

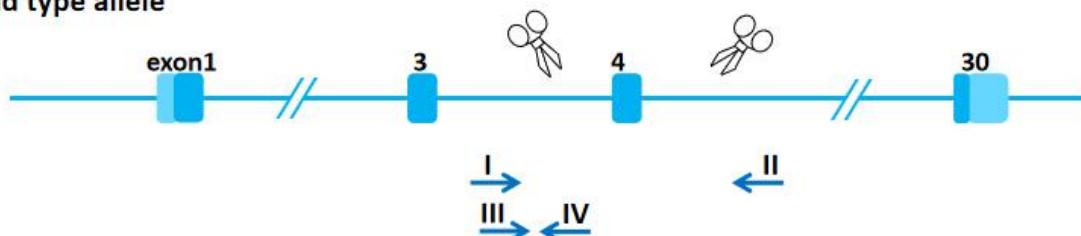
Itgav-KO Genotyping Protocol



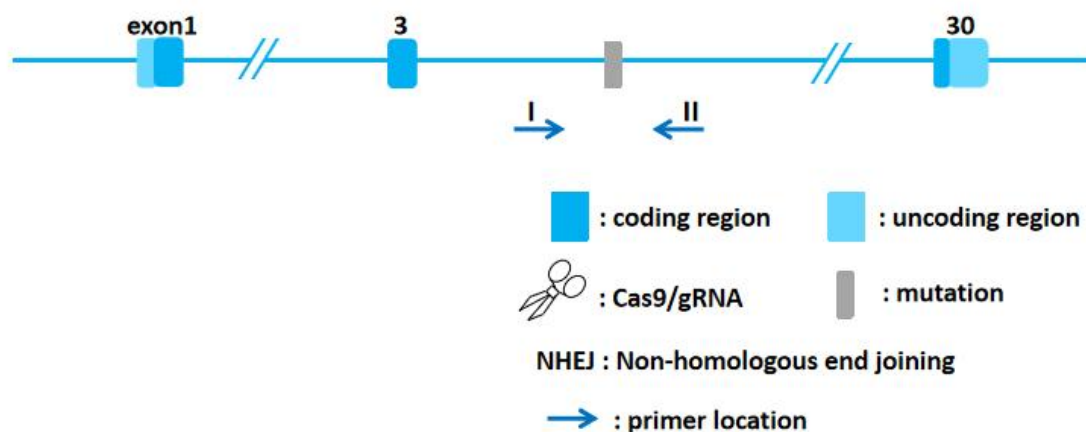
Common Name	Itgav-KO	Cat. NO.	NM-KO-205025
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	AATAGTTCAAGGCCAGGGTTTC	Forward
P2	AGACACGCAGGCTTTTCACTAATG	Reverse
P3	CTTGCTGCTTACTCGCCGTCTA	Forward
P4	GCTGCTGCTGTTGTTTTGGTTG	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE GQ Agarose #R0-491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P1/P2 HE HE WT M 1267bp 282bp P3/P4 HE HE WT M 268bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -985 bp Wild type: P1P2 =1267 bp; P3P4 =268 bp Heterozygote: P1P2 =1267 bp and 282 bp; P3P4 =268 bp Homozygote: P1P2 =282 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	