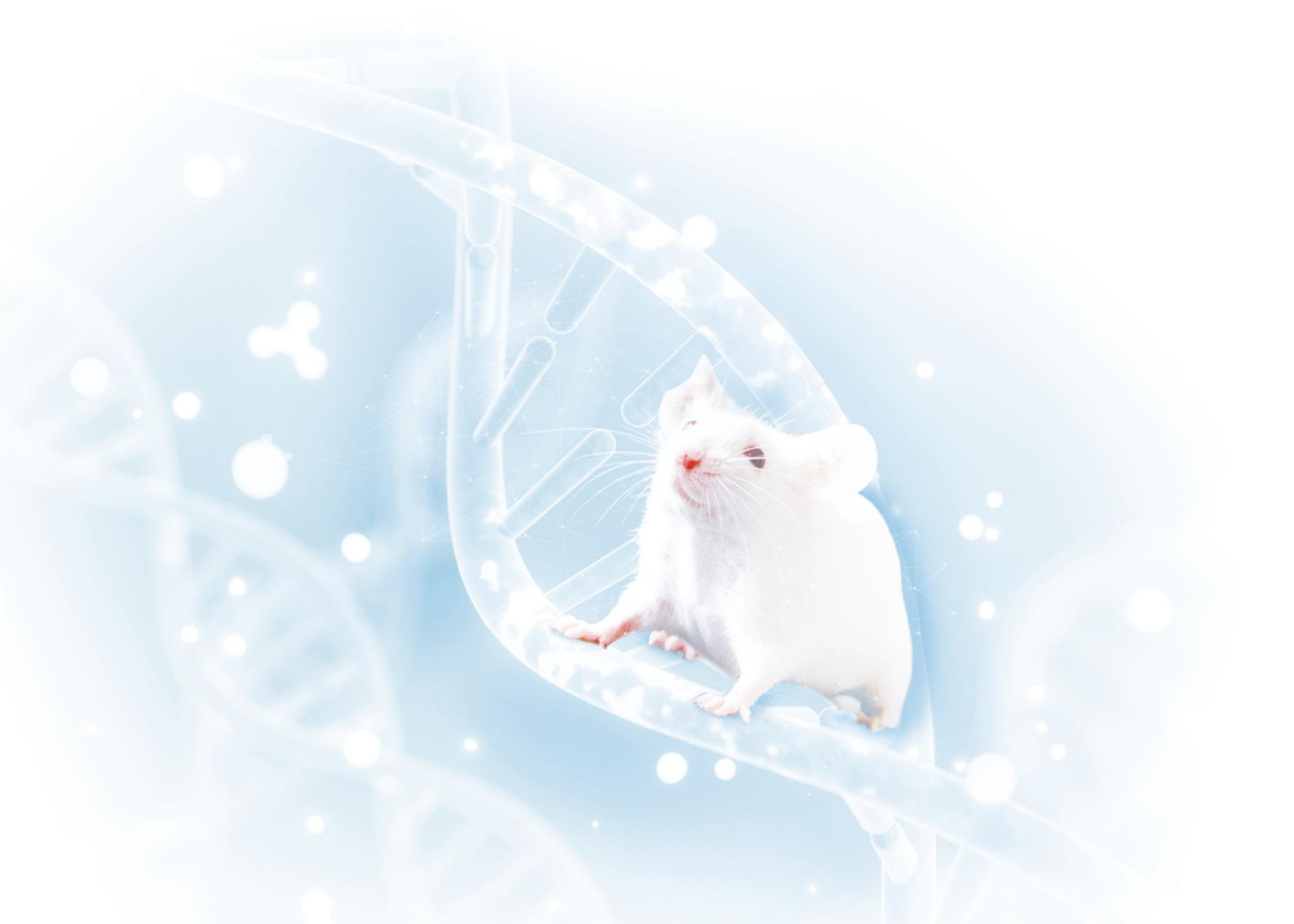


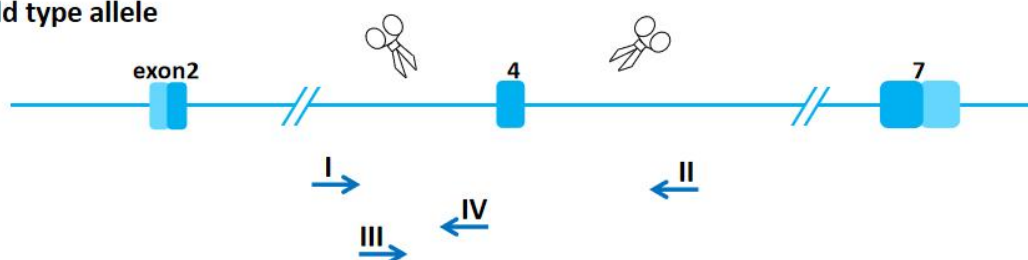
Tmem164-KO Genotyping Protocol



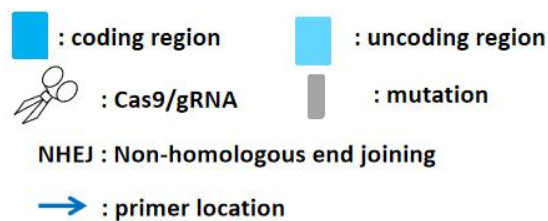
Common Name	Tmem164-KO	Cat. NO.	NM-KO-234906
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

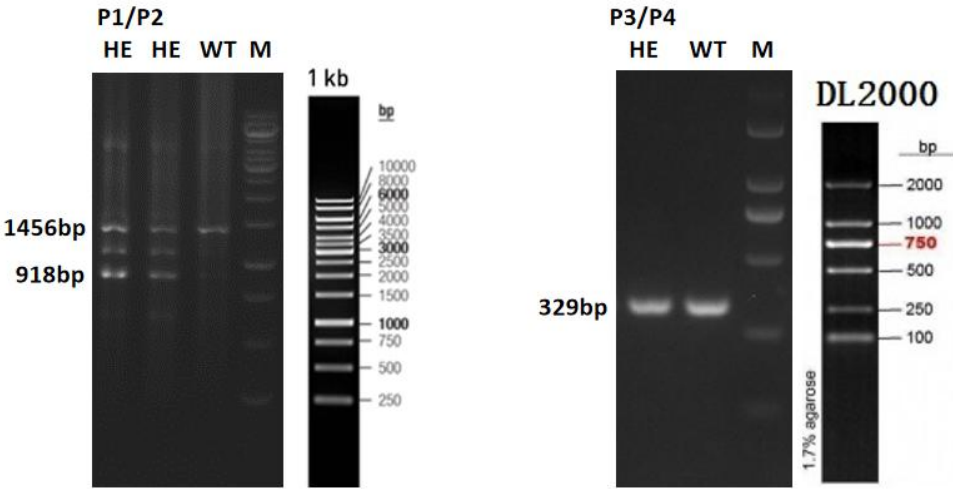


Primers

Primer	Sequence (5' → 3')	Primer type
P1	CATCACA TGCAGACCCTGT	Forward
P2	AGCCACCATGTGGTTACTGG	Reverse
P3	CAGCCTCTTCTGTCCAGGTC	Forward

P4	AGTGACCCTCGGTGAAAAAC	Reverse
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Expected results

Results	 P1/P2 HE HE WT M 1456bp 918bp 1 kb bp 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 P3/P4 HE WT M 329bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -538 bp Wild type: P1P2 =1456 bp; P3P4 =329 bp Heterozygote: P1P2 =1456 bp and 918 bp; P3P4 =329 bp Homozygote: P1P2 =918 bp 已设计 3 对以上 P1P2 引物, 无法摸到合适的阳性条带小于 500bp 的引物。

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 K0 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	