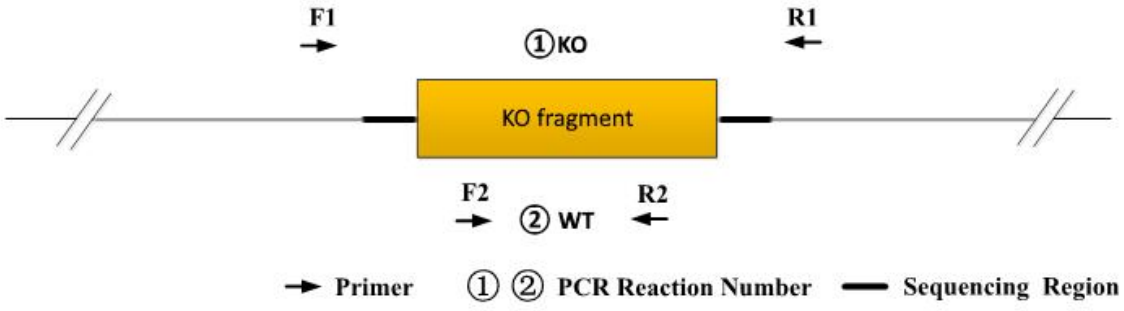
Genotyping Report

Strain ID	T053156	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Tiantian Sun	Gene Name	*Gdf11*		

1. Strategy of Genotyping



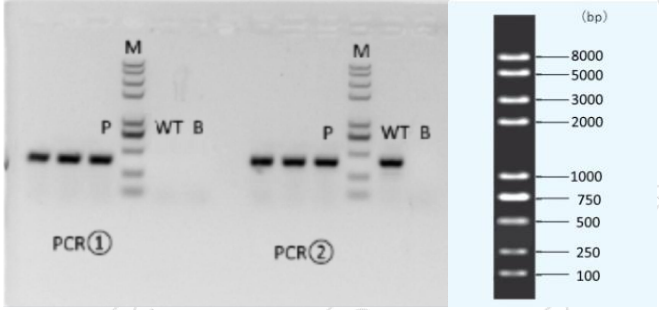
Wild type: ①PCR reaction obtains a single WT band; ②PCR reaction obtains a single WT band.
Heterozygote: ①PCR reaction obtains a WT band and a KO band; ②PCR reaction obtains a WT band.
Homozygote: ①PCR reaction obtains a single KO band; ② PCR reaction without product.
Note：1)The sizes of WT and Targeted 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.	Primer Name	Sequence	Band Size
PCR①	F1	JS02792-Gdf11-5wt-tF2	GCCAAGAGATTTGGAGACTGGAAG	WT:13493bp KO: 371bp
	R1	JS02792-Gdf11-3wt-tR2	GTTTGATTCCTTGTTCTACCTAACAACC	
PCR②	F2	JS12792-Gdf11-wt-F2	AGGCTTGGGAAGCAGGCAAGA	WT:372bp KO:0bp
	R2	JS12792-Gdf11-wt-R2	CCAAGAGGCCACTGCTGAGGTAT	

3. Gel Image

ggatgataaaacaagattggagtgctacatccaggta---13122bp---gaacctatttgccagagtatgttggagggatgtccccacttat



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% ⩾ 60% or GC% ⩽ 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	
2	98℃	30s	20×
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.