PCR protocol

RBRC 11099

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Strain name B6.129-Elovl1<tm1Akih>

Primer	primer_name	primer_seq	length		Reference
1	p1	GCGCGGCCGCGAGGAGAGTCTTGGAAAAGG	30		Mol. Cell. Biol., 33, 2787-2796 (2013).
2	p2	GCGGTCGACAACCCTAGATCCTGGGATACC	30		Mol. Cell. Biol., 33, 2787-2796 (2013).
3	p3	TCGCCTTCTTGACGAGTTCTTCTGAGG	27		Mol. Cell. Biol., 33, 2787-2796 (2013).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
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		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

(μL)

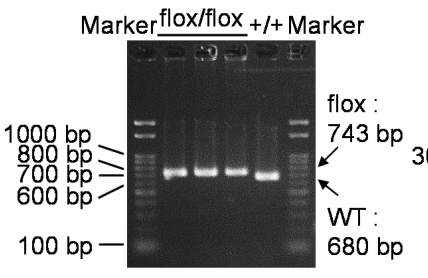
Reaction Conditions

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

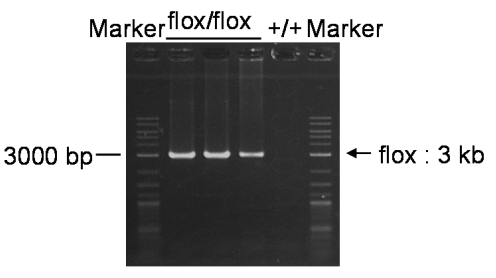
Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

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

【Reaction 1】



【Reaction 2】



primer_set		product_size	product (allele type)
primer 1	primer 2	680 bp	WT
primer 1	primer 2	743 bp	flox
primer 1	primer 3	3 kb	flox
primer 1	primer 3	529 bp	null (After cre mediated excision)

Reaction 1 +/+ (wild-type): 680 bp
flox/flox (homozygous): 743 bp

Reaction 2 flox/flox (homozygous): 3 kb

Comment Standard PCR

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