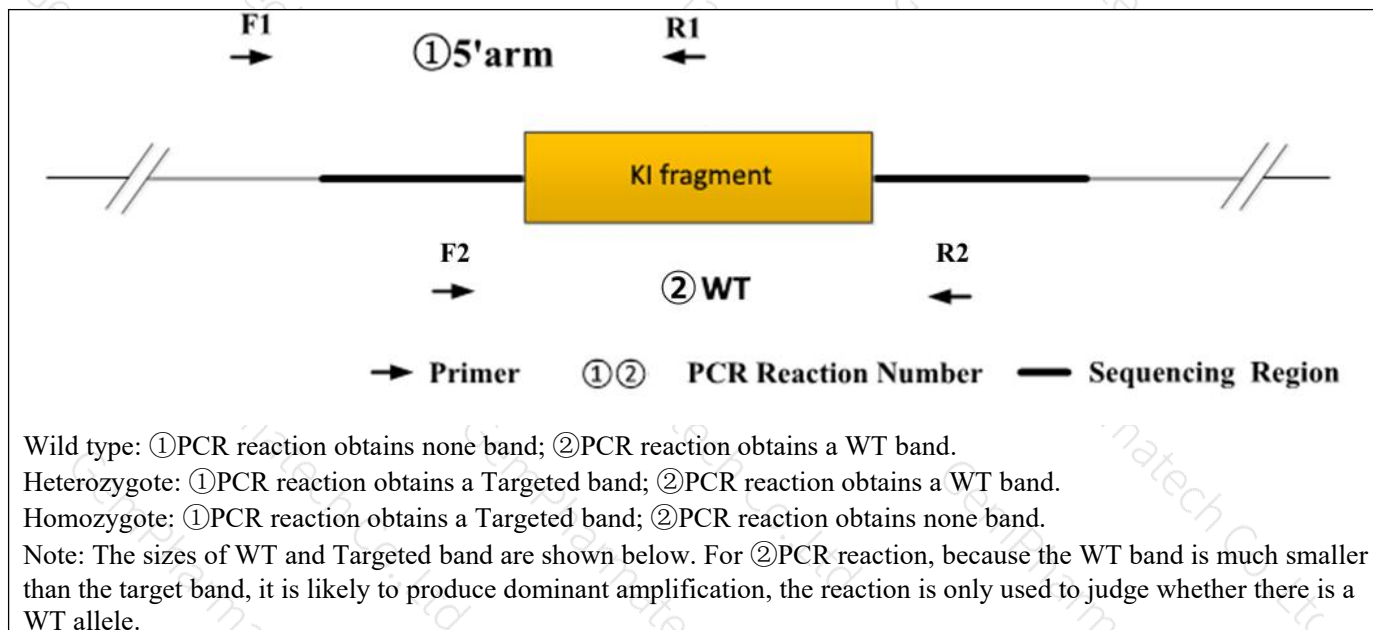


Genotyping Report

Strain ID	T003635	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Dongdong Zhang	Gene Name	<i>Ilf3</i>		

1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
① 5'arm GC%:37%	F1	T003635-F1A	GGGATCCACATACCTACCATGATC	WT:0bp Targeted:1255bp
	R1	T003635-R1A	GCTATGAACTAATGACCCCGTAATTG	
② WT	F2	H11-tF3	GGGCAGTCTGGTACTTCCAAGCT	WT:285bp Targeted:0bp
	R2	H11-tR3	ATATCCCCTTGTTCCCTTTCTGC	

备注：跨臂短方案，无法更换成初筛方案

3. Gel Image & Conclusion

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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

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Rapid Taq Master Mix (Vazyme P222)		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.