PCR protocol

Sheet ID PS_05905
Release date : 12/27/2017
Print date : 12/27/2017

RBRC 06336 **Strain name** NOD.Cg-Prkdc<scid> E2f1<tm1Meg>

Primer	primer_name	primer_seq	length		
1	scid_Wild	GGCCCCTGCTAACTTTCTCTTAGTA	25		RIKEN BRC
2	scid_Mut	GGCCCCTGCTAACTTTCTCTTAGTT	25		RIKEN BRC
3	common	GATATGTGCCATGTCAGTGTCTGAC	25		RIKEN BRC

Reaction Components

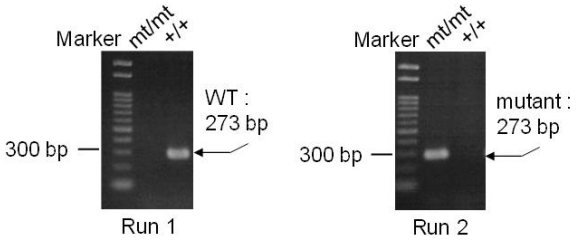
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.0	2.0		
Master Mix	2 X	5.0	5.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	0	1.0		
primer 3	10 pmol/μl	1.0	1.0		
DNA	10 pmol/μl	1.0	1.0		
	total	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	72	600	

Allele specific PCR (ASP)



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 3	273 bp	WT
primer 2	primer 3	273 bp	mutant

Comment

PCR protocol

Sheet ID PS_06706
Release date : 12/27/2017
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RBRC 06336 **Strain name** NOD.Cg-Prkdc<scid> E2f1<tm1Meg>

Primer	primer_name	primer_seq	length		
1	ex3_Fw	GAAGTCACGCTATGAAACCTCAC	23		RIKEN BRC
2	ex4_Rv	ATGTGCATCAGGTGATCCAGCTG	23		RIKEN BRC
3	Neo30	TTCTATCGCCTTCTTGACGAGTTCTTCTGA	30		

Reaction Components

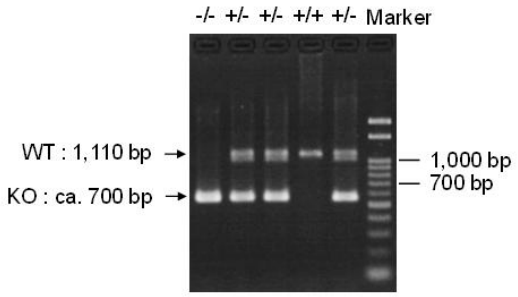
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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	90	* *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	1,110 bp	WT
primer 1	primer 3	ca 700 bp	KO

Comment

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