

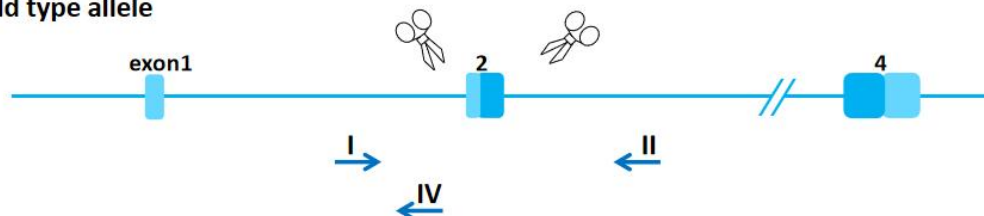
Atf3-KO Genotyping Protocol



Common Name	Atf3-K0	Cat. NO.	NM-K0-226260
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	GGACCGAGGCCACATTTGTA	Forward
P2	GCCTGCAGAGGATGTTGGAT	Reverse
P4	GTCCCATCCTAAACAGCACCC	Reverse

Expected results

Results	P1P2P4 HE HE WT H₂O M DL2000 614 bp 479 bp bp 2000 1000 750 500 250 100 1% agarose
Genotype	Knockout type: -1012bp Wild type: P1P4=479 bp Heterozygote: P1P2 =614 bp; P1P4=479 bp Homozygote: P1P2 =614 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 (μl)
	ddH ₂ O			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	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	