PCR protocol

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RBRC 00672 Strain name STAM1+/-, STAM2+/-

Primer	primer_name	primer_seq	length		
1	(S1)LH-2	CGGGACCAGAGGAAAAGCACCTGTCAC	27		Mol. Cell. Biol., 21, 3807-3819 (2001).
2	(S1)Wild-1	ATCAGTGTACAAATGGGAAGGTATTAT	27		Mol. Cell. Biol., 21, 3807-3819 (2001).
3	(S1)PGK-2	TGCGAGGCCAGAGGCCACTTGTGTAGC	27		Mol. Cell. Biol., 21, 3807-3819 (2001).
4	(S2)Wild-5'	GCCATAATGAAAAGGGTAAACCATAAGGTC	30		Depositor's primer
5	(S2)Wild-3'	AATCACGGGAACATACTTCTAAATGAAATA	30		Depositor's primer
6	(S2)mt-5'	TTCTAAGTACTGTGGTTTCCAAATGTGTCA	30		Depositor's primer

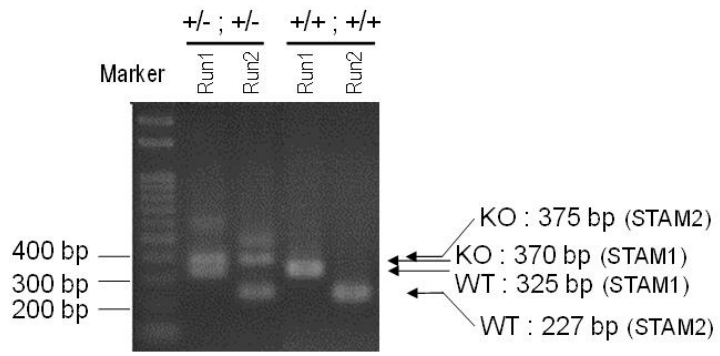
Reaction Components

Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	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	325 bp	WT (STAM1)
primer 1	primer 3	370 bp	KO (STAM1)
primer 4	primer 5	227 bp	WT (STAM2)
primer 6	primer 5	375 bp	KO (STAM2)

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