PCR protocol

Sheet ID PS_06212
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RBRC 06509 **Strain name** ICR.Cg-Tg(Oct4dPE-creERT2)4

Primer	primer_name	primer_seq	length		
1	Pou5f1_US	CATCCCTTGCAGACAGGCACTC	22		RIKEN BRC
2	cre 2	GCATAACCAGTGAAACAGCATTGCTG	26		Depositor's primer
3		CCAGGACTCGGTGGATATGGTC	22		RIKEN BRC
4	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
5	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

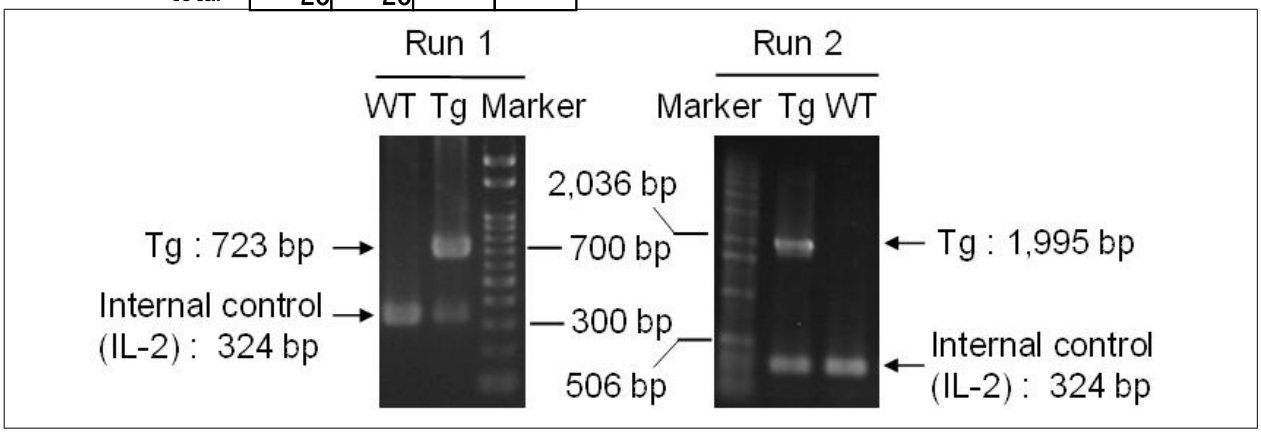
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8	0.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	1.0	1.0		
primer 5	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	120		<Run1>
2	98	10	*	
3	60	30	*	
4	68	60	*	*Repeat for 30 cycles
1	94	120		<Run2>
2	98	10	*	
3	60	30	*	
4	68	180	*	*Repeat for 30 cycles

Separated PCR



Marker
marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 1 kb

Gel
Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:1% agarose in 1 X TAE

PCR products	primer_set	product_size	product
primer 1	primer 2	723 bp	Tg
primer 1	primer 3	1,995 bp	Tg
primer 4	primer 5	324 bp	Internal control (IL-2)

Comment

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