PCR Genotyping with Rosa26 Reporter (R26R) Locus Specific Oligos

JDW 4/06
Pg. 1

Note:
This is from Soriano, P. 1999, Nature Genetics for 2 of the oligos, and the third is a different oligo from Charlie Murtaugh, which changes the scheme a little as far as what band sizes to look for, but the PCR works great.

JDW 4 (Rosa26 Forward) (Soriano, 1999):
5’- AAA GTC GCT CTG AGT TGT TAT

JDW 5 (Rosa26 Reverse) (LCM):
5’- TAA GCC TGC CCA GAA GAC TC

JDW6 (Knock in allele, reverse) (Soriano, 1999):
5’- GCG AAG AGT TTG TCC TCA ACC

The PCR should produce a band of ~ 309 bp should be produced if the Rosa26 (lacZ) allele is present. If the wildtype Rosa26 allele is present, PCR should yield a band of ~235 bp.

95°C – 2 minutes
95°C – 30 seconds
58°C – 30 seconds
68°C – 40 seconds
68°C – 7 minutes
4°C – forever

2.5 ul 10X HiFi Plat Taq Buffer
0.75 ul 50 mM MgSO4
0.75 ul 10 mM dNTPs
[0.3 uM] JDW4 (2.5 ul of a 3 uM stock)
[0.2 uM] JDW5 (1.66 ul of a 3 uM stock)
[0.2 uM] JDW6 (1.66 ul of a 3 uM stock)
0.25 ul HiFi Plat Taq
Q.S. to 23 ul H2O
2 ul gDNA (@ 1:50X)

A different set of primers is to use (as is on Jax’s website and in the original paper):
R26F2 / olMR8545 (WT For) 5’--AAAGTCGCTCTGAGTTTGATAT–3’,
R1295 / olMR8054 (MT For) 5’--GCGAAGAGTTTGTCCTCAACC–3’ and
RS23 / olMR8546 (WT Rev) 5’--GAGCAGCGAGAAATGGATATG–3’.

-PCR= 95-5:00 / 94-0:30, 58-0:30, 72-1.5--repeat for 40 cycles/ 72-10:00 / 4 for infinity. Do a 10 ul reaction and add 0.1 ul of each primer from a 100 uM stock. Use 1 ul of DNA.

WT band=550 bp and
Mutant/YFP or LacZ band=320 bp.