PCR protocol

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RBRC 04418

Strain name B6.B6C3-Tg(Barh1-lacZ)2Tets

Primer	primer_name	primer_seq	length		Reference
1	BGZA AS	CTGGGCGAAAGGGGGATGTGC	21		Depositor's primer
2	GSPFW2	AGCGGAGCGGAGCACACC	18		Depositor's primer
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).

Reaction Components

Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

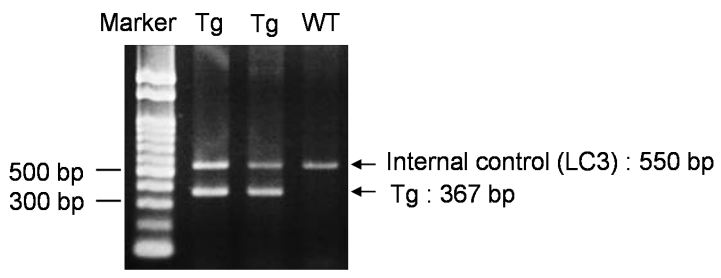
Reagent	Conc	Reaction 1	2	3	4	
H2O		11.3				(μL)
PCR buffer	10 X	2.0				
dNTP	2.5 mM	1.6				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq		0.1				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	65	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	367 bp	Tg
primer 3	primer 4	550 bp	Internal control (LC3)

Comment