PCR genotyping protocol for Rosa26$^{-228\text{-}DR5\text{-}TA\text{-}Cerulean}$ and Rosa26$^{-228\text{-}DR5\text{-}TA\text{-}Cerulean}$ alleles

PCR reagents:

1. oligo primers at 20 µM
   Amplification across lox2272 site: Rosa26.S11 + Rosa26.S2 yield a 523 bp Rosa26^{wt} amplicon & a 588 bp Rosa26$^{-228\text{-}DR5\text{-}TA\text{-}Cer(-H)}$ amplicon
   Rosa26.S11 5'- CGTGCTGAGCCAGACCTCCAT -3' (bottom)
   Rosa26.S2 5'- TCACAAGCAATAATAACCTGTAGT -3' (bottom)
2. Perkin Elmer PCR buffer with $MgCl_2$
3. 1.25mM dNTP premix (dNTP premix is made 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 (Rosa26.S1 or Rosa26.S10)
0.75 µl primer #2 (Rosa26.S11 or Rosa26.S2)
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 gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.