

RBRC 06322

Strain name B6.Cg-Edn1<tm1Ywa>

Primer	primer_name	primer_seq	length	Reference
1	5'_Fw	GCCCTGGAATGTGTCTGATCG	21	RIKEN BRC
2	5'_Rv	GAGACAAGTTGCCACTGACGGA	22	RIKEN BRC
3	ET-1AF and ET-1DR	AACCTCCCAGTCCATACGGTAC	22	J. Clin. Invest., 114, 4, 504-511 (2004).

Reaction Components

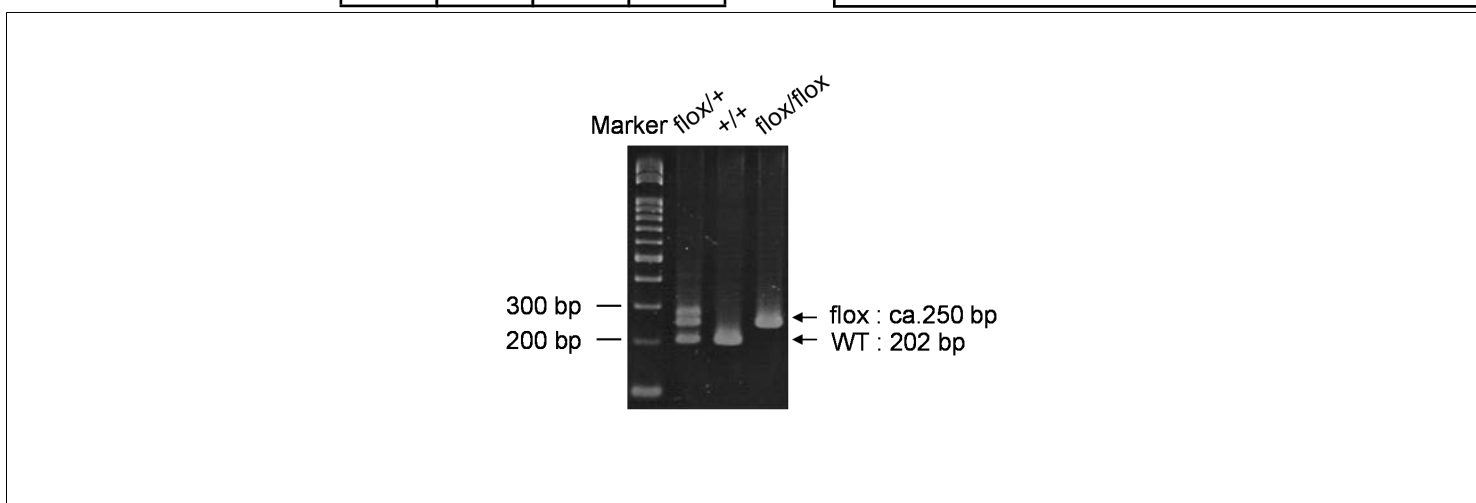
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(µL)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 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				

Marker	Gene Ladder 100 (Wako, Cat. 316-06951)
Gel	3% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set	product_size	product (allele type)
primer 1 primer 2	202 bp	WT
primer 1 primer 2	ca 250 bp	flox
primer 1 primer 3	430 bp	null

Comment [Standard PCR](#)

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

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2	5'_Rv	GAGACAAGTTGCCACTGACGGA	22	RIKEN BRC
3	3'_Fw	GCAGTGCACCAATATGCCAGGC	22	RIKEN BRC
4		AACCTCCCAGTCCATACGGTAC	22	J. Clin. Invest., 114, 4, 504-511 (2004).

Reaction Components

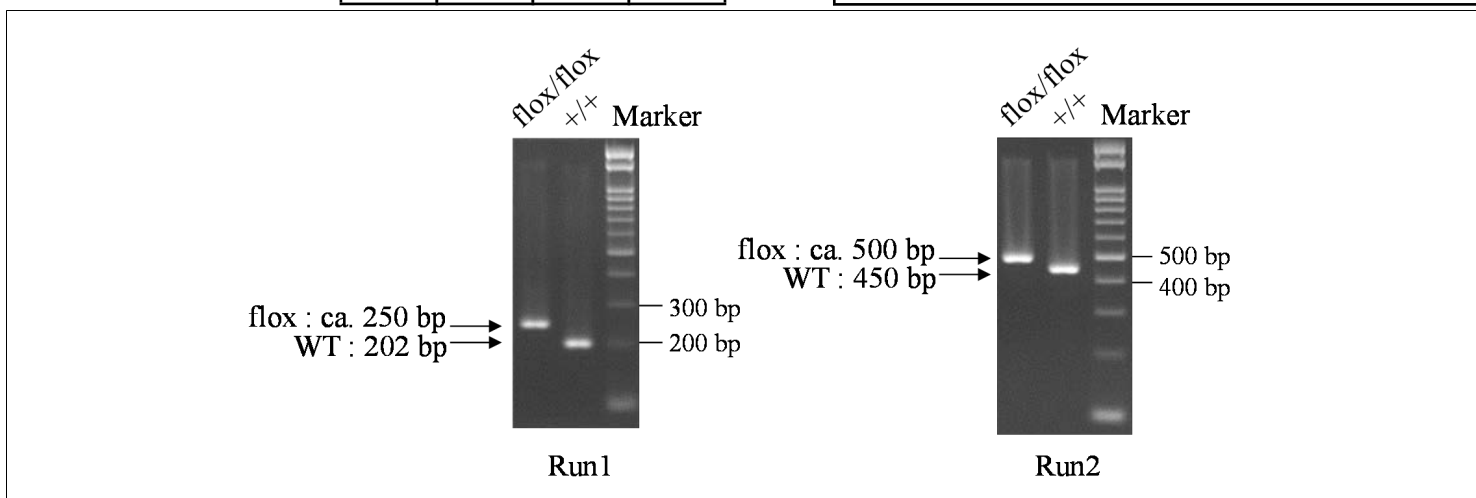
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(µL)
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			

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 3% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set	product_size	product (allele type)
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 Separated PCR

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