

RBRC 02704

Strain name B6.Cg-Tg(FucciS/G2/M)#474Bsi

Primer	primer_name	primer_seq	length	Reference
1	02704	CTGTGTTTGTGGATTGGCAGTGTG	25	RIKEN BRC
2	02704	GGCAGCGCCCTCAGAGTTC	19	RIKEN BRC
3	R201	CGCGGAACTCCATATATGGGCTATG	25	RIKEN BRC

Reaction Components

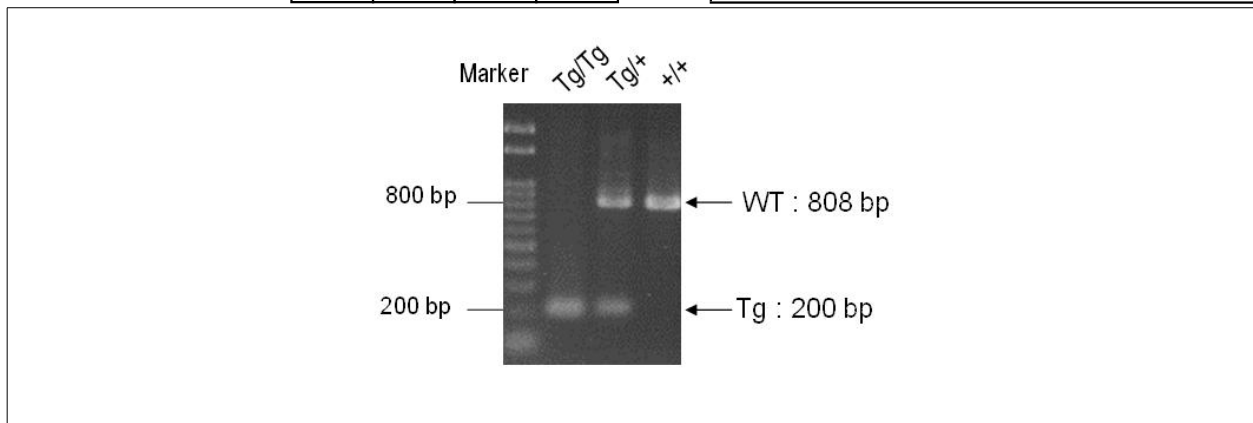
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

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	(μ L)
H ₂ O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 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				
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	808 bp	WT
primer 1 primer 3	200 bp	Tg
		+/+ : 808 bp
		Tg/+ : 808 bp, 200 bp
		Tg/Tg : 200 bp

Comment Standard PCR Zygoty determination PCR
Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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Strain name B6.B6D2-Tg(Fucci)474Bsi

Primer	primer_name	primer_seq	length	Reference
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21	Depositor's primer
2	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

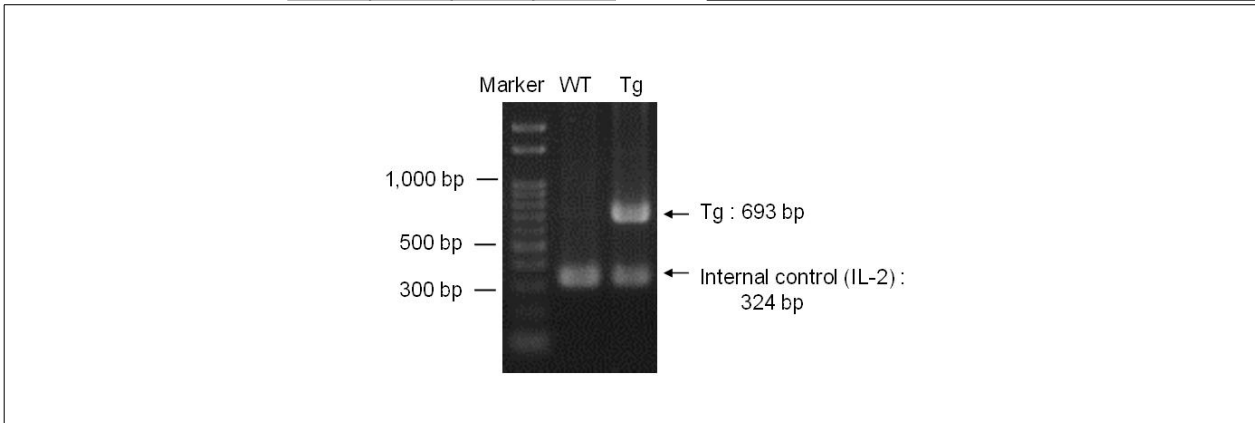
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	55	30*	
4	68	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		0.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	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	693 bp	Tg
primer 3	324 bp	Internal control (IL-2)

Comment Standard PCR