

PCR protocol

RBRC 06364

Strain name B6(D2)-Disc1 <Rgsc1390>/Rod

Primer	primer_name	primer_seq	length		
1	common	CCTGTCCAAGGACTGGCATC	21		RIKEN BRC
2	Rgsc1390	CAGGGACAAGGGAGCTCTTCG	21		RIKEN BRC
3	wild	CAGGGACAAGGGAGCTCTTCA	21		RIKEN BRC

Reaction Components

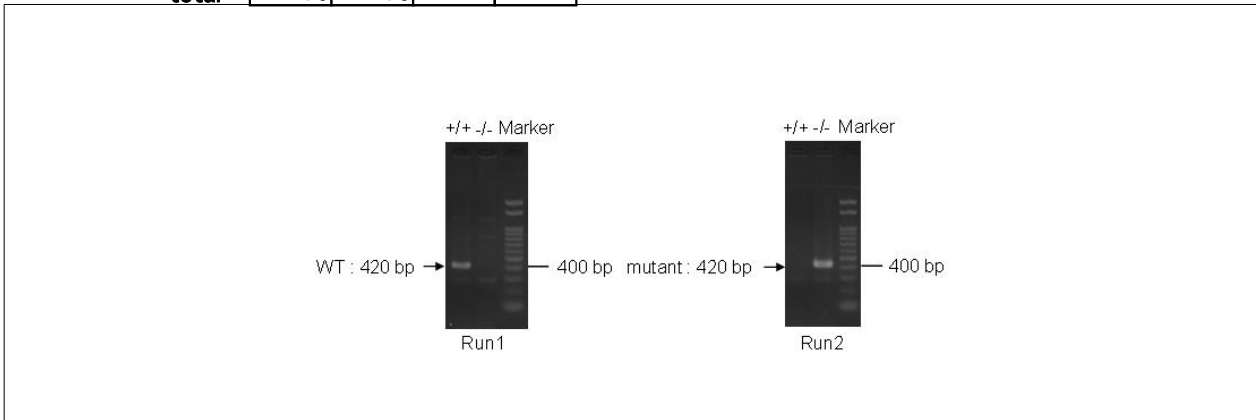
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles
5	72	600	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.0	2.0		
Master Mix	2 X	5.0	5.0		
primer 1	10 pmol/ μ l	1.0	1.0		
primer 2	10 pmol/ μ l	0	1.0		
primer 3	10 pmol/ μ l	1.0	0		
DNA		1.0	1.0		
total		10	10		

Allele specific PCR (ASP)



Marker
Gene Ladder 100 (Wako, Cat. 316-06951)

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

PCR products

primer_set	product_size	product
primer 1 primer 2	420 bp	mutant
primer 1 primer 3	420 bp	WT

Comment