Figure S1. **Detection of *FAM50B-AS* by Northern blot.** Using DIG-labeled probe complementary to the FAM50B-AS, generated using the FAM-19R primer, a band was detected between 1.0 and 2.0kb. The same band was observed when the probe generated using the FAM-19R primer (data not shown). M**,** 0.1-2kb RNA ladder; CB1 (96-day gestation), conceptus brain; CK, conceptus liver; CTe conceptus tesis; CL, conceptus liver; CB1 (88-day gestation), ATe, adult testis; AL, adult liver; AB, adult brain. The positions of the anticipated products are indicated by the arrows.

Figure S2. **Imprinting and paternal expression of *FAM50B.* A**, *FAM50B* imprinting in human adult tissues. Genomic DNA from a normal adult human heterozygous for polymorphism rs6597007 (G/C). cDNA sequencing demonstrates monoallelic expression of *FAM50B* in adult testis and liver. **B**, Paternal expression of *FAM50B*. Pedigree of a matched maternal genotype (C/C) with an informative (G/C) 113-day gestation human conceptus. The cDNA sequences from the brain of the conceptus demonstrate that *FAM50B* is expressed monoallelically from the paternal allele (representative DNA sequences from one individual out of four matched maternal/informative conceptuses that demonstrated paternal imprinted expression at the *FAM50B* locus). The position of SNP rs6597007 (G/C) used to determine allelic expression is indicated by the arrows.

Figure S3. **Allele-specific expression of *FAM50B* and *FAM50B-AS*. A**, Strand-specific RACE-PCR results of *FAM50B* and *FAM50B-AS* on an agarose gel. 3' RACE-PCR was performed by independently amplifying the 3' end of *FAM50B* and *FAM50B-AS* containing SNP rs6597007. The results show 526bp products of the *FAM50B* and 1310bp products of the *FAM50B-AS* in human tissues. M, low DNA mass ladder. N, control reactions performed using cDNA prepared in the

absence of primers to eliminate the possibility of genomic DNA and RNA contamination. CB, conceptus brain; AL, adult liver; ATe, adult testis. The positions of the anticipated products are indicated by the arrows. **B**, cDNA sequencing demonstrates monoallelic expression of *FAM50B* and *FAM50B-AS* in human tissues. S, *FAM50B* sense transcript; AS, *FAM50B* antisense transcript.

Figure S4. **Expression of *FAM50B* and *FAM50B-AS*.** Expression of *FAM50B* (**A**) and *FAM50B-AS* (**B**) is shown for a panel of human conceptus and adult tissues. Transcription levels were determined using quantitative RT-PCR, normalized to levels of *GAPDH* determined from parallel runs. CL, conceptus liver; CB, conceptus brain; CG, conceptus gut; CK, conceptus kidney; CP, placenta; CTh, conceptus thymus; CTe, conceptus testis; AB, adult brain; AL, adult liver; ATe, adult testis.

Figure S5. **Imprinting analysis of Human *C6orf145*.** Biallelic expression of *C6orf145* demonstrated in cDNA from brain, heart, kidney, lung, muscle, and placenta tissues from a human conceptus heterozygous for polymorphism rs226959 (C/G) in genomic DNA. The position of the SNP used to determine allelic expression is indicated by the arrows.

Figure S6. ***FAM50B* gene expression in opossum and mouse tissues.** Opossum *FAM50B* is expressed in kidney, liver, embryo and placenta (**A**), but expression is undetectable in the brain. Mouse *FAM50B is* expressed in liver, kidney, brain, and testes (**B**). M, 100 bp DNA ladder.

Figure S7. Allele-specific methylation at the *FAM50B* locus in testicular germ cell tumors (TGCTs). Clones obtained from bisulfite-treated DNA demonstrate that allele-specific methylation is partially lost in seminomas S2 (**A**) and S3 (**B**). CpG sites are represented by circles. Unfilled circle, unmethylated CpG site; filled circle, methylated CpG site.

Table S1. Primers used in this study for human *FAM50B*.

