

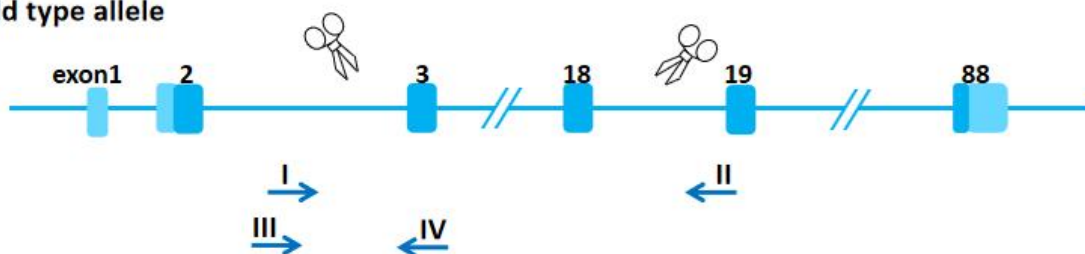
Zan-KO Genotyping Protocol



Common Name	Zan-KO	Cat. NO.	NM-KO-240426
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	TTTGTAAGGTGGGGAGGGGA	Forward
P2	TCCCAATCTGGGAGAGAGG	Reverse
P3	TTGAGTTTGTAAGGTGGGGAG	Forward
P4	CCAACTCACAATCCTCCCTCG	Reverse

Expected results

Results	
Genotype	Knockout type: -19240 bp Wild type: P3P4 =434 bp Heterozygote: P1P2 =417 bp; P3P4 =434 bp Homozygote: P1P2 =417 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			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	35 repeats to 2
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	20 sec	
	5	72°C	5 min	
	6	12°C	Hold	