PCR protocol

Sheet ID PS_05199
Release date : 3/3/2014
Print date : 3/3/2014

RBRC 01080 Strain name B6.129S-Drd1a<tm1Mok>

Primer	primer_name	primer_seq	length		
1		GGGAAGTCTGGCTAAGCCTGGCC	23		RIKEN BRC
2	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer
3	D1R3'60R	GTTGGAGAAGTTCTGTAACTGTCC	24		Depositor's primer

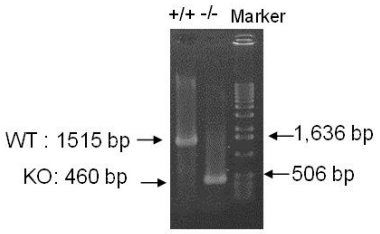
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	120	* *Repeat for 30 cycles



Marker
1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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

PCR products

primer_set		product_size	product
primer 1	primer 3	1,515 bp	WT
primer 2	primer 3	460 bp	KO

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

PCR protocol

Sheet ID PS_03150
Release date : 04/28/2010
Print date : 3/3/2014

RBRC 01080 Strain name B6.129S-Drd1a<tm1Mok>

Primer	primer_name	primer_seq	length		
1		GGGAAGTCTGGCTAAGCCTGGCC	23		RIKEN BRC
2	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer
3	D1R3'60R	GTTGGAGAAGTTCTGTAACTGTCC	24		Depositor's primer

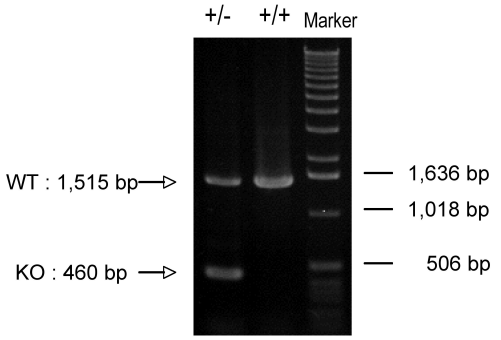
Reaction Components

Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	10 X	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	90	* *Repeat for 30 cycles
5	72	300	



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 3	1,515 bp	WT
primer 2	primer 3	460 bp	KO

Comment