PCR protocol

Sheet ID PS_06722
Release date : 5/6/2022
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RBRC 09377 **Strain name** B6.D2-(D6Mit188-D6Mit15)/Hkv

Primer	primer_name	primer_seq	length		Reference
1	D6Mit188-1	CTTTAGTCATTATTAGGATTGCCTATG	27		Depositor's primer
2	D6Mit188-2	TGGGATAGCATTGGAAACGT	20		Depositor's primer
3	D6Mit150-1	GGTAACAAAAAAAGGAAACCACC	23		Depositor's primer
4	D6Mit150-2	ATTATTGAGCATTTCCCCCC	20		Depositor's primer
5	D6Mit15-1	CACTGACCCTAGCACAGCAG	20		Depositor's primer
6	D6Mit15-2	TCCTGGCTTCCACAGGTACT	20		Depositor's primer

Reaction Components

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

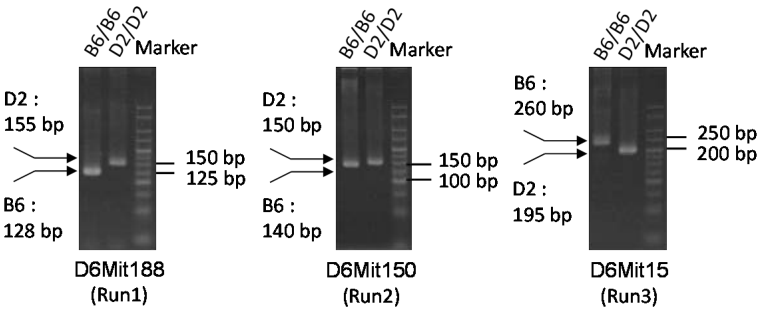
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0	2.0		(μL)
Master Mix	2 X	5.0	5.0	5.0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	0	1.0	0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	0	1.0		
primer 6	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
	total	10	10	10		

Reaction Conditions

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

Marker 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel 4% Nusieve in 0.5 X TBE (100 V, 80 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	155, 128 bp	DBA/2(D2), B6
primer 3	primer 4	150, 140 bp	DBA/2(D2), B6
primer 5	primer 6	195, 260 bp	DBA/2(D2), B6

Comment Separated PCR