

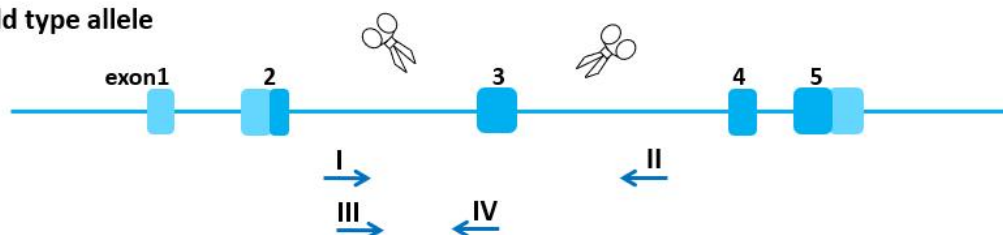
Wnt5b-KO Genotyping Protocol



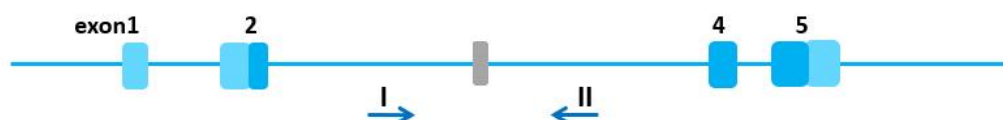
Common Name	Wnt5b-KO	Cat. NO.	NM-KO-240964
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



: coding region : uncoding region

: Cas9/gRNA : mutation

NHEJ : Non-homologous end joining

: primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	TTCAGCAGTGTCTGTACCCC	Forward
P2	TCCACCTCCACCTCTGACTT	Reverse
P3	CTCTGCCCACCTTCACCTTTTA	Forward
P4	AACTGACACAGCTTTCTCTGGC	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 HE HE WT M H 947bp 577bp P3/P4 HE HE WT H M 449bp DL2000 bp 2000 1000 750 500 250 100 1% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -370bp Wild type: P1P2 = 947 bp; P3P4 = 449 bp Heterozygote: P1P2 = 947 bp and 577 bp; P3P4 = 449 bp Homozygote: P1P2 = 577 bp

Note: In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band

Reaction &Cycling

PCR	Reaction Component	Volume (µl)
Reaction System	ddH ₂ O	8.0
	2×Rapid Taq Master Mix	10.0
	P1(10 pmol/µl) or P3(10 pmol/µl)	0.5

	P2(10 pmol/ μ l) or P4(10 pmol/ μ l)			0.5
	Genomic DNA			1.0
	Total			20
	2×Rapid Taq Master Mix from Vazyme(Code Number: P222-01)			
Cycling Reaction	Step	Temp	Time	Note
	1	95°C	5 min	
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	10 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	