

# PCR protocol

RBRC 01162

Strain name EGFP-EEA1-12 transgenic mouse

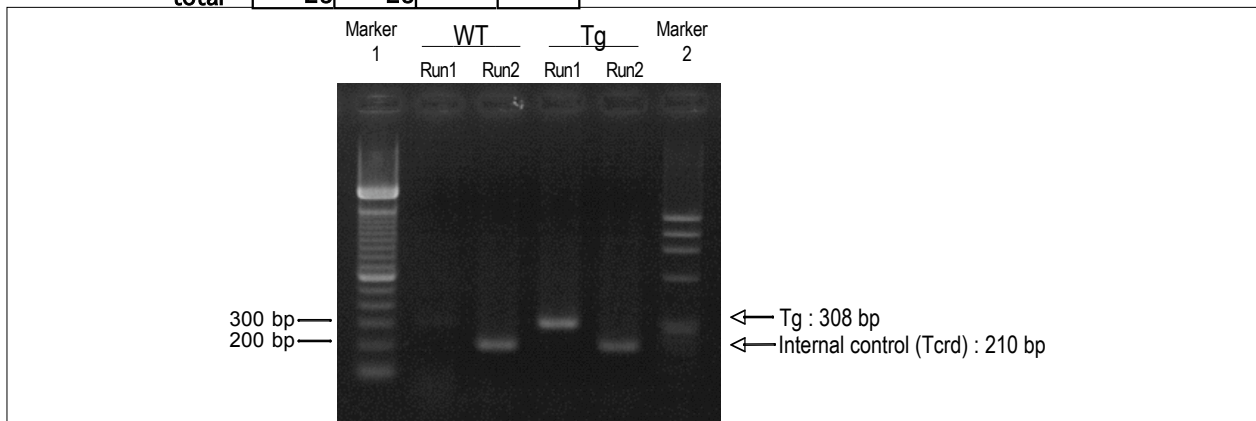
Primer	primer_name	primer_seq	length		
1	EGFP5'	TGAACCGCATCGAGCTGAAGGG	22		
2	EGFP3'	TCCAGCAGGACCATGTGATCGC	22		
3	oIMR0015	CAAATGTTGCTTGTCTGGTG	20	Internal control (Tcrd)	JAX Genotyping Protocol
4	oIMR0016	GTCAGTCGAGTGCACAGTTT	20	Internal control (Tcrd)	JAX Genotyping Protocol

**Reaction Components**

 Enzyme Roche Taq (Cat. No. BM1418432)
**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	65	30*	
4	72	30*	*Repeat for 35 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
<b>total</b>		<b>25</b>	<b>25</b>		


**Marker**

marker 1: 100 bp ladder (Invitrogen, Cat. 15628-050), marker2: φX174 Hae III

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

**PCR products**

primer_set		product_size	product
primer 1	primer 2	308 bp	Tg
primer 3	primer 4	210 bp	Internal control (Tcrd)

Comment