PCR protocol

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RBRC 02440

Strain name B6.129S-Drd1a<tm1Mok> Drd5<tm1Mok>

Primer	primer_name	primer_seq	length		Reference
1	D5R out1	TAGGGGTTTGGGATAAGGTTGTGA	24		Depositor's primer
2	D5R in1	CGTCCTGGAATTGTGACTCTGCTG	24		Depositor's primer
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer
4	D1TET1	CAGAAGACAGGTGGAAAGCA	20		Depositor's primer
5	D1R3' 60R	GTTGGAGAAGTTCTGTAACTGTCC	24		Depositor's primer

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

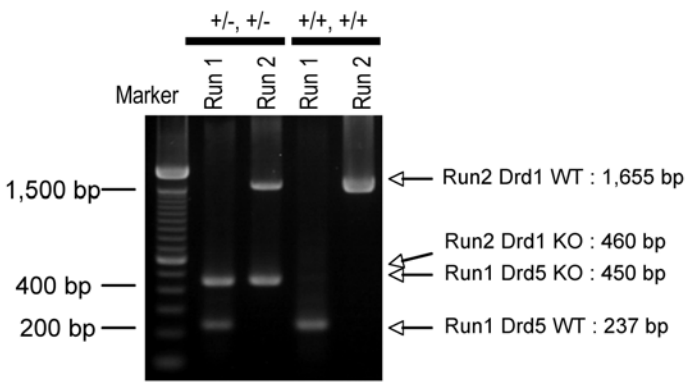
Reagent	Conc	Reaction 1	2	3	4	
H2O		16.3	16.3			(μL)
PCR buffer	10 X	2.5	2.5			
dNTP	2 X GC I	2.0	2.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	1.0	1.0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq		0.2	0.2			
	total	25	25			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 30 cycles
5	72	300	

Marker 100 bp ladder (Invitrogen, Cat. 15628-050)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	237 bp	WT (Drd5)
primer 1	primer 3	450 bp	KO (Drd5)
primer 3	primer 5	460 bp	KO (Drd1)
primer 4	primer 5	1,655 bp	WT (Drd1)

Comment Separated PCR