

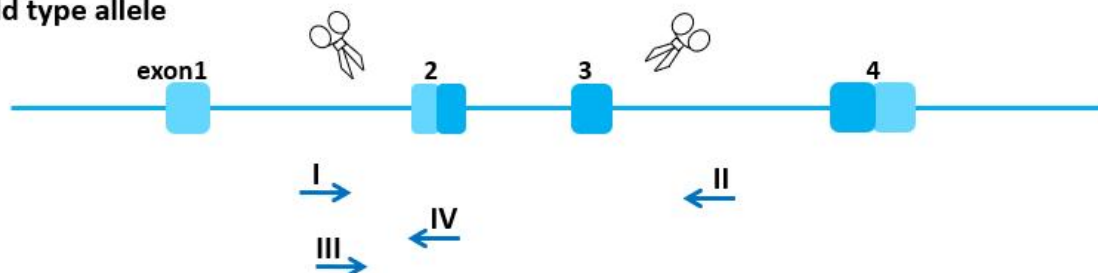
Tspo-KO Genotyping Protocol



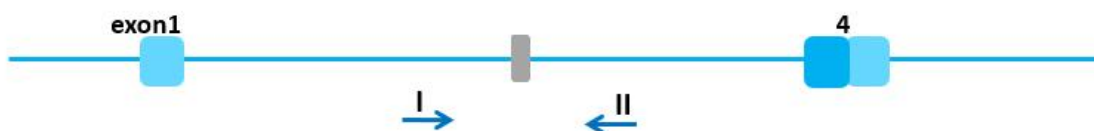
Common Name	Tspo-KO	Cat. NO.	NM-KO-240428
Strain of Origin	C57BL/6J	Version	V1

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	ACAATGCACAGACCTCCCAG	Forward
P2	CAGAACACAGGGCACCAGAT	Reverse
P3	GTCACACCAGGAGACCACAG	Forward
P4	CGGATGCCAAGAGGGTTTCT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% TgE buffer™ LE G-D Agarose #B0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -1062bp Wild type: P1P2 =1696 bp; P3P4 =433 bp Heterozygote: P1P2 =1696 bp and 634 bp; P3P4 =433 bp Homozygote: P1P2 =634 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	