

PCR protocol

RBRC 01273

Strain name sey (small eye) DBA/2 x B6

Primer	primer_name	primer_seq	length	
1	Sey_wt	caccaactccatcagttctaaTg	23	RIKEN BRC
2	Sey_mt	caccaactccatcagttctaaTt	23	RIKEN BRC
3	Sey	ccatacctgtattcttgcttcagg	24	RIKEN BRC

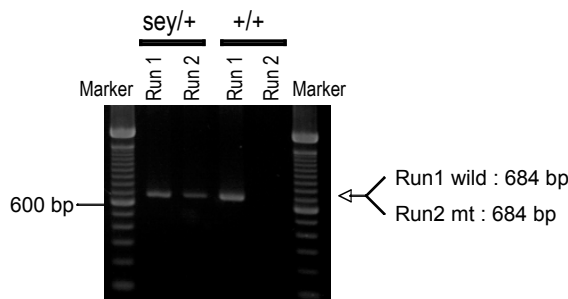
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

	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/ μ l	1.0	0		
primer 2	10 pmol/ μ l	0	1.0		
primer 3	10 pmol/ μ l	1.0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
total		25	25		

step	temp (°C)	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	684 bp	WT
primer 2	684 bp	mutant

Comment The allele-specific primers were designed using the sequence of Sey (Nature. 354(6354):522-525 (1991)).