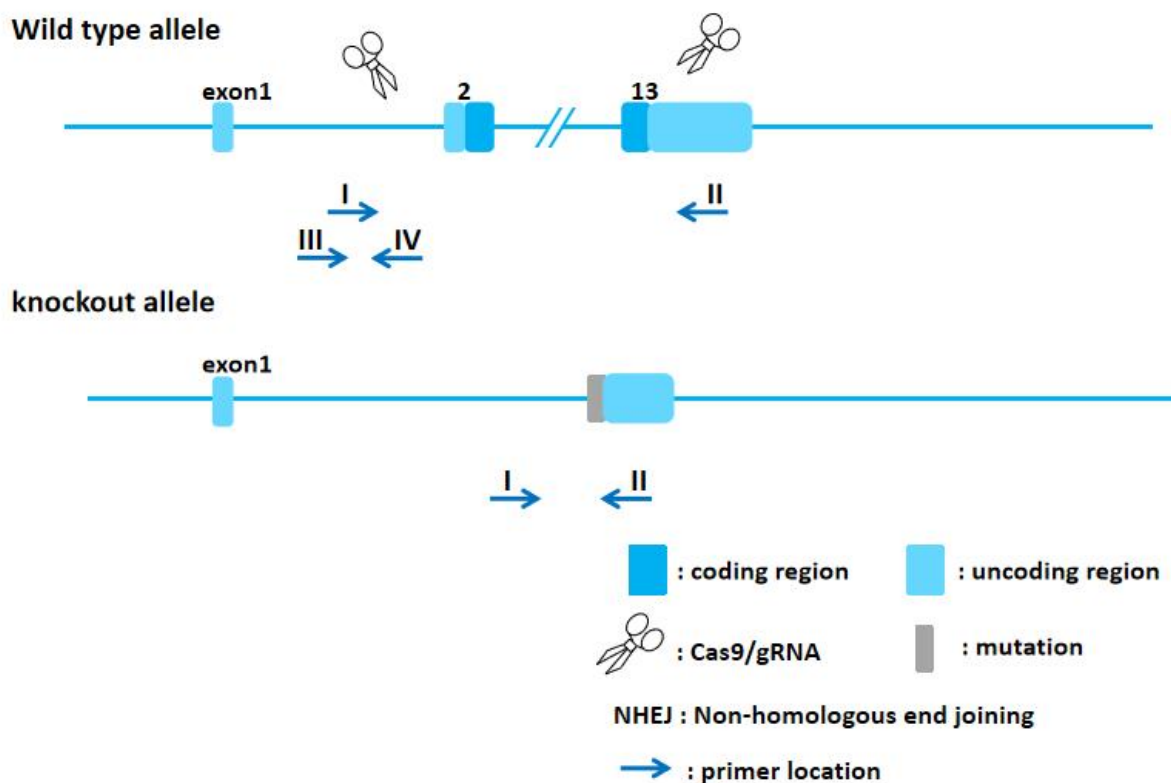


Atp6v1c1-KO Genotyping Protocol



Common Name	Atp6v1c1-KO	Cat. NO.	NM-KO-241375
Strain of Origin	C57BL/6J	Version	V1

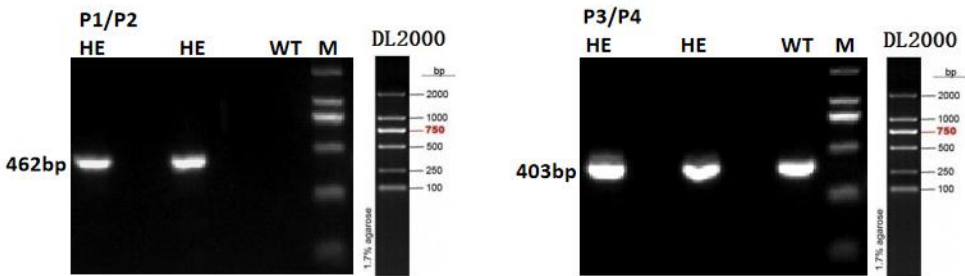
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	CCTGCTGTTGTAGCTGGGAA	Forward
P2	GGCTGTGCACAACAGTAACA	Reverse
P3	TAGCAGTGAAGAGGCCAACC	Forward
P4	AAAATGCCCCAGAAACCCCA	Reverse

Expected results

Results	
Genotype	Knockout type: -19114 bp Wild type: P3P4 =403 bp Heterozygote: P1P2 =462 bp; P3P4 =403 bp Homozygote: P1P2 =462 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	