國家實驗研究院
NARLabs 國家實驗動物中心 National Laboratory Animal Center

RMRC Genotyping Protocol-PCR

No.	RMRC13432	Strain Name	CD1(ICR)-*Akr1A1*$^{Tg(CAG-EGFP)Meltw}$/Narl

A. Primer

	Primer name	Sequence
1	Akr1A1 P1(+)	CTCGTCCCTCGATGTCATTT
2	Akr1A1 P2(-)	TCCTTAATGGAGGTGGCAAG
3	Akr1A1-EGFP P3(-)	TGCTGAAGATCAGTTGGGTG

B. Genomic extraction :

☐ Phenol-Chloroform extraction ■ commercial extraction kit _Geneaid,Genomic DNA Mini kit (Tissue), Cat.No. GT301_

☐ Lysis buffer _____ □ other _____

C. Reaction Conditions

Step 1. PCR

Component	Concentration		Volume		Step	Tempera-ture, °C	Time, sec	Number of Cycles
H_2O	--			µl	1	95	120	
PCR buffer	10x			µl	2	95	10	X35
PCR Dye Marker Mix	2x		10	µl	3	65	10	
Primer 1	10	µM(pmol/µl)	0.2	µl	4	72	1	
Primer 2	10	µM(pmol/µl)	0.2	µl	5	72	60	
DNA	10~50ng			µl	6	10	∞	
Total			20	µl				

The information of Taq, including company name and catalog number：Promega 10x PCR buffer

PCR Product

	Primer set		Product size	Target region
1	Akr1A1 P1(+)	Akr1A1 P2(-)	321 bp	
2	Akr1A1 P1(+)	Akr1A1-EGFP P3(-)	228 bp	

D. Gel Photo

Marker: Biomate. 1kb plus DNA RTU Ladder (100bp-10kb); Cat No.BR343.

