

RBRC 11400

Strain name C.Cg-Spib<tm2.1Ksho>

Primer	primer_name	primer_seq	length	Reference
1	Spib-4126	AACCAGGCCCAACTCCATTGTGAAAAC	27	Depositor's primer
2	Spib-4125	GCCAGCTTCTGATACGTCATGCGCTTG	27	Depositor's primer
3	Spib-4127	AGTCCTGTACACACCATTGGTTGCAGTG	27	Depositor's primer

Reaction Components

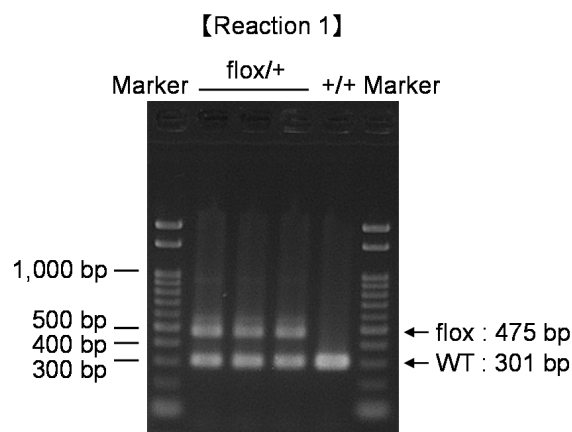
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

step	temp (°C)	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

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		

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	301 bp	WT
primer 1	primer 2	475 bp	flox
primer 1	primer 3	2,786 bp	WT
primer 1	primer 3	454 bp	null (After cre mediated excision)

Reaction 1
+/+ (wild-type): 301 bp
flox/+ (heterozygous): 301 bp and 475 bp

Comment [Standard PCR](#)

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