Primer Name	Nucleotide Sequence (5' to 3')	Position*
FAM-1F	AGGAAGAGAGTTTAGGAAAAGGTTTAGGTTTTTGG	-1406 to -1382
FAM-1R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACCTCTAACATACCAATTTCCAC	-1153 to -1128
FAM-2F	AGGAAGAGAGTTGGGGTTATAAGAAAGAATTATAGGT	-918 to -892
FAM-2R	CAGTAATACGACTCACTATAGGGAGAAGGCTAACCCTAAAAAAAATACCAAACTCC	-496 to -473
FAM-3F	AGGAAGAGAGTTAGGAGTTTGGTATTTTTTTTAGGG	-498 to -473
FAM-3R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAATAACCACCAACCTAACAATATC	-179 to -153
FAM-4F	AGGAAGAGAGGAAATTGGTGTTTGGTGATATTGTT	-194 to -168
FAM-4R	CAGTAATACGACTCACTATAGGGAGAAGGCTCTCCCTAACCAAAACCTCCTAC	237 to 260
FAM-5F	AGGAAGAGAGGGTAGGAGGTTTTGGTTAGGGA	238 to 260
FAM-5R	CAGTAATACGACTCACTATAGGGAGAAGGCTCAAAATAAAATCCTCCTTAATAAACATAA	714 to 743
FAM-6F	AGGAAGAGAGGAGGAGATGGAGGTTATTTTTAGTTA	558 to 284
FAM-6R	CAGTAATACGACTCACTATAGGGAGAAGGCTCATCAACAATAACACAACCAATATAAA	1118 to 1144
FAM-7F	ACAGCGGGGTAGCATCAG	-860 to -843
FAM-7R	CTGGAGGAGGCGCTTCTG	-641 to -624
FAM-8F	TTTTGAGGAGAGTGTTAGGTTTTTG	-472 to -495
FAM-8R	ACTACACTTTAACTCAATATCCCATAA	-998 to -971
FAM-9F	CTCATTTCTAGGCCCCATCA	1020 to 1039
FAM-9R	TGGTTTCAATACAGCTCAGCA	1205 to 1225
FAM-10R	TTTTACAAATATTAATTCA	1453 to 1471
FAM-11F	GCCTCCTCCTTGCTGTGTAG	1335 to 1354
FAM-11R	CCCAGCTAAAGCCCACTAGA	1652 to 1671
FAM-12F	AGATGGAGGTCACCTTCA	551 to 568
FAM-13F	CCTTGATTTCTTCCCCCAAA	1060 to 1079
FAM-14F	GCCTCCTCCTTGCTGTGTAG	1335 to 1354
FAM-14R	TCCATGCTCATAACCACCAG	1277 to 1296
FAM-15F	CGCGTTTTTCCTCTTTGCT	-28 to -46
FAM-15R	CCTCGTCCTTCACTTTCTCG	530 to 550
FAM-16F	GTCAGCTCCCGCGTGTCT	-367 to -351
FAM-16R	CGCATGGTGCCCTTGTACT	8 to 26
FAM-17F	GCGCATGGTGCCCTTGTA	10 to 25
FAM-17R	GGCGCAGAGCTGAGCATT	-79 to -96
FAM-18F	TCTGGCAGGAAGCTGGTGTCCA	443 to 464
FAM-18R	TAATACGACTCACTATAGGGCAGCTGGCCAAGCGCCAGCA	261 to 278
FAM-19F	GTGGGCCCAGTCATGGTTTCA	1218 to1238
FAM-19R	TAATACGACTCACTATAGGGCACCAGTCACTTGATTTCGTGA	1040 to1059
GAPDH-F	CTCTCTGCTCCTCCTGTTCGAC	
GAPDH-R	TGAGCGATGTGGCTCGGCT	

*Positions relative to the translational start codon

Table S2. Primers used in this study for opossum and mouse *FAM50B*.

