PCR genotyping Protocol for *Pdx1^{Cre}* mouse lines.

PCR reagents:

1. oligo primers at 20 µM

   Amplification across lox71/66 (5') site:

   Pdx1.5b 5'- TGA GAT TGT ATA TTG CGG TGC A -3'

   Pdx1.S3 5'- AGA ACT CTC TAG ATA GTA AGT -3'

   Pdx1.5b + Pdx1.S3 yields a **573 bp TM** (targeted mutant allele) band.

   Amplification across lox2272 site (3'):

   Pdx1.S5 5'- TGA GCA ATT CCA AGC AGC TGG A -3'

   Pdx1.3c 5'- ACC TTG CAG TCC TTC TGA AGT -3'

   Pdx1.S5 + Pdx1.3c yields a **426bp WT** (wild type allele) band and a **481bp 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/µl with sterile water

PCR reaction mixture:

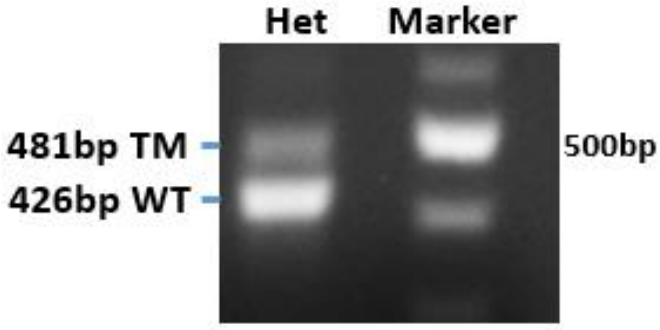
15.8 µl sterile water
2.5 µl 10X PCR buffer
4 µl dNTP premix
0.75 µl primer #1 (Pdx1.5b or Pdx1.S5)
0.75 µl primer #2 (Pdx1.S3 or Pdx1.3c)
1 µl dil. DNA template
0.2 µl Amplitaq Gold
25 µl total volume

Cycling conditions:

1 cycle - 94°C x 6 min.
40 cycles - 94°C x 1 min., 60°C x 30 sec., 72°C x 45 sec.
1 cycle - 72°C x 7 min.
hold at 4°C.

Analysis of PCR products:

Load 10 µl aliquots of reactions + 2 µl of 6X gel loading buffer in a 1.5% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.
Example 3' reaction:


Het
Marker
481bp TM
426bp WT
500bp