RIKEN BRC

Jul. 24, 2006

BRC_No 01607 **Strain name** PV449

Primer

	primer_name	primer_seq	length
1	Bai3-PV449-R1	ATTCAATAATATTTTGGTTGCTGAAAGTC	29
2	T/BAL	CTTGTGTCATGCACAAAGTAGATGTCC	27
3	Bai3-PV449-F1	CATGAACACCATTAATTTGGGAACTTC	27

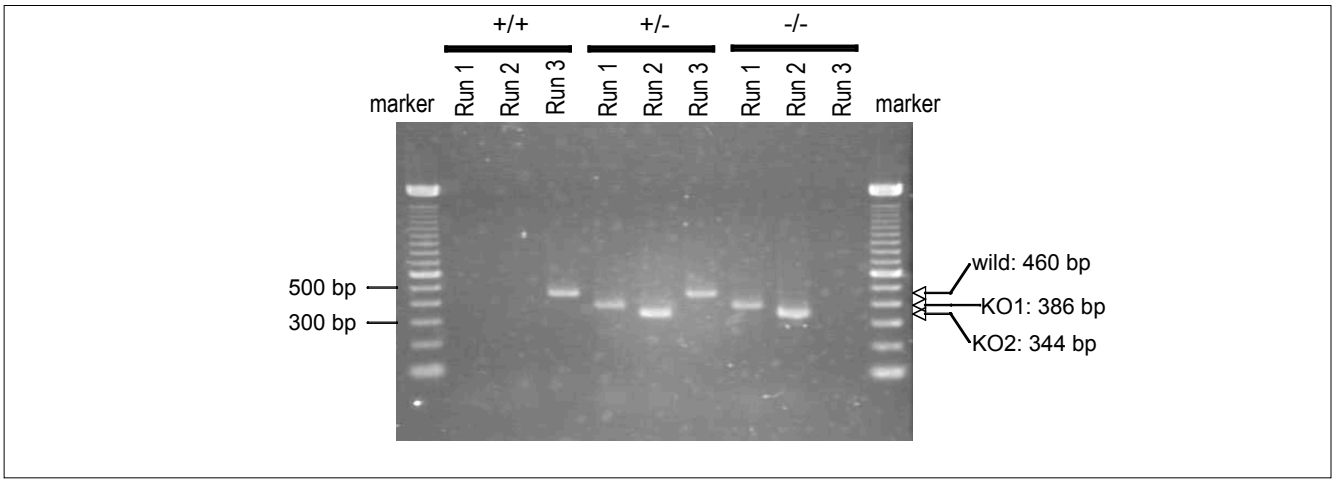
Reaction Components

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	1.0	0	1.0
primer 2	10 pmol/μl	1.0	1.0	0
primer 3	10 pmol/μl	0	1.0	1.0
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	55	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, 35 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	386 bp	KO1
primer 2	primer 3	344 bp	KO2
primer 1	primer 3	460 bp	wild

Comment