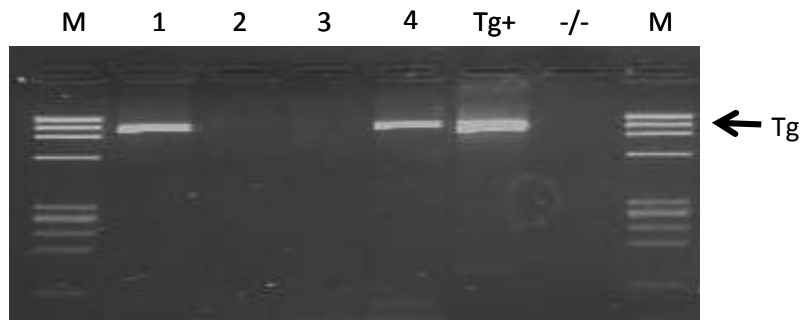


Transgene	Gnrh1-EGFP
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primer	Sequence(5'-3')	Size	target gene	accession
F2(Forward)	TACTATGGTCTATGCTGCACT	1015 bp	GFP	
ER1(Reverse)	ATCTGAAGAAGTCGTGCTGCT			
comment	F2 is designed at 3017 of rat GnRH promoter (Accession number: X62651) ER1 is designed at 334-356 of pEGFP1 (Accession number: U55761) GnRH-EGFP gene: rat GnRH promoter (3142 bp)-rabbit globin intron (640 bp)-EGFP (820bp)			

PCR condition							
Taq polymerase		BIOTAQ™ HS DNA Polymerase(BIOLINE, London, UK)					
Thermal cycler		PC-808(ASTEC)					
PCR buffer		Ampdirect® Plus (Shimadzu Corporation, kyoto, Japan)					
		first denature	denature	anneal	extension	cycle	final extension
PCR	°C	94	94	60	72	35	72
	min	3	0.5	0.5	1		3
comment		PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm FTA disc was removed from the bloody-stained region on FTA®card. Untreated sample discs were placed directly in 15 μ L PCR mixture containig 1 × Ampdirect®Plus, 0.2 μ M each primer and 0.4 units of BIOTAQ™ HS DNA Polymerase.					

Electrocataphoresis ; 4% agarose gel



Lane 1,4 ; Tg positive
 Lane 2,3 ; Tg negative
 Tg+ ; Tg positive control
 -/- ; Tg negative control (water)
 M; size marker ; ϕ X174-HaeIII

Strain	W-Tg(Gnrh1-EGFP)Nphy
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