



PCR protocol

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RBRC 10633

Strain name C57BL/6J-Tg(Chat-cre)609Sleep

Primer	primer_name	primer_seq	length	Reference
1	exon 3	TTCAGGCTCTGTGTGATGCTGAG	23	RIKEN BRC
2	CalcrJunc1Rv	CTAGAGCCTGTTTTGCACGTT	21	RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

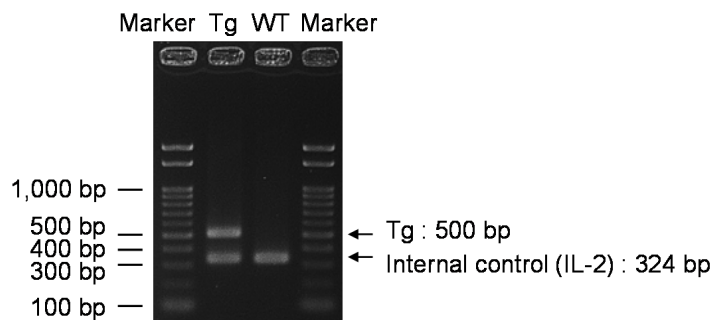
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
PCR buffer	2 X	10.0			
dNTP	2 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			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

Marker	Gene Ladder 100 (Wako, Cat. 316-06951)
Gel	2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	500 bp
primer 3	primer 4	324 bp

Non-Tg : 324 bp
Tg : 324 bp and 500 bp

Comment [Standard PCR](#)

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