

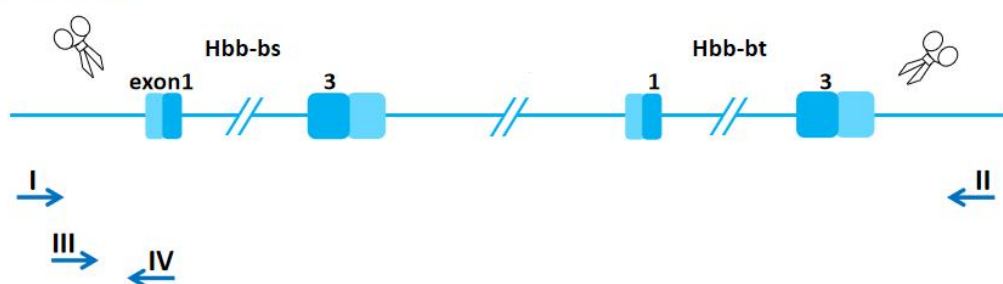
Hbb-bs/Hbb-bt-KO Genotyping Protocol



Common Name	Hbb-bs/Hbb-bt-KO	Cat. NO.	NM-KO-234923
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



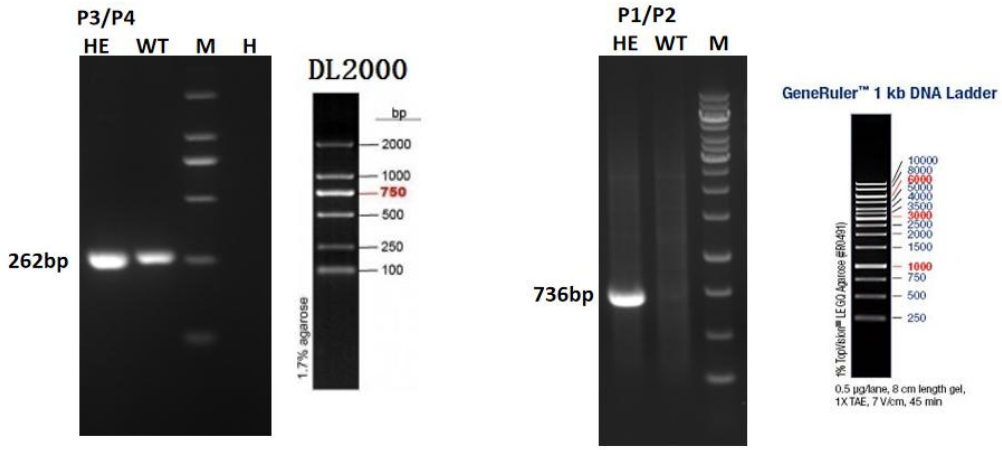
- : coding region
 : uncoding region
 : Cas9/gRNA
 : mutation
 NHEJ : Non-homologous end joining
→ : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGTACACTCCTCCCACACACA	Forward
P2	GGAAGGTTGCTGACGGAATA	Reverse

P3	CAGAGCTGAGACTCCTAAGCC	Forward
P4	GCAGCCTTCTCAGCATCAGT	Reverse

Expected results

Results	 P3/P4 HE WT M H 262bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose P1/P2 HE WT M 736bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1% Tris/Glycine/ED, Agarose #R10491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -15563 bp Wild type: P3P4=262 bp Heterozygote: P1P2 =736 bp; P3P4=262 bp Homozygote: P1P2 =736 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	