PCR protocol

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RBRC 05167 **Strain name** B6;B6CB-Slc17a7<tm1.2(IRES-tTAad)Imayo>

Primer	primer_name	primer_seq	length		
1	VGLUT1-WT-fw	CAGGTCTCAGTAGTGGCTGCCCAAAG	26		Depositor's primer
2	VGLUT1-KO-rv	CCCGGGCCCACGCGTTTAATTAAG	24		Depositor's primer
3	VGLUT1-WT-rv	GCCAGGCAGAACTTCCCAATAAACCAC	27		Depositor's primer
4	IRES	GGCAATGTGAGGGCCCGGAAACC	23		Depositor's primer
5	tTA-Ad-386bp-rv	CCAGGCCACATATGATTAGTTCCAG	25		Depositor's primer

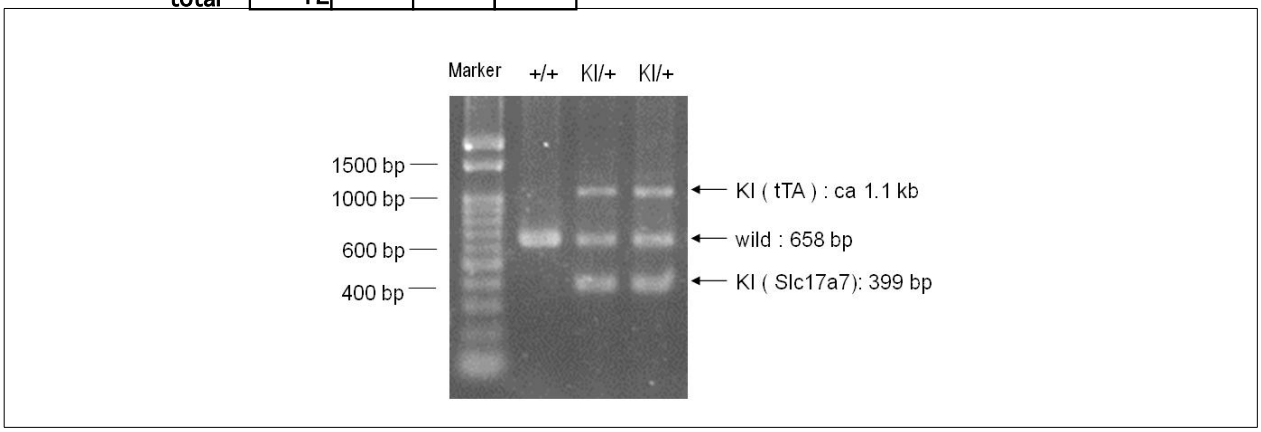
Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

	Conc	Run 1	Run 2	Run 3	Run 4
PCR buffer	2 X	6.0			
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			
primer 5	10 pmol/μl	1.0			
DNA		1.0			
	total	12			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	90	* *Repeat for 30 cycles
5	72	300	



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	399 bp	KI
primer 1	primer 3	658 bp	WT
primer 4	primer 5	ca 1.1 kb	KI (tTA)

Comment