PCR protocol

Sheet ID PS_08280
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RBRC 11555 **Strain name** C57BL/6-Hcrt<tm1.1(FLP)Ksak>

Primer	primer_name	primer_seq	length		Reference
1	kin19771F	CTCATTAGTACTCGGAAACTGCCC	24		Elife, 8:e44927 (2019).
2	kin20169R(9720R)	AAGCACTATCATGGCCTCAGTAGT	24		Elife, 8:e44927 (2019).
3	Venus262-R	AGAAGTCGTGCTGCTTCATG	20		Depositor's primer
4	SD223	GCATCTGGGAGATCACTGAG	20		Genesis, 28, 106-110(2000).

Reaction Components

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

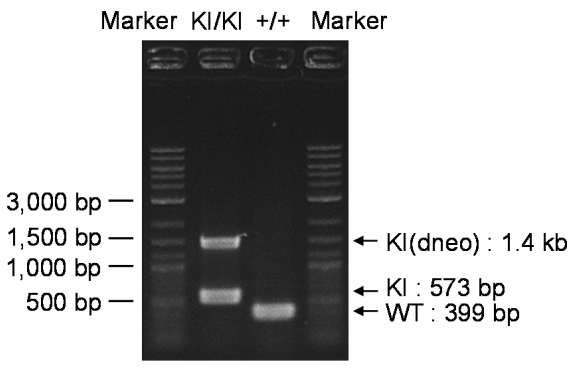
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	399 bp	WT	+/+ (wild-type): 399 bp
primer 1	primer 3	573 bp	KI	KI/KI (homozygous): 573 bp and 1.4 kb
primer 2	primer 4	1.4 kb	KI(dneo)	

Comment Standard PCR

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