

PCR protocol

RBRC 09859

Strain name C57BL/6J-Tg(CMV-cat,-SDHA<*>Y215F)1Hom

Primer	primer_name	primer_seq	length	
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23	RIKEN BRC
2	hSDHAPROBER1	CCCTGTAGTTGGTGGGAATG	21	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

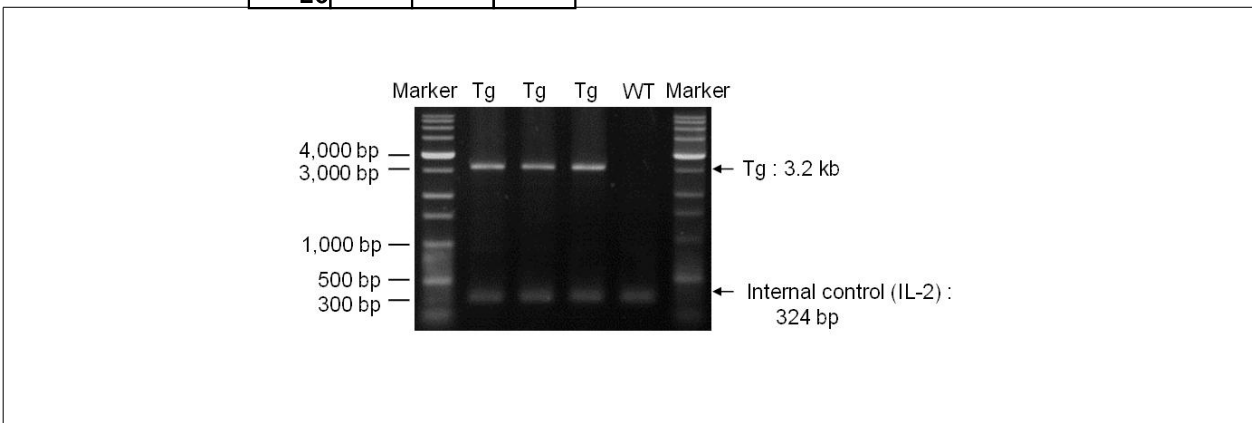
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	68	240*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.6			
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	0.4			
total		20			

Standard PCR



Marker	PCR products	primer_set	product_size	product
DNA Ladder, 1 kb Plus (Affymetrix, Cat. 76714 500 UL)	primer 1	primer 2	3.2 kb	Tg
Gel	primer 3	primer 4	324 bp	Internal control (IL-2)
1% agarose in 1 X TAE (100 V, 30 min)	primer 1	primer 2	1.4 kb	del (After cre mediated excision)

Comment

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