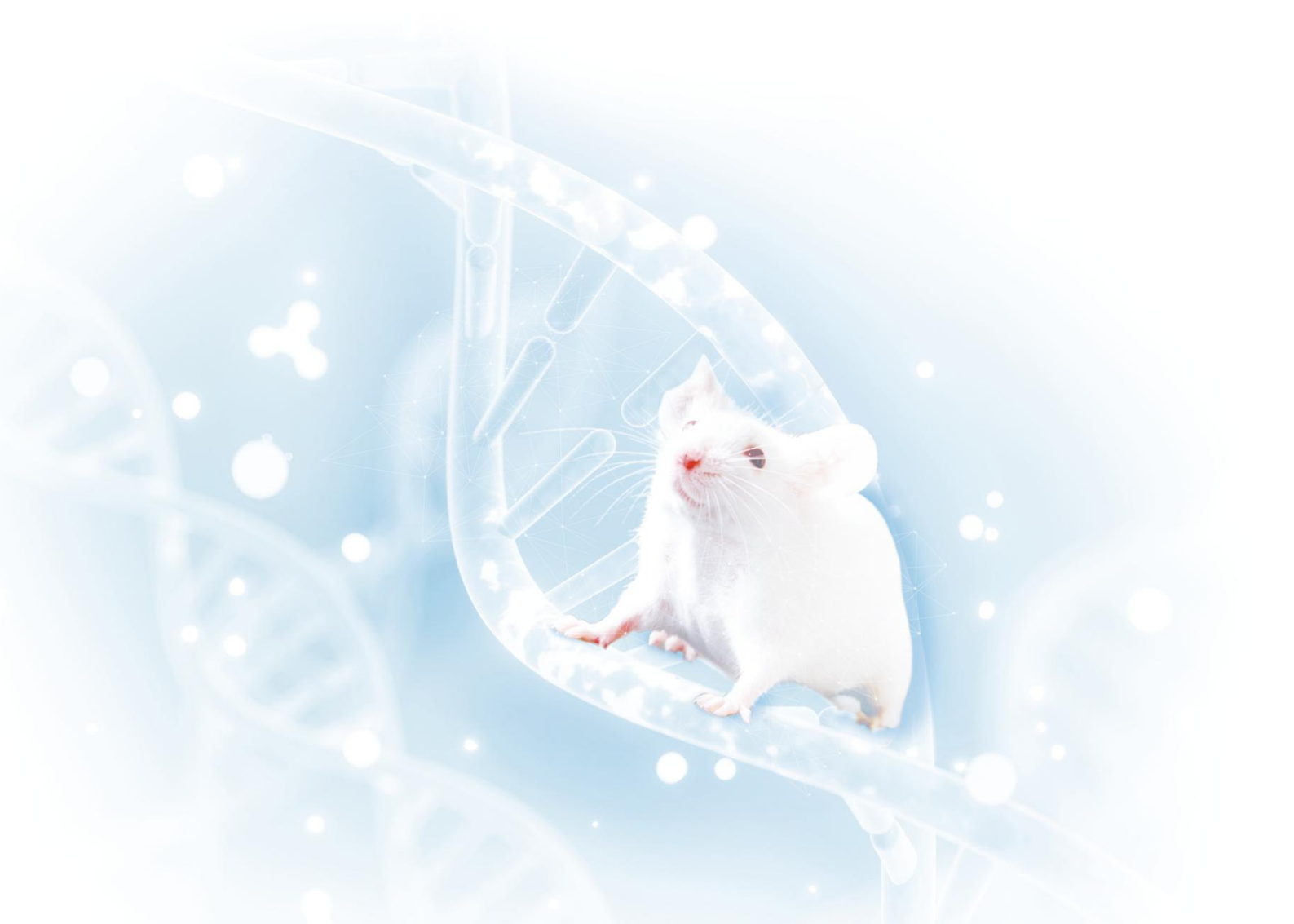


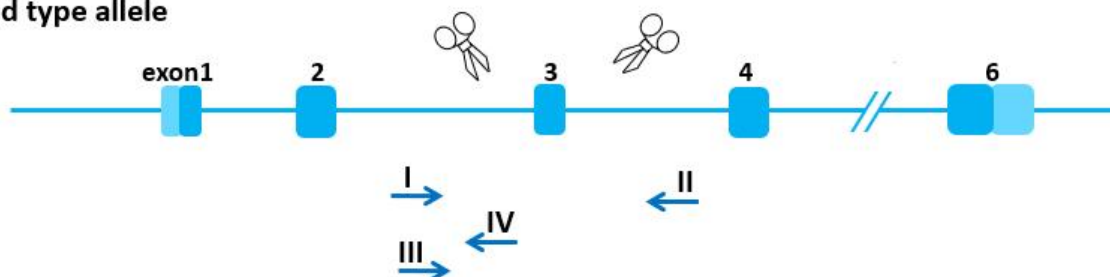
Vapb-KO Genotyping Protocol



Common Name	Vapb-KO	Cat. NO.	NM-KO-241164
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	ATTGCTTTTCCTGCTGTGGC	Forward
P2	AAGCAGGGCCTGTTCAGAGA	Reverse
P3	TGATGAGCTGCGTGGGAAAT	Forward
P4	CAGGTCAGCAGTTAAGGCCA	Reverse

Expected results

Results	P1/P2 HE HE WT M 1104bp 469bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7V/cm, 45 min P3/P4 HE HE WT H M 306bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -635bp Wild type: P1P2 =1104 bp; P3P4 =306 bp Heterozygote: P1P2 =1104 bp and 469 bp; P3P4 =306 bp Homozygote: P1P2 =469 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	