PDx1^{tm1.1Cvw} PCR Genotyping Protocol

Primers:

Pdx (-2377) - 5' - GGC TGA CTT TGG CCT CTC TA Pdx (-1878) - 3' - AGC GAG ACT TGG GAC AAA GA

Reaction mix:

0.5 ul DNA 2 ul Sigma Red Taq buffer 2 ul 10mM dNTPs 0.4 ul 20 uM Pdx (-2377)-5' 0.4 ul 20 uM Pdx (-1878)-3'

0.4 ul Gu Taq (a "homemade" taq polymerase. Others may work as well, though Sigma Red Taq does not).

14.3 ul H₂O

20 ul final volume

Program:

 $95^{\circ} - 7 \text{ min}$

 $95^{\circ} - 30 \text{ sec}$

 $58^{\circ} - 45 \text{ sec}$

72°-60 sec

 $72^{\circ} - 10 \text{ min}$

2-4 = 38 cycles

Band sizes:

wt = 499 bp

mut = 260 bp