PCR protocol

BRC_No 01514 **Strain name** Mnk1 and Mnk2 deficient mice

Primer

	primer_name	primer_seq	length	
1	Mnk1-sense primer	GACCCAGGAATGACACCTTC	20	
2	Mnk1-antisense	GCGCAAACCACATGTGCTTT	20	
3	NeoR primer	GATTCGCAGCGCATCGCCTTCTATCG	26	
4	Mnk2 sense primer	ATTGAGAAGCAGCTGGGCCACATCCGCAGC	30	
5	Mnk2-antisense	GTCGCAGCGCTTGTCGTAGATGCTGGC	27	

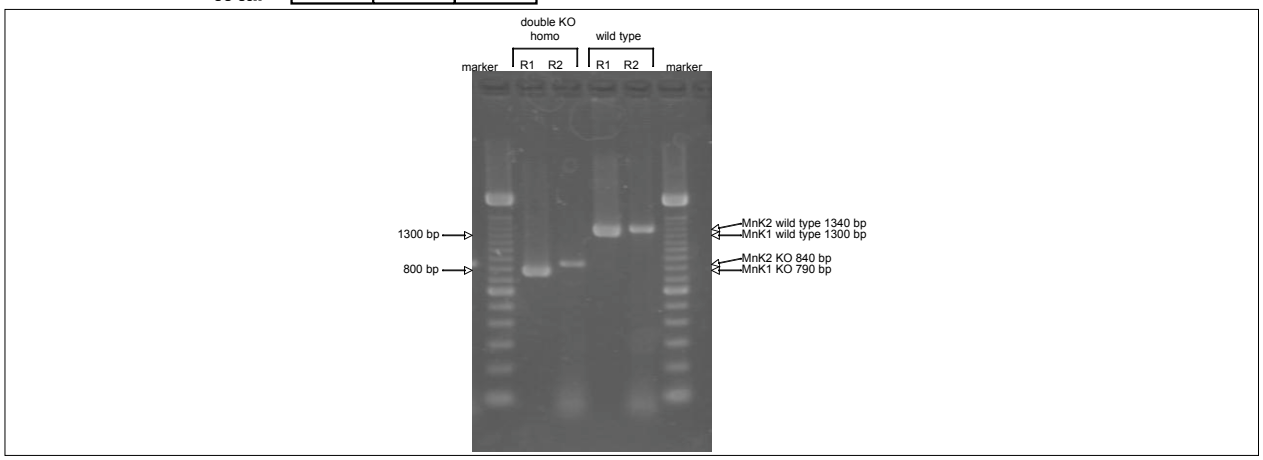
Reaction Components

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

	Conc	Run 1	Run 2	Run 3
H2O		3.8	3.8	
PCR buffer	2 X	10.0	10.0	
dNTP	2.5 mM	2.0	2.0	
primer 1	10 pml/μl	1.0	0	
primer 2	10 pml/μl	1.0	0	
primer 3	10 pml/μl	1.0	1.0	
primer 4	10 pml/μl	0	1.0	
primer 5	10 pml/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

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 1	primer 2	1300 np	MnK1 wild
primer 2	primer 3	790 bp	MnK1 KO
primer 3	primer 5	840 bp	MnK2 KO
primer 4	primer 5	1340 bp	MnK2 wild

Comment