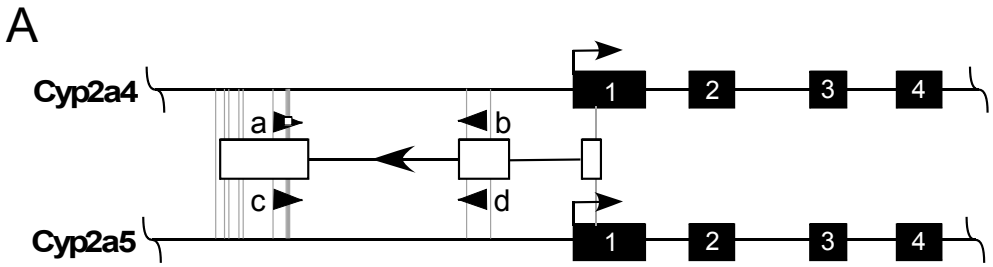
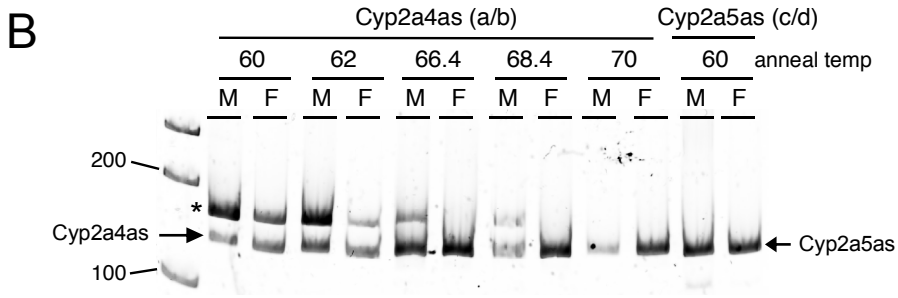
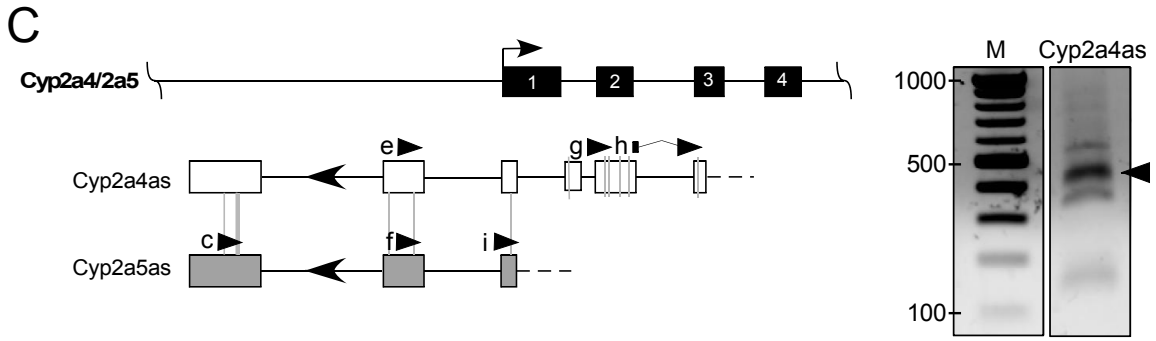
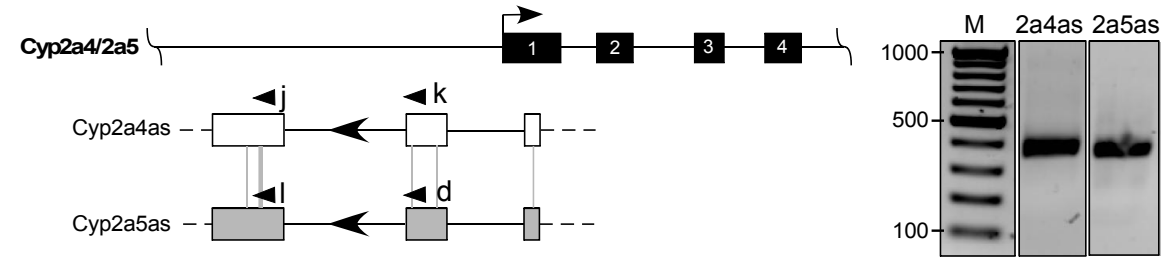
Supplemental Table 1. Primer sequences

