PCR for SMYD3-Tg

PCR REACTION:

10x taq buffer	5µl
genomic DNA	200ng
25mM $MgCl_2$	3µl
10mM dNTP	1µl
primer F (from 10mM stock)	2µl
primer R (from 10mM stock)	2µl
Taq	1µl
H_20	50µl total reaction

Forward: 5`-GAG AGT GAC ACA TGG CAG AGA AC-3`

Reverse: 5`-TGC AGT GAG CCA AGA TTG TGC CA-3`

PCR CONDITIONS:

1) 94.0, 5 min
2) 94.0, 30 sec
3) 56.0, 30sec
4) 72.0, 50sec, back to step 2 for 35 cycles
5) 72.0, 5 min (extension)

END

PCR PRODUCT: Band at ~431 bp.

Characterisation of SMYD3-Tg allelic composition

PCR REACTION:

10x taq buffer	5µl
genomic DNA	200ng
25mM $MgCl_2$	3µl
10mM dNTP	1µl
SMYD 3 primer F (from 10mM stock)	2µl
SMYD 3 primer R (from 10mM stock)	2µl
MEH primer F (from 10mM stock)	2µl
MEH primer R (from 10mM stock)	2µl
Taq	1µl
H_20	50µl total reaction

SMYD3 Forward: 5`-GAG AGT GAC ACA TGG CAG AGA AC-3`
SMYD3 Reverse: 5`-TGC AGT GAG CCA AGA TTG TGC CA-3`
NOTE: The particular set of primers is designed to only amplify the region of the human SMYD3 gene that is integrated in the mouse genome as shown in the sequence provided below. These primers cannot amplify any region of the mouse Smyd3 gene.

mEH Forward: 5'-AAG TGA GTT TGCA TGG CGC AGC-3'
mEH Reverse: 5'-CCC TTT AGC CCC TTC CCT CTG-3'
NOTE: mEH (official gene name: Ephx1) is used as a reference gene because it is known to exist as a single copy in the mouse genome.

PCR CONDITIONS:

1) 94.0, 5 min
2) 94.0, 30 sec
3) 56.0, 30sec
4) 72.0, 35sec, back to step 2 for 21 cycles
5) 72.0, 5 min (extension)

END

PCR PRODUCTS: SMYD3 band: ~431 bp
mEH band: ~341bp

Band Analysis:

PCR samples were separated on a 24x34 cm, 6% polyacrylamide gel. The gel was incubated for 20min in SYBR green (2µl of 10.000x SYBR green diluted in 40ml TBE buffer) and the amplified, stained products were quantified using Image Quant. Comparison of the corresponding band numbers in every lane conclude the allelic composition of the SMYD3 transgene taking into consideration that the band number for the mEH gene corresponds to a single copy gene.

SMYD3 transgene sequence

ATGGAGCCGCTGAAGGTGGAAAAGTTCGCAACCGCCAAGAGGGGAAACGGGCTGCGCGCCGTGACCCCGC

TGCGCCCCGGAGAGCTACTCTTCCGCTCGGATCCCTTGGCGTACACGGTGTGCAAGGGGAGTCGTGGCGT

XhoI

CGTCTGCGACCGCTGCCTTCTCGGGAAGGAAAAGCTGATGCGATG **CC CTCGAG** CG ATG CT

CTCTCAGTGCCGCGTC GCCAAATAC

TGTAGTGCTAAGTGTCAGAAAAAAGCTTGGCCAGACCACAAGCGGGAATGCAAATGCCTTAAAAGCTGCA

AACCCAGATATCCTCCAGACTCCGTTCGACTTCTTGGCAGAGTTGTCTTCAAACTTATGGATGGAGCACC

TTCAGAATCAGAGAAGCTTTACTCATTTTATGATCTGGAGTCAAATATTAACAAACTGACTGAAGATAAG

AAAGAGGGCCTCAGGCAACTCGTAATGACATTTCAACATTTCATGAGAGAAGAAATACAGGATGCCTCTC

AGCTGCCACCTGCCTTTGACCTTTTTGAAGCCTTTGCAAAAGTGATCTGCAACTCTTTCACCATCTGTAA

TGCGGAGATGCAGGAAGTTGGTGTTGGCCTATATCCCAGTATCTCTTTGCTCAATCACAGCTGTGACCCC

AACTGTTCGATTGTGTTCAATGGGCCCCACCTCTTACTGCGAGCAGTCCGAGACATCGAGGTGGGAGAGG

AGCTCACCATCTGCTACCTGGATATGCTGATGACCAGTGAGGAGCGCCGGAAGCAGCTGAGGGACCAGTA

CTGCTTTGAATGTGACTGTTTCCGTTGCCAAACCCAGGACAAGGATGCTGATATGCTAACTGGTGATGAG

CAAGTATGGAAGGAAGTTCAAGAATCCCTGAAAAAAATTGAAGAACTGAAGGCACACTGGAAGTGGGAGC

AGGTTCTGGCCATGTGCCAGGCAATCATAAGCAGCAATTCTGAACGGCTTCCCGATATCAACATCTACCA

GCTGAAGGTGCTCGACTGCGCCATGGATGCCTGCATCAACCTCGGCCTGTTGGAGGAAGCCTTGTTCTAT

GGTACTCGGACCATGGAGCCATACAGGATTTTTTTCCCAGGAAGCCATCCCGTCAGAGGGGTTCAAGTGA

TGAAAGTTGGCAAACTGCAGCTACATCAAGGCATGTTTCCCCAAGCAATGAAGAATCTGAGACTGGCTTT

Forward SMYD3 primer

TGATATTAT**GAGAGTGACACATGGCAGAGAAC**ACAGCCTGATTGAAGATTTGATTCTACTTTTAGAAGAA

XbaI ***BamHI***

TGCGAC GCCAACATCAGAGCATCC **T** CTAGA GGATCC CGG GCT GAC TAC AAA GAC CAT

3FlAG

GAC GGT GAT TAT AAA GAT CAT GAC ATC GAC TAC AAG GAT GAC GAT GAC AAG

TAG TGA T ***CC CGG GTG GCA TCC CTG TGA*** CCC CTC CCC AGT GCC TCT CCT GGC

CCT GGA AGT TGC CAC TCC AGT GCC CAC CAG CCT TGT CCT AAT AAA ATT AAG

TTG CAT CAT TTT GTC TGA CTA GGT GTC CTT CTA TAA TAT TAT GGG GTG GAG GG

GGT GGT ATG GAG CAA GGG GCA AGT TGG GAA GAC AAC CTG TAG GGC CTG CGG

GGT CTA TTG GGA ACC AAG CTG GAG TGC AGT GGC ACA ATC TTG GCT CAC TGC

Reverse SMYD3 primer

AAT CTC CGC CTC CTG GGT TCA AGC GAT TCT CCT GCC TCA GCC TCC CGA GTT

GTT GGG ATT CCA GGC ATG CAT GAC CAG GCT CAG CTA ATT TTT GTT TTT TTG

GTA GAG ACG GGG TTT CAC CAT ATT GGC CAG GCT GGT CTC CAA CTC CTA ATC

TCA GGT GAT CTA CCC ACC TTG GCC TC Bstxi

The coding sequence following the *BamHI* restriction site including the 3 FLAG sequence originates from the pCMV14 vector. This sequence was also integrated in the mouse genome.