PCR protocol

RBRC 00144 **Strain name** C57BL/6-Ly-5.1

Primer	primer_name	primer_seq	length		
1	common	caggtccactgaaacagaggatg	23		RIKEN BRC
2	Ptprc_a	CTGAGCCTGcATCTAAACCTGAtc	24		RIKEN BRC
3	Ptprc_b	CTGAGCCTGtATCTAAACCTGAgt	24		RIKEN BRC

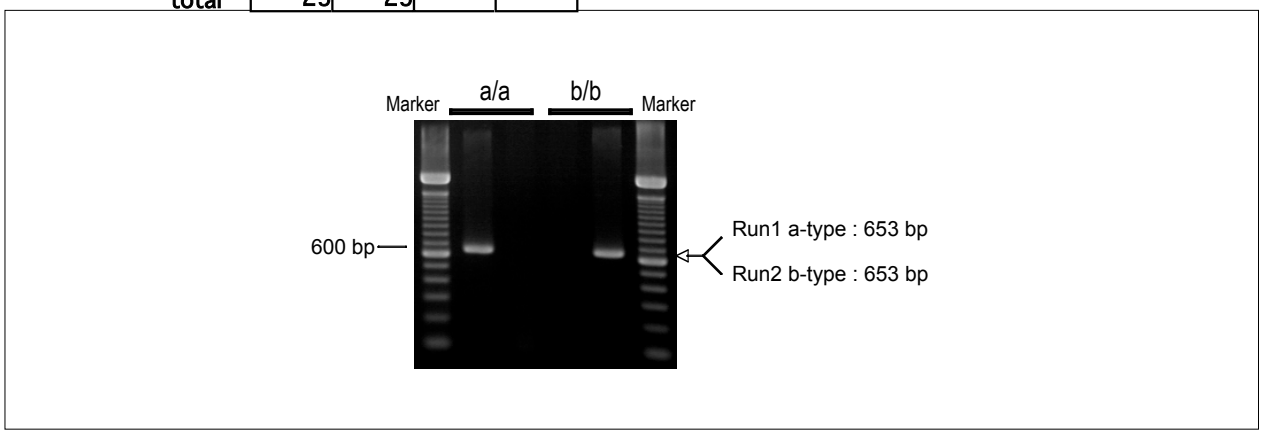
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	25	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	653 bp	a type
primer 1	primer 3	653 bp	b type

Comment The allele-specific primers were designed using the sequence of Ly5a (Zebedee *et al.*, *Dev. Immunol.*, 1 (1991) 243-254).