

RMRC Genotyping Protocol-PCR

種原編號	RMRC13030	品系名稱	C57BL/6-Tg(CD3S-Cybr)C51Wcy
種原編號	RMRC13031	品系名稱	C57BL/6-Tg(CD3S-Cybr)C56Wcy

A. Primer

	Primer-name	Sequence	Length	Note
1	Primer-1	CTCGAGTGAAGATGACCTCCTCAGAAA	27	Forward
2	Primer-2	GAATTCACAGGAACATCGTCTGCTT	25	Reverse

B. Reaction Components

Chemical	Conc.		Run1	Run2	Run3	Run4
H ₂ O	--		12.75μl			
PCR buffer	10x		2μl			
dNTP	2.5	mM	2μl			
Primer 1	10	pmol/μl	0.5μl			
Primer 2	10	pmol/μl	0.5μl			
DNA	150ng/μl		2μl			
Tag	2	U/μl	0.25μl			
Total	20μl		20μl			

C. Reaction Conditions

Step	Temp.(°C)	Time(Sec)	Note
1	94	420	
2	94	60	Repeat for 30 cycles
3	57	30	
4	72	40	
5	72	600	
6	4	∞	
7			
8			

D. PCR product

	Primer set		Product size	Product
1	Primer-1	Primer-2	280bp	C51,C56 (WT no product)
2				

E. Gel photo