

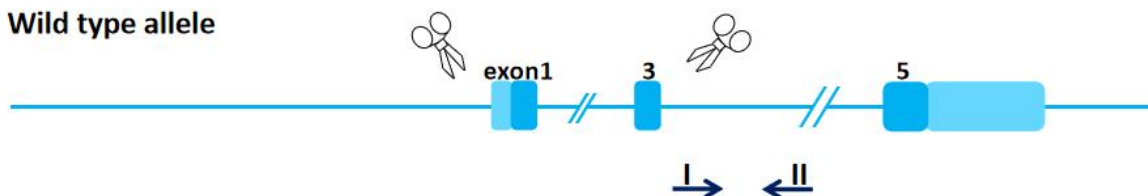
Cd8a-Flox Genotyping Protocol



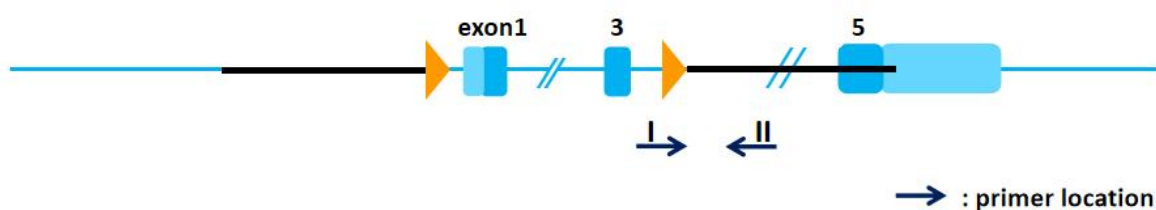
Common Name	Cd8a-Flox	Cat. NO.	NM-CK0-234419
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



Targeted allele



→ : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	GACAGCCCTTCAGGGAGATT	Forward
P2	CAGGGCTATGTAAACCCCTCC	Reverse

Expected results

Results	Agarose gel electrophoresis image. The gel has four lanes labeled HE, HE, WT, M, and H. A DNA ladder (DL2000) is on the right with markers at 2000, 1000, 750, 500, 250, and 100 bp. The HE lanes show two bands at 313bp and 248bp. The WT lane shows a single band at 248bp. The M lane shows a single band at 248bp. The H lane shows a single band at 313bp. The gel is stained with 1.7% agarose.
Genotype	Wild type: P1P2 =248 bp Heterozygote: P1P2 =248 bp and 313 bp Homozygote: P1P2 =313 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			8.0
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
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	