PCR protocol

RBRC 01945 **Strain name** P2EmB1D enhancer knockout mouse

Primer	primer_name	primer_seq	length		
1	RP213	CAGGACAGCCACTGAGCTGCAGGCCTGA	28		Depositor's primer
2	RP220	CCCAGTGGTCGAGACCCTGGAGGCTCTGA	29		Depositor's primer
3	Fesneo	CGCCTTCTATCGCCTTCTTGACGAG	25		Depositor's primer
4	LP236	GAGGCACGGGATAGACATCCAGGTACCCA	29		Depositor's primer

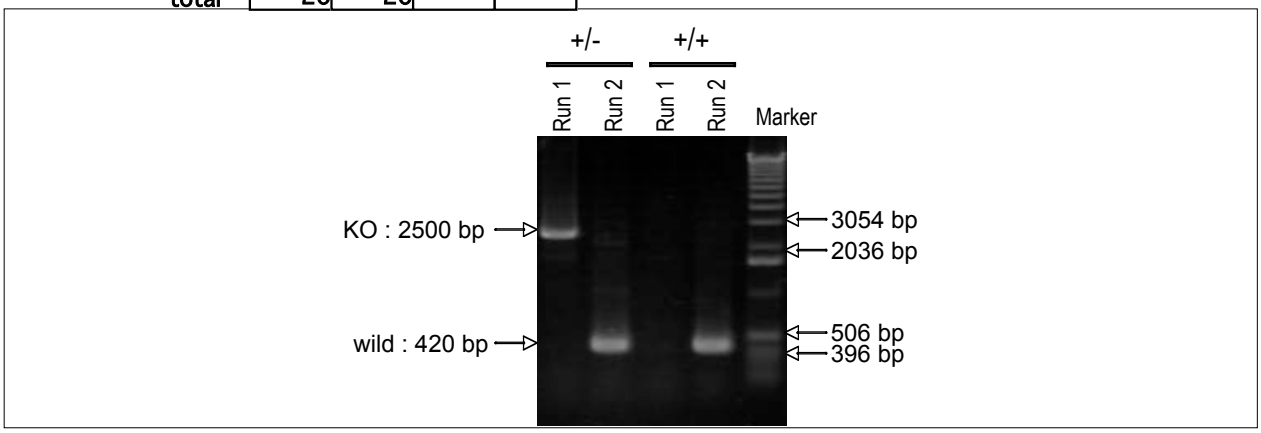
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.8	4.8		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	0	1.0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	
4	72	120	*	*Repeat for 30 cycles
5	72	300		



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 3	2500 bp	KO
primer 2	primer 4	420 bp	WT

Comment