PCR protocol

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RBRC 00443 **Strain name** B6D2-Tg(CAG-Nbl1)1Saki/Rbrc

Primer	primer_name	primer_seq	length		Reference
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23		RIKEN BRC
2	exon 4	CAATCTTCTCTACCAGCTTGTC	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)

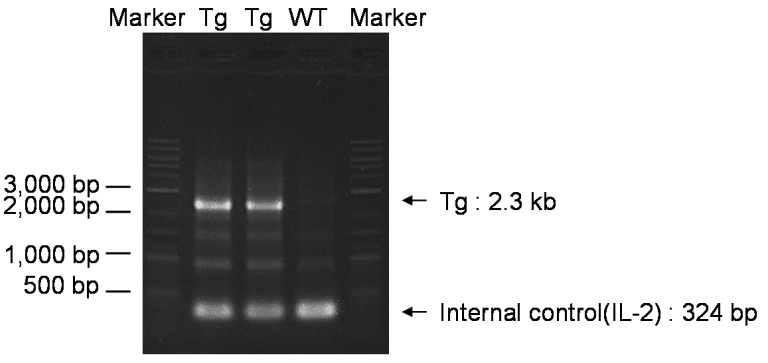
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	63	30	*
4	68	180	* *Repeat for 30 cycles

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	Tg	Non-Tg : 324 bp Tg : 324 bp and 2.3 kb
primer 3	primer 4	324 bp	Internal control (IL-2)	

Comment Standard PCR

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