

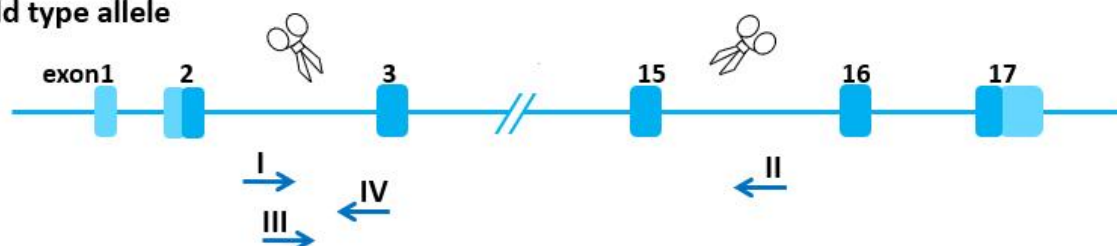
Abcb8-KO Genotyping Protocol



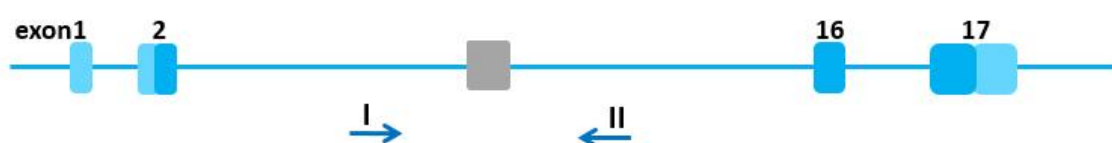
Common Name	<i>Abcb8</i> -KO	Cat. NO.	NM-KO-240865
Strain of Origin	C57BL/6J	Version	V1

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	TCTTCCCGAGGTCCTGAGTT	Forward
P2	TTTCTTGTCCTGGGCTGTGG	Reverse
P3	TGATGTGTCTGAAGTCAGCTAAGG	Forward
P4	TACAGTCCGCAGGAGGTGTG	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder DL2000 8328bp 650bp 425bp 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1% Tris/Glycerol LE G0 Agarose #60491 0.5 µl/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 1.7% agarose 2000 1000 750 500 250 100 bp
Genotype	Knockout type: -7678bp Wild type: P1P2 =8328 bp; P3P4 =425 bp Heterozygote: P1P2 =8328 bp and 650 bp; P3P4 =425 bp Homozygote: P1P2 =650 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

	Reaction Component	Volume (µl)
PCR	ddH ₂ O	8.0
Reaction	2×Rapid Taq Master Mix	10.0
System	P1(10 pmol/µl) or P3(10 pmol/µl)	0.5
	P2(10 pmol/µl) or P4(10 pmol/µl)	0.5

	<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>1min 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>	