MEF2C southern probe creation and genotyping

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I. Probe generation:

PCR primer list:
Neo1 5′-tgt agc gcc aag tgc cag cgg g
Neo2 5′-gct aaa gcg cat gct cca gac tg
Mef2C 5′ Pst for1 5′—cag atg cta agg cag tga tag c
Mef2C 5′ Pst for2 5′—gag att atc tgg gtt aat gtg ggc

Nested PCR strategy.
PCR round 1:

25ng/ul Neo1	1ul
25ng/ul Mef2c 5′ Pst for1	1ul
10mM dNTPS	1ul
10x Expand buffer 2	2.5ul
1:4 diluted Expand pol	2ul
Tm1 mouse tail DNA	1ul
dH20	16.5ul
total	25ul

1) 99.5C — 5 min
2) 95C — 30 sec - add Expand
3) 95C — 30 sec.
4) 52C — 45 sec.
5) 72C — 1 min
6) go to step 3 X 29 cycles
7) 72C — 7 min.

PCR round 2:
Same as above except use primer combination Neo2 and Mef2C 5′ Pst for2 and 1ul of Rd. 1 PCR reaction as template.

T/A clone PCR product from 2nd round ~1.3kb into pCR2.1 (invitrogen)

To purify 600bp TM15′ southern fragment:
Sequential digest of pCR2.1 + Tm15′ with BglII and XmaI. Expect 4 bands, purify 600bp fragment.

II. Southern blot genotyping

-Digest DNA with PstI. Run gel long enough to get good separation ~45 min @120V
-Hybridize radiolabelled TM15′ probe at 58C O/N.
-Quick wash in 2X SSC/0.1% SDS (Blot wash buffer).
-Wash 2X @ 58C for ~7 min in blot wash buffer.

Expected band sizes with PstI digest:
WT – 3.3kb
TM1 – 1.8kb
Flox – 2.7kb
Recomb – 1.3 kb

Alternatively, you may digest using SacI to yield following band sizes, but this is no good for discriminating the Tm1 and floxed alleles:

WT – 7.1kb
TM1 – 3.6kb
Flox – 3.6kb
Recomb – 6kb