PCR protocol

Sheet ID PS_08020
Release date : 4/15/2020
Print date : 4/15/2020

RBRC 10754 **Strain name** C57BL/6J-Tg(Rspo2-cre)BIto

Primer	primer_name	primer_seq	length		Reference
1	exon 1	CGATCTACACCTGGTGCTGGGGC	23		Dev. Growth. Differ., 50, 85-95 (2008).
2	Ex2_Rv	CTCGCTTATTGCGTCTCCATCGG	23		RIKEN BRC
3	CW-Cre2	ACCTGATGGACATGTTCAGGGATCG	25		Nature, 406(6797): 726-731 (2000).

Reaction Components

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

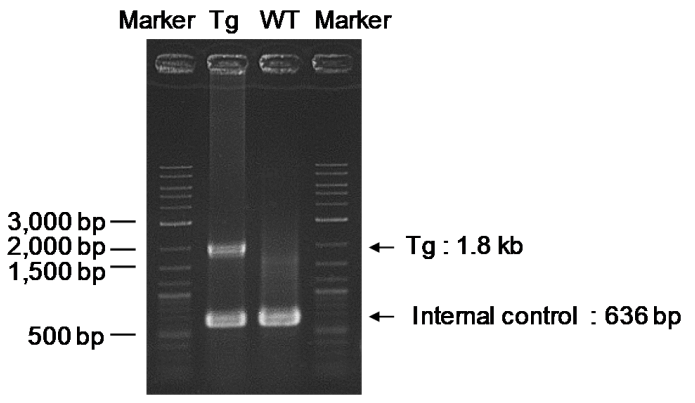
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	636 bp	Internal control	Non-Tg : 636 bp
primer 2	primer 3	1.8 kb	Tg	Tg : 636 bp and 1.8 kb

Comment Standard PCR

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

PCR protocol

Sheet ID PS_08004
Release date : 3/6/2020
Print date : 4/15/2020

RBRC 10754

Strain name C57BL/6J-Tg(Rspo2-cre)BIto

Primer	primer_name	primer_seq	length		Reference
1	exon 1	CGATCTACACCTGGTGCTGGGGC	23		Dev. Growth. Differ., 50, 85-95 (2008).
2	CW-Cre2	TCCGGTTATTCAACTTGCACCATGC	25		Nature, 406(6797): 726-731 (2000).

Reaction Components

Enzyme: Roche Taq (Cat. No. BM1418432)

Reagent	Conc	Reaction 1	2	3	4
H2O		13.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
DNA		1.0			
Taq	5 U/μl	0.1			
	total	20			

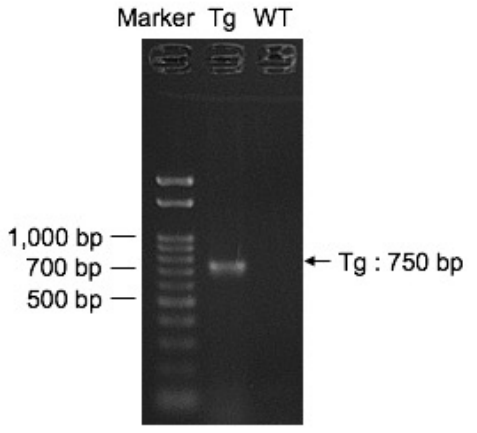
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 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	750 bp	Tg

Comment

PCR protocol

Sheet ID PS_07882
Release date : 9/25/2019
Print date : 4/15/2020

RBRC 10754 **Strain name** C57BL/6J-Tg(Rspo2-cre)BIto

Primer	primer_name	primer_seq	length		Reference
1	promoter	CAGTAACTTCAACTCTGGCTCAC	23		RIKEN BRC
2	Ex2_Rv	CTCGCTTATTGCGTCTCCATCGG	23		RIKEN BRC
3	CW-Cre2	ACCTGATGGACATGTTCAGGGATCG	25		Nature, 406(6797): 726-731 (2000).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	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			

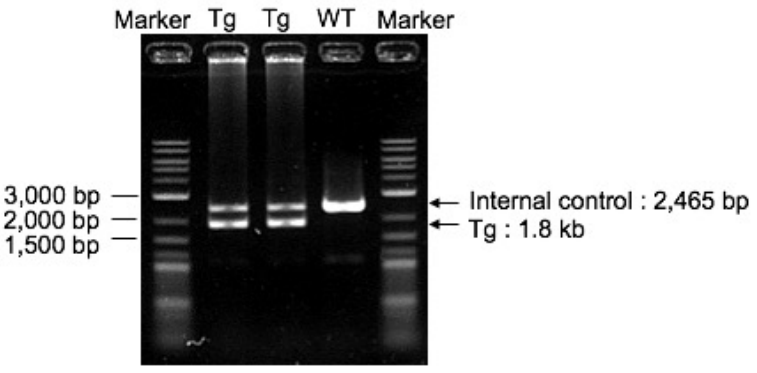
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	2,465 bp	Internal control
primer 2	primer 3	1.8 kb	Tg

Comment Standard PCR

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