





Genotyping Report

Strain ID	T019062	Strain Type	CKO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Ngb		

1. Strategy of Genotyping



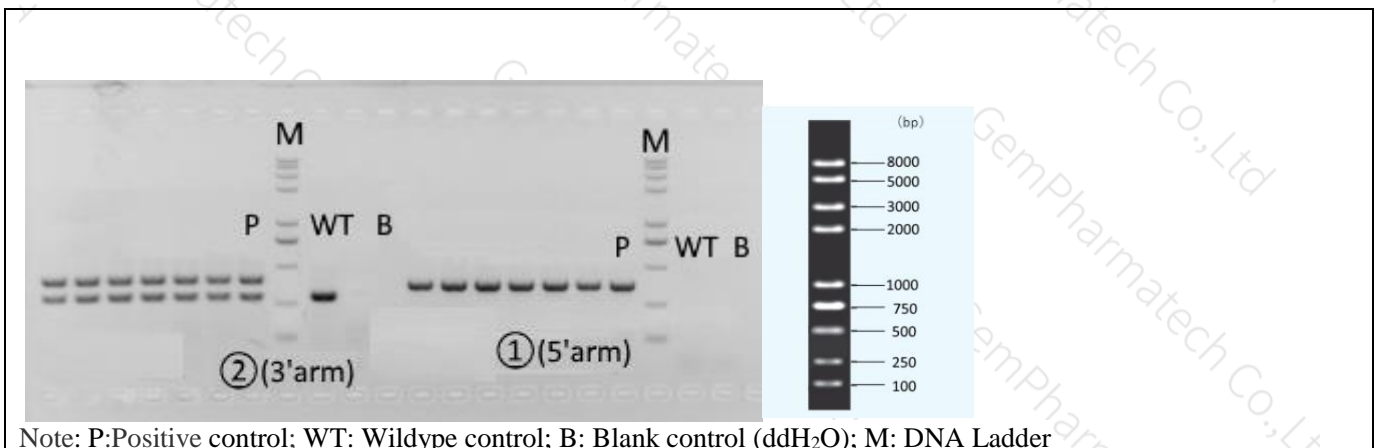
 **Loxp**  **Primer** ① ② **PCR Reaction Number**  **Sequencing Region**

Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a single WT band.
 Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band and a Targeted band.
 Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a single Targeted band.
 Note: The sizes of WT and Targeted band are shown below.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①(5'arm)	T019062-F1	CTGAACTGGTTGAGGTCCGATG	WT:0bp Targeted:322bp
	T019062-R1	CCAACTGACCTTGGGCAAGAACAT	
②(3'arm)	T019062-F2	GCCGTGGGATCTGTAGAAAGTAGG	WT:286bp Targeted:387bp
	T019062-R2	CTTCCCTCAAGGAATACGTGCTG	

3. Gel Image & Conclusion





4. PCR Condition

PCR Reaction Component			
Seg.	reaction component	Volume (μl)	
1	2 × Taq Master Mix , Dye Plus, (Vazyme P112-03)	12.5	
2	ddH2O	9.5	
3	Primer A(10pmol/μl)	1	
4	Primer B(10pmol/μl)	1	
5	Template(≈100ng/μl)	1	
PCR program			
Seg.	Temp.	Time	Cycle
1	95℃	5min	
2	98℃	30s	20×
3	65℃ (-0.5℃/cycle)	30s	
4	72℃	45s	
5	98℃	30s	20×
6	55℃	30s	
7	72℃	45s	
8	72℃	5min	
9	10℃	hold	

Reviewer:Ting Sun

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