RIKEN BRC

Jun. 13, 2006

BRC_No 01572 **Strain name** PV630

Primer

	primer_name	primer_seq	length	
1	Ppfia4-PV630-F1	CAGCTGGGAGTTTACACTGTTGTCCAG	27	
2	Ppfia4-PV630-R1	GTCTCTCTACCTGGCTGCCCTCATG	25	
3	T/BAL	CTTGTGTCATGCACAAAGTAGATGTCC	27	

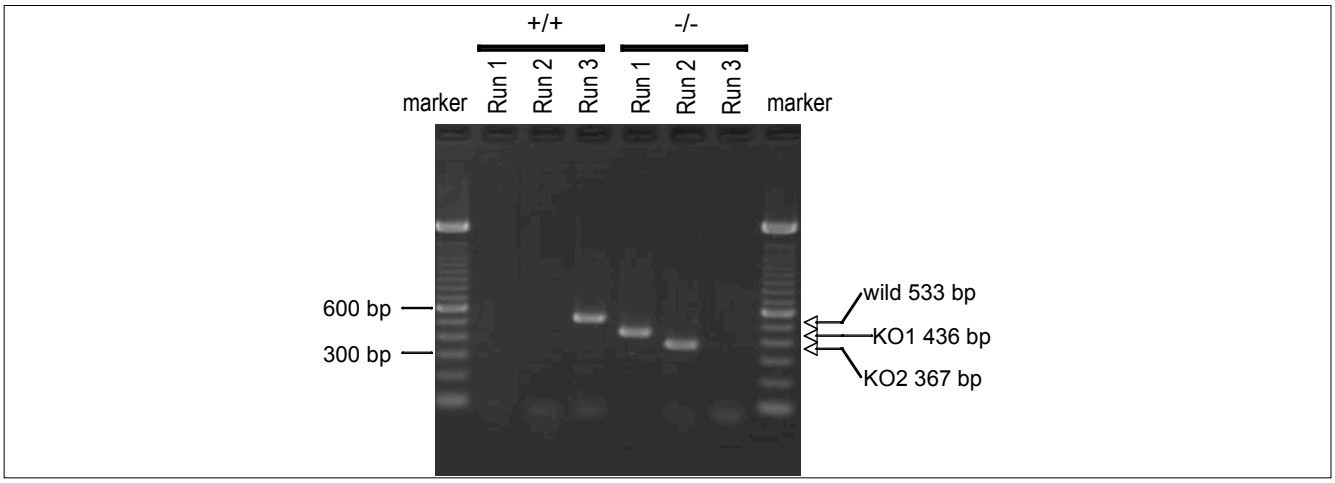
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3
H2O		17.3	17.3	17.3
PCR buffer	10 X	2.5	2.5	2.5
dNTP	2.5 mM	2.0	2.0	2.0
primer 1	10 pmol/μl	0	1.0	1.0
primer 2	10 pmol/μl	1.0	0	1.0
primer 3	10 pmol/μl	1.0	1.0	0
DNA		1.0	1.0	1.0
Taq		0.2	0.2	0.2
	total	25	25	25

Reaction Conditions

step	temp (℃)	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, 40 min)

PCR products

primer_set		product_size	product
primer 2	primer 3	436 bp	KO 1
primer 1	primer 3	367 bp	KO 2
primer 1	primer 2	533 bp	wild

Comment