

RBRC 10635

Strain name C57BL/6J-Tg(Chat-cre)615Sleep

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

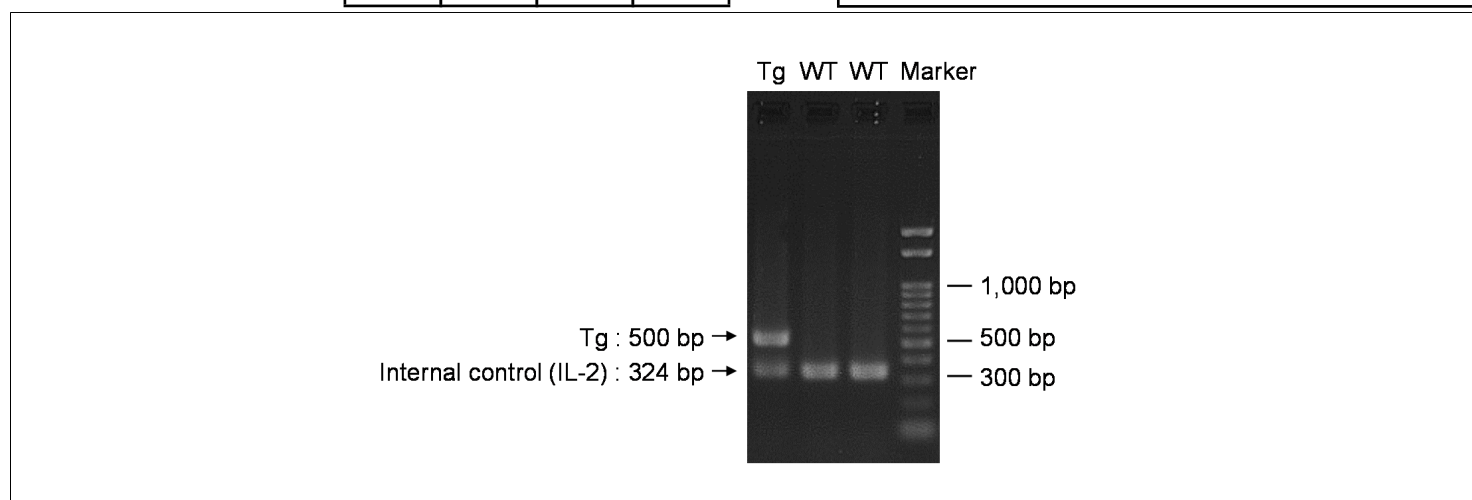
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

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	(μ L)
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	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

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.