PCR protocol

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RBRC 11423

Strain name B6-Tg(IVL-Cyp4f39)6Akih

Primer	primer_name	primer_seq	length		Reference
1	Involucrin-Tg-F	CTAGGAGGACCTTTCTCTGC	20		Depositor's primer
2		TTGCGACAGGTAATGCGGAAGTCAC	25		Elife. 9:e53582 (2020).
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
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			

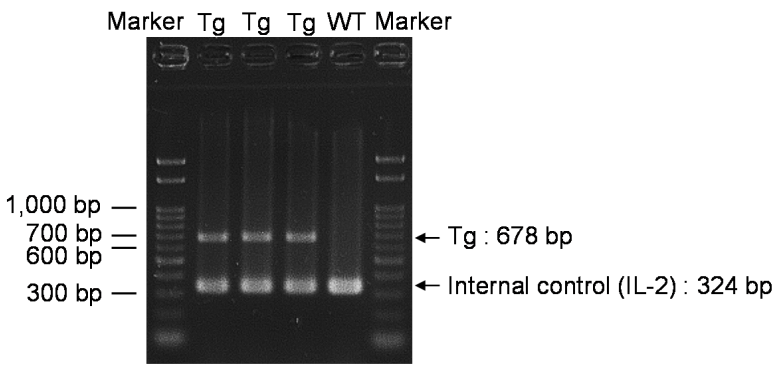
(μL)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	678 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Non-Tg : 324 bp
Tg : 324 bp, 678 bp

Comment Standard PCR

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