

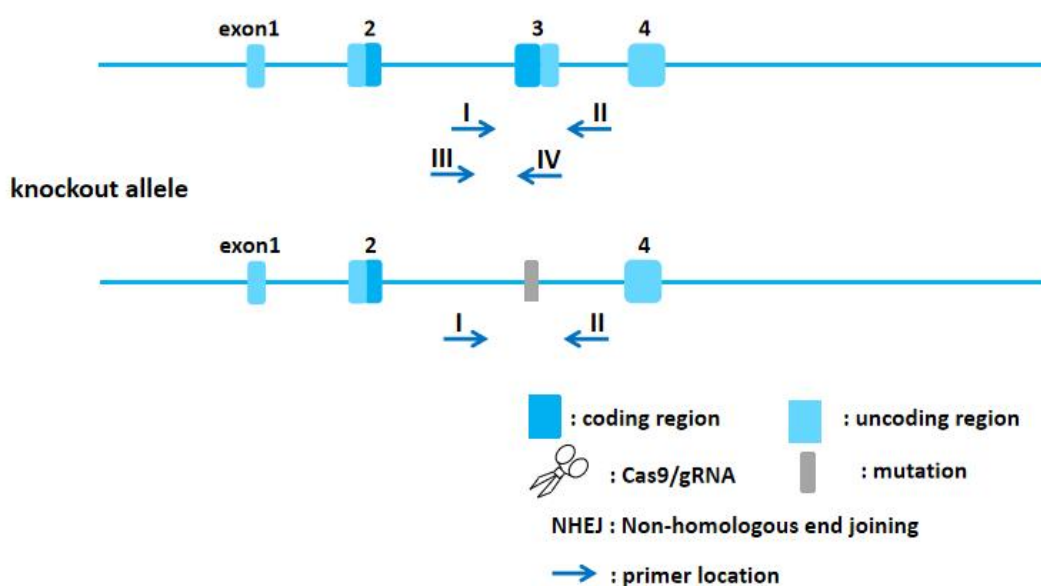
Apela-KO(2) Genotyping Protocol



Common Name	Apela-KO(2)	Cat. NO.	NM-KO-210328
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	GTCTTGCTGCTGAAAAGTCAGC	Forward
P2	GTGCATATCCTAGCCAGTGT	Reverse
P3	ACGGAAGATGGAGTGTCTTGG	Forward
P4	AAGGAGAAAGGGGCAGCATC	Reverse

Expected results

Results	
Genotype	Knockout type: -601 bp Wild type: P1P2 = 971 bp; P3P4 = 438 bp Heterozygote: P1P2 = 971 bp and 370 bp; P3P4 = 438 bp Homozygote: P1P2 = 370 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 (μ l)
	ddH ₂ O	8.0
	2 × Rapid Taq Master Mix	10.0
	P1(10 pmol/ μ l) or P3(10 pmol/ μ l)	0.5
	P2(10 pmol/ μ l) or P4(10 pmol/ μ l)	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	