PCR protocol

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RBRC 05861 **Strain name** B6.Cg-Tg(CAG/Su9-DsRed2,Acr3-EGFP)RBGS002Osb

Primer	primer_name	primer_seq	length		
1	A	GCTCTAGAGCCTCTGCTAACC	21		FEBS Letters, 500, 7-11, (2001).
2	DsRed2-R	TGCTCCACGATGGTGTAGTCC	21		Mol Neurodegener, 6:75 (2011).
3	acro	GTGGAGCTTTGTGAGGTCACAG	22		Depositor's primer
4		CTTTGCTCAGGGCGGACTGGGT	22		RIKEN BRC
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

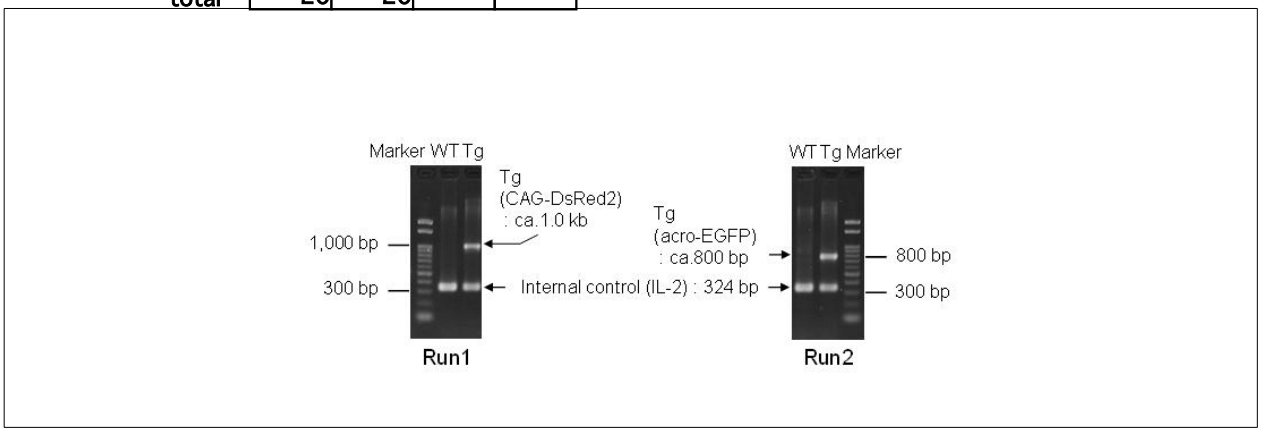
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8	0.8		
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	90	* *Repeat for 30 cycles

Standard PCR



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	ca. 1.0 kbp	Tg(CAG-DsRed2)
primer 3	primer 4	ca. 800 bp	Tg(acro-EGFP)
primer 5	primer 6	324 bp	Internal control (IL-2)

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