Primer Name	Nucleotide Sequence (5' to 3')
Opossum-1F	CACGGAGGAGAACATGATGA
Opossum-1R	GTTTTTCCCGCTCCTTTACC
Opossum-2F	TGAATGACATGAAGGCCAAA
Opossum-2R	CAAGAACTGCTGCATGGTGT
Opossum-3F	ACACCATGCAGCAGTTCTTG
Opossum-3R	TGGATCATAAGGCTCCCAAC
Opossum-RT-S	TGATCTGATGGTGTACTTG
Opossum-4F	AGGAAGAGAGTTAGGTAATGATGGTGTTTGAGGTT
Opossum-4R	CAGTAATACGACTCACTATAGGGAGAAGGCTATTAAACTTCATCATATTCTCCTCC
Opossum-5F	AGGAAGAGAGTTGGTTTTTATGTTATTTAGGGTGA
Opossum-5R	CAGTAATACGACTCACTATAGGGAGAAGGCTATCACATTTAAATCCAAAACCTCCT
Opossum-6F	AGGAAGAGAGGGTTATGGTTTAATATAAGGGGGT
Opossum-6R	CAGTAATACGACTCACTATAGGGAGAAGGCTTCCTACTTAACTTCCCATTCCTACC
Opossum-7F	AGGAAGAGAGGGTTTTTTGTAAGAATTGTTGTATGG
Opossum-7R	CAGTAATACGACTCACTATAGGGAGAAGGCTTCACCCTAAATAACATAAAAACCAAA
Mouse-1F	TGACGCAGCTGTACTGGTTT
Mouse-1R	CTTGGCCTTCATGTCATTCA
Mouse-2F	GACAAGAAGTTCTCGGCACA
Mouse-2R	GAGCCATCCCAGTAGCTGAA
Mouse-3F	GAAGCGCGAGAAGGTGAAG
Mouse-3R	ACGCCAGGTACAGAACCAAC
Mouse-4F	AAAAGCCTTGCGCTGAACT
Mouse-4R	CTTGGCCTTCATGTCATTCA
Mouse-RT-S	GTATTTATTAATTTTCGAT
Mouse-5F	AGGAAGAGAGAAGGTTTTGTGTTGTTATTTAGTGG
Mouse-5R	CAGTAATACGACTCACTATAGGGAGAAGGCTAATTCCCTAACCTAAATTTCTCCCT
Mouse-6F	AGGAAGAGAGGTGTTAAAGTTTAGGGTTTTGGTGA
Mouse-6R	CAGTAATACGACTCACTATAGGGAGAAGGCTAACCCACAAATTCAAAAATCTATCC
Mouse-7F	AGGAAGAGAGTTTTTTGGAATTGGAGTTAGAGATG
Mouse-7R	AGGAAGAGAGTTAGTTGTGTGTGTTTGTTTGGATT
Mouse-8F	CAGTAATACGACTCACTATAGGGAGAAGGCTTCCAACAACCCTACAATAAAAAAAA
Mouse-8R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAACATTACCCCAAAAACAAAAT
Mouse-9F	AGGAAGAGAGTAAATGTTATGGGAAATTGGAAAGA
Mouse-9R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAAAAACAAAACTCCTTCCTACC
Mouse-10F	AGGAAGAGAGTTGAGAAATATTTTGGGTTTGGTTA
Mouse-10R	CAGTAATACGACTCACTATAGGGAGAAGGCTACTCTAACATTCCATATCATTCCAAAA
Mouse-11F	AGGAAGAGAGGATTTTTGGAATGATATGGAATGTT
Mouse-11R	CAGTAATACGACTCACTATAGGGAGAAGGCTAATCCCAAAAAACTTTCATCCTAAC
Mouse-12F	AGGAAGAGAGAGGGGAAGTTTTTAAGGATTTTTTT
Mouse-12R	CAGTAATACGACTCACTATAGGGAGAAGGCTTTTTAACCTACCCAATCCCTATTTC
Mouse-13F	AGGAAGAGAGAGAAATAGGGATTGGGTAGGTTAAA
Mouse-13R	CAGTAATACGACTCACTATAGGGAGAAGGCTTTACCCAAATTCTTCTTAACTCCAA
Mouse-14F	AGGAAGAGAGGGAGATTATTTTTAGTTATTGGGATGG
Mouse-14R	CAGTAATACGACTCACTATAGGGAGAAGGCTACACAAAAAAACCACAAACAAAAAC
Mouse-15F	AGGAAGAGAGTTAGTTTGGTGGGTTAGTAGATTGG
Mouse-15R	CAGTAATACGACTCACTATAGGGAGAAGGCTCAAAAACAAAACAAAAAATCCAAAA
Mouse-16F	AGGAAGAGAGATAATTTTGGTGATATTGGGTGTTG
Mouse-16R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAAACAAACATCTAACCAACTAAA
Mouse-17F	AGGAAGAGAGTAGGAAAATTTTATGGGAGGATTGT
Mouse-17R	CAGTAATACGACTCACTATAGGGAGAAGGCTAATAAAAAAAACAAACCCCAATTTC
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Mouse-18R	CAGTAATACGACTCACTATAGGGAGAAGGCTTACCCACTATAAAATATCCAACCCA

