PCR protocol

Sheet ID PS_00245
Release date : 11.19.2008
Print date : 8/1/2012

RBRC 01260 Strain name Xist^{1loxGFP}mice

Primer	primer_name	primer_seq	length		
1	Xist-GFP W	TTCTGCCCAGAACAGACCAC	20		Depositor's primer
2	Xist-GFP F	ATCACATGGTCCTGCTGGAG	20	gfp	Depositor's primer
3	XistGFP-R	TACAACAATGGTCCTAGTTTCC	22		Depositor's primer

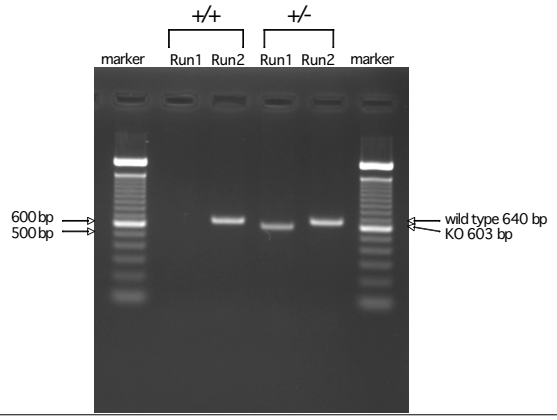
Reaction Components

Enzyme: Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	25	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30	*
3	62	60	*
4	72	120	* *Repeat for 30 cycles
5	72	300	



Marker: 100 bp ladder (Invitrogen, Cat. 15628-050)

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

PCR products

primer_set		product_size	product
primer 2	primer 3	603 bp	KO
primer 1	primer 3	640 bp	WT

Comment

PCR protocol

Sheet ID PS_04103
Release date : 07/05/2011
Print date : 8/1/2012

RBRC 01260 Strain name Xist^{1loxGFP}mice

Primer	primer_name	primer_seq	length		
1	Xist-GFP W	TTCTGCCCAGAACAGACCAC	20		Depositor's primer
2	EGFP5'	TGAACCGCATCGAGCTGAAGGG	22		Depositor's primer
3	XistGFP-R	TACAACAATGGTCCTAGTTTCC	22		Depositor's primer

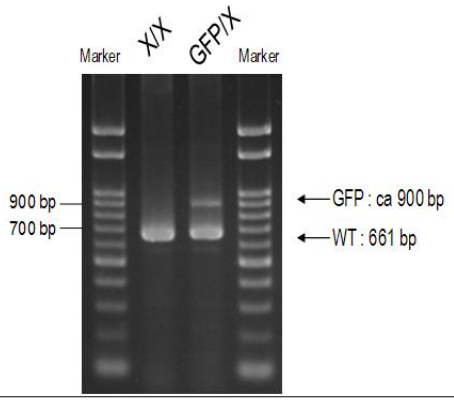
Reaction Components

Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.8			
PCR buffer	2 X	2.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/μl	0.5			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	62	60	*
4	72	60	* *Repeat for 35 cycles
5	72	300	



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

primer_set		product_size	product
primer 2	primer 3	ca 900 bp	GFP
primer 1	primer 3	661 bp	WT

Comment

PCR protocol

Sheet ID PS_04221
Release date : 08/01/2012
Print date : 8/1/2012

RBRC 01260 Strain name Xist^{1loxGFP}mice

Primer	primer_name	primer_seq	length		
1	Xist-GFP W	TTCTGCCCAGAACAGACCAC	20		Depositor's primer
2	XistGFP-R	TACAACAATGGTCCTAGTTTCC	22		Depositor's primer
3	EGFP5'	TGAACCGCATCGAGCTGAAGGG	22		Depositor's primer

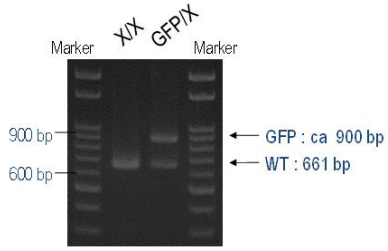
Reaction Components

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	2.0 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		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



Marker

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

Gel

2% agarose in 1 X TAE (100 V, 35 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	661 bp	WT
primer 3	primer 2	ca 900 bp	GFP

Comment