

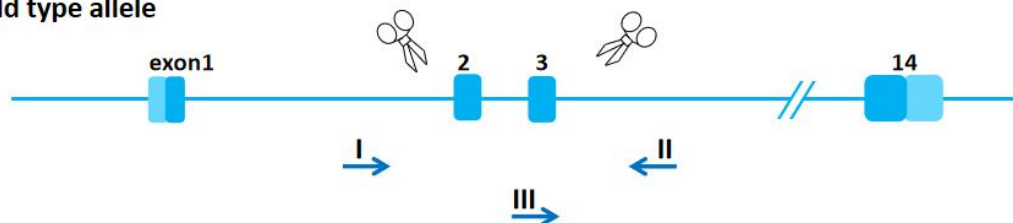
Ubash3b-K0 Genotyping Protocol



Common Name	Ubash3b-KO	Cat. NO.	NM-KO-233125
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



: coding region : uncoding region

: Cas9/gRNA : mutation

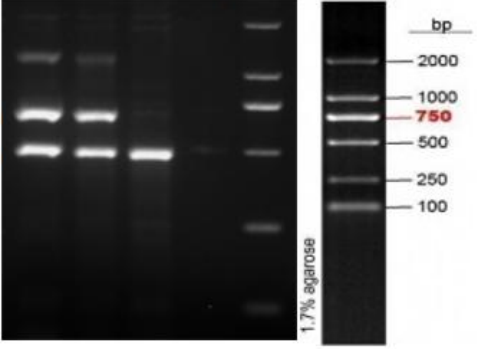
NHEJ : Non-homologous end joining

: primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGATGATGGATCCCCCTGG	Forward
P2	TCTACCCACCAAGGGTCAGG	Reverse
P3	CTTATCTCCACAGGTTGTTCTCC	Forward

Expected results

Results	P1P2P3 HE HE WT H₂O M DL2000 
Genotype	Knockout type: -1042bp Wild type: P3P2=480 bp Heterozygote: P1P2 =684 bp; P3P2=480 bp Homozygote: P1P2 =684 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			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	