

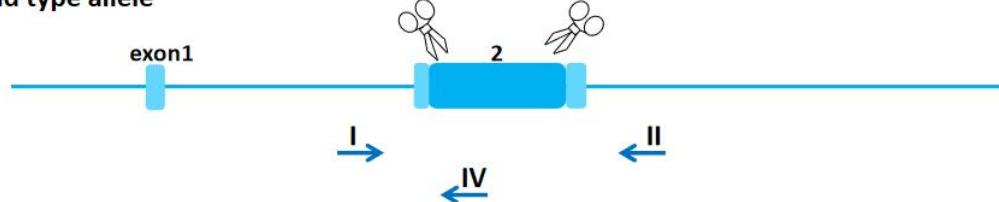
# Trex1-KO Genotyping Protocol



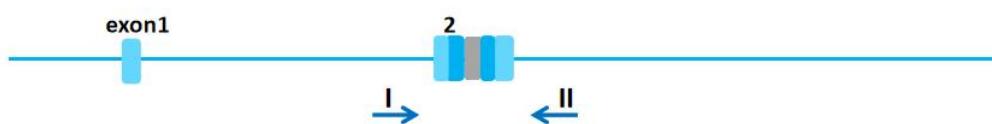
Common Name	Trex1-KO	Cat. NO.	NM-KO-232136
Strain of Origin	C57BL/6J	Version	V1

### Genotyping strategy

#### Wild type allele



#### knockout allele



 : coding region       : uncoding region  
 : Cas9/gRNA       : mutation

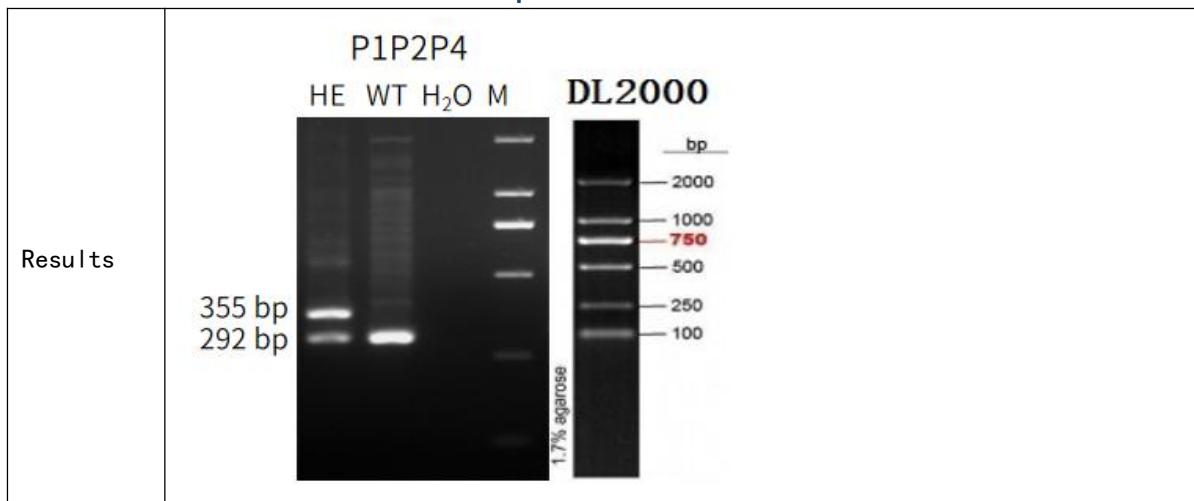
NHEJ : Non-homologous end joining

→ : primer location

### Primers

Primer	Sequence (5' → 3' )	Primer type
P1	TCCCACTAGAACAAACCCCTGC	Forward
P2	CCCGCTTCCCCTTCTTCAG	Reverse
P4	AGTGTCTCCAGAGCACGTC	Reverse

### Expected results



	Knockout type: -998bp
Genotype	Wild type: P1P4=292 bp Heterozygote: P1P2 =355 bp; P1P4=292 bp Homozygote: P1P2 =355 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 ( $\mu$ l)
	ddH2O		7.5
	2 $\times$ Taq Plus Master Mix		10.0
	P1(10 pmol/ $\mu$ l)		0.5
	P2(10 pmol/ $\mu$ l)		0.5
	P4(10 pmol/ $\mu$ l)		0.5
	Genomic DNA		1.0
	Total		20
2 $\times$ Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
Cycling Reaction	Step	Temp	Time
	1	95° C	5 min
	2	95° C	20 sec
	3	60° C	20 sec
	4	72° C	20 sec
	5	72° C	5 min
	6	12° C	Hold
			Note
			35 repeats to 2