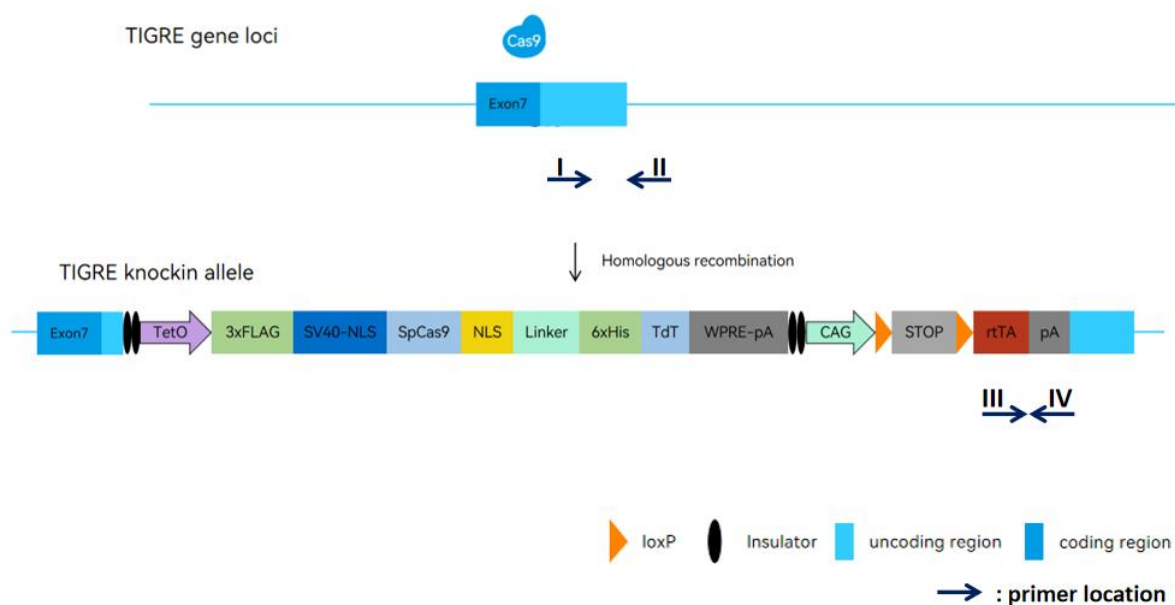


TIGRE-TetO-Cas9TdT-CAG-LSL-rtTA Genotyping Protocol



Common Name	TIGRE-TetO-Cas9TdT-CAG-LSL-rtTA	Cat. NO.	NM-KI-240155
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GCTTCCCCAACGGTCACTTA	Forward
P2	TCCCAAGTCATCCCTCCCAT	Reverse
P3	GCAATTGAGCTGTTCGACCG	Forward
P4	GGGCAAACAACAGATGGCTG	Reverse

Expected results

Results	
Genotype	Wild type: P1P2 =461 bp; Heterozygote: P1P2 =461 bp; P3P4=283 bp Homozygote: P3P4 =283 bp

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	