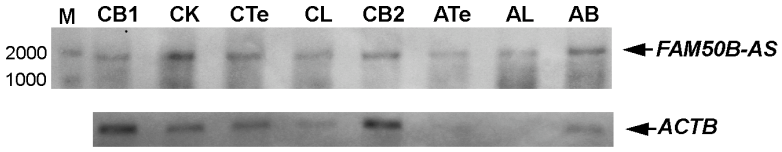
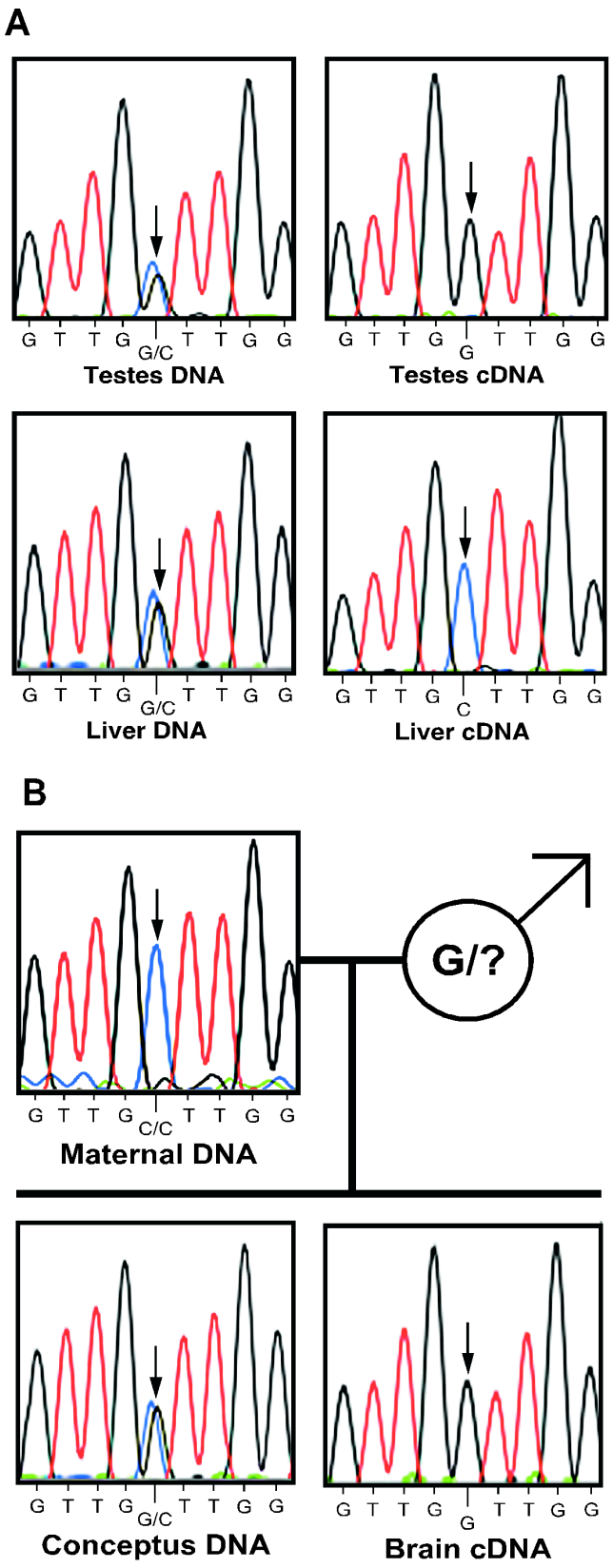


