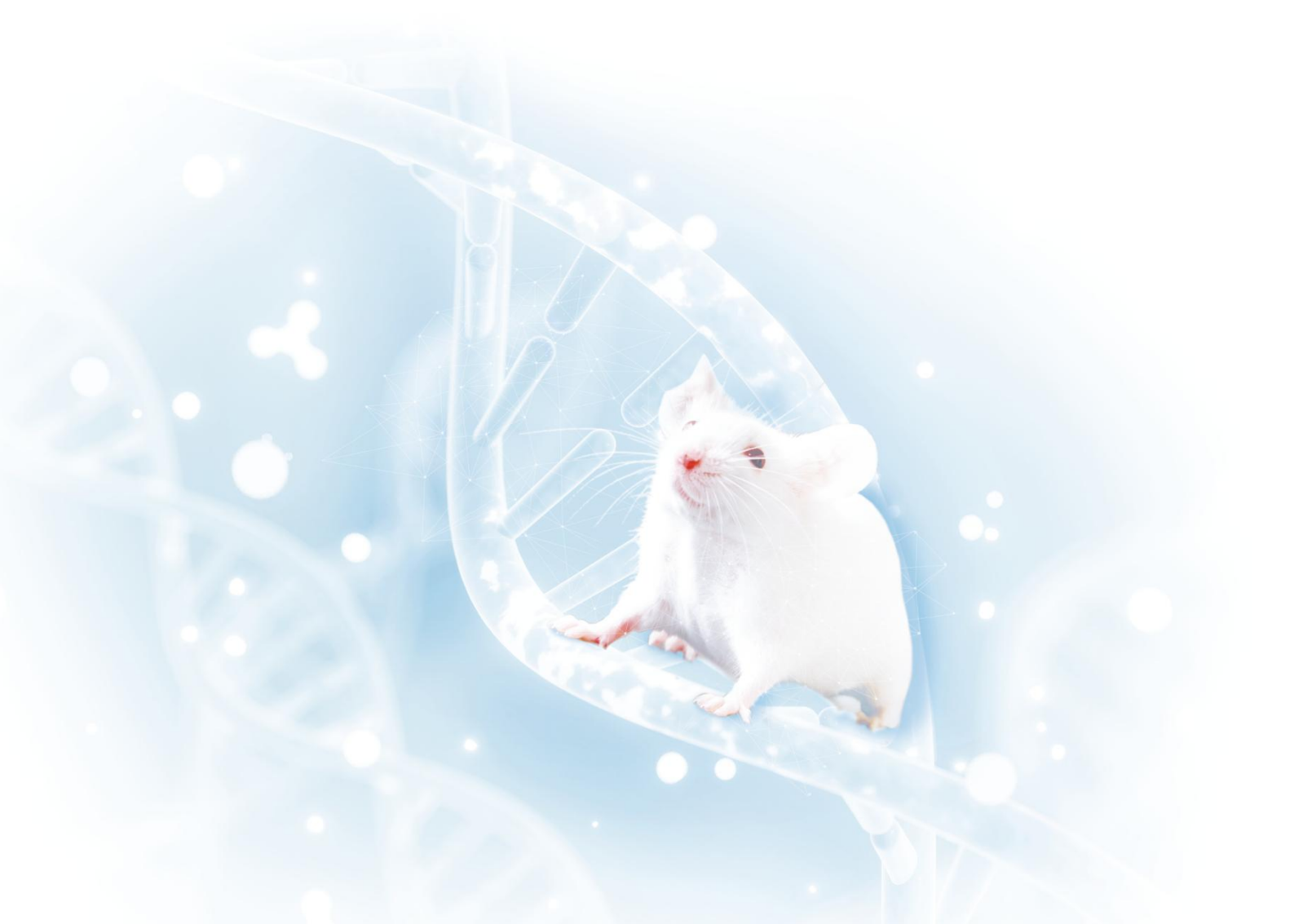
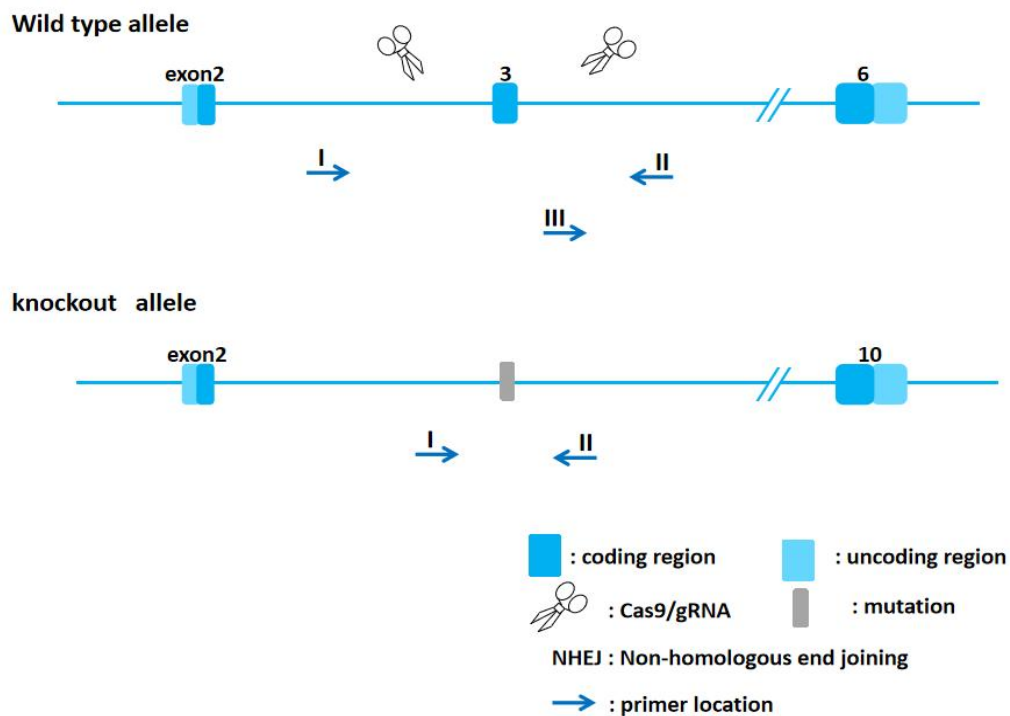


Cdc42 Genotyping Protocol



Common Name	Cdc42-KO	Cat. NO.	NM-KO-226250
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCAGCCGAAGTCTGTGAA	Forward
P2	CACCTGTGAGGCCAGAACA	Reverse
P3	AGGAGGCAAAGGCAGTTGGA	Forward

Expected results

Results	P1P2P3 475 bp 352 bp bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -991 bp Wild type: P2P3 =352 bp Heterozygote: P1P2 =475 bp; P2P3=352 bp Homozygote: P1P2 =475 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)
	ddH2O			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P3 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
Cycling Reaction	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
	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	