PCR protocol

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RBRC 02684 **Strain name** B6J.C-Atp2b2<wri>/Oda

Primer	primer_name	primer_seq	length		Reference
1	common	AGGTGGCTCTTGATGTACCTGTG	23		RIKEN BRC
2	Atp2b2	GTGACAGCCAGAGGGAGGCCCGC	23		RIKEN BRC
3	Atp2b2<wri>	GGTGCTGGTGGTCGCTGTGCCTA	23		RIKEN BRC
4		GGGACTTGCCCACACGCTCT	20		Depositor's primer

Reaction Components

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

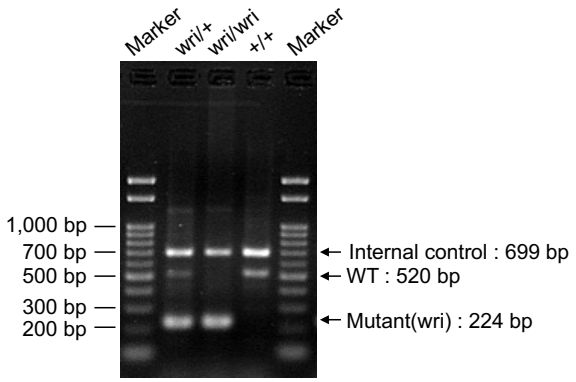
Reagent	Conc	Reaction 1	2	3	4	
primer 1	10 pmol/μl	1.0				(μL)
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
Master Mix	2 X	5.0				
DNA		1.0				
	total	10				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	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	520 bp	WT
primer 3	primer 4	224 bp	mutant
primer 1	primer 4	699 bp	Internal control

+/+ (wild-type): 699 bp and 520 bp
wri/+ (heterozygous): 699 bp, 520 bp and 224 bp
wri/wri (homozygous): 699 bp and 224 bp

Comment Standard PCR