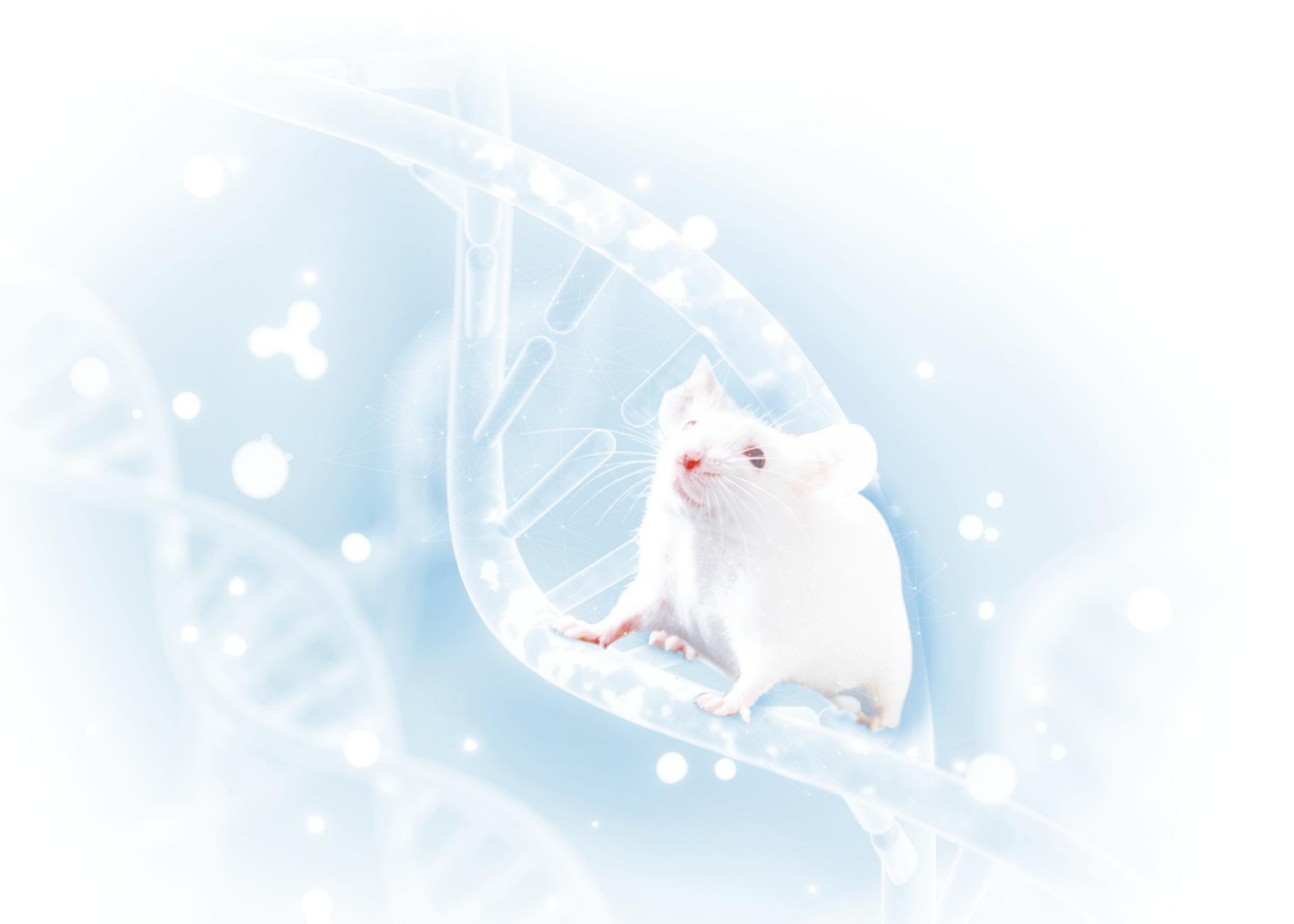


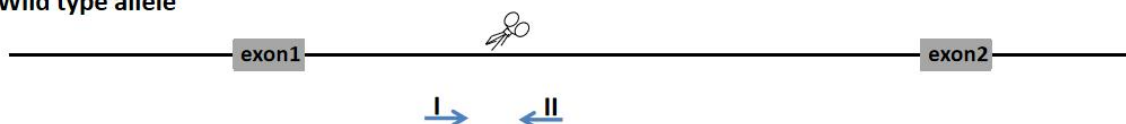
R26-CAG-Luc-2A-tdTomato(M-NSG) Genotyping Protocol



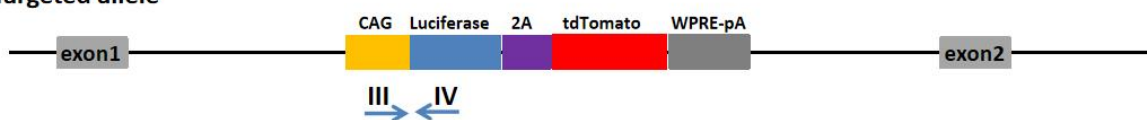
Common Name	R26-CAG-Luc-2A-tdTomato(M-NSG)	Cat. NO.	NM-KI-233090
Strain of Origin	M-NSG	Version	V1

Genotyping strategy

Wild type allele



Targeted allele



→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	AGGCAGGGTCTCACTATGTAT	Forward
P2	TTGATGGGGGAAAACCTGAATG	Reverse
P3	TGTCCCAAATCTGTGCGGAG	Forward
P4	TTCGAGTGGGTAGAATGGCG	Reverse

Expected results

Results	DL2000 bp 2000 1000 750 500 250 100 1.7% agarose P1/P2 451bp He WT M P3+P4 HE HE WT M 433bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1% TopVision™ LE G0 Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Wild type: P1P2 =451 bp; Heterozygote: P1P2 =451 bp; P3P4 =433 bp Homozygote: P3P4 =433 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	