PCR protocol

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RBRC 09645 **Strain name** B6-Tg(Slc32a1-YFP*)39Yyan

Primer	primer_name	primer_seq	length		
1	VGAT-1tg	CGCTCACCTTGGCCTGGGACTTGTT	25		Neuroscience., 164, 1031-1043 (2009).
2	Venus-3tg	TGAGCTACCAGTCCGCCCTGAGCAA	25		Neuroscience., 164, 1031-1043 (2009).
3	TR-1b	GGCACAGCTCTCCCTTCTGTTTGC	24		Neuroscience., 164, 1031-1043 (2009).
4	TR-3	GCTCTCCTTTCGCGTTCCGACAG	23		Neuroscience., 164, 1031-1043 (2009).

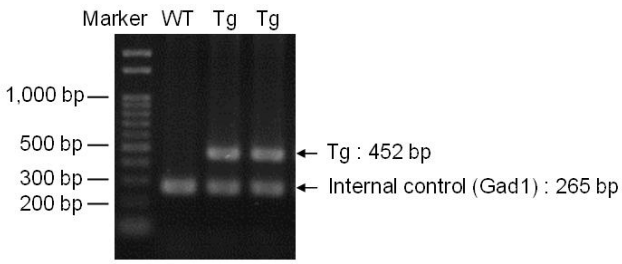
Reaction Components

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/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	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)

PCR products	primer_set	product_size	product
primer 1	primer 2	452 bp	Tg
primer 3	primer 4	265 bp	Internal control (Gad1)

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