

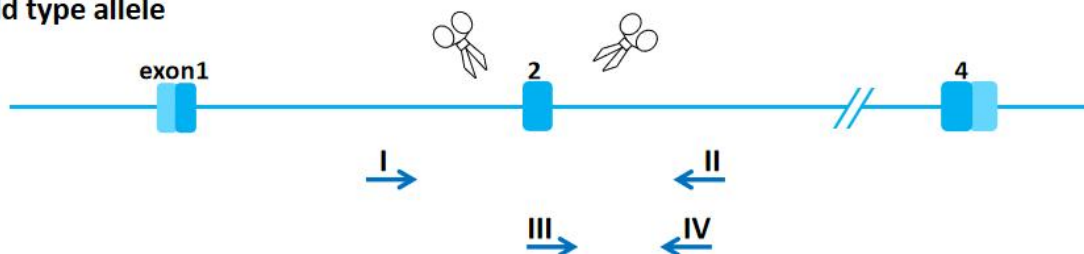
Wnt3a-KO Genotyping Protocol



Common Name	Wnt3a-KO	Cat. NO.	NM-KO-234571
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	CACTGAACCCAGAACTCCCC	Forward
P2	AAAGAAGCCTTTGCCCACT	Reverse
P3	CTTCTGCAGGAACCTACGTGGA	Forward
P4	CTCCCCAACATACTCCAAGCC	Reverse

Expected results

Results	
Genotype	Knockout type: -392bp Wild type: P1P2 =1056 bp; P3P4=324 bp Heterozygote: P1P2 =1056 bp and 664 bp; P3P4=324 bp Homozygote: P1P2 =664 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 K0 band

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			8.0
	2×Taq Plus 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×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	