

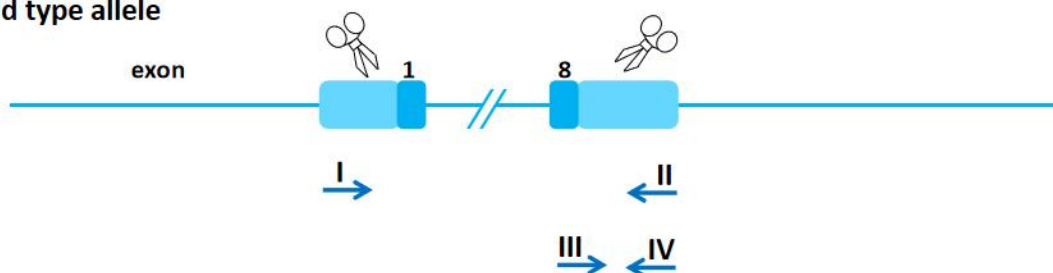
Ust-K0 Genotyping Protocol



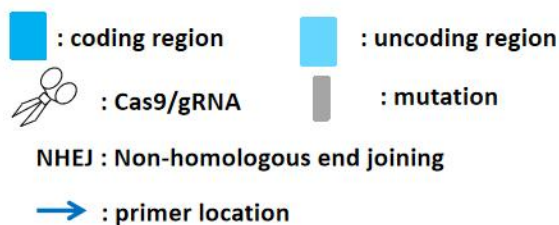
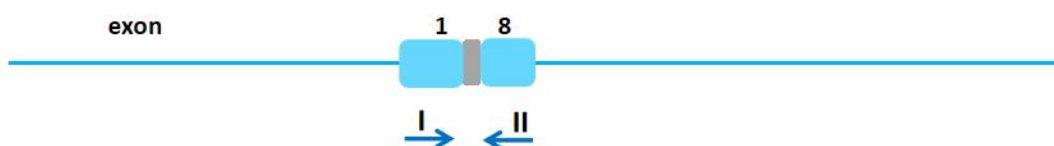
Common Name	Ust-K0	Cat. NO.	NM-K0-240280
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

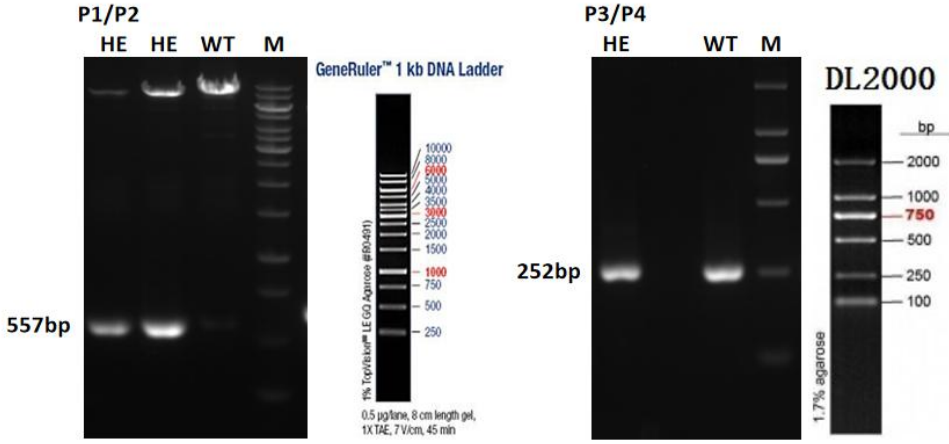


Primers

Primer	Sequence (5' → 3')	Primer type
P1	CAGGCGTGGGTGCGA	Forward
P2	CAGTGGATTCTCCCTCTTGTC	Reverse
P3	GATACCTGCGCCAGATTGTG	Forward

P4	AGGTGCCTTGTGGTAGAGC	Reverse
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Expected results

Results	 GeneRuler™ 1 kb DNA Ladder DL2000 557bp 252bp 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -311181 bp Wild type: P3P4 =252 bp Heterozygote: P1P2 =557 bp; P3P4 =252 bp Homozygote: P1P2 =557 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 Component	Volume (µl)
Reaction	ddH2O	8.0
System	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	
	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	