PCR protocol

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RBRC 11422 **Strain name** B6-Cyp4f39<em1Akih>

Primer	primer_name	primer_seq	length		Reference
1	exon 11	CATCAAGCTGCCAGATGGACG	21		RIKEN BRC
2		TCATGTCTCTGTCACTCTGACTCCTCTGC	29		J. Invest. Dermatol.,140,319-326 (2020).
3	Cyp4f39_p1	GGATCCTTGTGATTGGTGGATGGACAGATAG	31		Depositor's primer
4	mut	AGCAGGCACCTTTGGGGATGACATC	25		RIKEN BRC

Reaction Components

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

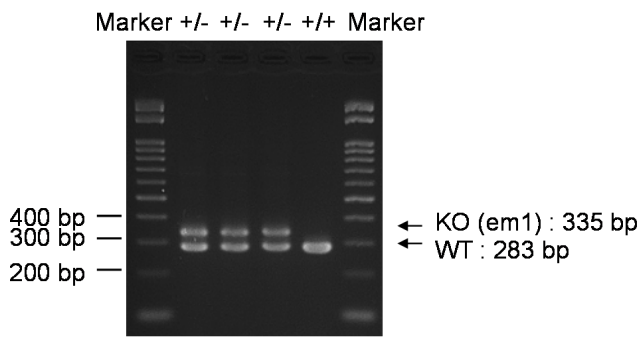
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 3% Nusieve in 0.5 X TBE (100 V, 50 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	283 bp	WT
primer 3	primer 4	335 bp	KO(em1)

+/+ (wild-type): 283 bp
+/- (heterozygous): 283 bp and 335 bp

Comment Standard PCR

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