

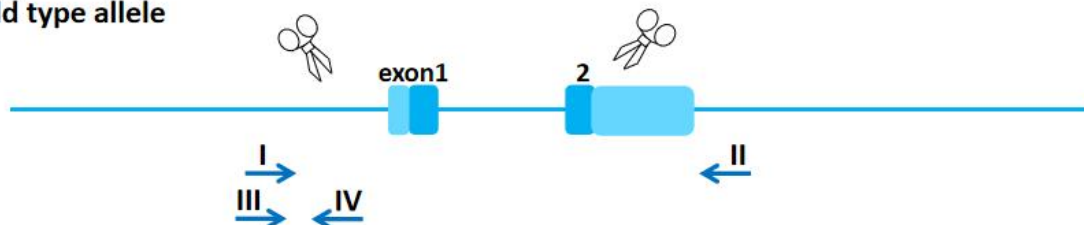
Atp6v1f-KO Genotyping Protocol



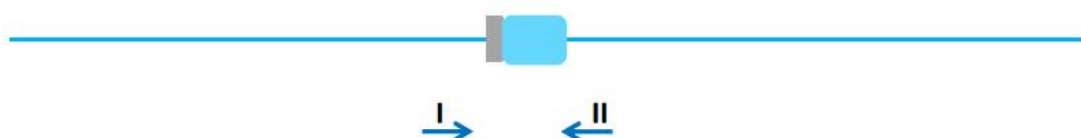
Common Name	Atp6v1f-KO	Cat. NO.	NM-KO-241374
Strain of Origin	C57BL/6J	Version	V1

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	CACAAACTTGTGGCCTGCTC	Forward
P2	AGGCAGTCTCTGATCCAGGT	Reverse
P3	CTCACAAACTTGTGGCCTGC	Forward
P4	ACTACGTCTCCAGAAGGCT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 HE HE WT M 3406bp 401bp 0.5 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 410bp DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -3005 bp Wild type: P1P2 =3406 bp; P3P4 =410 bp Heterozygote: P1P2 =3406 bp and 401 bp; P3P4 =410 bp Homozygote: P1P2 =401 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	