PCR protocol

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RBRC 09735 **Strain name** C57BL/6N-Tg(CAG-EGFP,-IL6/mCherry)56Kenw

Primer	primer_name	primer_seq	length		
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23		RIKEN BRC
2	mCherry FW Primer	CCCATGGTCTTCTTCTGCAT	20		RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

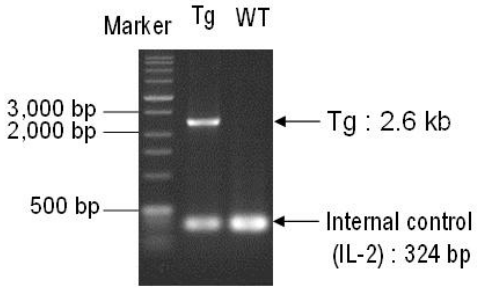
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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/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	21			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	120		
2	98	10	*	
3	60	30	*	
4	68	240	*	*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	2.6 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

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