



Models to
Accelerate Innovation



Pi16-SA-IRES-CreERT2-WPRE-polyA Mouse Model Strategy CRISPR-Cas9 Technology

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Reviewer:

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Date:

2025-9-16



Project Overview

Project Name

Pi16-SA-IRES-CreERT2-WPRE-polyA

Project Type

Cas9-KI

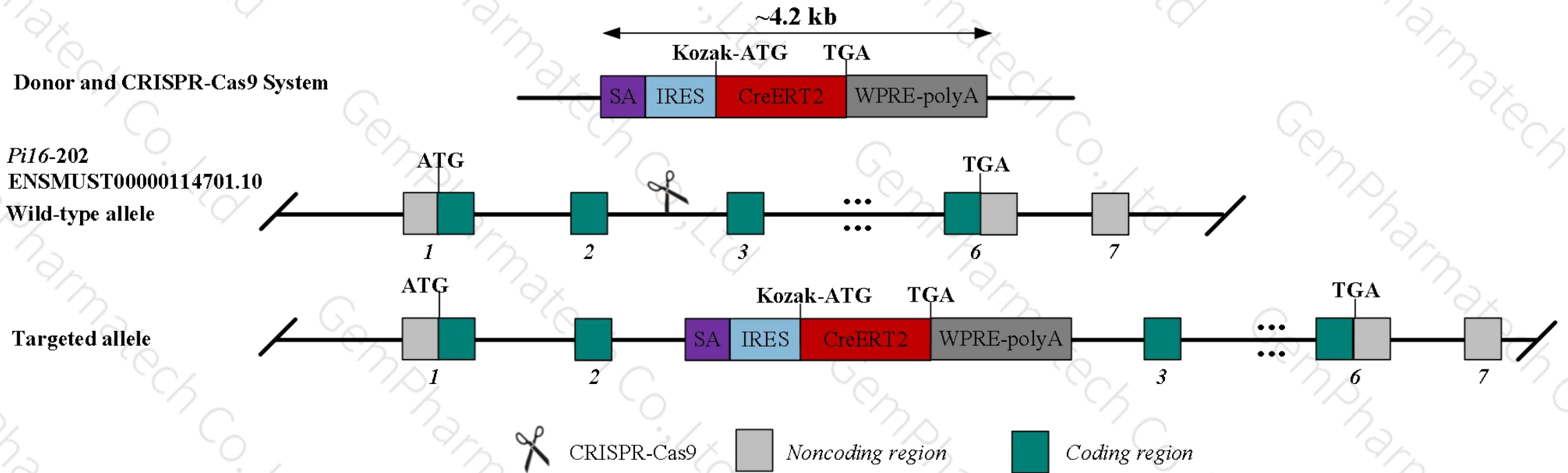
Background

C57BL/6JGpt



Strategy

This project will use CRISPR-Cas9 technology to edit the mouse *Pi16* gene and the schematic diagram is as follows:





Technical Description

- Based on the data in Ensembl, the *Pi16* gene has 6 transcripts. The transcript *Pi16*-202(ENSMUST00000114701.10) is selected for this strategy. This transcript has 7 exons and codes 498 aa. The ATG is located in exon 1 and the TGA is located in exon 6.
- According to the structure of *Pi16* gene and customer's requirements, the *SA-IRES-CreERT2-Wpre-polyA* element will be inserted immediately into the intron 2-3. *CreERT2* will be expressed under the direction of endogenous regulatory mechanism.
- In this project we use CRISPR-Cas9 technology to modify *Pi16* gene. The brief process is as follows: CRISPR-Cas9 system and Donor will be microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs will be transplanted to obtain positive F0 mice which will be confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain will be obtained by mating positive F0-generation mice with C57BL/6JGpt mice. Confirmation of the desired mutant allele will be carried out by PCR and on-target amplicon sequencing.



Note

- According to the data of MGI, homozygous mutant mice exhibited numerous immunological abnormalities during tissue-specific FACS analyses including an increased percentage of CD25+ cells in lymph node and B cell compartment differences in bone marrow spleen and lymph node.
- *Pi16* and *CreERT2* will be linked by IRES, which will be transcribed together, but translated separately (non-fusion expression pattern). Usually the gene behind IRES is expressed at a lower level than that in front of IRES.
- Insertion of the SA-IRES-CreERT2-Wpre-polyA cassette into the intron 2-3 may knock down the *Pi16* gene.
- The effect of the exogenous knock-in fragment on *Gm46603* lncRNA gene function is unknown.
- The *Pi16* gene is located on Chr 17. Shall the mutant mice be crossed with other strains to obtain double homozygous mutant offspring, be mindful about linkage effect when the second mutation is also on Chr 17.
- This strategy is designed based on the currently available information in the existing databases. Due to the complexity of gene expression regulation, insertion of exogenous sequences between the gene coding and non-coding regions may affect biological processes such as gene expression and mRNA splicing. The effect of this strategy on gene expression cannot be completely predicted at the present technology level.

