08.05.10 MS
01.30.12 MS updated

Genotyping Protocol: **MMRRC 246**

Assay Type: PCR, can distinguish transgene positive from transgene negative animals; cannot distinguish hemizygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat#69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain contains multiple copies of a modified BAC in which an EGFP reporter gene is inserted immediately upstream of the coding sequence of the LIM homeobox protein 6 gene (*Lhx6*). Details can be found in Gong S, et al. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature. 2003 Oct 30;425(6961):917-25.

Primer Information:
1) Name: M246 (Lhx6) F T2 Sequence: 5'- TCT CCA AGT TTG TCG GGA CCT TCT-3'
Genomic location : Chromosome 2; upstream of exon 1 in *Lhx6* transcript
2) Name: M246 (Lhx6) R T2 Sequence: 5'- AAG AAG TCG TGC TGC TTC ATG TGG -3'
Binds to GFP transgene

Assay Name: MMRRC line 246 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M246 (Lhx6) F T2	IDT	25 µM	0.3
M246 (Lhx6) R T2	IDT	25 µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

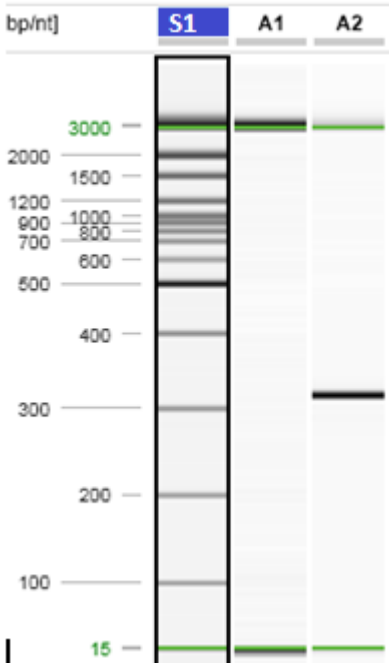
Cycle Parameters:
1) 95°C 5 minutes
2) 94°C 1 minute
3) 66°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 minutes
7) 4°C hold until refrigerate product

Product Analysis:
Expected product: ~330 bp

Transgene + = ~330 bp product
Transgene - = no product

08.05.10 MS
01.30.12 MS updated

Example gel:



Lane A1 displays a transgene negative sample (no product)
Lane A2 displays a transgene positive sample (330bp product)
Lane S1 displays a 15bp-3kb size marker.

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.