

PCR protocol

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RBRC 06040

Strain name ICR.Cg-Hcn4<tm1(tTA)>/1Kyo

Primer	primer_name	primer_seq	length	
1		GCTGCAGAAGCCGCTCCTATGGC	23	RIKEN BRC
2		GATGAGCCGCGCTTCCTCCGC	21	RIKEN BRC
3	d2tTA-645 as	CGACTTGATGCTCTTGATCTTCCAA	25	Depositor's primer

Reaction Components

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

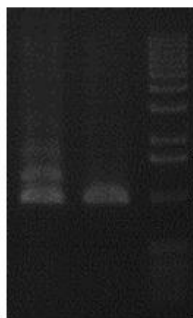
Reaction Conditions

	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	120*	*Repeat for 30 cycles

KI/+ +/- Marker

KI : ca. 1.3 kbp →
WT : 1,056 bp →



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	1,056 bp	WT
primer 1	ca. 1.3 kbp	KI

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