Target Gene



Gene name	<i>mouse Pi16</i>
Gene ID (NCBI)	74116
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/74116
Gene link (Ensembl)	https://www.ensembl.org/Mus_musculus/Gene/Summary?db=core;g=ENSMUSG0000024011;r=17:29536654-29549567
Chromosome location	Chr 17



Gene Information (NCBI)

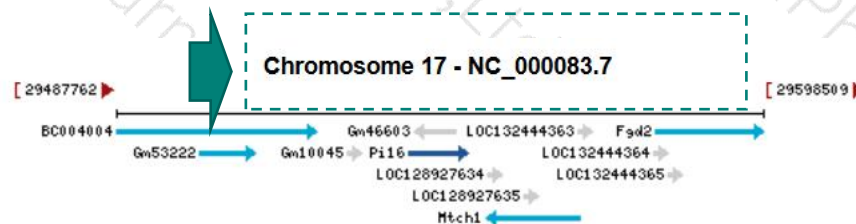
Pi16 peptidase inhibitor 16 [*Mus musculus* (house mouse)]

Gene ID: 74116, updated on 3-May-2025

Download Datasets

Summary

Official Symbol Pi16 provided by [MGI](#)
Official Full Name peptidase inhibitor 16 provided by [MGI](#)
Primary source [MGI:MGI:1921366](#)
See related [Ensembl:ENSMUSG00000024011](#) [AllianceGenome:MGI:1921366](#)
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 Cripi; PI-16; 1200009H11Rik
Summary Predicted to enable peptidase inhibitor activity. Acts upstream of or within negative regulation of cell growth involved in cardiac muscle cell development. Located in extracellular space. Is expressed in cranial ganglion; dorsal root ganglion; medulla oblongata basal plate mantle layer; and neural retina. Orthologous to human PI16 (peptidase inhibitor 16). [provided by Alliance of Genome Resources, May 2025]
Expression Biased expression in bladder adult (RPKM 160.4), mammary gland adult (RPKM 72.5) and 6 other tissues [See more](#)
Orthologs [human](#) [all](#)



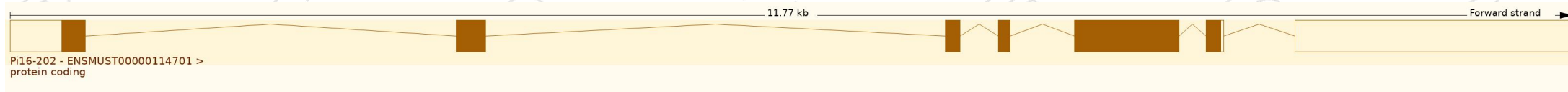


Transcript Information (Ensembl)

The gene has 6 transcripts, as shown below:

