

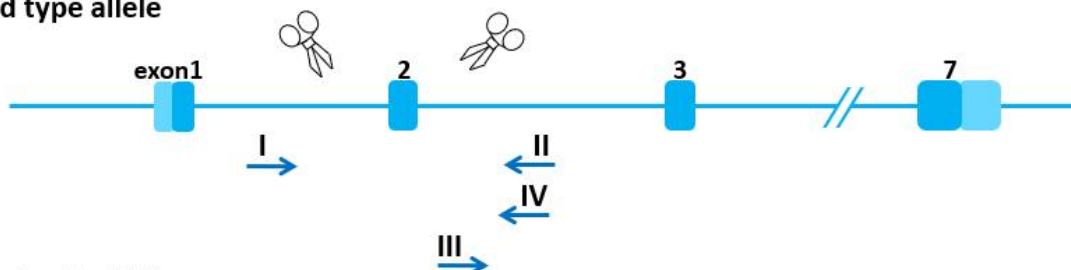
Vnn1-KO Genotyping Protocol



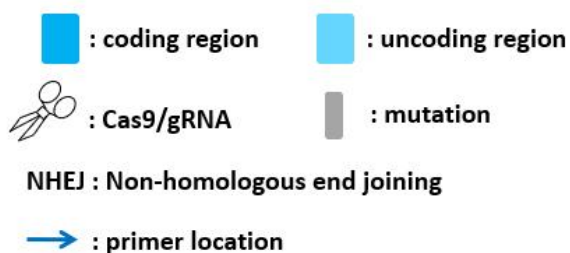
Common Name	Vnn1-KO	Cat. NO.	NM-KO-241530
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	GCTAGGTGACCACTGTACGG	Forward
P2	TGGACTGGAGGGAACTGGA	Reverse
P3	CACGCCCAGCTCAGTTATGA	Forward
P4	CCATTCAACAGCCCACAACG	Reverse

Expected results

Results	
Genotype	Knockout type: -1280bp Wild type: P1P2 =1745 bp; P3P4 =365 bp Heterozygote: P1P2 =1745 bp and 465 bp; P3P4 =365 bp Homozygote: P1P2 =465 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 System	Reaction Component			Volume (μl)
	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/μl) or P4(10 pmol/μl)			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	20 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	