PCR protocol

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RBRC 06164 **Strain name** B6;129-Edn2<tm1Ywa>

Primer	primer_name	primer_seq	length		
1	ET-2 Geno F ko	AGGTGACACAAAATATTCTGTTCAGTCCAC	30		J. Clin Invest., 123, 2643-2653 (2013).
2	ET-2 Geno R WT	GCAGTGTTCACCCAGATGATGTCCAGGTG	29		J. Clin Invest., 123, 2643-2653 (2013).
3	M11	CTTGGGTGGAGAGGCTATTCGGCT	24		

Reaction Components

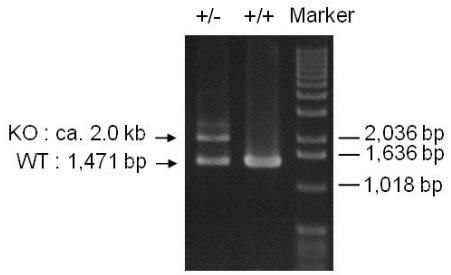
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Standard PCR



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,471 bp	WT
primer 1	primer 3	ca 2.0 kb	KO

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