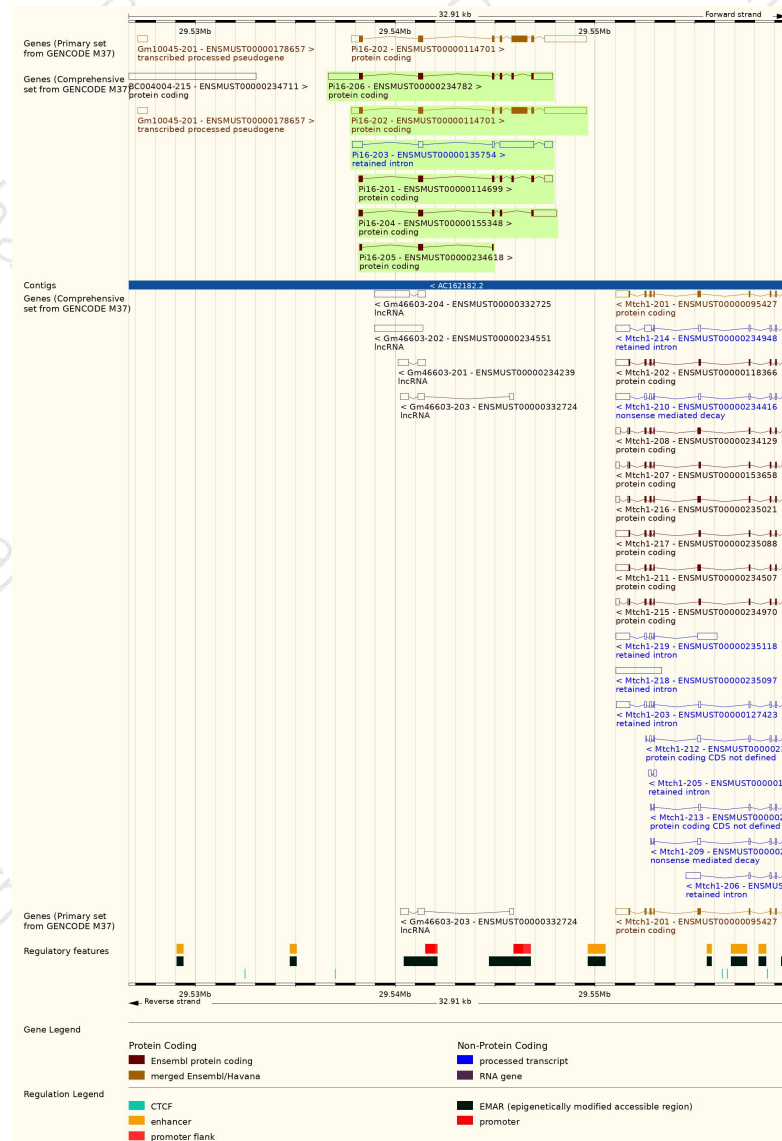
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000114699.9	Pi16-201	1244	269aa	Protein coding		D3Z6T6	GENCODE Basic APPRIS ALT2 TSL:1
ENSMUST00000114701.10	Pi16-202	3994	498aa	Protein coding	CCDS28595	Q9ET66-1	Ensembl Canonical GENCODE Primary GENCODE Basic APPRIS P3 TSL:1
ENSMUST00000135754.2	Pi16-203	2932	No protein	Retained intron		-	TSL:1
ENSMUST00000155348.3	Pi16-204	1875	236aa	Protein coding		Q9ET66-2	GENCODE Basic APPRIS ALT2 TSL:1
ENSMUST00000234618.2	Pi16-205	397	132aa	Protein coding		A0A3Q4EC30	CDS 5' and 3' incomplete
ENSMUST00000234782.2	Pi16-206	3288	269aa	Protein coding		D3Z6T6	GENCODE Basic APPRIS ALT2

The strategy is based on *Pi16-202*, which contains 7 exons, is 3994 bps long, and encodes 498 amino acids.



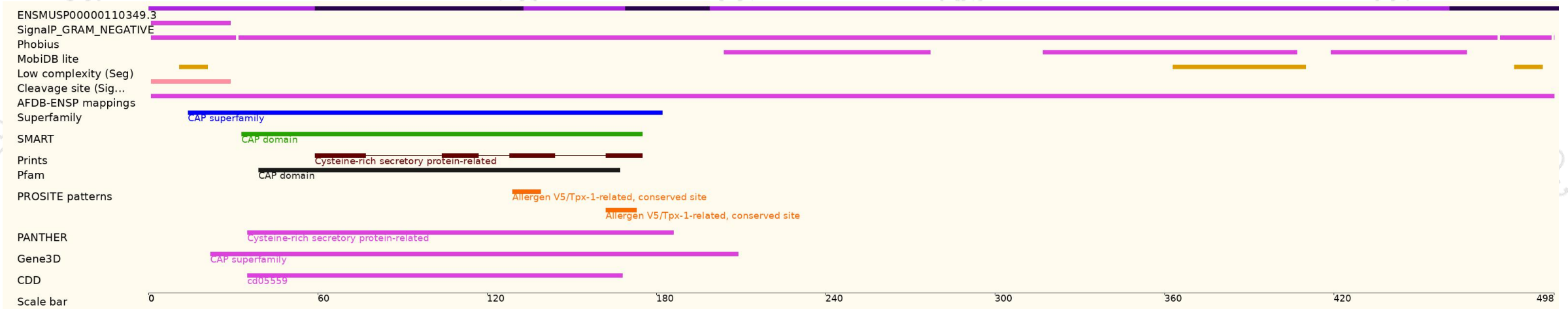


Genomic Information





Protein Information





Exciting Model (MGI)

URL link is as follows: <https://www.informatics.jax.org/allele/summary?markerId=MGI:1921366&alleleType=Targeted>

