

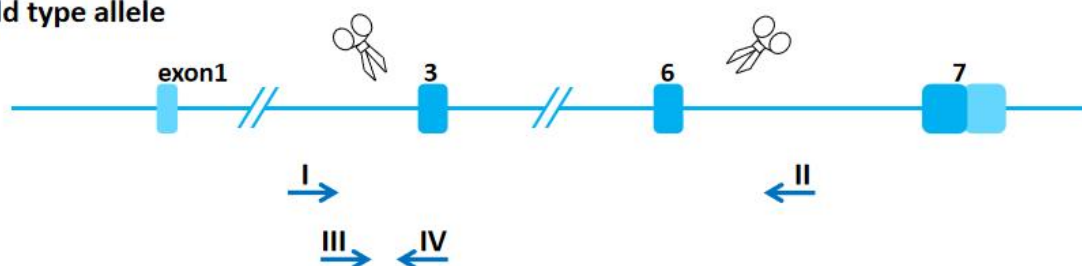
Calu-K0 (2) Genotyping Protocol



Common Name	Calu-K0 (2)	Cat. NO.	NM-K0-234852
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



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

Primers

Primer	Sequence (5' → 3')	Primer type
P1	AAGTGAGAACCCAGGTGGAC	Forward
P2	GAGACCAGACAGTGCTACCC	Reverse
P3	GGCCGTGATCTGTTACCTTT	Forward
P4	CCAGCGCTTTTGTGCAAATT	Reverse

Expected results

Results	
Genotype	Knockout type: -11501bp Wild type: P1P2 =11777 bp; P3P4=250 bp Heterozygote: P1P2 =11777 bp and 276 bp; P3P4=250 bp Homozygote: P1P2 =276 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

	Reaction Component	Volume (μl)
PCR Reaction System	ddH2O	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	