

Genotyping Report

Strain ID	T003005	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Fut2		

1. Strategy of Genotyping

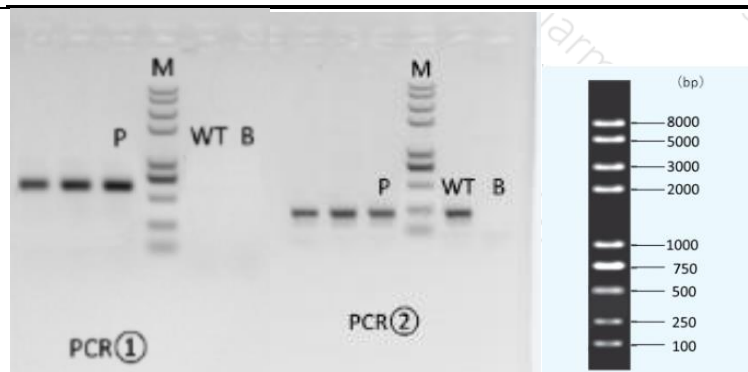


2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
PCR①	T003005-F1	GCAGAGTCTGCAAAGGCCAGT	WT:4573bp KO:641bp
	T003005-R1	TTAGAAGGCTAGTGCAGGCTTGG	
PCR②	T003005-F2	CTCACTTTGTAGACTTGTAGCTCTCC	WT:229bp KO:0bp
	T003005-R2	GCTAAGTAACAGTACACCGGGCTT	

3. Gel Image

aagctggaactaatttatctccatcaaccagtgga---3932bp---tgtgggagcccatgcaccaattgagcatgctag
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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	
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.