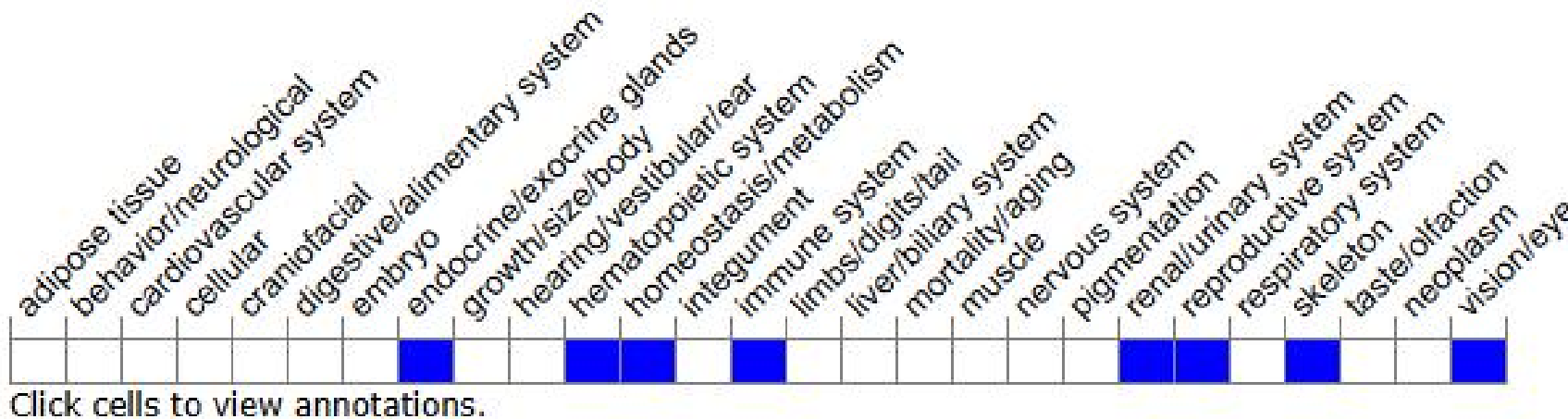
Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems
Pi16^{tm1.2Engl} peptidase inhibitor 16; targeted mutation 1.2, Stefan Engelhardt	17		Targeted (Null/knockout)	homeostasis, immune
Pi16^{tm1a(KOMP)Mbp} peptidase inhibitor 16; targeted mutation 1a, Mouse Biology Program, UC Davis	17		Targeted (Conditional ready, Null/knockout, Reporter) (Cell Line)	
Pi16^{tm1e(KOMP)Mbp} peptidase inhibitor 16; targeted mutation 1e, Mouse Biology Program, UC Davis	17		Targeted (Null/knockout, Reporter) (Cell Line)	
Pi16^{tm2a(KOMP)Mbp} peptidase inhibitor 16; targeted mutation 2a, Mouse Biology Program, UC Davis	17		Targeted (Conditional ready, Null/knockout, Reporter) (Cell Line)	
Pi16^{tm2e(KOMP)Mbp} peptidase inhibitor 16; targeted mutation 2e, Mouse Biology Program, UC Davis	17		Targeted (Null/knockout, Reporter) (Cell Line)	



Mouse Phenotype Description (MGI)

URL link is as follows: <https://www.informatics.jax.org/marker/MGI:1921366>

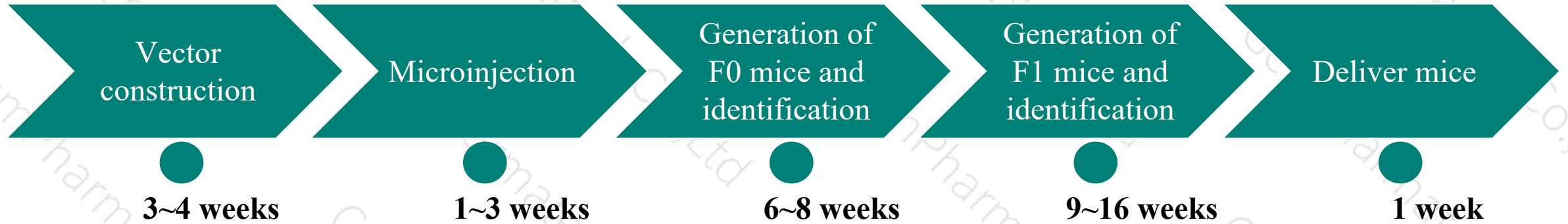
Phenotype Overview ?



Homozygous mutant mice exhibited numerous immunological abnormalities during tissue-specific FACS analyses including an increased percentage of CD25+ cells in lymph node and B cell compartment differences in bone marrow spleen and lymph node.



Work Flow





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