PCR protocol

Sheet ID PS_07585
Release date : 7/25/2019
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RBRC 06233 **Strain name** DS/Shi-Trpv3<Nh>/Shi

Primer	primer_name	primer_seq	length		Reference
1	common	GGTACTACTCATCCTTCGTAGTG	23		RIKEN BRC
2	wild	CACGAGAGGCTTCCAGTCTATAG	23		RIKEN BRC
3	mut	CACGAGAGGCTTCCAGTCTATAA	23		RIKEN BRC

Reaction Components

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

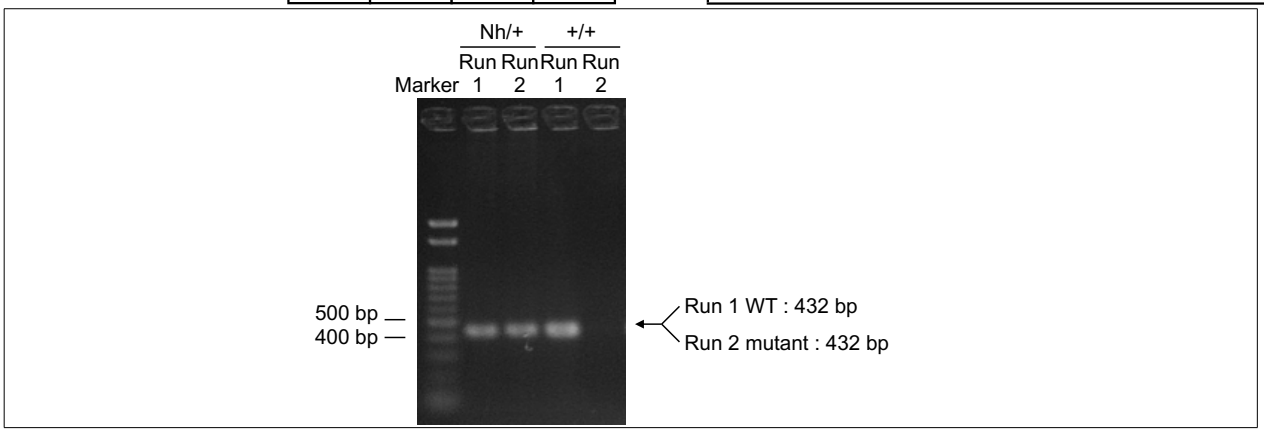
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
primer 1	10 pmol/μl	1.0	1.0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Master Mix	2 X	5.0	5.0			
		total	10	10		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	63	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	432 bp	WT
primer 1	primer 3	432 bp	mutant

Reaction 1 (Run1)
Nh/+ : 432 bp
+/+ : 432 bp

Reaction 2 (Run2)
Nh/+ : 432 bp
+/+ : none

Comment Allele specific PCR (ASP)