

Genotyping Report

Strain ID	T015600	Strain Type	KO(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Zifan Lin	Gene Name	Gpr31b		

1. Strategy of Genotyping

Wild type: 0 copy.

Heterozygote: 1 copy.

Homozygote: 2 copy.

NOTE:

Because the sequence of the gene is very nonspecific, 4 other sequences with only base mismatches can be found in the genome. Therefore, it is necessary to use the heterozygous mice as a single copy control, and detect the copy number of KO genotype through qPCR to judge the genotype.

由于该基因的序列非常不特异，在基因组上能找到其他4处仅存在个别碱基错配的序列，所以需要使用交付的杂合子小鼠作为单拷贝对照，通过QPCR检测KO基因型的拷贝数，来进行基因型判断。

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
KO	T015600-q-tF1A	AGGCACTATGAGGGTAACCTCTTC	146bp
	T015600-q-tR1A	TCACACCCTGGTCAGGCTGTAGTT	
IC	Gapdh-Q-TF1	GCCTAGTTGGCTTCATCTCAAGG	77bp
	Gapdh-Q-TR1	TGAGGGCAAAGAGAGACCCATC	

3. Conclusion

	Result	
Sample No.	2-ΔΔCT	Genotype
1	1.00	Homo
2	0.67	Hetero
3	0.55	Hetero
4	0.57	Hetero
5	0.49	Hetero
6	0.59	Hetero
7	0.53	Hetero

4. PCR Condition

PCR Reaction Component		
Seg.	reaction component	Volume (μl)
1	2X SYBR Mix	5
2	Primer	0.2

3	Primer	0.2	
4	Rox	0.2	
5	Template+ddH ₂ O	4.4	
PCR program			
Seg.	Temp.	Time	Cycle
1	95°C	30s	40×
2	95°C	5s	
3	60°C	30s	

T015600-wt:

AAACTGCACTCCAGAGGGTCCCTAGCACATGGAACAAGAGCTGCTGCTGAAGGCTGACACATCTG
CAGGCAGGACAGTAGTAGATCAAGTGTGGTGGCCAAGGCAGGGTCTGCCACAAGAAAGTGCA
CATTGGGAGCAGGGGCACAGTGGGCTCTGGGTAGCACATTGCAGGACGTGTCTTGTAACAGCCC
TGGCTGCAGCTGACAATTCCAGAAAGGTGCAGGCATAGACAATGCACCAACCTAAGGCAGATGC
TCAAGACCAAAAGTCTTAAGGTCCAGCTCCTTCCTCTGTAAATGTTAGTCCCTCACTTCCCTCCTTCT
GAGCATCAGAGGATCTTTCTCCCTGGACCCAGACAGAAGACTTAAATTATATTGGCTTCAGCCTTT
GCATGATTAAGGAATTAAATTAATAATGAAGAATTCAGTATGAAATGTAGTTCCCATCTGGAA
ACTCTTGCTTGCAGGGCACAAAACCCAGTGTTCAGGTCCTATGTAGA

T015600-wt similar sequence 1 (Lowercase letters are inconsistent bases 小写碱基为不一致碱基):

AAACTGCACTCCAGAGGGTCCCTAGCACATGGAACAAGAGCTGCTGCTGAAGGCTGACACATCTG
CAGGCAGGACAGTAGTAGcTCAAGTGTGGcGGCCAAGGCAGGGTCTGCCACAAGAAAGTGCA
ATTGGGAGCAGGGGCACAGTGGGCTCTGGGTAGCACATTGCAGGACGTGTCTTGTAACAGCCCT
GGCTGCAGCTGACAATTCCAGAAAGGTGCAGGCATAGACAATGCACCAACCTAAGaCAGATGgTC
AAGACCAAAAGTCTTAAGGTCCAGCTCCTTCCTCTGTAAATGTTAGTCCCTCACTTCCCTCCTTCTG
AGCATCAGAGGATCTTTCTCCCTGGACCCAGACAGAAGACTTAAATTATATTGGCTTCAGCCTTTG
CATGATTAAGGAATTAAATTAATAATGAAGAATTCAGTATaAAATGTAGTTCCCATCTGGAAA
CTCTTGCTTGCAGGGCtCAAACCCAGTGTTCAGGTCCTATGTAGA

