PCR protocol

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RBRC 02121 **Strain name** B6.Cg-Tg(BiPRO2)2Htan

Primer	primer_name	primer_seq	length		Reference
1	promoter	CCAAGTCCCGCTAGGCATCTG	21		RIKEN BRC
2	EGFP3'	TCCAGCAGGACCATGTGATCGC	22		Biochem. Biophys. Res. Commun. 321, 275–279 (2004).
3	exon 1	CGACCGCGGGTGAGCCTGTG	20		RIKEN BRC
4	DsRed2-R	TGCTCCACGATGGTGTAGTCC	21		Mol Neurodegener, 6:75 (2011).
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

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

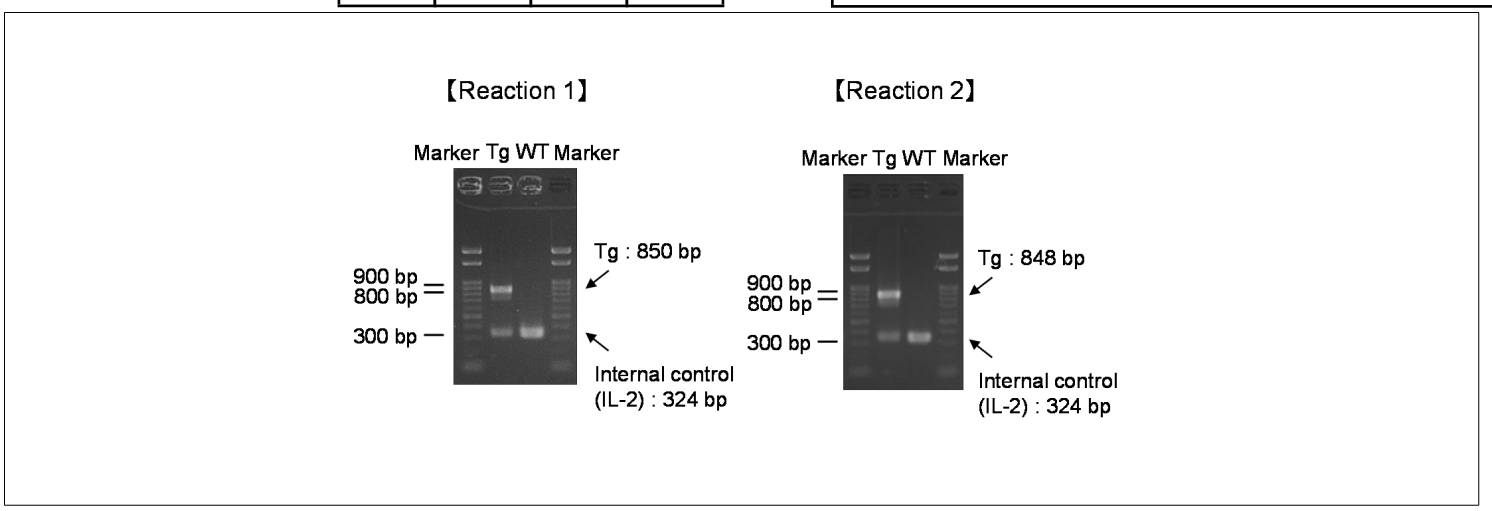
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8	0.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	1.0	1.0			
primer 6	10 pmol/μl	1.0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

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

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	850 bp	Tg
primer 3	primer 4	848 bp	Tg
primer 5	primer 6	324 bp	Internal control (IL-2)

Reaction 1
Non-Tg : 324 bp
Tg : 324 bp, 850 bp
Reaction 2
Non-Tg : 324 bp
Tg : 324 bp, 848 bp

Comment

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