PCR protocol

Sheet ID PS_06275
Release date : 6/18/2015
Print date : 6/18/2015

RBRC 05885 Strain name B6.Cg-Vdr<tm1Ska>

Primer	primer_name	primer_seq	length		
1		GCATACCGTCTCTTCTGATGAGG	23		RIKEN BRC
2		GTTGCTCTCAGTAACCGGGAG	21		RIKEN BRC
3	VDR_NeoR-1	AAGGTGAGATGACAGGAGATC	21		Endocrinology, 141, 1317-1324 (2000).

Reaction Components

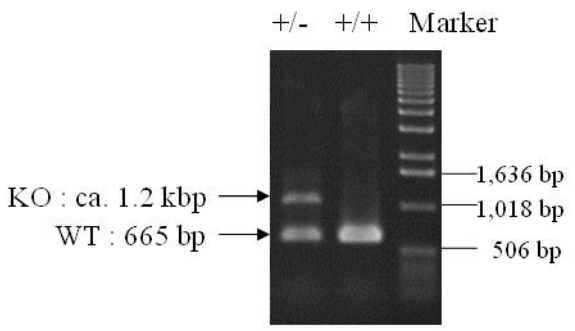
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 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

Standard PCR



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 2	665 bp	WT
primer 1	primer 3	ca. 1.2 kbp	KO

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

PCR protocol

Sheet ID PS_05370
Release date : 11/19/2013
Print date : 6/18/2015

RBRC 05885 Strain name B6.Cg-Vdr<tm1Ska>

Primer	primer_name	primer_seq	length		
1	VDR-2	CAAAGAACTGCCACCCACTC	20		Endocrinology, 141, 1317-1324 (2000).
2	VDR-1	CCTCCATCCCTGTAAGAAGA	20		Endocrinology, 141, 1317-1324 (2000).
3	VDR_NeoR-1	AAGGTGAGATGACAGGAGATC	21		Endocrinology, 141, 1317-1324 (2000).

Reaction Components

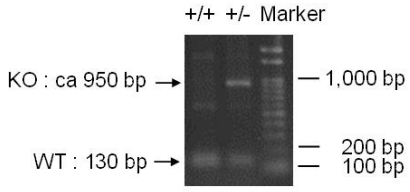
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 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	60	* *Repeat for 30 cycles

Standard PCR



Marker: Gene Ladder 100 (Wako, Cat. 316-06951)

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

PCR products

primer_set		product_size	product
primer 1	primer 2	130 bp	WT
primer 1	primer 3	ca 950 bp	KO

Comment