

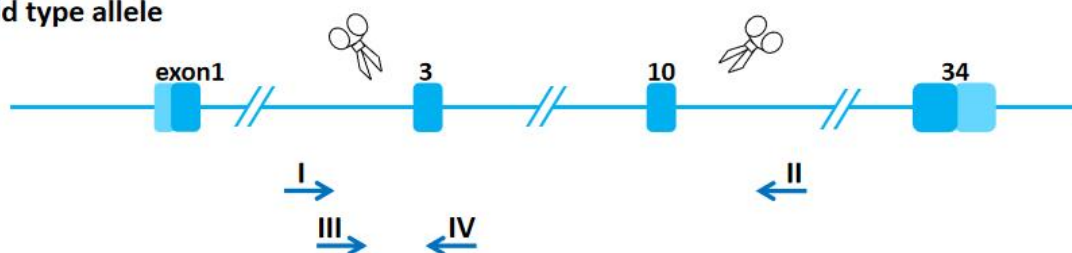
Mybpc3-K0 Genotyping Protocol



Common Name	Mybpc3-KO	Cat. NO.	NM-KO-234766
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	GCCAGGTAAGAGCTTGCTGA	Forward
P2	CCCAACCTCAACTCACCTCC	Reverse
P3	GCTCGGAAAGGGACAACAGA	Forward
P4	CTCGTTGTCCAGCCCTCTTT	Reverse

Expected results

Results	P1/P2 HE WT M 3239bp 756bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE WT M 361bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2484+1bp Wild type: P1P2 =3239 bp; P3P4=361 bp Heterozygote: P1P2 =3239 bp and 756 bp; P3P4=361 bp Homozygote: P1P2 =756 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 Component	Volume (µl)
Reaction	ddH2O	8.0
System	2×Taq Plus 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×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	