

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
dH2O	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