T015600-wt similar sequence 2 (Lowercase letters are inconsistent bases 小写碱基为不一致碱基):

AAACTGCACTCCAGAAgGTCCCTAGCACATGGAACAAGAGCTGCTGCTGAAGGCcGACACATCTG
CAGGaAGGACAGTAGTAGATCAAGTGTGGcGGCCAAGGCAGGGTCTGCCACAAGAAAGTGCA
ATTaGGAGCAGGGGCACAGTGGGCTCTGGGTAGCACATTGCAGGACGTGTCTTaTAAACAGCCCT
GGCTGCAGCTGACAATTCCAGAAAGGTGCAGGCATAGACAATGCACCAACCTAAGGCAGATGgTC
AAGACCAAAAGTCTTAAGGTCCAGCTCCTTCCTCTGTAAATGTTAGTCCCTCcCTTCCCTCCTTCTGA
GCATCAGAGGATCTTTCTCCCTGGACCCAGACAGAAGACTTAAATTATATTGGCTTCAGCCTTTGC
ATGATTAAGGAATTAAATTAATAATGAAGAATTCAGTATaAAATGTAGTTCCCATCTGGAAAC
TCcTGCTTGCAGGGCtCAAACCCAGTGTTCAGGTCCTATGTAGA

T015600-wt similar sequence 3 (Lowercase letters are inconsistent bases 小写碱基为不一致碱基):

AAACTGCACTCCAGAAgGTCCCTAcCACATGGAACAAGAGCTGCTGCTGAAGGCcGACACATCTGC
AGGaAGGACAGTAGTAGATCAAGTGTGGcGGCCAAGGCAGGGTCTGCCACAAGAAAGTGCA
TTaGGAGCAGGGGCACAGTGGGCTCTGGGTAGCACATTGCAGGACGTGTCTTaTAAACAGCCCTG
GCTGCAGCTGACAATTCCAGAAAGGTGCAGGCATAGACAATGCACCAACCTAAGGCAGATGgTCA
AGACCAAAAGTCTTAAgGTCCAGCTCCTTCCTCTGTAAATGTTAGTCCCTCcCTTCCCTCCTTCTGAG
CATCAGAGGATCTTTCTCCCTGGACCCAGACAGAAGACTTAAATTATATTGGCTTCAGCCTTTGCA
TGATTAAGGAATTAAATTAATAATGAAGAATTCAGTATaAAATGTAGTTCCCATCTGGAACT
CcTGCTTGCAGGGCtCAAACCCAGTGTTCAGGTCCTATGTAGA

T015600-wt similar sequence 4 (Lowercase letters are inconsistent bases 小写碱基为不一致碱基):

AAACTGCACTCCAGAAgGTCCCTAcCACATGGAACAAGAGCTGCTGCTGAAGGCcGACACATCTGC
AGGaAGGACAGTAGTAGATCAAGTGTGGcGGCCAAGGCAGGGTCTGCCACAAGAAAGTGCA
TTaGGAGCAGGGGCACAGTGGGCTCTGGGTAGCACATTGCAGGACGTGTCTTaTAAACAGCCCTG
GCTGCAGCTGACAATTCCAGAAAGGTGCAGGCATAGACAATGCACCAACCTAAGGCAGATGgTCA
AGACCAAAAGTCTTAAgGTCCAGCTCCTTCCTCTGTAAATGTTAGTCCCTCcCTTCCCTCCTTCTGAG



CATCAGAGGATCTTTCTCCCTGGACCCAGACAGAAGACTTAAATTATATTGGCTTCAGCCTTTGCA
TGATTAAGGAATTAAATTAAATGAAGAATTCACTGAT_aAAATGTAGTTCCCATCTGGAAACT
CcTGCTTGCAGGGCtCAAAACCCAGTGTTCAGGTCCTATGTAGA