

RMRC Genotyping Protocol-PCR

種原編號	RMRC13063	品系名稱	B6.129- <i>Dlgap5</i> ^{tm2Tfs}
------	-----------	------	---

A. Primer

	Primer name	Sequence
1	Hurp- Forward	5' ctc aca tga ttt gct cgc ttg ag 3'
2	Hurp- Reverse	5' tac ttg ttt tgg agc tgg cct tc 3'
3	IRES- Forward	5'cgt tac tgg ccg aag ccg ctt g 3'
4	IRES- Reverse	5'gag gga tct tgg cca tgg taa gc 3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		13.3	μl	1	95	300	1
PCR buffer	10x		2	μl	2	95	30	33
dNTP	2.5	mM	1.2	μl	3	71	30	33
Primer 1	10	μM(μmol/μl)	1	μl	4	72	60	33
Primer 2	10	μM(μmol/μl)	1	μl	5	72	300	1
DNA	--		1	μl	6	4	∞	1
Taq	6	U/μl	0.5	μl	7			
Mg ²⁺	0	mM	0	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	IRES-F	IRES-R	603 bp	KO allele
2	Hurp-F	Hurp-R	363 bp	WT allele

D. Gel Photo

