

Genotyping Report

Strain ID	T033727	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	<i>Kcmf1</i>		

1. Strategy of Genotyping



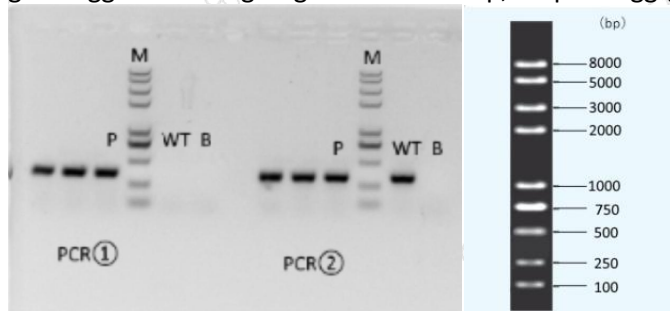
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ②PCR reaction without product.
Note: 1)The sizes of WT and Targeted 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.	Sequence	Band Size
PCR①	JS24661-Kcmf1-5wt-tF1	TTCAAGGTCATTTTGGGCTTCC	WT: 22979bp KO: 387bp
	JS24661-Kcmf1-3wt-tR1	GCACACGGAATCAACTCATAAACC	
PCR②	JS34661-Kcmf1-wt-tF1	TTGTTTCAGTCACCCACCTTACGAC	WT: 307bp KO:0bp
	JS34661-Kcmf1-wt-tR1	CATCAGCACTCCTCCTATGGAATTG	

3. Gel Image

gttcttaggattttatactgccagataat---22594bp,+2bp---CGggtgtcacggctctgcacccagtatccct



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% $\geq$ 60% or GC% $\leq$ 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	20×
2	98℃	30s	
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	35×
2	98℃	30s	
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.