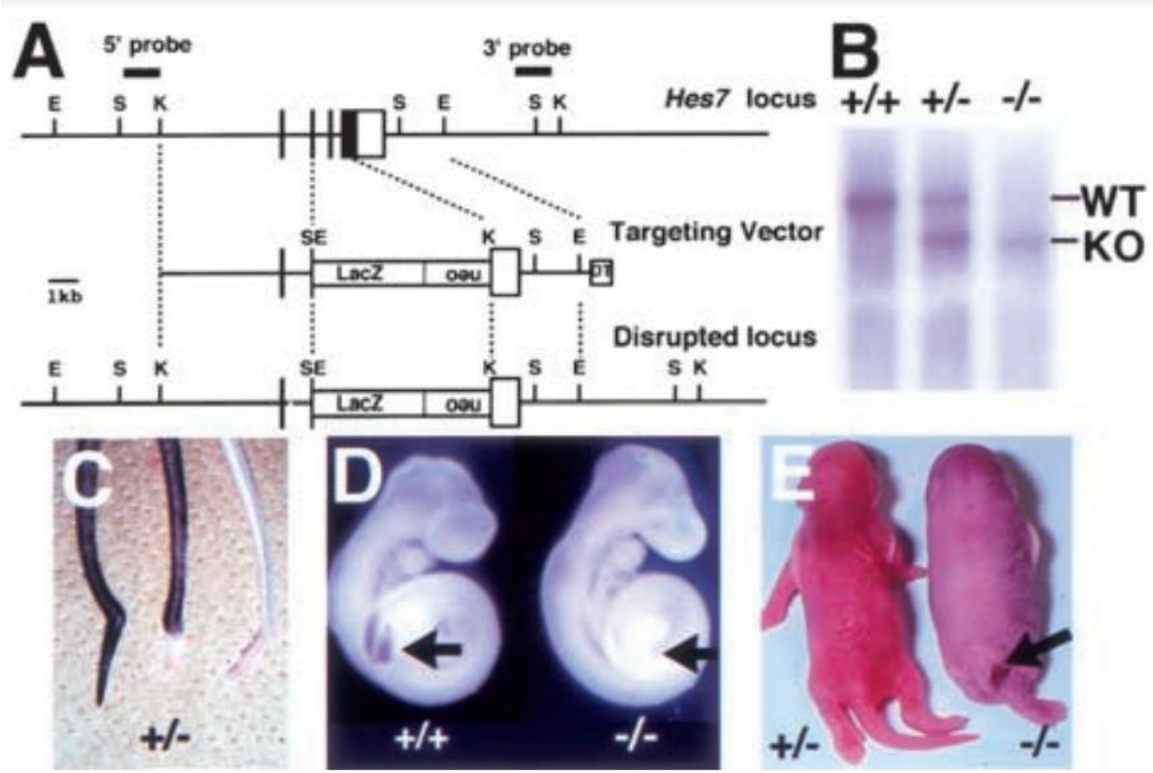


Figure 2. Generation of *Hes7*-null mice. (*A*) Targeting strategy. The *top* line shows the structure of the wild-type *Hes7* gene and the *middle* line shows the structure of the targeting vector. Most of the coding region of *Hes7* was replaced by IRES–*LacZ* and PGK–*neo* (inverted orientation). The *bottom* line indicates the resultant disrupted locus. *Diphtheria toxin* gene (DT) was used for negative selection. (E) *Eco*RV; (S) *Sac*I; (K) *Kpn*I. The positions of 5′-external and 3′-external probes are indicated on *top*. (*B*) Southern blot analysis. The 5′-external probe detected 10-kb wild-type and 7-kb mutant bands of *Sac*I-digested genomic DNA. (*C*) Kinked tails of adult heterozygous mutants. (*D*) Whole-mount in situ hybridization for *Hes7* of wild-type (*left*) and homozygous-mutant (*right*) embryos at E9.5. In the mutant embryo, *Hes7* expression is completely missing (arrows). (*E*) The appearance of heterozygous- (*left*) and homozygous-mutant (*right*) neonates. A homozygous mutant has a short trunk and a short tail (arrow).