PCR protocol

Sheet ID PS_04461
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RBRC 05264 **Strain name** B6.MSM-(D16Mit71-D16Mit106)/Hkv

Primer	primer_name	primer_seq	length		Reference
1	D16Mit71-1	TAGAAAATCTTCAAATAGGATCTGTTC	27		Depositor's primer
2	D16Mit71-2	GAGCATTTCCCTTTTACCTGG	21		Depositor's primer
3	D16Mit106-1	GTTGTTGGTCCACCATACCC	20		Depositor's primer
4	D16Mit106-2	GGGTATGAGGTACAATGCTTTACC	24		Depositor's primer

Reaction Components

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

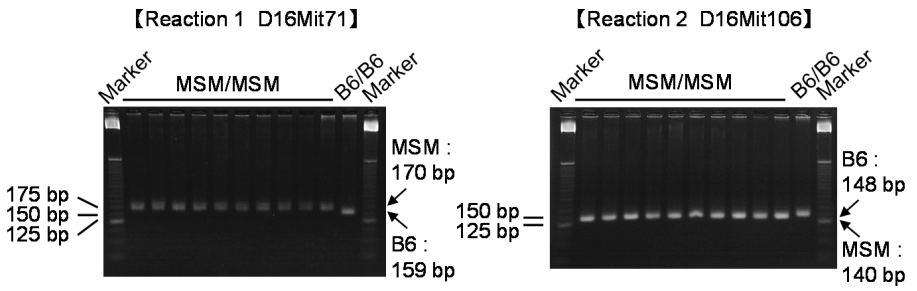
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
Master Mix	2 X	5.0	5.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA	10 pmol/μl	1.0	1.0			
	total	10	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	63	90	*
4	72	60	* *Repeat for 30 cycles
5	60	1800	

Marker 25 bp DNA ladder (Invitrogen, Cat. 10597-011)

Gel 4% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	159 bp	B6
primer 1	primer 2	170 bp	MSM
primer 3	primer 4	148 bp	B6
primer 3	primer 4	140 bp	MSM

Reaction 1
MSM/MSM: 170 bp
B6/B6: 159 bp
Reaction 2
MSM/MSM: 140 bp
B6/B6: 148 bp

Comment Standard PCR