

RBRC 09370

Strain name 129S6/SvEvtac-Epb4.1I2<tm1Tfru>

Primer	primer_name	primer_seq	length	
1	KO-check-4.1G_F2	CTGCTGAAGAAGTGAGGAAGG	21	Depositor's primer
2	KO-check-4.1G_R2	TAACAGGGAGGCAGAAGGTG	20	Depositor's primer
3	PGK R2	CTACCCGGTAGAATTGACCTGCA	23	Depositor's primer

Reaction Components

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

Reaction Conditions

	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/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		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)

PCR products			
	primer_set	product_size	product
primer 1	primer 2	400 bp	WT
primer 2	primer 3	250 bp	KO

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