PCR Protocol: Screening for Rosa26 $^{MafA.cherry(-hygro)}$ RMCE
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The following conditions are used to screen across the lox sites for the Rosa26 $^{MafA.cherry(-hygro)}$ RMCE into the Rosa26.LCA targeted locus.

PCR reagents:

1. oligo primers at 20 µM
   Amplification across lox71 (5') site: Rosa26.S1 + Cherry-2F yield a 1233 bp Rosa26 $^{MafA.cherry(-hygro)}$ amplicon (reaction screens for hygro deletion)
   Rosa26.S1 5'- AGA CTT ATC TAC CTC ATA GGT G -3'
   Cherry-2F 5'- CAG TTC ATG TAC GGC TCC -3'
   Amplification across lox2272 site (3'): Rosa26.R1 + Rosa26.S2 yields a 512 bp amplicon for the tm (either hygro + or hygro -).
   Rosa26.R1 5'- GAG GAT CAT AAT CAG CCA TAC C -3'
   Rosa26.S2 5'- TCA CAA GCA ATA ATA ACC TGT AGT -3'
   Internal control primers: amplify a 150 bp fragment in all genomic DNA samples
   oMIR015 5'-CAA ATG TTG CTT GTC TGG TG-3'
   oMIR016 5'-GTC AGT CGA GTG CAC AGT TT-3'
   The PCR conditions outlined here are optimum for no more than two different fragment amplifications per reaction. Hence, the internal control primers can be used when screening for RMCE events on both the 5' & 3'ends.
2. Perkin Elmer PCR buffer with $MgCl_2$
3. dNTP premix (I make my own dNTP premix 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:

15.5 µl sterile water
2.5 µl 10X PCR buffer
4 µl dNTP premix
0.75 µl primer #1 (Rosa26.S1 or Rosa26.R1)
0.75 µl primer #2 (Cherry-2F or Rosa26.S2)
0.15 µl oMIR015
0.15 µl oMIR016
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 1 min. for reaction #1 and 45 sec. for reaction #2.
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.