

RBRC 05518

Strain name B6;129S4-ECAT15-1<tm1Yam> ECAT15-2<tm1Yam>

Primer	primer_name	primer_seq	length	Reference
1	15-1G-s1	ACAACAGGAAGTAGGAGGAGATTTT	25	Mol. Cell. Biol., 31, 4366-4378 (2011).
2	15-1G-as1+	GAAGTGACAATTTAGCATTTCCTGT	25	Mol. Cell. Biol., 31, 4366-4378 (2011).
3	15-2Reds	GCACAGGCTGGTCTTGAGTATTTT	24	Mol. Cell. Biol., 31, 4366-4378 (2011).
4	15-2Reds3-as	AGACAAGCCCGAGACACATCCGTC	24	Mol. Cell. Biol., 31, 4366-4378 (2011).
5	PGK-as-seq	ACCGGTGGATGTGGAATGTG	20	Mol. Cell. Biol., 31, 4366-4378 (2011).

Reaction Components

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

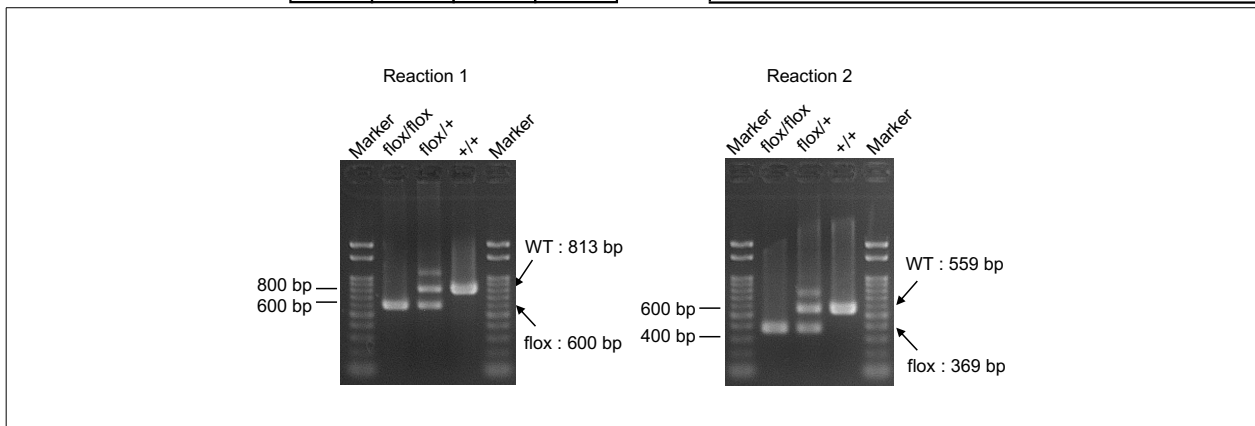
Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
primer 4	10 pmol/ μ l	0	1.0		
primer 5	10 pmol/ μ l	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/ μ l	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	813 bp	WT (ECAT15-1)
primer 1	600 bp	flox (ECAT15-1)
primer 3	559 bp	WT (ECAT15-2)
primer 3	369 bp	flox (ECAT15-2)

Reaction 1 flox/flox: 600 bp
flox/+ : 600 bp, 813 bp
+/: 813 bp

Reaction 2 flox/flox: 369 bp
flox/+ : 369 bp, 559 bp
+/: 559 bp

Comment Standard PCR

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