PCR protocol

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RBRC 09643 **Strain name** Tg(SOD1*H46R)LAra

Primer	primer_name	primer_seq	length		Reference
1	intron 1	CCACTCCCAAGTCTGGCTGC	20		RIKEN BRC
2	intron 2	GCATGAGGATCAATGGAGCCTG	22		RIKEN BRC
3	intron 3	GTGTGTAGACGTGAAGCCTTG	21		RIKEN BRC
4	Human SOD1 Reverse	CAAGGGGAGGTAAAAGAGAGC	21		Human Molecular Genetics., 24, 3427–3439 (2015).
5	oIMR0015	CAAATGTTGCTTGTCTGGTG	20		JAX Genotyping Protocol
6	oIMR0016	GTCAGTCGAGTGCACAGTTT	20		JAX Genotyping Protocol

Reaction Components

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

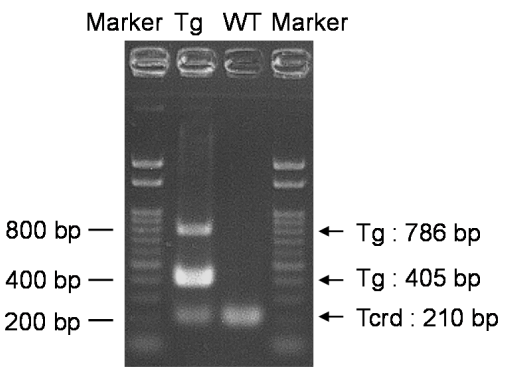
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	1.0				
primer 6	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	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	405 bp	Tg (SOD1)
primer 3	primer 4	786 bp	Tg (SOD1)
primer 5	primer 6	210 bp	Internal control (Tcrd)

Non-Tg : 210 bp
Tg : 210 bp, 405 bp, 786 bp

Comment Standard PCR

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