PCR protocol

BRC_No 01058 **Strain name** ICR-Nrp1^{tmHfu}

Primer

	primer_name	primer_seq	length	
1	P1	CCTCACTGTCTTCTGAAGTGAC	22	
2	P2	GATTTTTATGGTCCCCGCCACA	22	
3	322	TGATATTGCTGAAGAGCTTGG	21	

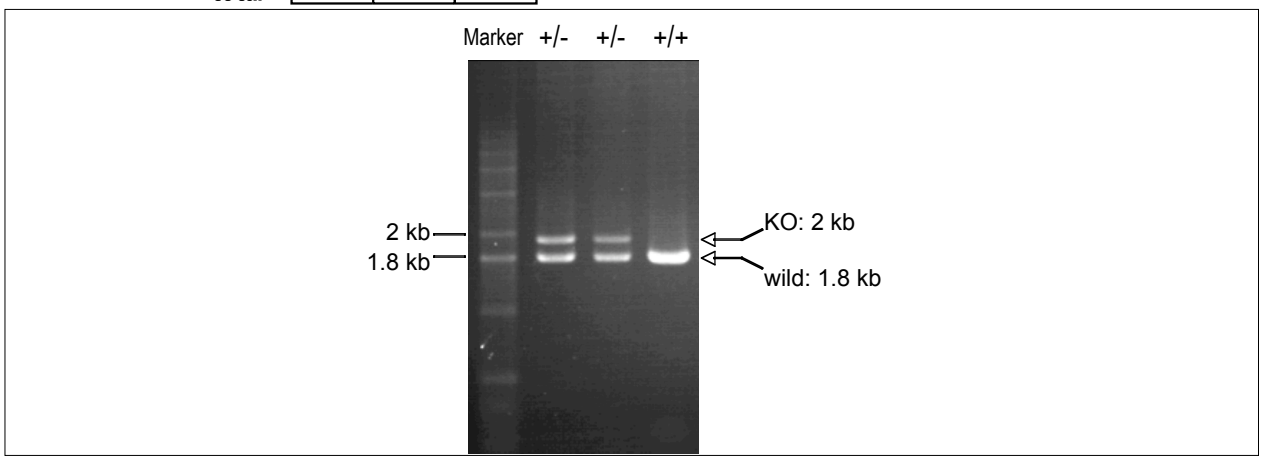
Reaction Components

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

	Conc	Run 1	Run 2	Run 3
H2O		3.8		
PCR buffer	2 X	10.0		
dNTP	2.5 mM	2.0		
primer 1	10 pmol/μl	1.0		
primer 2	10 pmol/μl	1.0		
primer 3	10 pmol/μl	1.0		
DNA		1.0		
Taq		0.2		
	total	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30*	
3	55	30*	
4	72	180*	*Repeat for 35 cycles
5	72	300	



Marker

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

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 3	2 kb	KO
primer 1	primer 2	1.8 kb	wild

Comment