

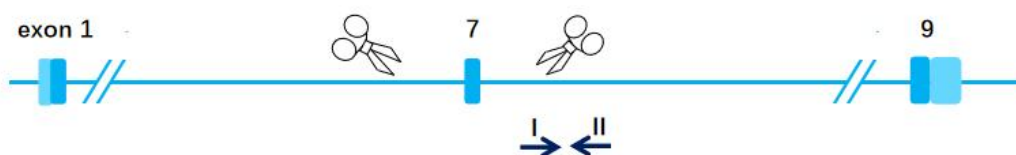
Znrf3-Flox Genotyping Protocol



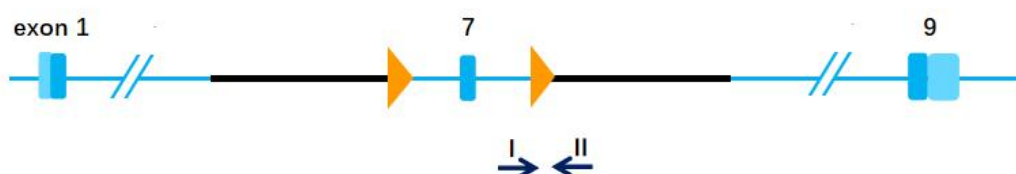
<i>Common Name</i>	<i>Znrf3-Flox</i>	<i>Cat. NO.</i>	<i>NM-CKO-240898</i>
<i>Strain of Origin</i>	<i>C57BL/6J</i>	<i>Version</i>	<i>V1</i>

Genotyping strategy

Wild type allele



Targeted allele

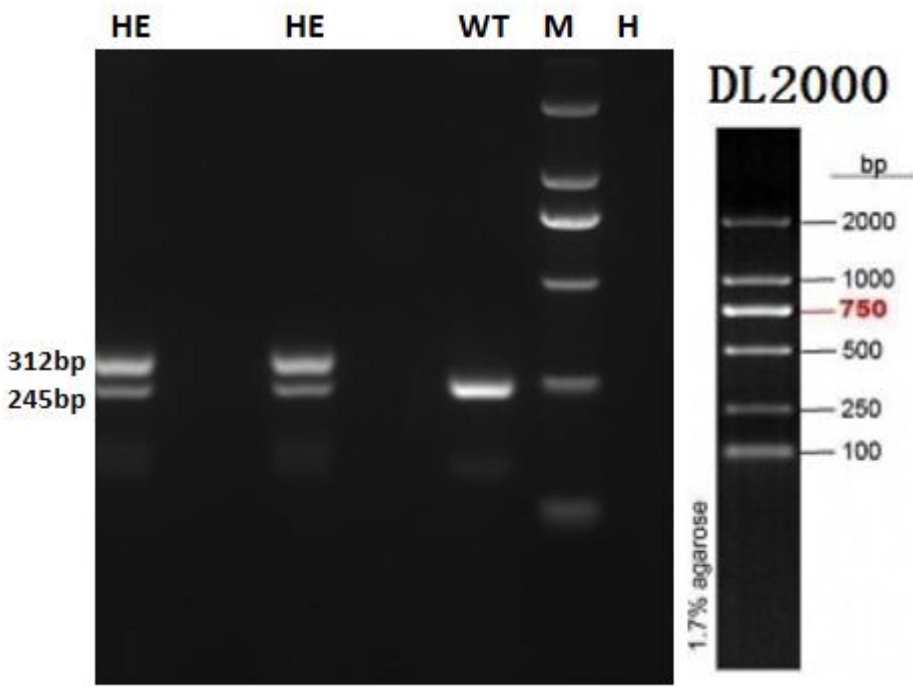


→ : primer location

Primers

<i>Primer</i>	<i>Sequence (5'→3')</i>	<i>Primer type</i>
P1	GAGCTGCAAGTGACATGGTG	Forward
P2	TGGTCTCCAACTTGCCACAA	Reverse

Expected results

Results	 DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 312bp 245bp HE HE WT M H
Genotype	Wild type: P1P2 =245 bp Heterozygote: P1P2 =245 bp and 312 bp Homozygote: P1P2 =312 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)	0.5
	P2(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	