

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: FVB.Cg-Tbx1^{tm1Rak}/Mmcd MMRRC # 036811-UCD

Protocol: (PCR protocol provided by Donating Investigator)

Reagent/Constituent	Volume (μL)	Final Concentrations
Water	0.75	
10x Buffer	2.5	1x
DMSO	2.5	10%
MgCl ₂	3.0	3mM
dNTPs	4.0	0.2mM
Primer 1 (Tbx1KOF)	5.0	1 μM
Primer 2 (Tbx1KOR)	2.5	0.5 μM
Primer 3 (Mut2R)	2.5	0.5 μM
Hot-start Taq Polymerase	0.25	1.25 Units
Genomic DNA	2.0	Variable
TOTAL VOLUME OF REACTION:	25.000 μL	

Comments on protocol:

Homozygotes and heterozygotes can be distinguished by Southern blot (Merscher et al., 2001)

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	94	10:00	1
2. Denaturation	94	1:00	} 24
3. Annealing	55	1:00	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	20	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Tbx1KO1F	TTGGTGACGATCATCTCGGT
2. Tbx1KO2R	ATGATCTCCGCCGTGTCTAG
3. Mut2R	AGGTCCCTCGAAGAGGTTCA

Electrophoresis Protocol:

Agarose: 1.5% V: 100

Primer Combination	Expected Bands	Genotype
Tbx1KO1F/Tbx1KOR	410 bp	WT +/+
	410/233 bp	HET +/-
Tbx1KOF/Mut2R	233 bp	KO -/-