

RBRC 05763

Strain name STOCK Izumo1 <tm1Osb> Tg(Clgn-Izumo1/mCherry)Osb Tg(CAG/Acr-EGFP)C3-N01-

Primer	primer_name	primer_seq	length	
1	Pr-B	GGGTTCACTCTCCAGCTACCCCAAACCTCAC	30	
2	O2097_1	GTGAAGCATTAGCGCTTGAGGGCG	24	RIKEN BRC
3	LCB355(NEO 602)	GTCTTGTTCGATCAGGATGATCTGGA	25	
4	196	CCTTCCTGCGGCTTGTCTCT	21	Depositor's primer
5	IZUMO1-TGR	GGTCTCAGAAGTCTCCCAAACCCTGTA	30	Depositor's primer
6	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
7	oIMR0043	GTAGGTGGAATTCTAGCATCATCC	25	JAX Genotyping Protocol
8	acro	GTGGAGCTTTGTGAGGTCACAG	22	
9	gfp	CAGCTTGCCGGTGGTGCAGATG	22	

Reaction Components

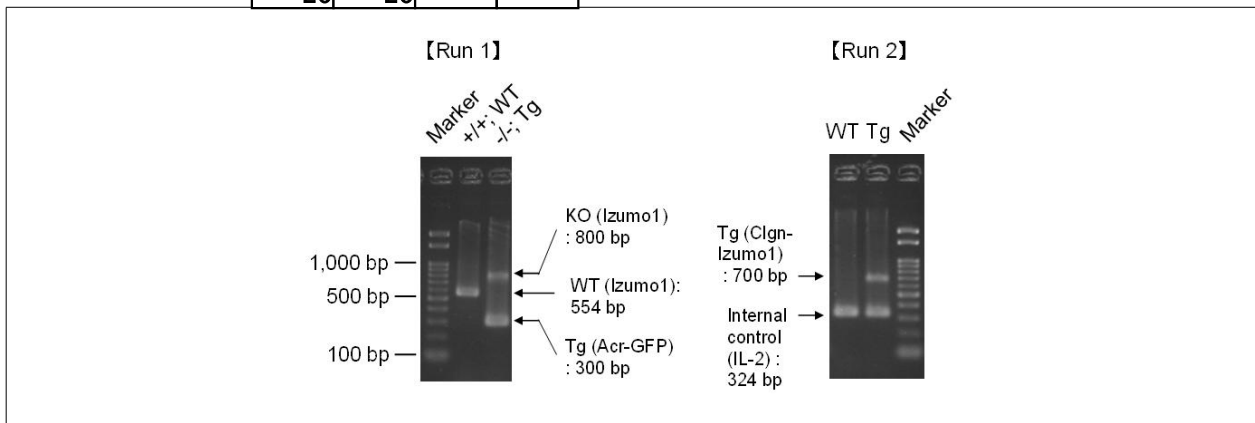
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8	0.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	1.0	0		
primer 4	10 pmol/ μ l	0	1.0		
primer 5	10 pmol/ μ l	0	1.0		
primer 6	10 pmol/ μ l	0	1.0		
primer 7	10 pmol/ μ l	0	1.0		
primer 8	10 pmol/ μ l	0.5	0		
primer 9	10 pmol/ μ l	0.5	0		
DNA		1.0	1.0		
Taq	1 U/ μ l	0.2	0.2		
total		20	20		

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

Standard PCR



Marker

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

Gel

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

Marker	primer_set	product_size	product
primer 1	primer 2	554 bp	WT
primer 3	primer 2	800 bp	KO
primer 4	primer 5	700 bp	Tg (Clgn-Izumo1)
primer 6	primer 7	324 bp	Internal control (IL-2)
primer 8	primer 9	300 bp	Tg (Acr-GFP)

Comment

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