

RMRC Genotyping Protocol-PCR

No.	RMRC13249	Strain Name	B6.129-Gucy2g ^{tm2Rbya}
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A. Primer

	Primer name	Sequence
1	CU	5'-ttt gcg gcc gca tgg tta aca taa caa gcc tgt g-3'
2	FD	5'-tca gtc gac att tct tgc ctt agt tag ggt gtg tgc tac t-3'
3.	JD	5'-ata gtc gac agg cca tca agt cca tag agg aag-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		12	μl	1	94	180	
PCR buffer	10x		2	μl	2	94	30	35
dNTP	10	mM	0.5	μl	3	66(CU-FD) 60(CU-JD)	30	35
Primer 1	10	μM(μmol/μl)	1	μl	4	72	60	35
Primer 2	10	μM(μmol/μl)	1	μl	5	72	300	
DNA	100 ng/ul		1	μl	6	4	Hold	
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺	15	mM	2	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	CU	FD	636 bp	Wt region
2	CU	JD	635 bp	Mutant region

D. Gel Photo

