

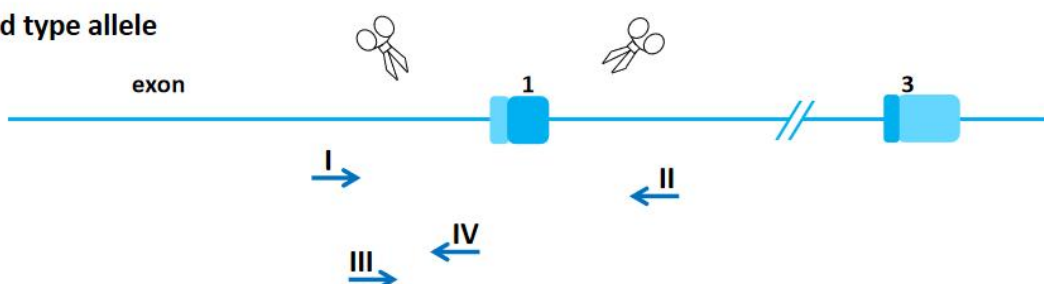
Cdkn2a-K0 (2) Genotyping Protocol



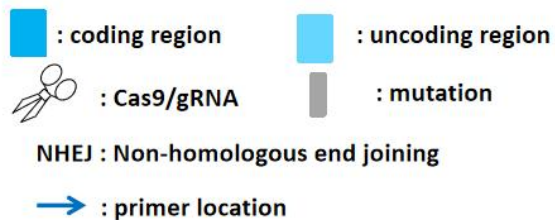
Common Name	Cdkn2a-K0 (2)	Cat. NO.	NM-K0-240239
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGGGGTCAGTTCTCTGTCTGT	Forward
P2	GAAGGCACCTTCCAGCCTTT	Reverse
P3	GCCTTTAATCCTGGCACCCA	Forward

P4	TAAATGAGTTGGTGTGCTGCG	Reverse
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Expected results

Results	
Genotype	Knockout type: -1312 bp Wild type: P1P2 =1797 bp; P3P4 =247 bp Heterozygote: P1P2 =1797 bp and 485 bp; P3P4 =247 bp Homozygote: P1P2 =485 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 Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	