PCR protocol

RBRC 02700 **Strain name** B6.Cg-Rapgef2<tm1Tkat> Rapgef6<tm1.1Tkat>

Primer

	primer_name	primer_seq	length	
1	mRA-GEF-1 loxpE2833	cgagtcctgaacataagggcaggg	24	
2	mRA-GEF-1 loxP F	gtcagactagcagcagaccacctag	25	
3	mGEF2-right loxp3'	CTAGGGAGGTGTCAGCAAAG	20	
4	mGEF2-5'-R-MfeI	GAGCCTTGAGATACAGAAACTTG	23	

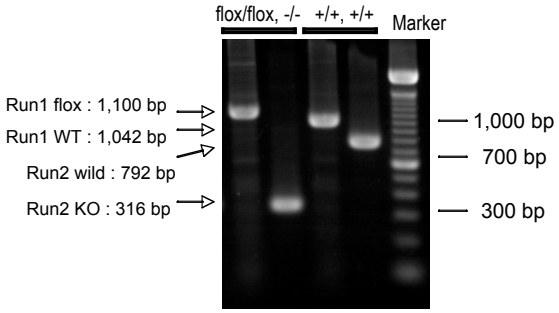
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	25	25		

Reaction Conditions

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



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,100	flox
primer 1	primer 2	1,042	wild
primer 3	primer 4	316	KO
primer 3	primer 4	792	wild

Comment