Primer Name	Sequence (5' to 3')
a	ACTACAGGCACTGACTG
b	CCTGGGAGGAACACACT
c	GCAGGCACTGACTCCTG
d	CCTGGGAGGAACACACC
e	ATAGGCCTATAATCCCAGCACTCAGG
f	ATAGGCCTATAATCCCAGCACTCAGA
g	TGGTCCATCAGATCAGCCAACGTTA
h	GTGAGAGGGTCTTCCCAGGCATAG
i	TGGCCTTCCTCAGCGTCCTGGTC
j	AGACACAGACACATGAACAGTC
k	AGTGCCTGGGAGGAACACACT
l	AGACACAGACACATGAACCAGG
m	GTGACTACAGGCACTGACTG
n	TAGTCAAAGCGGTCCCCGAAGACG
Cyp2a4-F	GGAAGACGAACGGTGCTTTC
Cyp2a4-R	TTCCCAGCATCATTCTAAGA
Cyp2a5-F	GGAAGACGAACGGTGCTTTT
Cyp2a5-R	TTCCCAGCATCATTCGAAGC
Sfrs4-F	CTCGCACAGAGTACAGACTTAT
Sfrs4-R	TTGCGTCCCTTGTGAGCATCT
L30-F	ATGGTGGCCGCAAAGAAGACGAA
L30-R	CCTCAAAGCTGGACAGTTGTTGGCA
Sds-F	GTCACCCTGCCCAAGATC
Sds-R	TTCTCAAGAGCAGACACAGC
Oat-F	AAGCAGGCGTTATCGTTCC
Oat-R	ATCCACAGCCAGCCATCTAC
L30-U-F	GGCAAAGCGAAGTTGGTTATC
L30-U-R	CTTCTTCCCAATGCTGGATACT
U6-F	GTGCTCGCTTCGGCAGCACATATA
U6-R	CGAATTTGCGTGTCATCCTTGCG


A
Cyp2a4
1
2
3
4
a
b
c
d
Cyp2a5
1
2
3
4


B
Cyp2a4as (a/b)
Cyp2a5as (c/d)
60
62
66.4
68.4
70
60
anneal temp
M F M F M F M F M F M F
200
*
Cyp2a4as
100
Cyp2a5as


C
Cyp2a4/2a5
1
2
3
4
e
g
h
Cyp2a4as
c
f
i
Cyp2a5as
M
Cyp2a4as
1000
500
100


D
Cyp2a4/2a5
1
2
3
4
j
k
Cyp2a4as
l
d
Cyp2a5as
M
2a4as
2a5as
1000
500
100

Supplemental Figure 1. Temperature titration of Cyp2a4 and Cyp2a5 antisense RT-PCR reactions to ensure amplification specificity. A. The 5' ends of the *Cyp2a4* and *Cyp2a5* genes are shown; the black boxes are the exons and the arrows denote the transcriptional direction. The EST that was annotated antisense to the *Cyp2a4* gene is shown as open boxes, lines are the introns and the arrowhead within the intron denotes the transcriptional direction. Vertical thin gray lines designate the nucleotide differences between the two genes in this region; primers to detect the potential antisense transcripts are diagrammed as arrowheads. Primers a and c differ by a 4 bp deletion in Cyp2a4 relative to Cyp2a5. **B**. cDNA from a male (M) or female (F) liver was amplified with Cyp2a4 (a/b) primers with annealing temperatures that ranged from 60^{o}C to 70^{o}C and the products were separated on a 6% polyacrylamide gel, isolated, cloned and sequenced. The slower migrating amplicon (marked with *), which is gradually lost with increasing annealing temperature, was found to be non-specific. In contrast, the faster migrating Cyp2a4 amplicon, which is predominant at higher temperatures was from the predicted Cyp2a4 antisense transcript. The same cDNA amplified with Cyp2a5 (c/d) primers at 60^{o}C resulted in a single product that was confirmed by sequence analysis to be from a Cyp2a5 antisense RNA. **C.** 5' RACE analysis of Cyp2a4as and Cyp2a5as transcripts. Shown below the combined Cyp2a4/2a5 gene diagram are the tentative structures of the Cyp2a4 (open boxes) and Cyp2a5 (gray boxes) antisense transcripts (Cyp2a4as and Cyp2a5as, respectively), based on initial 5' RACE reactions. Additional Cyp2a4as and Cyp2a5as 5' RACE reactions were performed using the gene-specific primers shown. Nucleotide differences between Cyp2a4as and Cyp2a5as transcripts are marked by gray lines. The 3'-most primers (e and c) were used for cDNA synthesis and the other primers were used for the first and nested amplifications. The resulting products from the Cyp2a4as reaction were separated on a 1% agarose gel, shown to the right. Multiple bands were cloned and sequenced; the amplicon matching Cyp2a4as is marked with an arrow. The 100 bp ladder is marked; the lanes shown are non-adjacent lanes from the same gel. The 5' RACE reaction using the Cyp2a5as primers (f and i) did not result in a Cyp2a5as-specific product (not shown). **D**. The 3' RACE analysis primer strategy is shown. Tagged oligo dT was used for cDNA synthesis and the primers shown were used for the first and

nested PCR reactions. To the right, the resulting products were separated on a 1% agarose gel. The 100 bp ladder is marked; the lanes shown are non-adjacent lanes from the same gel.