

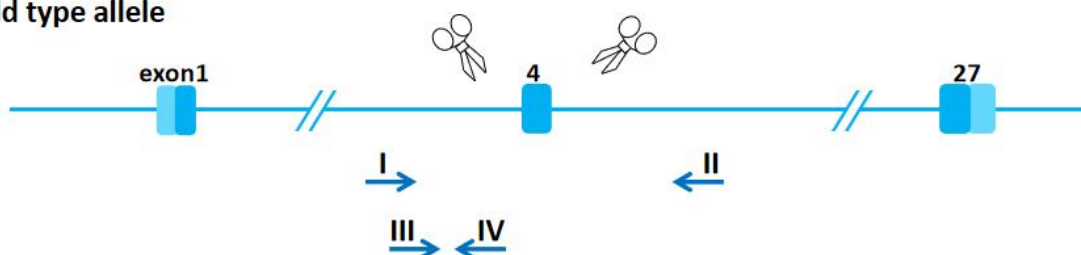
Ano3-K0 Genotyping Protocol



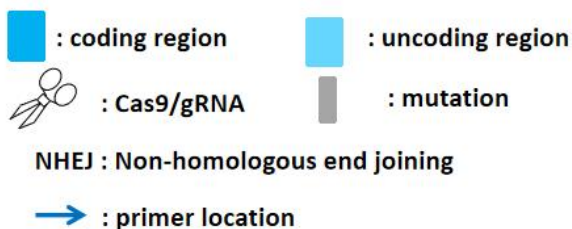
Common Name	Ano3-KO	Cat. NO.	NM-KO-240968
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCAGCTTGCTTGGCTTCTAC	Forward
P2	AGCTGCCCTTTACCTAACC	Reverse
P3	ACTGGGACCAGAGGATGCTA	Forward
P4	GCCTTCAGGTGGATCAGACG	Reverse

Expected results

Results	
Genotype	Knockout type: -1299bp Wild type: P3P4 =253 bp Heterozygote: P1P2 =564 bp; P3P4 =253 bp Homozygote: P1P2 =564 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 K0 band

Reaction &Cycling

PCR	Reaction Component	Volume (μl)
Reaction	ddH2O	8.0
System	2×Taq Plus 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×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
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	