

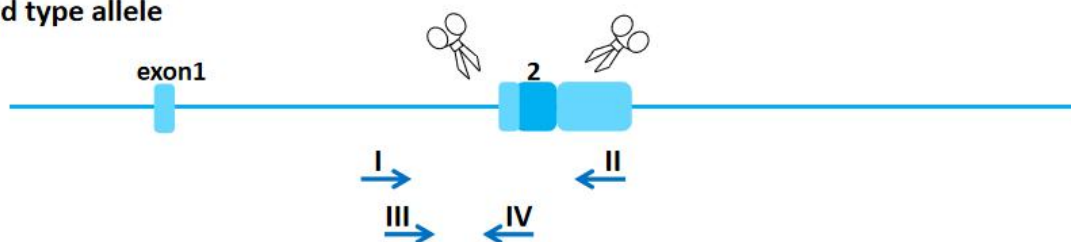
Nat2-K0 Genotyping Protocol



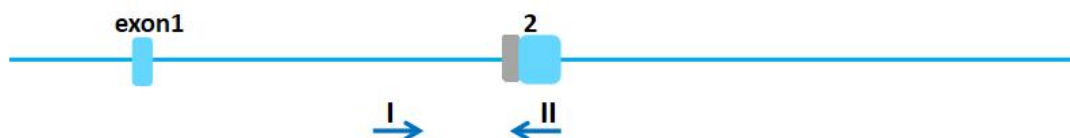
Common Name	Nat2-KO	Cat. NO.	NM-KO-240374
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

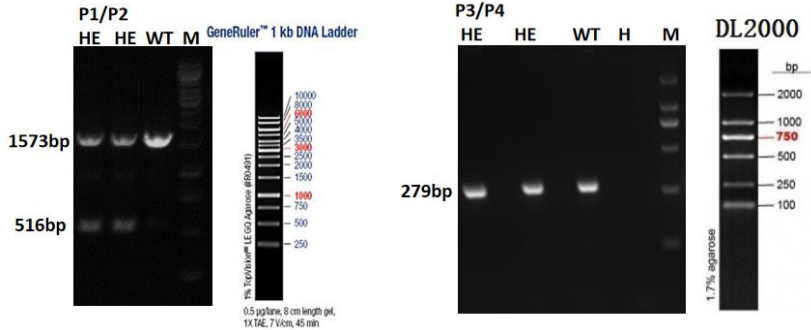


 : coding region  : uncoding region
 : Cas9/gRNA  : mutation
 NHEJ : Non-homologous end joining
 : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGGGCAGAGCATACTACGTC	Forward
P2	TTGGTGTTTGTCCACAGT	Reverse
P3	GCTCACCATTGACCTGCAT	Forward
P4	TACGCTTCGATGTCCATGGTT	Reverse

Expected results

Results	 GeneRuler™ 1 kb DNA Ladder DL2000
Genotype	Knockout type: -1057bp Wild type: P1P2 =1573 bp; P3P4=279 bp Heterozygote: P1P2 =1573 bp and 516 bp; P3P4=279 bp Homozygote: P1P2 =516 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

	Reaction Component	Volume (μl)
PCR	ddH2O	8.0
Reaction	2×Rapid Taq Master Mix	10.0
System	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	