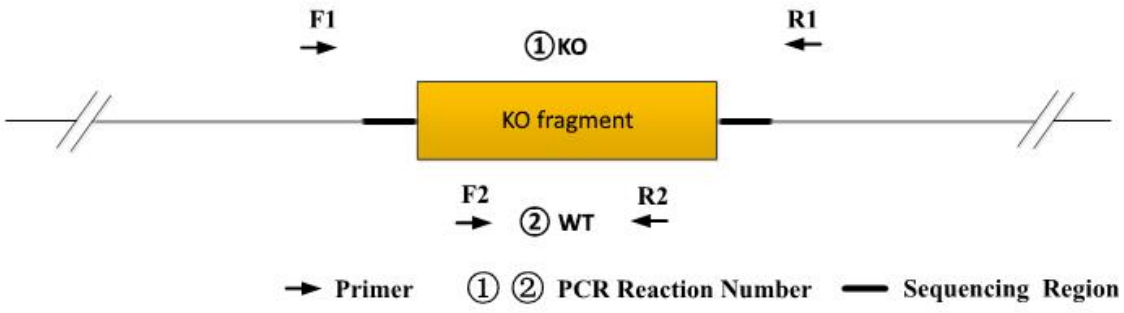
Genotyping Report

Strain ID	T006071	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	*Gprc5a*		

1. Strategy of Genotyping



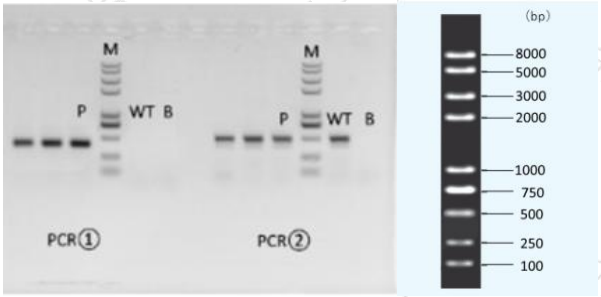
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted band are shown below.
2) If the WT band is too large, it may not be possible to obtain a WT band.

2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	JS00553-Gprc5a-ssDNA-5wt-tF2	AAGGCCAAAGTCCATTAGAAGTCC	WT:2397bp KO:440bp
	R1	JS00553-Gprc5a-ssDNA-3wt-tR2	TGCTGGGAAGCAAACCTGGTC	
PCR②	F2	JS10553-Gprc5a-wt-tF2	CCTTCATCATCAAGCTGGATGG	WT:491bp KO:0bp
	R2	JS10553-Gprc5a -wt-tR2	TGTGCTGAGAATGGTATCATCCC	

3. Gel Image

tttgggctgacggtccagtttctcaagcccctg---2013bp,+56bp---CATCGCATTGTCTGAGTAGGTGATAACTTCG
TATAATGTATGCTATACGAAGTTATgaggatcagaaattcactgtcatcctgggcta



Note: P: Heterozygous samples; WT: Wildtype control; B: Blank control (ddH2O); M: DNA Ladder

① Control (WT) : It is an important reference mark for whether the PCR reaction is successful and whether the product band position and size meet the theoretical requirements.
② Control (B) : PCR amplification was performed without template in the PCR reagent to monitor whether the reagent was contaminated.

4. PCR Condition

(Generally recommend to use Vazyme P222;If the sequences contain special structures such as GC% ⩾ 60% or GC% ⩽ 40%，recommend to use Vazyme P515.)

PCR Reaction Component		
Seg.	**reaction component**	**Volume (μl)**
1	2 × Rapid Taq Master Mix(Vazyme P222) or 2 × Phanta Max Master Mix (Vazyme P515)	12.5
2	ddH2O	9.5
3	Primer A(10pmol/μl)	1
4	Primer B(10pmol/μl)	1
5	Template(20~80ng/μl)	1

PCR program Ⅰ (priority selection)			
Seg.	**Temp.**	**Time**	**Cycle**
1	95℃	5min	
2	98℃	30s	20×
3	65℃*（-0.5℃/cycle）	30s	
4	72℃	45s*	
5	98℃	30s	15×
6	55℃*	30s	
7	72℃	45s*	
8	72℃	5min	
9	10℃	hold	

PCR program Ⅱ (the second choice)			
Seg.	**Temp.**	**Time**	**Cycle**
1	95℃	5min	
2	98℃	30s	35×
3	58℃*	30s	
4	72℃	45s*	
5	72℃	5min	
6	10℃	hold	

Note*: Annealing temperature and extension time can be determined according to the actual amplification situation and amplification enzyme efficiency.