PCR protocol

Sheet ID PS_06127
Release date : 5/14/2015
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RBRC 06322 **Strain name** B6.Cg-Edn1<tm1Ywa>

Primer	primer_name	primer_seq	length		
1	5'_Fw	GCCCTGGAATGTGTCTGATCG	21		RIKEN BRC
2	5'_Rv	GAGACAAGTTGCCACTGACGGA	22		RIKEN BRC
3	3'_Fw	GCAGTGCACCAATATGCCAGGC	22		RIKEN BRC
4		AACCTCCCAGTCCATACGGTAC	22		J. Clin. Invest., 114, 4, 504-511

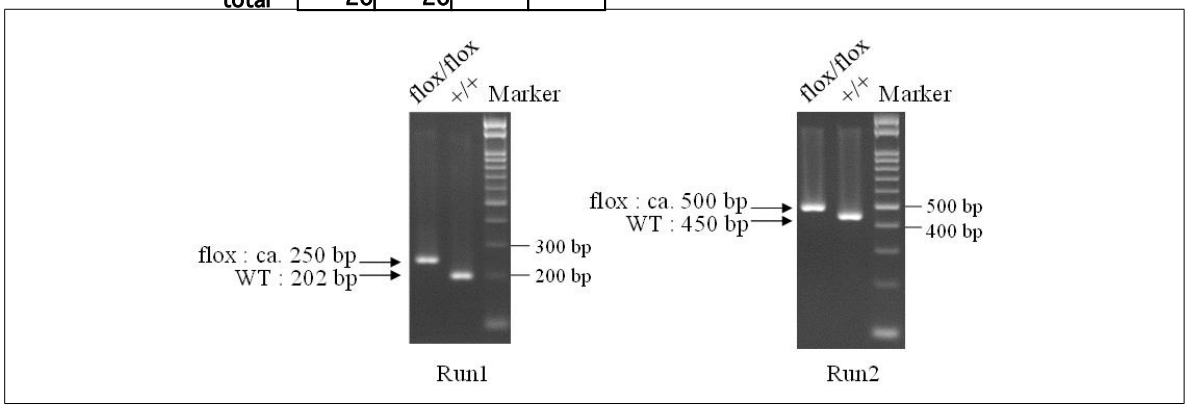
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8	2.8		
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	0	1.0		
primer 4	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

3% Nusieve in 0.5 X TBE (100 V, 60 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	202 bp	WT (5' site)
primer 1	primer 2	ca. 250 bp	flox (5' site)
primer 3	primer 4	450 bp	WT (3' site)
primer 3	primer 4	ca. 500 bp	flox (3' site)

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