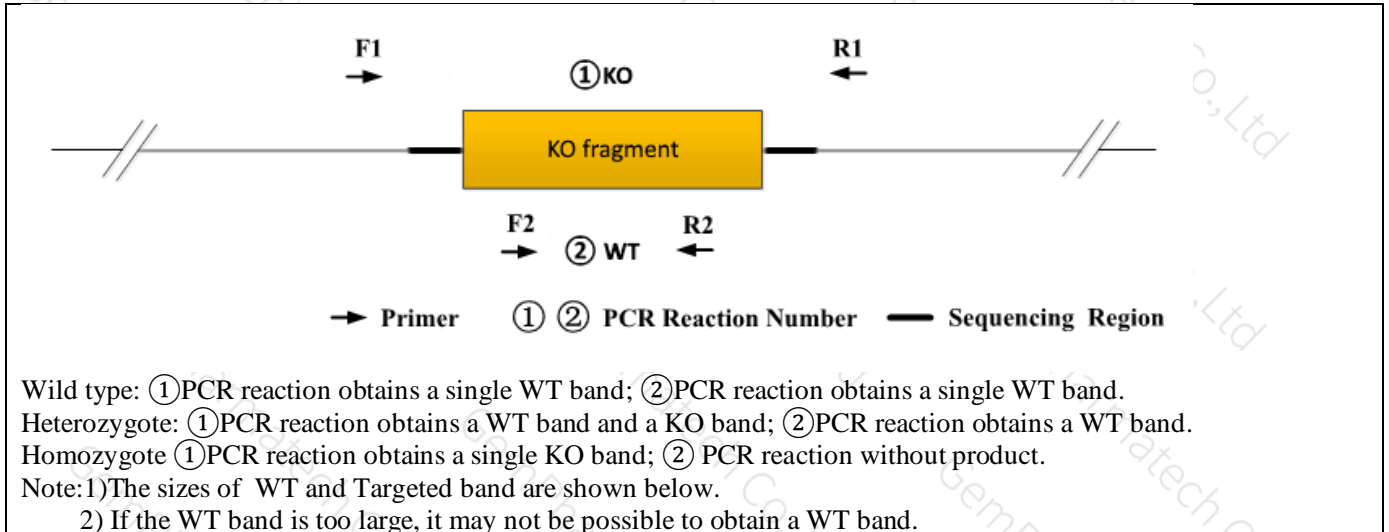


Genotyping Report

Strain ID	T014527	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Binjie Jiao	Gene Name	<i>Cd200</i>		

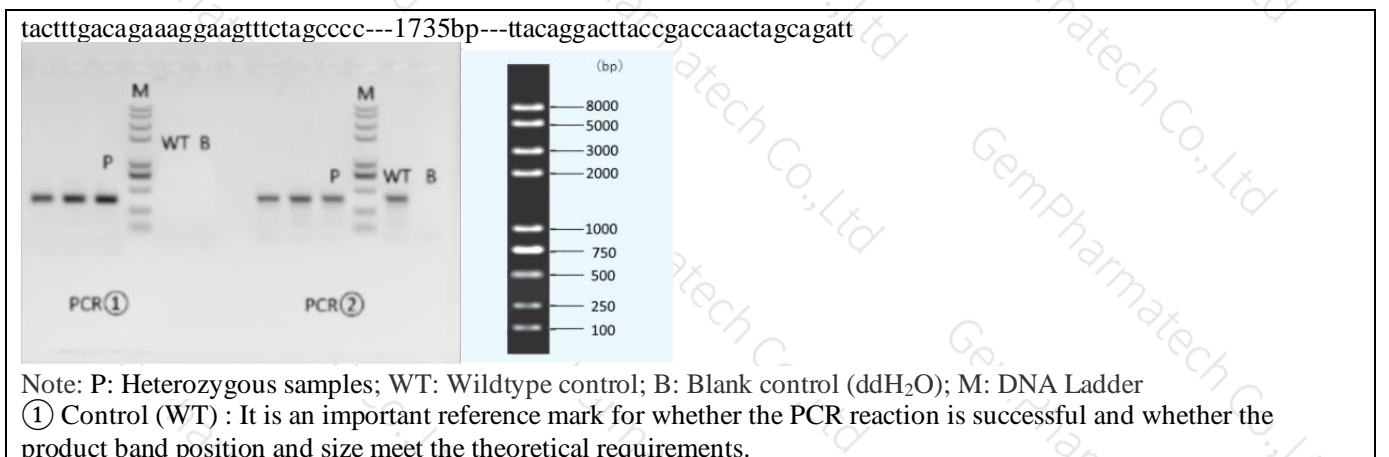
1. Strategy of Genotyping



2. Primer Information

PCR No.	Primer No.	Primer Name	Sequence	Band Size
PCR①	F1	JS03779-Cd200-5wt-tF1	GAAAGATGGCCTTGGTGACTGAG	WT:2101bp KO:366bp
	R1	JS03779-Cd200-3wt-tR1	ATGTGCTAGGCTATGCTTGCC	
PCR②	F2	JS13779-Cd200-wt-tF1	TGGTGGGTAAACACTTGACTCCTG	WT:397bp KO:0bp
	R2	JS13779-Cd200-wt-tR1	CTACTGGAATTGCTTCTGAGTGCC	

3. Gel Image



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 (μ l)
1	2 \times Rapid Taq Master Mix (Vazyme P222) or 2 \times Phanta Max Master Mix (Vazyme P515)		12.5
2	ddH ₂ O		9.5
3	Primer A (10pmol/ μ l)		1
4	Primer B (10pmol/ μ l)		1
5	Template (20~80ng/ μ l)		1
PCR program I (priority selection)			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	20 \times
3	65°C* (-0.5°C/cycle)	30s	
4	72°C	45s*	
5	98°C	30s	
6	55°C*	30s	15 \times
7	72°C	45s*	
8	72°C	5min	
9	10°C	hold	
PCR program II (the second choice)			
Seg.	Temp.	Time	Cycle
1	95°C	5min	
2	98°C	30s	35 \times
3	58°C*	30s	
4	72°C	45s*	
5	72°C	5min	
6	10°C	hold	

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