PCR protocol

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RBRC 09291 **Strain name** A.SM-(D12Mit270)

Primer	primer_name	primer_seq	length		Reference
1	D12Mit270-1	AGGCATCTTTTTGAATAGTTTTATACA	27		Depositor's primer
2	D12Mit270-2	ATTAAGGCATTGGTAAAGTGATATATG	27		Depositor's primer
3		GGTAGCTGAGAGTTCTACATCCG	23		RIKEN BRC
4	rs29184291	GGACGAACTGGATGGACTGTGAGC	24		RIKEN BRC
5	rs29184291	GGACGAACTGGATGGACTGTGAGT	24		RIKEN BRC

Reaction Components

Enzyme Run1: QIAGEN Multiplex PCR Kit (Cat. No. 206143) Run2, 3: Roche Taq (Cat. No. BM1418432)

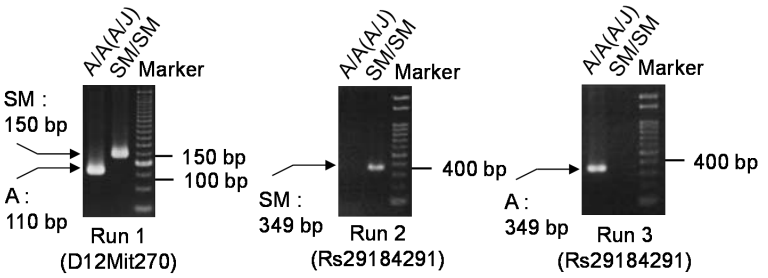
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	13.3	13.3		(μL)
Master Mix	2 X	5.0	0	0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	0	1.0	1.0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
PCR buffer	10 X	0	2.0	2.0		
dNTP	2.5 mM	0	1.6	1.6		
Taq	5 U/μl	0	0.1	0.1		
	total	10	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	[Reaction 1]
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 30 cycles
5	60	1800	
1	94	180	[Reaction 2, 3]
2	94	30	*
3	60	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	

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

Gel Run1:4% Nusieve in 0.5 X TBE (100 V, 60 min),Run2,3:2 % agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	150 bp	SM
primer 1	primer 2	110 bp	A
primer 3	primer 4	349 bp	SM
primer 3	primer 5	349 bp	A

Comment Allele specific PCR (ASP)