

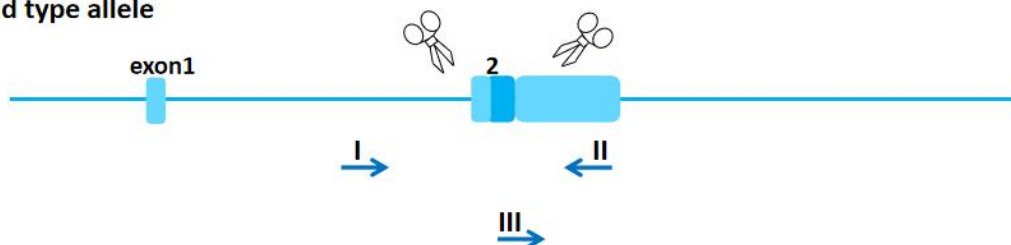
Xcr1-KO Genotyping Protocol



Common Name	Xcr1-K0	Cat. NO.	NM-K0-200568
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	ACAAGGGCACAGTAGTGGGA	Forward
P2	AGTTTGGGGCAACCCTATGT	Reverse
P3	GGGACCCTCCTTCTACTGAG	Forward

Expected results

Results	P1P2P3 HE HE WT H₂O M DL2000 475 bp 346 bp bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -1206bp Wild type: P3P2=346 bp Heterozygote: P1P2 =475 bp; P3P2=346 bp Homozygote: P1P2 =475 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)
	ddH ₂ O			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P3 (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	