PCR protocol

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RBRC 02431

Strain name B6.129-Drd2<tm1Mok> Drd3<tm1Mok> Drd4<tm1Mok>

Primer	primer_name	primer_seq	length		Reference
1	D2R5' C	CACTCCTTGTATTCATTTCTCCCG	24		Depositor's primer
2	D2G	ACCTCCAGATAGACGACCCAG	21		Depositor's primer
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Oncogene, 15, 1151-1159 (1997).
4	01082F	CTGATGATCTCTACCTCTGCTAGCC	25		RIKEN BRC
5	01082R	ACAGCCAGGCTCACCACTAGGTAG	24		RIKEN BRC
6	5' bamD	TGAAAGGATGTCCTGCAGAG	20		Depositor's primer
7	3' bamA	AGGCACCTTTTGGAGAAAGG	20		Depositor's primer
8	Pol II 160R	CAGTTCACCGCTGAATATGC	20		Depositor's primer

Reaction Components

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

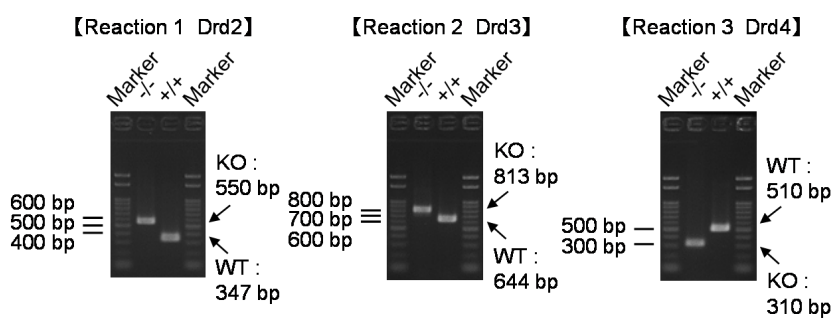
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8	1.8	1.8		(μL)
PCR buffer	2 X	10.0	10.0	10.0		
dNTP	2.0 mM	4.0	4.0	4.0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	1.0	1.0	0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	1.0	0		
primer 6	10 pmol/μl	0	0	1.0		
primer 7	10 pmol/μl	0	0	1.0		
primer 8	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
Taq	1 U/μl	0.2	0.2	0.2		
	total	20	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)



primer_set		product_size	product (allele type)
primer 1	primer 2	347 bp	WT (Drd2)
primer 1	primer 3	550 bp	KO (Drd2)
primer 4	primer 5	644 bp	WT (Drd3)
primer 3	primer 4	813 bp	KO (Drd3)
primer 6	primer 7	510 bp	WT (Drd4)
primer 7	primer 8	310 bp	KO (Drd4)

Comment Standard PCR

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