PCR protocol

Sheet ID PS_05371
Release date : 09/13/2013
Print date : 9/13/2013

RBRC 04720 **Strain name** B6;129S-Rap1a<tm1Morz> Rap1b<tm1Morz>

Primer	primer_name	primer_seq	length		
1	Rapa1	CCAAGGCTCTCAGTTGATTTCTA	23		Depositor's primer
2	Rapa2	TATCTGCACATAATCTGCATGCC	23		Depositor's primer
3	Rapb1	CCCTCTCATGCTATTCCTAATGT	23		Depositor's primer
4	Rapb2	GCCACCTCAGTAGAAAAGACC	21		Depositor's primer

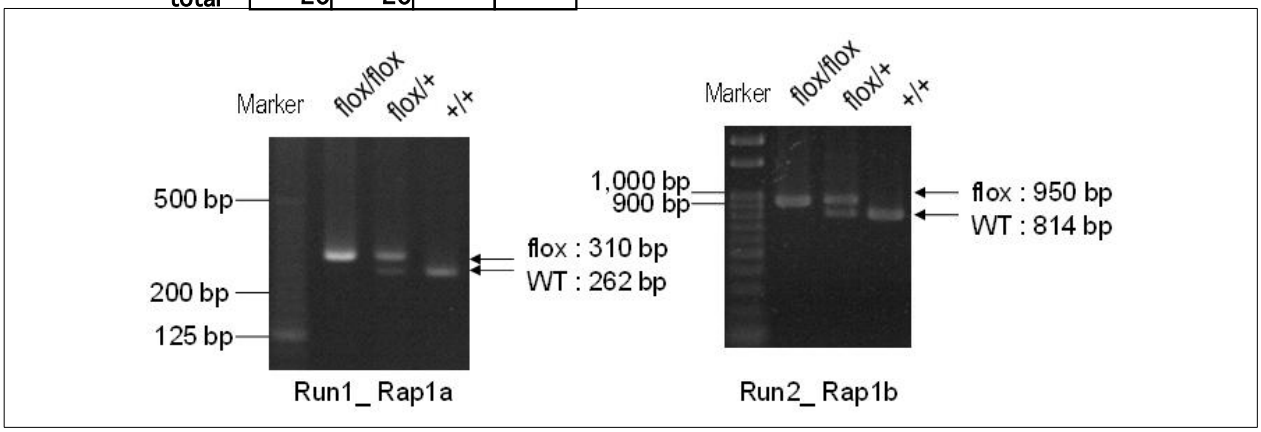
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
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	5 U/μl	0.1	0.1		
	total	20	20		

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

marker Run1: 25 bp ladder (Invitrogen, Cat. 10416-014) , marker Run2: Gene Ladder

Gel

Run1: 3% Nusieve in 0.5 X TBE (100 V, 50 min) Run2: 2%

PCR products

primer_set		product_size	product
primer 1	primer 2	262 bp	WT (Rap1a)
primer 1	primer 2	310 bp	flox (Rap1a)
primer 3	primer 4	814 bp	WT (Rap1b)
primer 3	primer 4	950 bp	flox (Rap1b)

Comment