

RBRC 09305

Strain name Clart knockout

Primer	primer_name	primer_seq	length	
1	Chrono WT Fw2	TAGCGGGTGCTGTTTTAGG	20	Depositor's primer
2	gm129-genetrap-RV	ATGAGGTGCTGTATGTTGGTTAC	23	Depositor's primer
3	AGN	TCGTGCTTTACGGTATCGCCG	21	

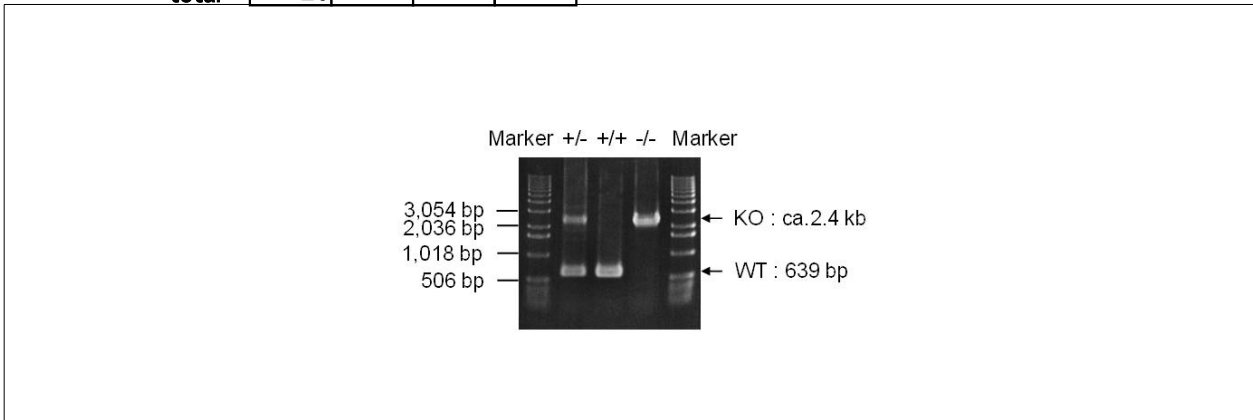
Reaction Components

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	180*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
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
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	639 bp	WT
primer 3	primer 2	ca. 2.4 kbp	KO

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