PCR protocol

Sheet ID PS_08541
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RBRC 10747

Strain name C57BL/6-Rapgef3<tm1.2Ito> Rapgef4<tm1.1Ito>

Primer	primer_name	primer_seq	length		Reference
1	GEF1-P1	GGAAATGGGCTTAGCTATGC	20		Depositor's primer
2	GEF1-P2	AGATGGCCGGCACTTCGCAC	20		Depositor's primer
3	GEF1-P3	CCCAGAGCCTGTGTAGGGTT	20		Depositor's primer
4	GEF2p2	TGTTTCGCCAAGGGGATATTG	21		Depositor's primer
5	GEF2p3	CTGGTGCTCACACCTCGTAC	20		Depositor's primer
6	intron 2	GGAAACGTGTTACTCTAGAAACGAC	25		Depositor's primer

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

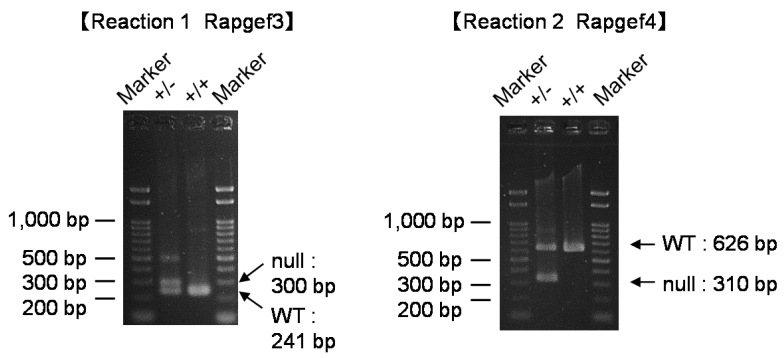
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8	1.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	1.0	0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	0	1.0			
primer 6	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10 *	
3	60	30 *	
4	68	60 *	*Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	241 bp	WT
primer 1	primer 3	300 bp	null
primer 4	primer 5	626 bp	WT
primer 6	primer 5	310 bp	null

Comment

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.