



PCR protocol

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RBRC 11185

Strain name C57BL/6-Gt(ROSA)26Sor^{tm1.1(CAG-tdiRFP713NLS)Imayo}

Primer	primer_name	primer_seq	length	Reference
1	RCE-Rosa1	CCCAAAGTCGCTCTGAGTTGTTATC	25	Depositor's primer
2	RCE-Rosa2	GAAGGAGCGGGAGAAATGGATATG	24	Depositor's primer
3	olMR0214	CCGGAGAACCTGCGTGCAATCC	22	JAX Genotyping Protocol
4	iRFP-365bp-fw	GAGCTGGAACCACTCAGAGAGACGTG	27	Depositor's primer

Reaction Components

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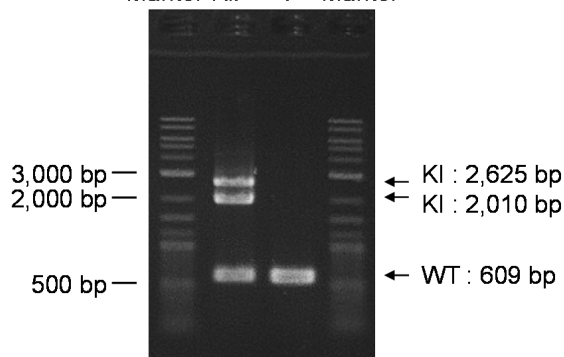
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	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/μl	1.5			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
primer 4	10 pmol/μl	0.5			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

Marker	2-Log DNA Ladder (NEB, Cat. N3200)
Gel	1% agarose in 1 X TAE (100 V, 30 min)

Marker KI/+ +/+ Marker



primer_set	product_size	product (allele type)	
primer 1	primer 2	609 bp	WT
primer 1	primer 3	2,625 bp	KI
primer 2	primer 4	2,010 bp	KI

+/+ (wild-type): 609 bp
KI/+ (heterozygous): 609 bp, 2625 bp and 2010 bp

Comment

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