

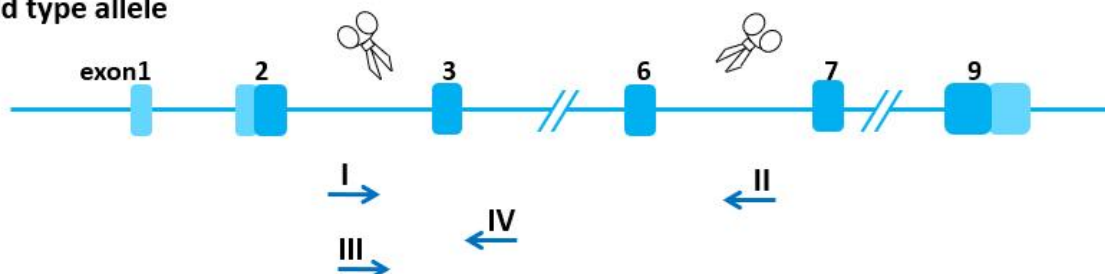
Tdh-KO Genotyping Protocol



Common Name	Tdh-KO	Cat. NO.	NM-KO-240553
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	ATTCTCGTCTTTGGAGCTGGG	Forward
P2	GGCACAGAGTGTGGCTGTTA	Reverse
P3	CCTGCATGGAAAGCAACTCC	Forward
P4	TGTTTGTGCTAAGGAGGCA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 WT M HE HE 2817bp 705bp P3/P4 HE HE WT H M 424bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -2112bp Wild type: P1P2 =2817 bp; P3P4 =424 bp Heterozygote: P1P2 =2817 bp and 705 bp; P3P4 =424 bp Homozygote: P1P2 =705 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 System	Reaction Component			Volume (µl)
	ddH ₂ O			8.0
	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	