PCR protocol

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RBRC 05519 **Strain name** B6;129S4(Cg)-ECAT15-1<tm1.1Yam> ECAT15-2<tm1.2Yam>

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	15-1G-as1-	GAGCTCAGACCATAACTTCGTATAA	25		Mol. Cell. Biol., 31, 4366-4378 (2011).
4	15-2Reds	GCACAGGCTGGTCTTGAGTATTTT	24		Mol. Cell. Biol., 31, 4366-4378 (2011).
5	15-2Reds3-as	AGACAAGCCCGAGACACATCCGTC	24		Mol. Cell. Biol., 31, 4366-4378 (2011).

Reaction Components

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

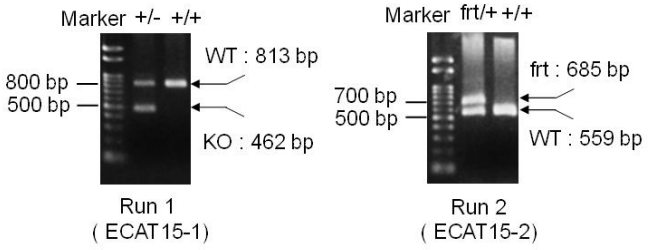
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8	2.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	1.0	0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	0	1.0			
DNA	10 pmol/μl	1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	63	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, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	813 bp	WT (ECAT15-1)
primer 1	primer 3	462 bp	KO (ECAT15-1)
primer 4	primer 5	559 bp	WT (ECAT15-2)
primer 4	primer 5	685 bp	frt (ECAT15-2)

Comment Standard PCR

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