PCR protocol

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RBRC 10045 **Strain name** B6(D2)-Sufu<Rgsc2027>

Primer	primer_name	primer_seq	length		Reference
1		AACCCTGTTCCCAGAGGAGT	20		PLoS One. 10(3):e0119455 (2015).
2		TGCTCATATATAGTTCACCCACA	23		PLoS One. 10(3):e0119455 (2015).
3	Rgsc2027_WT	GCTCAGGTTAGAACCGTCTGTCAC	24		RIKEN BRC
4	Rgsc2027_MT	GCGCGGCCGCAGCAGGAGAGAGTTGACAAAGGCATC	37		RIKEN BRC

Reaction Components

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

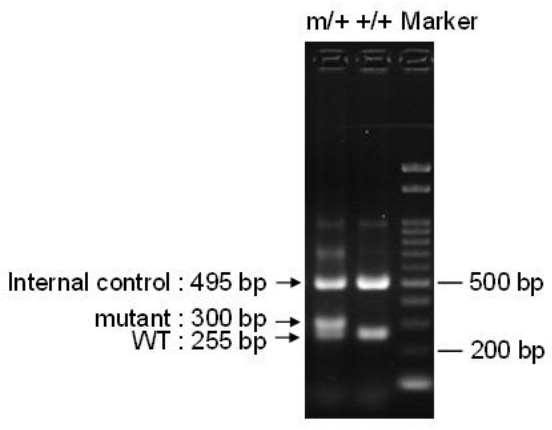
Reagent	Conc	Reaction 1	2	3	4	
primer 1	10 pmol/μl	1.0				(μL)
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Master Mix	2 X	5.0				
	total	10				

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	495 bp	Internal control
primer 1	primer 3	255 bp	WT
primer 2	primer 4	300 bp	mutant

Comment Allele specific PCR (ASP)