

PCR protocol

RBRC 00062

Strain name BALB-PC(-)

Primer	primer_name	primer_seq	length
1	a	CCGGATTCTACTGAAGCAGCACaA	24
2	b	CCGGATTCTACTGAAGCAGCACaG	24
3	common	gtgagttgcaggacagctaggg	22

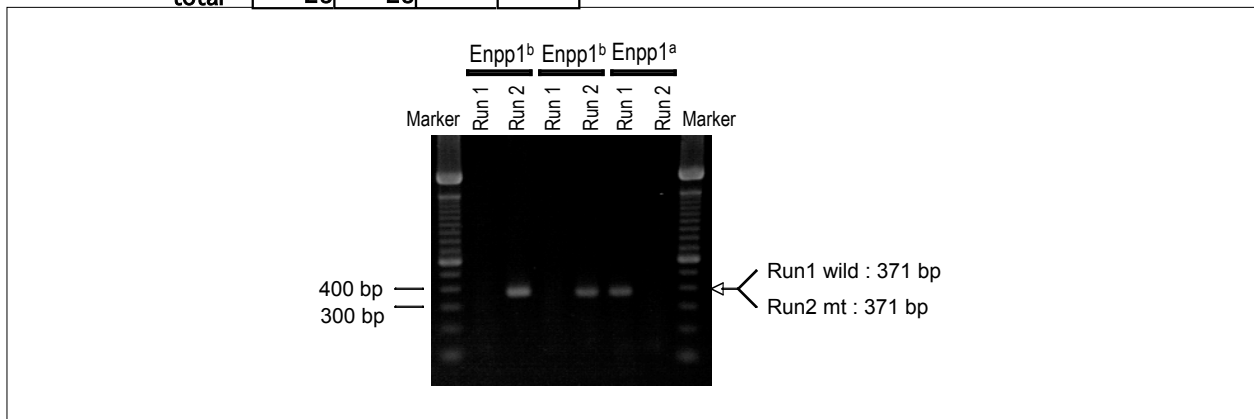
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

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	

Conc	Run 1	Run 2	Run 3	Run 4
H2O	17.2	17.2		
PCR buffer	2.5	2.5		
dNTP	2.0	2.0		
primer 1	1.0	0		
primer 2	0	1.0		
primer 3	1.0	1.0		
DNA	1.0	1.0		
Taq	0.3	0.3		
total	25	25		



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	371 bp	WT
primer 2	371 bp	mutant

Comment The allele-specific primers were designed using the sequence of Enpp1 (Banakh *et al.*, *Eur. J. Immunogenetics*, 29 (2002) 307-313). These primers can detect a point mutation at codon 650 of Enpp1.