

PCR protocol

RBRC 06379

Strain name C57BL/6J-Tg(Trpv5-NCX1.3)2

Primer	primer_name	primer_seq	length	
1	FpV5-3	GCCATAAAGACAATGCAAGA	20	Depositor's primer
2	R1NheI	GGTCTCCAATGAAAGCTGTCAGTAT	25	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

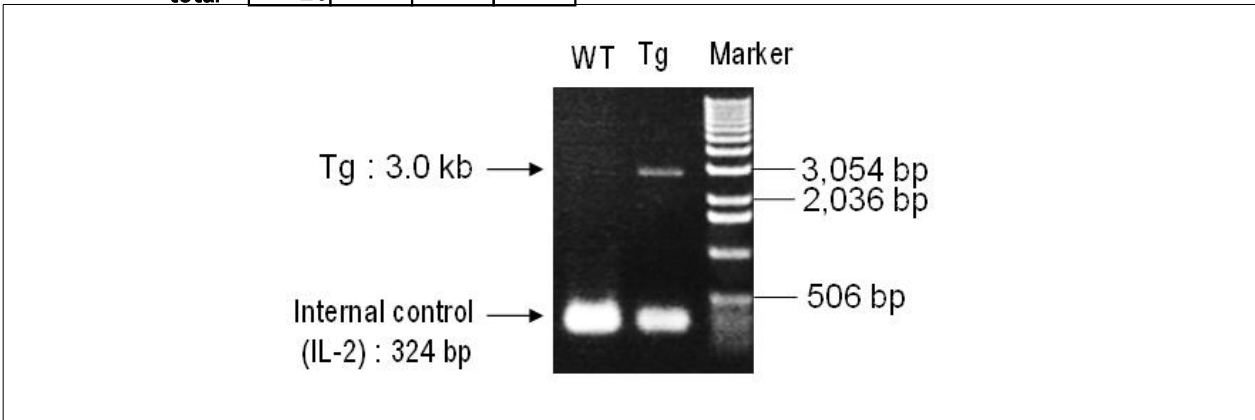
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
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			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set	product_size	product
primer 1 primer 2	ca 3.0 kb	Tg
primer 3 primer 4	324 bp	Internal control (IL-2)

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