

RMRC Genotyping Protocol-PCR

No.	RMRC13428	Strain Name	NOD.Cg-Prkdc ^{scid} Il2rg ^{em1Narl} B2m ^{em1Narl} /YckNarl
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A. Primer

	Primer name	Sequence
1	B2m#67	TAAGATGGTGCACGGTGCAG
2	B2m#68	GGCATGCTTAACCTCTGCAGG

B. Genomic extraction :

- ☐ Phenol-Chloroform extraction
 ☐ commercial extraction kit _____
☐ Lysis buffer _____
 ☐ other _____

C. Reaction Conditions

Step 1. PCR

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, sec	Number of Cycles
H ₂ O	--		32	μl	1	1	94	3’
PCR buffer	10x		2.5	μl	2	2	94	30’’
PCR Dye Marker Mix	2x		12.5	μl	3	3	60	30’’
Primer 1	10	μM(ρmol/μl)	1	μl	4	72	30’’	
Primer 2	10	μM(ρmol/μl)	1	μl	5	72	3’	
DNA	--		1	μl	6			
Total			50	μl				

The information of Taq, including company name and catalog number :

Step 2. Enzyme cut

Component	Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	7.7	μl	1	60	30'	1
NEB cutsmart buffer	2	μl				
BsaJI	0.3	μl				
PCR product	10	μl				
Total	20	μl				

D. PCR Product

	Primer set		Product size	Target region
1	B2M#67	B2M#68	309 bp	
2			bp	

E. Gel Photo

