PCR protocol

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RBRC 10983

Strain name B6.Cg-Hcn4<tm1(tTA)Nnka> Hcn4<tm1.1(Luc/EGFP)Nna>

Primer	primer_name	primer_seq	length		Reference
1	promoter	GCTGCAGAAGCCGCTCCTATGGC	23		RIKEN BRC
2	exon 1	GATGAGCCGCCGTTCCTCCGC	21		RIKEN BRC
3	d2tTA-645 as	CGACTTGATGCTCTTGATCTTCCAA	25		Depositor's primer
4	luc_f	CGTTCGGYTGGCAGAAGCTATGAA	24		RIKEN BRC

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		0.6			
PCR buffer	2 X	10.0			
dNTP	2 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.4			
	total	20			

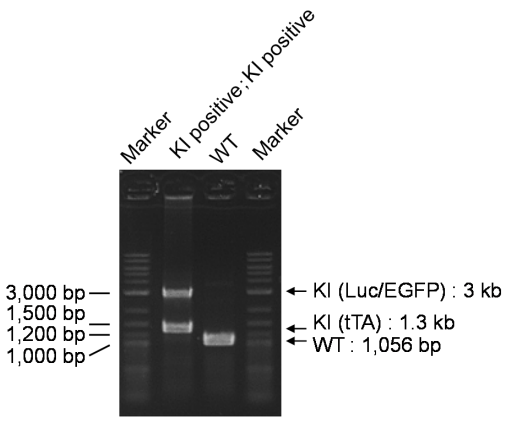
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	68	240*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	1,056 bp	WT
primer 1	primer 3	1.3 kb	KI
primer 2	primer 4	3 kb	KI

Comment Standard PCR

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