PCR protocol

RBRC 02442 **Strain name** MRL/MpJ-lpr-Tg(dLck-Fas)

Primer	primer_name	primer_seq	length		
1	Antisense	CACAGTGTTCACAGCCAGGA	20		
2	Secse	GGAATTCCGCTGTTTTCCCTTGCTGCA	27		
3		GTAAATAATTGTGCTTCGTCAG	22		
4		TAGAAAGGTGCACGGGTGTG	20		
5		CAAATCTAGGCATTAACAGTG	21		

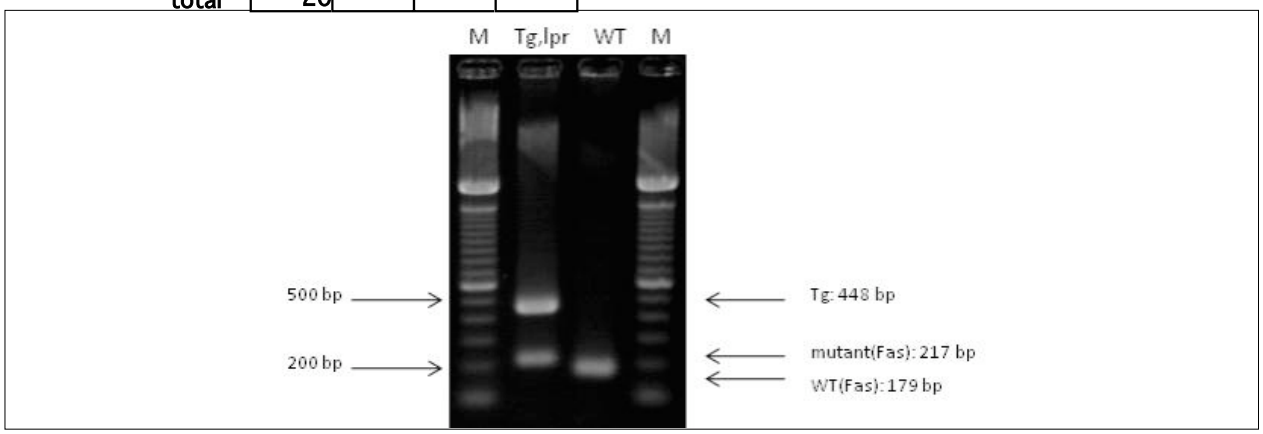
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	2.0			
primer 4	10 pmol/μl	1.0			
primer 5	10 pmol/μl	1.0			
DNA		1.0			
Master Mix	2 X	10.0			
	total	20			

Reaction Conditions

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



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	448 bp	Tg
primer 3	primer 5	179 bp	WT (Fas)
primer 3	primer 4	217 bp	mutant (Fas)

Comment