Figure S1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Testes cDNA
G T T G G/C T T G G
Liver DNA
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Liver cDNA
B
G/?
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G T T G G/C T T G G
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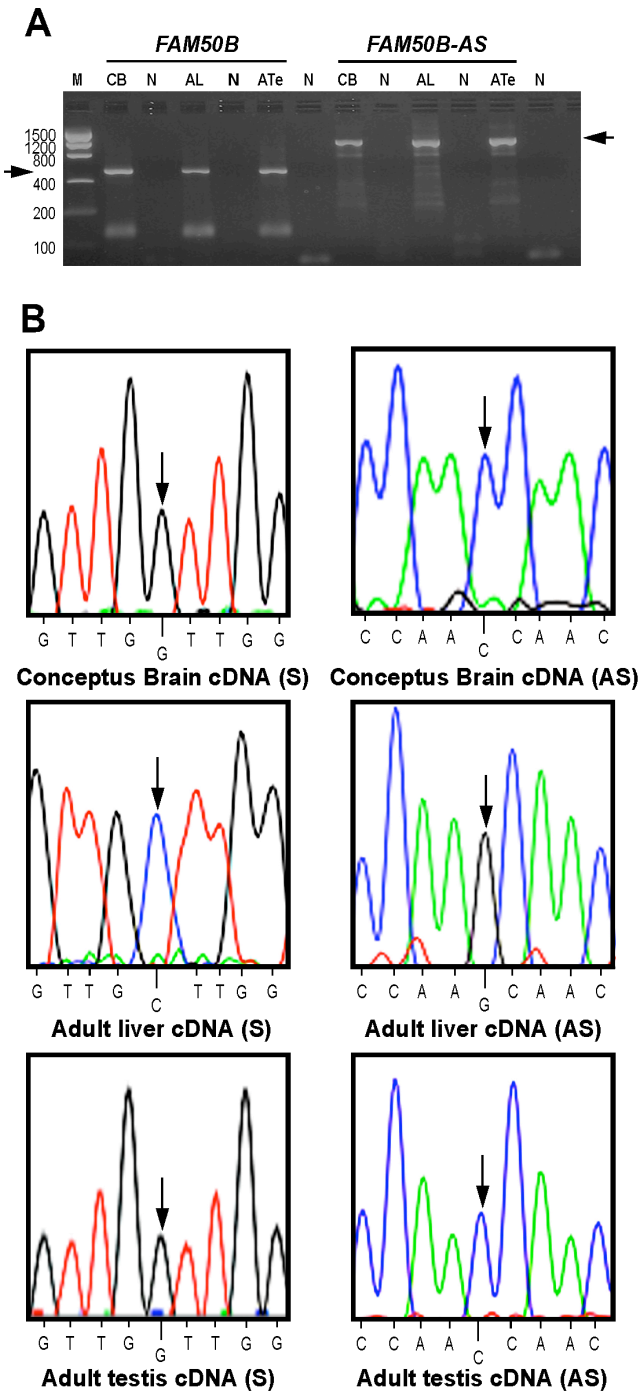
Brain cDNA

Figure S2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FAM50B-AS
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1200
800
400
200
100
B
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Conceptus Brain cDNA (S)
C C A A C A A C
Conceptus Brain cDNA (AS)
G T T G C T T G G
Adult liver cDNA (S)
C C A A G C A A C
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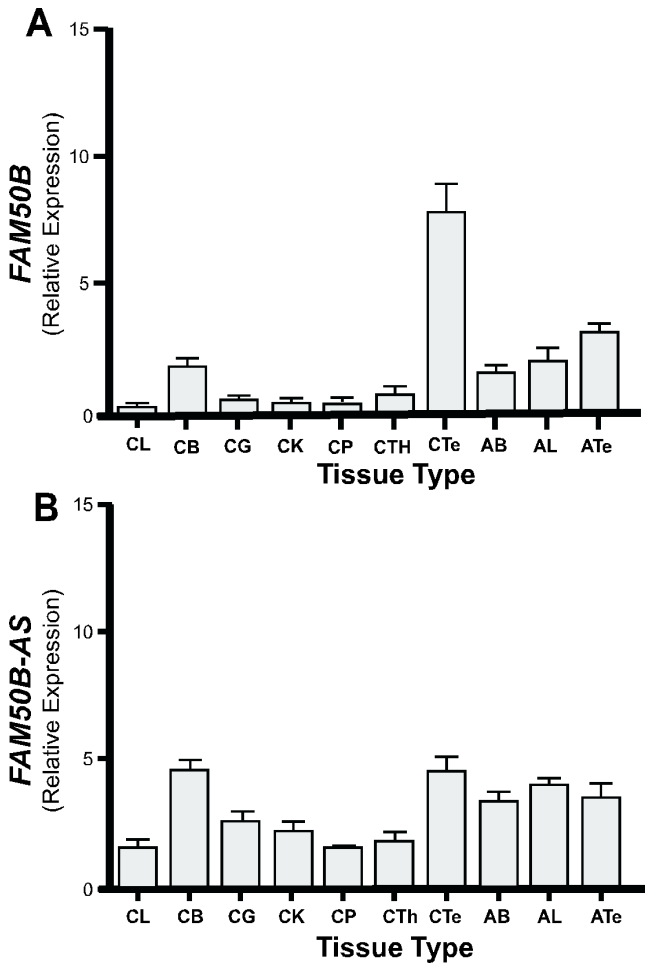
C C A A C A A C
Adult testis cDNA (AS)

