PCR protocol

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RBRC 09806 Strain name B6.Cg-Tg(CAG-cat,-EGFP)39Miya

Primer	primer_name	primer_seq	length		
1	CAGp-F/CA1	CTGCTAACCATGTTCATGCC	20		Oncogene, 25, 7673-7679 (2006).
2	C2ven-R	GTAGGTCAGGGTGGTCACGA	20		
3	oIMR0015	CAAATGTTGCTTGTCTGGTG	20		JAX Genotyping Protocol
4	oIMR0016	GTCAGTCGAGTGCACAGTTT	20		JAX Genotyping Protocol

Reaction Components

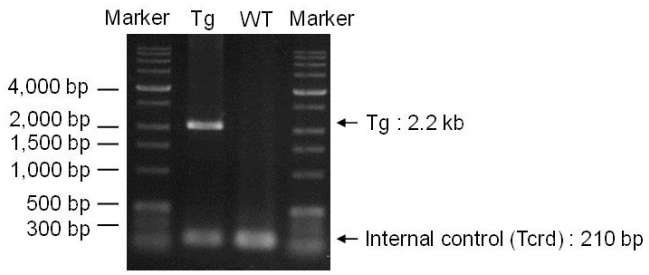
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Standard PCR



Marker

DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	2.2 kb	Tg
primer 3	primer 4	210 bp	Internal control (Tcrd)
primer 1	primer 2	0.4 kb	del (After cre mediated excision)

Comment

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