PCR genotyping protocol for *Neurog3*$^{HA.LCA}$ mouse line

PCR reagents:

1. oligo primers diluted to a 20 uM working concentration

Ngn3HA-F 5'-TGCAGTGACCTCTAAGTCAGAGGCT-3'
Ngn3HA-R 5'- ACCCACTTCTGCTTCGGAGCAGT-3'

Ngn3HA-F+ Ngn3HA-R yields a **230bp WT** (wild type allele) band and **330bp TM** (targeted mutant allele) band.

2. Perkin Elmer PCR buffer with $MgCl_2$
3. 1.25mM dNTP premix (dNTP premix is made by using 100 mM NEB dNTP's. The premix contains 250 µl of each dNTP - A, C, G, &T - and 19 ml sterile water and is stable at -20°C. I freeze this in 1 ml aliquots and thaw and refreeze as needed.)
4. Perkin Elmer Amplitaq Gold
5. genomic DNA samples diluted to 50 ng/ul with Promega Nuclease-Free water

PCR reaction mixture:

15.8 ul sterile water
2.5 ul 10X PCR buffer
4 ul dNTP premix
0.75 ul primer Ngn3HA F
0.75 ul primer Ngn3HA R
1 ul dil. DNA template
0.2 ul Amplitaq Gold
25 ul total volume

Cycling conditions:

1 cycle - 94° x 5 min.
35 cycles - 95° x 30 sec., 59° x 30 sec., 72° x 30 sec.
1 cycle - 72° x 7 min.
hold at 4°C.

Analysis of PCR products:

Load 10 ul aliquots of reactions + 2 ul of 6X gel loading buffer in a 1.5% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down with a DNA size marker appropriate for distinguishing the amplicons you expect.