

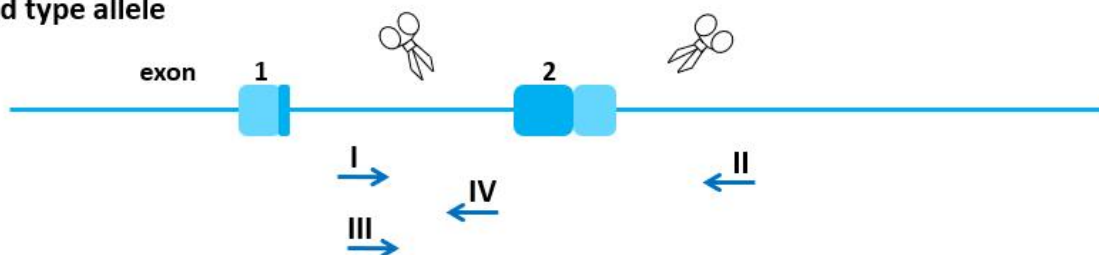
Ifit3-KO Genotyping Protocol



Common Name	<i>Ifit3</i> -KO	Cat. NO.	NM-KO-232784
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	AGAACAGGGATAAAGAATGACAACC	Forward
P2	CAGTGCAGACATAGATGCTAAACAC	Reverse
P3	GTGCACCTGCCACCTTGTAAC	Forward
P4	ACAGCCAGTGCAGGAGGTTT	Reverse

Expected results

Results	P1/P2 HE HE HE WT M 2873bp 379bp GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE GG Agarose (#60491) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 311bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2494bp Wild type: P1P2 =2873 bp; P3P4 =311 bp Heterozygote: P1P2 =2873 bp and 379bp; P3P4 =311 bp Homozygote: P1P2 =379 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 KO band

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

	<i>P2(10 pmol/μl) or P4(10 pmol/μl)</i>			<i>0.5</i>
	<i>Genomic DNA</i>			<i>1.0</i>
	<i>Total</i>			<i>20</i>
	<i>2×Rapid Taq Master Mix from Vazyme(Code Number: P222-01)</i>			
<i>Cycling Reaction</i>	<i>Step</i>	<i>Temp</i>	<i>Time</i>	<i>Note</i>
	<i>1</i>	<i>95°C</i>	<i>5 min</i>	
	<i>2</i>	<i>95°C</i>	<i>20 sec</i>	
	<i>3</i>	<i>60°C</i>	<i>20 sec</i>	
	<i>4</i>	<i>72°C</i>	<i>30 sec</i>	<i>35 repeats to 2</i>
	<i>5</i>	<i>72°C</i>	<i>5 min</i>	
	<i>6</i>	<i>12°C</i>	<i>Hold</i>	