

RMRC Genotyping Protocol-PCR

No.	RMRC13102	Strain Name	B6.129- <i>Hnrnpa1</i> ^{tm1Cyf}
-----	-----------	-------------	--

A. Primer

	Primer name	Sequence
1	Ex-hnA1 20147 U	tat agc ggg atg tga cgt gtt ttg
2	In-hnA1 20597 L	aat gaa tca aca ccc cgc aac aac
3	Ex-hnA1 22732 L	act gca ccc aca atg ctt taa gag

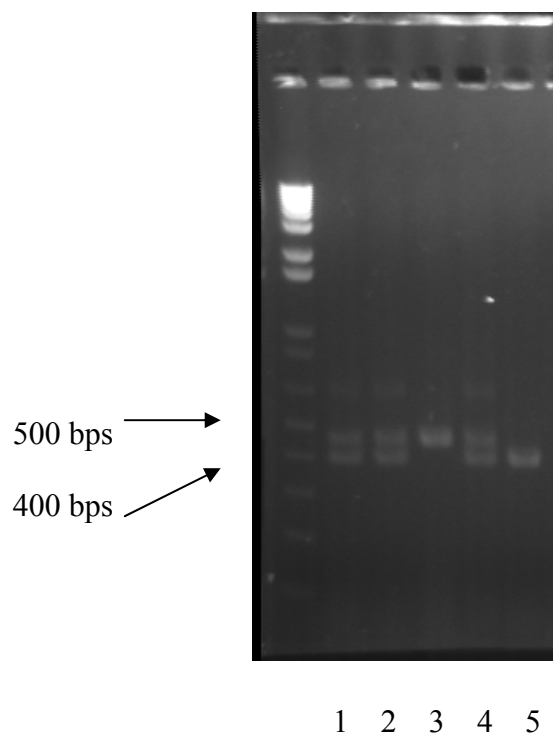
B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		10.3	μl	1	95	600	1
PCR buffer	10x		2.5	μl	2	95	30	35
dNTP	2.5	mM	5	μl	3	58	45	35
Primer 1	10	μM(μmol/μl)	1	μl	4	72	60	35
Primer 2	10	μM(μmol/μl)	0.5	μl	5	72	420	1
Primer 3	10	μM(μmol/μl)	0.5	μl				
DNA	--		5	μl	6			
Taq	2.5	U/μl	0.2	μl	7			
Mg ²⁺	0	mM	0	μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	20147	20597	451 bp	Target hnRNP A1 gene (have band have hnRNP A1 gene)
2	20147	22732	379 bp	Target hnRNP A1 knockout region (have band no hnRNP A1 gene)

D. Gel Photo



1. hnRNP A1^{+/-}
2. hnRNP A1^{+/-}
3. hnRNP A1^{+/+}
4. hnRNP A1^{+/-}
5. hnRNP A1^{-/-}