Figure S3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FAM50B
(Relative Expression)
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CTe
AB
AL
ATe
Tissue Type
B
15
10
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(Relative Expression)
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CTe
AB
AL
ATe
Tissue Type

Figure S4

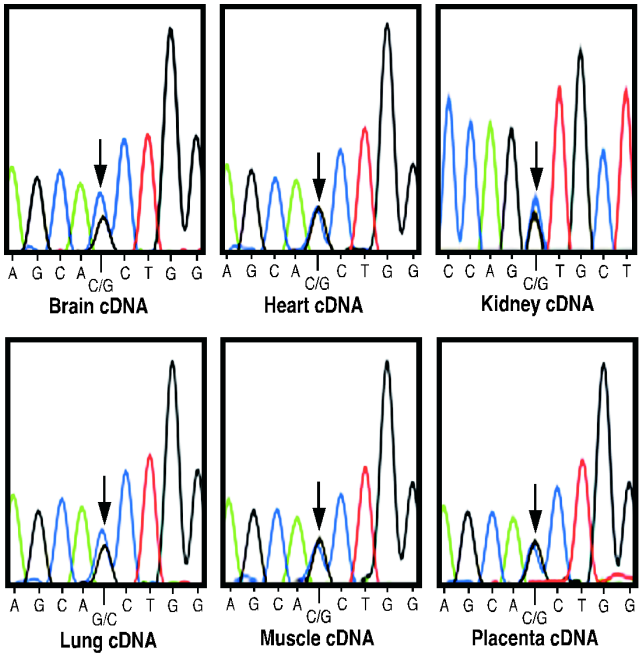
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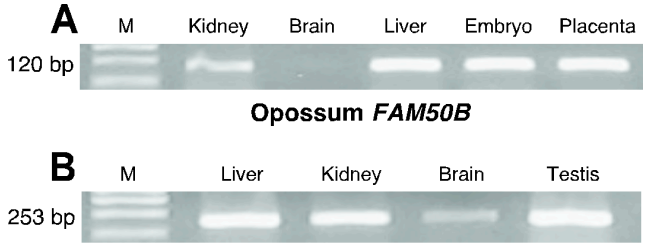
Figure S5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Kidney
Brain
Liver
Embryo
Placenta
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Opossum FAM50B
B
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Liver
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Brain
Testis
253 bp
Mouse FAM50B

Figure S6

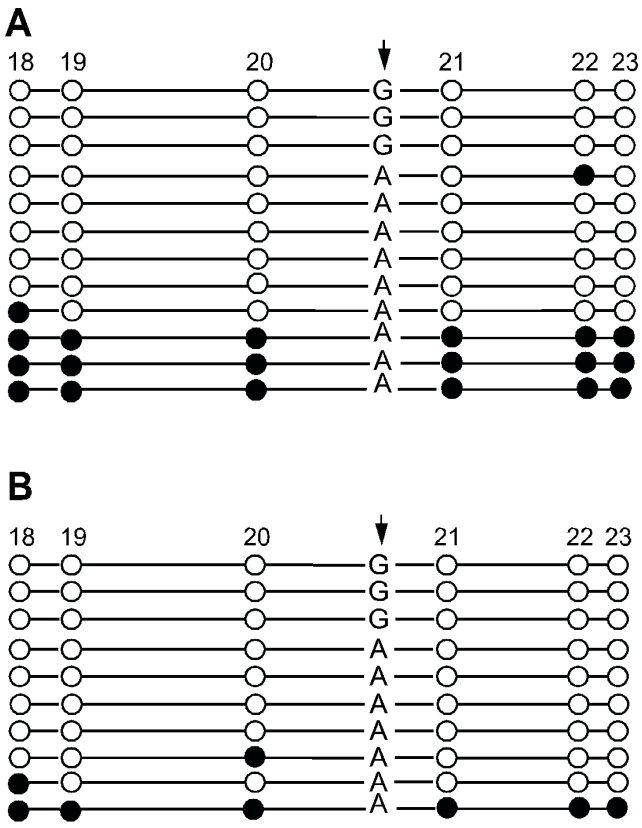

Figure S7