



PCR protocol

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Release date : 8/17/2022
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RBRC 05668

Strain name B10.Cg-H2<k> Tg(H2-Ea-Cd74/MCC*T102E)29Nnak

Primer	primer_name	primer_seq	length	Reference
1	promoter	GGCTTGAACATTGGCAAGCAG	21	RIKEN BRC
2	3CAG	AGCCAGAAGTCAGATGCTCAAGGGGC	26	RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

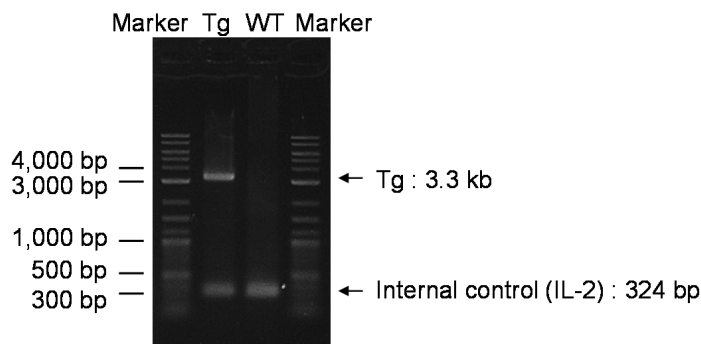
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	63	30*	
4	68	240*	*Repeat for 30 cycles

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/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

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	3.3 kb
primer 3	primer 4	324 bp

Non-Tg : 324 bp
Tg : 324 bp, 3.3 kb

Comment

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

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Primer	primer_name	primer_seq	length	Reference
1	TG-u	GCAACGTGCTGGTTATTGTGCTGTCT	26	rabbit beta-globin intron II
2	TG-d	TTCGTTAGCTCCGGACTTCTTCTCCT	26	
3	olMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	olMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

Enzyme	TaKaRa Ex Taq (Cat. No. RR001A)
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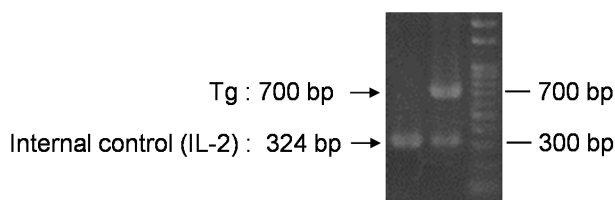
Reagent	Conc	Reaction 1	2	3	4
H2O		11.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	5 U/ μ l	0.1			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Marker	Gene Ladder 100 (Wako, Cat. 316-06951)
Gel	2% agarose in 1 X TAE (100 V, 30 min)

+/- Tg Marker



primer_set	product_size	product (allele type)
primer 1	primer 2	700 bp
primer 3	primer 4	324 bp

Comment Standard PCR