

RBRC 10626

Strain name C57BL/6J-Tg(Atoh1-cre/Esr1<\*>)676Sleep

Primer	primer_name	primer_seq	length	Reference
1		GACTGAGAGGTGAAGATCGCGG	22	RIKEN BRC
2		CCAGGACTCGGTGGATATGGTC	22	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)

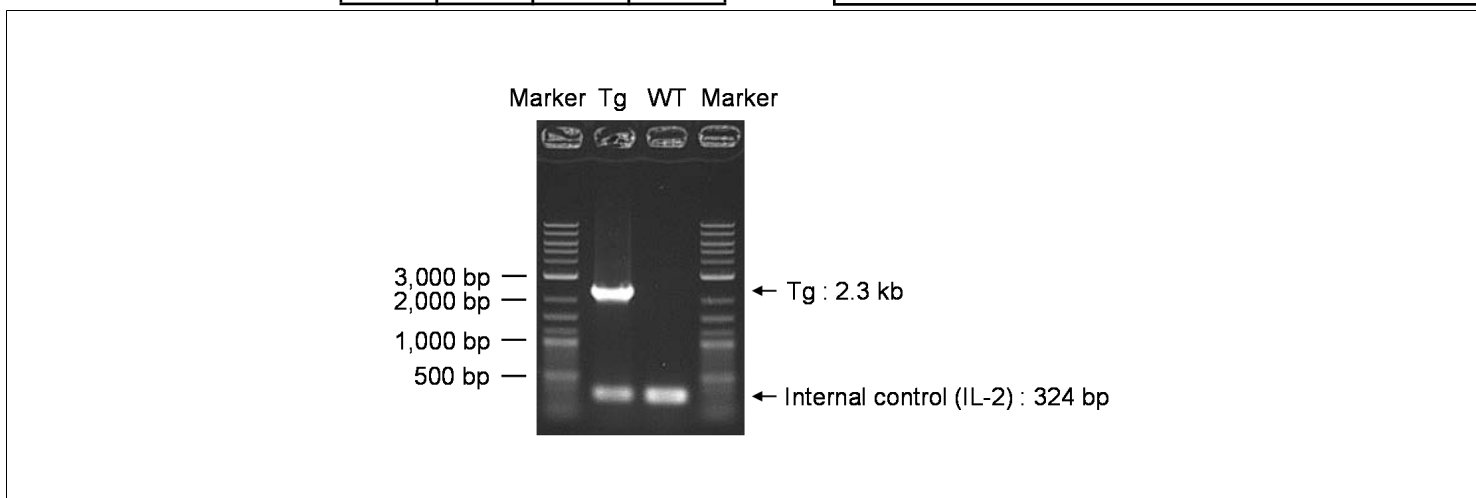
### Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	63	30*	
4	68	240*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	( $\mu$ L)
H2O		0.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ $\mu$ l	1.0				
primer 2	10 pmol/ $\mu$ l	1.0				
primer 3	10 pmol/ $\mu$ l	1.0				
primer 4	10 pmol/ $\mu$ l	1.0				
DNA		1.0				
Taq	1 U/ $\mu$ l	0.2				
<b>total</b>		20				

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	2.3 kb
primer 3	primer 4	324 bp

Non-Tg : 324 bp  
Tg : 324 bp, 2.3 kb

Comment Standard PCR

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