PCR protocol

RBRC 03165 **Strain name** B6J.B6D2-Tg(CAG-Wld*W258A)1Ara

Primer

	primer_name	primer_seq	length		
1	TA1_pCAGGS5' fwd	gaccggcggctctagagcctctgctaa	27		Depositor's primer
2	TA3_NMNAT1_5' rev	cctgaggtgcatgttggtgatggggtta	28		Depositor's primer
3	5_NMNAT1_3' fwd	cagaggaacgccgcagaggccaagcacaa	29		Depositor's primer
4	TA2_pCAGGS3' rev	gctcaaggggcttcatgatgtccccata	28		Depositor's primer
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

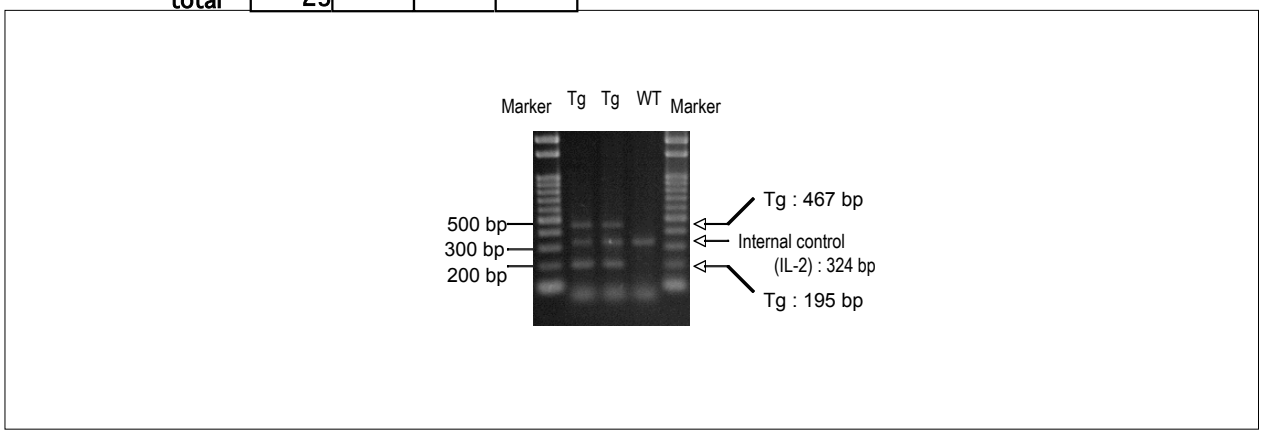
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.5			
primer 2	10 pmol/μl	1.5			
primer 3	10 pmol/μl	1.0			
primer 4	10 pmol/μl	1.0			
primer 5	10 pmol/μl	0.5			
primer 6	10 pmol/μl	0.5			
DNA		1.0			
Taq		0.2			
	total	25			

Reaction Conditions

step	temp (℃)	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)

PCR products

primer_set		product_size	product
primer 1	primer 2	467 bp	Tg
primer 3	primer 4	195 bp	Tg
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment