SUPPLEMENTARY ONLINE MATERIAL

List

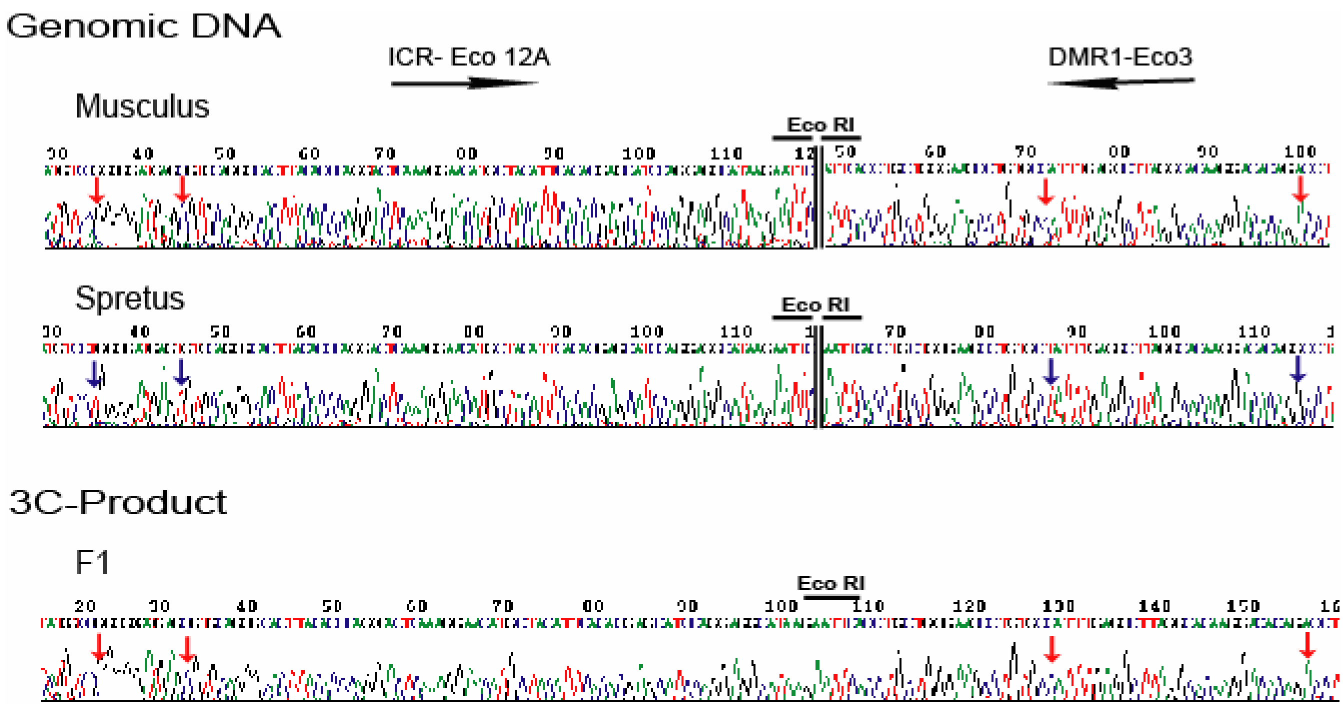

Figure S-1. DNA sequencing of the DMR1-ICR (Eco #3-Eco #12A) cross-linked product. Top, genomic sequences of the ICR and DMR1 fragments of *M. musculus* and *M. spretus*. Note the SNPs marked by arrows (red, Musculus; blue, Spretus). Bottom, the two Eco RI fragments were cross-linked in 3C products of F1 newborn liver. Both ICR and DMR1 fragments were from the maternal allele (*Musculus*).

Note N1: *M. Spretus* sequencing and 3C primer design

We first used “Gene sorter” to sort the mouse *Igf2* in the USC data base (http://genome.ucsc.edu/). We then mapped the Eco RI sites and selected 37 RI sites. We used *primer3* program (*http://www-genome.wi.mit.edu/genome_software/other/primer3.html*) to design primers across the selected sites to amplify ~ 500 bp DNA fragments from *M. spretus* liver DNA. We obtained 33 *Spretus* PCR products and sequenced these products by direct PCR sequencing.

To design 3C primers the *M. spretus* sequences encompassing the 32 RI sites were aligned with the *M. musculus* sequences using BLAT (http://genome.ucsc.edu/). We employed the *primer3* program and chose 3C primers (Tm ~ 74˚C) across the 32 RI sites for both mouse strains in the homologous sequence regions avoiding any SNP sites (that are abundant) in the selected primers. Samples of the BLAT results using the mouse genome (Assembly Feb 2006) to design primers across the Eco #3 (near the *Igf2* DMR1) and Eco #12A and #12B (ICR) sites are listed below. The RI sites (yellow) and the SNPs (blue) are showed. The designed primers are underlined and the polymorphic restriction sites used to distinguish the two parental alleles are highlighted (green).

Spretus sequence Eco #3 (9f) (DMR1, *Stu*I-site AGGCCT in *Spretus*)
taagcactgntggactctgccgaggaagctc
tgctgtttggtggccctgcagttcactggtcaggtactgggtagggaccagggaggtag
aggctgtgtctggtggaatactggagactgggggcagcatgtaggcctgtgtccttgtg
cctaagcctcaaatagcacaggcttcccagcaggtgaattccacccctcttccagcctat
catgctcatgccagaaagaagccaagaatcttctttcaaatgttggttcagacactaaaa
caattacatagtggtatggcttctgtctatctaGacccaGgacactggccttccctgctg
acccctctccagtgGcccacagcaacccttacttacttttggtgtga

Spretus-Alignment of Eco3 - chr7:142475848-142476222

```
tAAGCACTgn TGGACTCTGC CGAGGAAGCT CTGCTGTTTG GTGGCCCTGC  50
AGTTCACTGG TCAGGTACTG GGTAGGGACC AGGGAGGTAG AGGCTGTGTC  100
TGGTGGAATA CTGGAGACTG GGGGGCAGCA TGTAGGCCTG TGTCCTTGTG  150
CCTAAGCCTC AAATaGCACA GGCTTCCCAG CAGGTGAATT CCACCCCTCT  200
TCCAGccTAT CATGCTCATG CCAGAAAGAA GCCAAGAATC TTCTTTCAAA  250
TGTTGGTTCA GACACTAAAA CAATTACATA GTGGTATGGC TTCtGTCTAT  300
CtAGACCCAG GACACTGGCC TTCCCTGCTG ACCCCTCTCC AGTGgCCCAC  350
AGCAACCCTT ACTTACTTTT GGTGTGa
```

Musculus-Genomic chr7 (reverse strand):

```
ccttaggaga gtgtgtgtcc taattctatt gtgttctgaa ggccatgggt  142476273
ttcctgtgtg tgaatggggt gggaagcagc tagagagggc acggggccag  142476223
AAGCACTgTG GACTCTGCCG AGGAAGCTCT GCTGTTTGGT GGCCCTGCAG  142476173
TTCACTGGTC AGGTACTGGG TAGGGACCAG GGAGGTAGAG GCTGTGTCTG  142476123
GTGGAATACT GGAGACTGGG GGCAGCATG TAGGtCTGTG TCCTTGTGCC  142476073
TAAGCCTCAA ATgGCACAGG CTTCCCAGCA GGTGAATTCC ACCCCTCTTC  142476023
CAGgaTATCA TGCTCATGCC AGAAAGAAGC CAAGAATCTT CTTTCAAATG  142475973
TTGGTTCAGA CACTAAAACA ATTACATAGT GGTATGGCTT CaGTCTATCc  142475923
AGACCCAGGA CACTGGCCTT CCCTGCTGAC CCCTCTCCAG TGccCCCACA  142475873
GCAACCCTTA CTTACTTTTG GTGTGggttg tgttgatcag tttggtgcaa  142475823
tcctcatgtt ggtggttcag gtagtgactt cttcccaggc ttgcagattg  142475773
gcatagagca ttgcttgggt cttcc
```

Side by Side Alignment (Spretus, top /Musculus, bottom)

```
000000002 aagcact 000000008
<<<<<<<<< ||||||| <<<<<<<<<
142476222 aagcact 142476216
```

```
000000011 tggactctgccgaggaagctctgctgtttggtggccctgcagttcactgg 000000060
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142476214 tggactctgccgaggaagctctgctgtttggtggccctgcagttcactgg 142476165

000000061 tcaggtactgggtagggaccagggaggtagaggctgtgtctggtggaata 000000110
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142476164 tcaggtactgggtagggaccagggaggtagaggctgtgtctggtggaata 142476115

000000111 ctggagactggggggcagcatgtaggcctgtgtccttgtgcctaagcctc 000000160
<<<<<<<<< |||||||||||||||||||||||||| ||||||||||||||||||||||| <<<<<<<<<
142476114 ctggagactggggggcagcatgtaggtctgtgtccttgtgcctaagcctc 142476065

000000161 aaatagcacaggcttcccagcaggtgaattccacccctcttccagcctat 000000210
<<<<<<<<< |||| ||||||||||||||||||||||||||||||||||||||||  ||| <<<<<<<<<
142476064 aaatggcacaggcttcccagcaggtgaattccacccctcttccaggatat 142476015

000000211 catgctcatgccagaaagaagccaagaatcttctttcaaatgttggttca 000000260
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142476014 catgctcatgccagaaagaagccaagaatcttctttcaaatgttggttca 142475965

000000261 gacactaaaacaattacatagtggtatggcttctgtctatctagacccag 000000310
<<<<<<<<< ||||||||||||||||||||||||||||||||| ||||||| |||||||| <<<<<<<<<
142475964 gacactaaaacaattacatagtggtatggcttcagtctatccagacccag 142475915

000000311 gacactggccttccctgctgacccctctccagtg 000000344
<<<<<<<<< |||||||||||||||||||||||||||||||||| <<<<<<<<<
142475914 gacactggccttccctgctgacccctctccagtg 142475881
```

```
000000346 cccacagcaacccttacttacttttggtgtg 000000376
<<<<<<<<< ||||||||||||||||||||||||||||||| <<<<<<<<<
142475878 cccacagcaacccttacttacttttggtgtg 142475848
```

Spretus sequence-Eco #12A, #12B (12f)
(CTCF binding site #1 in bold, polymorphic Hpa II is hi-lited (green))

ttagtactttctgccctggcagtccccagattgacaaaggcatgcctaacttgtttctgg
aagaagagatctagctctatcccatcgaaatgcaaatgaaccactaggagtttagcctga
ccaaggaagctttcctgctcactgtccatgcaatgcagtcaaaagtgctgtgactataca
ggaggaacatagcagtgctgtgactatacaggaggaacatagccgaggctaaagggccat
ggtgccatgtaagtgtgttctgtgcagcAActgatgaccagacagtactgagtctgcctg
gagcctgagttaaaaccgagaaaatagccattgcctacagttcccgaatcaccacaagga
aagaaaaaggttggtgagaaaatagagattctattttcatgtcctggggatgagtgtgca
gggcacttacacccaggactcaaaggaacatgctacattcacacgagcatccaggaggca
taagaattctgcaaggagaccatgccctattcttggacgtctgctgaatcagttgtgggg
tttatacgcgggagttg**ccgcgtggtgg**cagcaaaatcgattgcgccaaacctaaagagc
cccccgacccccggaattggaattcacaaatggcaatgctgtgggtcacccaagttcagt
acctcagggggtcacaaatgccacta

Spretus, Alignment of Eco12A12B - chr7:142391308-142392040

```
TTaGTACTTT CTGCCCTGGC AGTCCCCAGA TTGACAAAGG CATGCCTAAC  100
TTGTTTCTGG AAGAAGAGAT CTAGCTCTAT CCCATCGAAA TGCAAATGAA  150
CCACTAGGAG TTTAGCCTGA CCAAGGAAGC TTTCCTGCTC ACTGTCCATg  200
CAATGCAGTC AAAAGTGCTG TGACTATACA GGAGGAACAT AGCAGTGCTG  250
TGACtATACA GGAGGAACAT AGCcGAGGCT AAAGGGCCAT GGTGCCATGT  300
AAGTGTGTTC TGTGCAGCAA CTGATGACCA GACAGTACTG AGTCTGCCTG  350
GAGCCTGAGT TAAAACCGAG AAAATAGCCA TTGCCTACAG TTCCCGAATC  400
ACCACAAGGA AAGAAAAAGG TTGGTGAGAA AATAGAGATT CTATTTTCAT  450
GTCCtGGGGA TGAGtGTGCA GGGCACTTAC ACCCAGGACT CAAAGGAACA  500
TGCTACATTC ACACGAGCAT CCAGGAGGCA TAAGAATTCT GCAAGGAGAC  550
CATGCCCTAT TCTTGGACGT CTGCTGAATC AGTTGTGGGG TTTATACGCG  600
GGAGTTGCCG CGTGGTGGCA GCAAAATCGA TTGCGCCAAA CCTAAAGAGC  650
CCCCCgACCC CcGGaATTGG AATTCACAAA TGGCAATGCT GTGGGTCACC  700
CAAGTTCAGT ACCTCAGGGG GTCACAAATG CCACTA
```

Musculus, Genomic chr7 (reverse strand):

```
ttagagtacc atgcttcagt taggttctgt ttgcccacca gctgctagcc  142392091
atcacctagt cctcaatgtc acgtactatt acaatggcca aaacagacta  142392041
GACTTGACCC CAAGAGCCCC CCTCCCCGCC AAAGCTTACT GCCCTCATTG  142391991
TACTTTCTGC CCTGGCAGTC CCCAGATTGA CAAAGGCATG CCTAACTTGT  142391941
TTCTGGAAGA AGAGATCTAG CTCTATCCCA TCGAAATGCA AATGAACCAC  142391891
TAGGAGTTTA GCCTGACCAA GGAAGCTTTC CTGCTCACTG TCCATtCAAT  142391841
GCAGTCAAAA GTGCTGTGAC TATACAGGAG GAACATAGCA GTGCTGTGAC  142391791
cATACAGGAG GAACATAGCa GAGGCTAAAG GGCCATGGTG CCATGTAAGT  142391741
GTGTTCTGTG CAGCAACTGA TGACCAGACA GTACTGAGTC TGCCTGGAGC  142391691
CTGAGTTAAA ACCGAGAAAA TAGCCATTGC CTACAGTTCC CGAATCACCA  142391641
CAAGGAAAGA AAAAGGTTGG TGAGAAAATA GAGATTCTAT TTTCATGTCC  142391591
gGGGGATGAG cGTGCAGGGC ACTTACACCC AGGACTCAAA GGAACATGCT  142391541
ACATTCACAC GAGCATCCAG GAGGCATAAG AATTCTGCAA GGAGACCATG  142391491
CCCTATTCTT GGACGTCTGC TGAATCAGTT GTGGGGTTTA TACGCGGGAG  142391441
TTGCCGCGTG GTGGCAGCAA AATCGATTGC GCCAAACCTA AAGAGCCCCC  142391391
CcACCCCtGG tATTGGAATT CACAAATGGC AATGCTGTGG GTCACCCAAG  142391341
TTCAGTACCT CAgGGGGGTC ACAAATGCCA CTAgggggggc aggacacatg  142391291
cattttctag gctggtacct cgtggactcg gactcccaaa tcaacaaggt  142391241
cggcttactc tctgcaaaga atcctttgtg tgt
```

Side by Side Alignment (Spretus, top /Musculus, bottom)

```
000000051 ttagtactttctgccctggcagtccccagattgacaaaggcatgcctaac 000000100
<<<<<<<<< || ||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391993 tt.gtactttctgccctggcagtccccagattgacaaaggcatgcctaac 142391945

000000101 ttgtttctggaagaagagatctagctctatcccatcgaaatgcaaatgaa 000000150
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391944 ttgtttctggaagaagagatctagctctatcccatcgaaatgcaaatgaa 142391895

000000151 ccactaggagtttagcctgaccaaggaagctttcctgctcactgtccatg 000000200
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||  <<<<<<<<<
142391894 ccactaggagtttagcctgaccaaggaagctttcctgctcactgtccatt 142391845

000000201 caatgcagtcaaaagtgctgtgactatacaggaggaacatagcagtgctg 000000250
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391844 caatgcagtcaaaagtgctgtgactatacaggaggaacatagcagtgctg 142391795

000000251 tgactatacaggaggaacatagccgaggctaaagggccatggtgccatgt 000000300
<<<<<<<<< |||| |||||||||||||||||| |||||||||||||||||||||||||| <<<<<<<<<
142391794 tgaccatacaggaggaacatagcagaggctaaagggccatggtgccatgt 142391745

000000301 aagtgtgttctgtgcagcaactgatgaccagacagtactgagtctgcctg 000000350
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391744 aagtgtgttctgtgcagcaactgatgaccagacagtactgagtctgcctg 142391695

000000351 gagcctgagttaaaaccgagaaaatagccattgcctacagttcccgaatc 000000400
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391694 gagcctgagttaaaaccgagaaaatagccattgcctacagttcccgaatc 142391645

000000401 accacaaggaaagaaaaaggttggtgagaaaatagagattctattttcat 000000450
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391644 accacaaggaaagaaaaaggttggtgagaaaatagagattctattttcat 142391595

000000451 gtcctggggatgagtgtgcagggcacttacacccaggactcaaaggaaca 000000500
<<<<<<<<< |||| ||||||||| ||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391594 gtccggggatgagcgtgcagggcacttacacccaggactcaaaggaaca 142391545

000000501 tgctacattcacacgagcatccaggaggcataagaattctgcaaggagac 000000550
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391544 tgctacattcacacgagcatccaggaggcataagaattctgcaaggagac 142391495

000000551 catgccctattcttggacgtctgctgaatcagttgtggggtttatacgcg 000000600
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391494 catgccctattcttggacgtctgctgaatcagttgtggggtttatacgcg 142391445

000000601 ggagttgccgcgtggtggcagcaaaatcgattgcgccaaacctaaagagc 000000650
<<<<<<<<< |||||||||||||||||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391444 ggagttgccgcgtggtggcagcaaaatcgattgcgccaaacctaaagagc 142391395

000000651 cccccgacccccggaattggaattcacaaatggcaatgctgtgggtcacc 000000700
<<<<<<<<< ||||| ||||| || ||||||||||||||||||||||||||||||||||| <<<<<<<<<
142391394 ccccccacccctggtattggaattcacaaatggcaatgctgtgggtcacc 142391345

000000701 caagttcagtacctca.ggggtcacaaatgccacta 000000736
<<<<<<<<< ||||||||||||||| |||||||||||||||||||| <<<<<<<<<
142391344 caagttcagtacctcagggggtcacaaatgccacta 142391308
```

Note N2: *M. Spretus* sequences

Spretus sequences across the 29 RI sites (yellow hi-lited) are listed below. Three RI sequences (Eco #3, Eco #12A and 12B are listed in Supplementary Material, Note N1). Sequences were read by forward (f) and/or reverse (r) PCR primers. There were two polymorphic RI sites and 3 large insertion/deletions (22 bp and 44 bp). To find *Spretus/Musculus* SNPs and polymorphic sites, simply BLAT the sequence to the Mouse genome using BLAT (http://genome.ucsc.edu/) as described in Supplementary Material, Note N1.

mLit1 (#34f)
tgcctcatttggaggctggaaatctgttacctgcaggcactaagggagccaagctaccat
gggatgggttcctgcgttgactatggatgcctgatacagtaggaatatggggtggtagcg
gggaggtacagggtgaggtagactctcaaggccacttcaaaagatccagctaatgtcatc
tatatcccttgcccccatagaaatggacctggcaggaaagccagtacttgttcaaagtaa
catgggaatggagtttgggagtcaggctgtcatctatgaattcccagcctccactcatac
caggggtccctgggggcccatctggtgagtgacaggctgtgctgtgcctcccacctggcc
tctggcacccacactgcacctgtaacaacagggcgtcggagctggcgtccgattacggcc
ccctcgcggcctgctctgaattcctgcggagagccggcacatcgaggtcctgggccgggc
aggaggacaatccccacagtgtctgtgcagctggcactgtagcatgtggcctgctgagag
ggcagcccggcccagcccagggcttgggtccaaccggcatctcccagcccccaacaagg

mAscl2 (#1f)
ggtatgcttgtgtg
tgcaaggatgagcacagcagtgggtatggaggttggaggatatcttggatgttggtcctt
cccttccagcctgtttaagatagacgttgttgttcattattgcagtcctggggctagctg
gtctggaagtttgaaggaattctcttgttctcttctttgcattttgggattctatgggtt
ctgaggatttgaactcaggtcctcacacttgcacagcaagtgctttacttaacaatctcc
ccagaccaactggattgctccaaacagcagagaagtgactagactcatccaagtgctctt
agcagacacccgggaaatttcgactcactgcccacttcccacagcaaggttatcaaaagg
ctggattaggatgatacagccttgatgaatcctggagagtcatggaacctgcaggcagcc
ccacactggcagtacatgctccaaggcagtgcgaaagcagagagaagcctggttttggta
gccctcccccagtcggatatttacaaagaaagtaactccaggttact

mTh (#3f)
cctgaatgcctaagaaagacctaaagccctagtatgactct
ggaacaggtacaagttaaaccaaatggaaggccttaggccatagtaagatgctaagaaa
gatagtagacctctgtgaGaccctggggaaacCttAGgCtcccaaaagactctggggaag
gccttaggcaccagagagaccctgcaaagcctcaggtcatagtgaaactcagcgaaggcc
atacactctgctgagatccaaggaggatcttaggcccctgtgagaccctggaggaggata
caggttctaataagactccagggaagaagcccttaggactcggtgagagtgaggataggga
attccactttcagagcctaccatgactttaggccacagtgagagtctgggaaaggcctca
ggctctagaagatctaggaagtgcctcagaccacaaagaggaaggctatagatcac

Ins-a (#4f)
ccaattagaatctaagcaaggccttaggacatattg
agaaaatggagaaggttttaaagcccagtgacagccttggaagtccttaggccctagtga
aaatcttggaagtccataggccccagtgagaacctgttgaaagttttagaccccagtgag
accctgtaggaggacttagaattcagtgacactttgggagagtccttgtgtcccactggg
aacctggagaagcttttaagtaccagtgagaacctggttatagagtgagagcatttaaga
ggcctagggacccagtgaaaatggtcaaaggcctgtgaaaatttgggaaaatttttagac
atcagtgagaccctggttatattttaggcctattgagagcttatgtaatgccttaggctt
cagtgagaacctgggcaaggctttaagacccagtgagaa

Ins-b (#5f)
ggcaccaagtgtttttgctggttgcctaggga
acagggcagtgccaaatcaggaacagaaagagtcaaggaacccccaaccactccaagcgg
aggctgggaaaggttttgtagctggaatagagcatgcactaacagatggagacagctggc
tttgagctctgaagcaagtattacatatggagacttgcttggccttcaggtacttatctt
gttattggatactgcaggaggatgtaccacagggcttcagctcagctgacccccaagtgg
gctatggaaagagagatagaggaggagggaccattaagtgtcttgctgcctgaattctgc
tttccttctacctctgagagagagctggggactcagctgagttaagaacccagctatcaa
tgggaactgtgaaacagtccaagggacaaagatactaggtctccaactgcaacttcctgg
ggaatgatgtggaaaaatgctcaGccaaggacaaagaaagcatcAcccactctgggacan
tgtcccctgctgtgaactggttcatcaggctatcagggccccttgttaGaactc

Ins-c (#6f)
caggTAggtactcttctcagtgggcctggctccccagctaagacctcagggact
tgaggtaggatatggcctcctctcttacgtggaacttttgctatcctcaacccagcctat
cttccaggtcattgtttcaacatggccctgtggatgcgcttcctgcccctgctggccctg
ctcttcctctgggagtcccaccccacccaggcttttgtcaagcagcacctttgtggttcc
cacctggtggaggctctctacctggtgtgtggggagcgtggcttcttctacacacccatg
tcccgccgtgaagtggaggacccacaaggtgagttctgccactgaattctgtccccagtg
ctaactaccctggttttcttcacacttgggacattgtaaattgtgtcctaggtgtggagg
gtctcgggataaccagggagtagggacacgtttctgggggaaactagacatatgtaaaca
tggcagctgccaggaatgagtaagaatcctgccttaagggttccttggtggtagtaactt
gggatatgtgactagatcccaggatgggtacctatttagggccctcatagagcactgcac
tgactgaggatgagtaggctttagaggcccatgtgtccatccatgaccagtgacttgtcc

Eco #1(7f)
cagagagtagtttcattttattgttttttctgtgctgtgtcttcatagtt
acacctcaatatttgttcttttctttaatggtcccttcctcctgtgtcccctagactgtg
tccatggaacatgaaataaaattttgaagggctatgcccttcaaactctaactcactgat
actttgtgccccattcttgcctcagtggcactatggtttagttactcctccatgggcact
tgttacctgcattcttcccttccttaaacaatcagtgtttaaaataaaataaaataaaat
aaaataaaataaaataaaataaaataaaataaatagctctgggcctattctgagggaat
tcaagggaatagagcttggatacatttaagaaaaataaagtttttcttctctggctatga
cctctaggatatagccattttttctttattgcctcttatccttctaccagctcagaggaa
gcgcctctcttggagctttcatggaatctcttcacaggtttggagaaatccttcactttc
ttcatgtcaacctctggcttagtaaatcacagccaaacattctcagagagccttagtggt
agcagtttgcaatgtcaagcatgttgctgtggacaatagagggcagcacttgtcacatac
ttctagtctcagggcctagagtaggtctagaatc

Eco #2 (8r)
catgggattgacgccatgtgcaaacaccatgcaccccac
gtgcgtaatgtcgtgangtggcgtggcacaggtaaaaggggcgtggcttatgcaaatta
gggtccggatttaaaataaatattaaaattactttctaaggtcttccgggtgtggtgtat
ccttgtggcacancgccatcttgtcgcctcatgttataaggacactcaatttagaattct
ctgtcatcagtcatctgacctggccccctgccctttatgagtggtgccaaacactaagca
tactggtgcagatggcatgtatagtgacacttgttcactcttttggggatgttatttggg
tgggttctcaccccttttagccgagttgcagtgctgtttaaggcacgtactcaaacgct
gtttaaggaacggactcaaatgtccttcttaaaggacagctgctgagatggggctcagat
aacaagtattgcctgaagtcacatttaaactctgagacctttgttcctggctttgaggga

Eco #4
ccctacacccgaggacttaccggcttctctgaacctcaggagttagtttga
ctgagatccttgaaatttaagcattgctttggggggtgggggtgggtgctccaaaatcgg
tcagcatttgttctgttcacagggaaaaggttttgttgttgttgttgttgttgtttggtt
tttattttgttttgttttgtttttgatacttgatttccaaatattttaaagggtgggaat
tctgcctctgctgactcctcacaattagaatattttggcttgggcccccaagtttgcttc
tggctcctaaccattttaaatgcaagctcctatatatagatataaaaccaccattctcag
ttccctaaaatttaaagtaagcctgggtccttagtccttttgacaaaatttccttacttg
ggagagtggacattttgacagcatttaggcactgtctccaggggatgaggtagcggggtg
ttttccccatg

Eco #5
ggaatctgtttaggaaattaaaatattaaaagacattgatttttctggct
ttctcaaatggactgcttgcacttctaattccaccacatttagacagcatttcctcattc
tcnagaggacttctctgaagccacagaaattagaggtgatttttcccgggtgttgtaggg
cccactggcttcagggtgaaccctagtctccactcccacccctcaaaagaactgacctga
attccctaaaatccatctgttgtttttcatctcccatttttaagcaattaaaattaagct
cccccaaatttcatgttgggaattttcttaaacccaacagcaacaacaaaccacctaact
tctacaaattagtctaggattttgtgggttccccaagctttctaaccaagatattgggtt
cccctcgttaggcctggatcaagatgccccccatttttgcatagaataattttcctcatt
ccccaacttggattctaatttttctggaatctc

Eco #6
cctcataaagagcaggcctgggctcatctgc
tggtggggttgaagggaaagcaaagagtactgtctccngccctgtaggccattgggggct
gcccttggcctgccttgcaggctcctcttgctggcctagaggattgcttttgggaaagtc
tgggcagtacctctacttccccccctcccttgcctgccatccctggcctcccctcgatcag
gaccagatgtgccttggagcaggaattctagctattttgttagccagccgcttgctggac
tttctgataaggcaaattggttctctacagagttgggcgtggaagagtaggagaaggagg
agagagaacaatatgcccaccctctcatcttcctgtgccagtgacgccccagagaggttc
ctggcccaactccagaagcttctttctgacacatactagggtgataaaaggtatgctagt
gcccaggagatgcaagtggg

Eco #6x (11f)
gacactcctaccgtgataaaaaaattcattaaagagtga
gaaaagaatcctaaaaagtggtcaaataaaatatactagttacttttgatgttgctgtc
accaaatgcttgacaagaagcaacttggggaagggaagtttgtttgggcttagatttaag
ggagtgtagtcagtcacagtcacagtggggtaaccatggtagcaagagcagtttacagcg
gcctccatggaagctttctcacatctggatgggtcaagaatcagagagatcagaagcggt
gctgggggatgcctggttacttgctgttggatatgcatcaccctatctagcaagcacctc
tgaattcaggaacaggcaggaggtactgcctggggcttggaagacctagcaaagaggga
gaaggctatctaggaggaaagggcccagagacctgtgttcttctacattataagaaaggc
aagggaccactgaggagcctgtggcctaagcaggatggaaGaaagagtgaccctcgggcc
ttacacatgccgaagctctcctttctgcagcgtgtgacctcccAccatggacccct

Eco #7 (12f)
tagaaggTgagagaGgctgtgtgcagtatgactccctgttgtgagatggcag
gagcctggtgacctttgaggcaggcgcggggaccctggcacattcactaggggtttccaa
actagggcagggcagcagaagagggtttttctgggctgctggggccatgaagaaggaca
gaacttggactcttagaccaaggtggtctttatgatctgtatgtgtgtgtgtgtttgtgt
gtgtagaatccaaaacagggtgagtctttaaaccttcatctctcaggcaccttctggtct
tcctgtgtgcatacttctgtggaattctcttcatctaacaatagtcaaggtgctggctct
gcctgacggggttcaggctgtggcactggcttcagctttgtccacccgacaacagcaggt
tgaagggatgcaaggccagttctgtgcagcttcccatgtgcttcttcagaatcctacagg
caggcgggcaacagtgtaGaatggccaggttctattactaagggctggagctgctggctc
tctctcagcctagggagactgagaaaagcagttccatggaagagcttccgttccctgctt
gtgacatgatggcacacagtttcaactgtctcatgctatggtgagatatgagaatcacta
ccatgcctggccatggaggaggcatgaggaagg

Eco #7x (13f)
tttagcaactgaccttctggtcagtcctggt
tgacctactattacaaaataccagttgccctactattataaaatacaagtcaggagagga
gccctacttcgagtctcaagaaaagagcaggtggcagaagaatggattcaaagtctccac
aacctccttgaaagaagaaaaggaaagctttaggacctgtggtgtgagctcttcgagaat
tctcagcttgaggtatgatgtgggagaaggccagtgacttcttctttgagttttcaaact
gttctggttgtaattaaacacaaggcatcccactgtctctctgaagtccggtgtgtctgc
tttggaataagacaagagtgggatgggaagccatggagatgttacagctcgtgtaacata
cacaaagttcctcgcattccagtatacatctgctccagtttgatatttctatggaacctt
tggggagatttgccccattcaaaggccttccctaggtctgagaaactcaagatcaggtta
tggggtaatggctcagtgtcagggcatgtctcccatggtggctttggttgggaactcagg
tggaaggggtttgtagagaaagttttggtttcccaagggcc

Eco #8 (14f)
cagacctncatttcccactttggccactgccctctgttgttgcccaccttccccgcccaa
gttcatcaaacaaaattcactgaaaagtaagcaggcttgcatcattgggagttatat
atggggatccaagtctatctgaaggctttctctttggaaGagaaaattaggtgtccttga
aaGagaacaaggaattctaaggcccaaaGaaGagcctcttgggaaggagtcaacctacaa
atgccatctaaacatatctAgtgtttgcttgaggtaactttatggggagcaaGagtgggt
agggttggcatcccaaaatagcaTatgggtcgacttcatgataccgggtgtctaacacaa
gtaGagggtcaaggacagtcctanctccagttccatcggcagtggctgctctggtcccca
ccattccctgagttacttccttcatagccTgccttgttccacctatcctttaaccttagg
gaacttgctacctttgcttctaGaacattcctgaGaacactgggtaggcccatgaagcca
ctgatgataGacacactcaTggggacaaaatgt

Eco #8-9 (15f) –present in *M. musculus* but mutated in *M. Spretus*)
gtggagagcacaacatgntagaacaaatagattgtg
acgaatatattatggtgtccagggaatagaaaaactgtgtagaacggtgtgtggagta
tagagctgagaacagactgtggaagcagagaacggagctttgtgtgtaaagagagactcg
agggtagaaggtagaatatctcatattccatatggggtgtaaagaacataatatggtcca
cagcaaatgaagctagagcatgcacgatggcgaatggagaaactaggaaacattacagaa
t**a**ctaagtaagaatgccacaaagtttccctctggccttcacacatgtgctatgatatgca
tatccaggcacataggcacagactcacaataaacaaaaataaatacaacttaaaaacaca
aataaatggaggtagaaaatagtgtatgggtaacgaggaaggaaatgcagagtttataaa
acatatacaaaacatgcaaacatgtagtataaaatacagtataggaaatggcatgaagag
gagaagggctgggagatagattacagaatggaaaagtgagaggaagcaatgaggtataca
atatgaaggtgggaagggaaatgtgatatgtaa

Eco #9 (16f)
gtggcccagcagtggccactagggtccttcagcctcagtttctccctg
gcacaatcacctacaaaggtatactattacacaggacaagcatgggctttcccagaggag
ctgaacctacctggttcctaccaagttctccaccgggcctccctgcttccatgcttgctc
ctgacctgactgtgaagactttcacggctcctcaggtttggacaaatgaccaacctcagt
aaccccatgaggagctttctgctggccactccacttagccttgtgggccactgaattcac
tgtccaccctgtattcctcattcttcaggactgtcccccacgagtgcctccactgagatt
ctaagctgggaaaaaagaagaaatcgtctggctgtcctccaatcaagtgtgcttacagca
tcctgggagtcctgggaactgtcacctcaagcctagagtgcagccagtgctgagaggaag
aggcatccgcctggggacacgtggaaggacatagaaaggtgagggacaaattcagcaagg
gtagtgatctgtcctggaaaggacatgctagggagtttgctttagaactttccttggagg
aacaaggccc

Eco #9x (18f)
gtccgtattctcagatctgacggggccagagccaggacagaggaagagggcttg
ttgcagccagcccagctgggacagcaaacaaacctctggggtctctcctttcccttgcct
gcccaccacaggggtctggctggggccagagctgggggagacttcctcgatttatctctg
cagccggtggccagccctaagcctggaatcccgggtcaggctaaggtttgcctgccctgc
tgtgagcagcttggagctggtcagaaggcccaggctgagcagggggctgggattgaaaga
attctgggaaaaattgcttggggcctgtgtttggggacccaacaaaggcaatgaaggcaa
tggttacatcggttttttttttttnggttttttt
Eco #9x (18r)
ggtccttcttttgcccatctagaaggttctggaagccctctggcttctcttttgctgtg
ggcacaaccgntgntcccttcttgctaactctgccttctacatgggactatagccaaagt
aatcacgcccctgacctgcttacctttactgcagactaggccatgtcaacattgagcagc
accagccctgctcaatagaccctgactacaaaaaaaaacaacaaaaaaaanccaaaaaaaa

Eco #10 (19f)
ccacatggttggaggaagcaatatccctagcagatctctgcttattggttaactagcct
caatacaggataatactgtgtgcttggagcttagatccccaagtctataggaccccaggc
tcccagtctgaagctcccccacccccaccccaagtgctggacttcctaccttgggctggc
aggaccccctggaagcctcttgtgcccccatgccagttctgcacatacaaacccactact
agcaacactccccaaataatcaccctatcatacaaagctgccctgttgcaagggccccca
ttgggcctcagggtcaataagactgaattccaagacctaagaccctggcagagctgctcg
actcagggaagccatttatcactattagctctggctcacccatctgctctgccaccctgg
ccaggatcctacagccccactatcaacccacccagactccccttggccagagctccttca
ccatcatctgccatgatttgttgtctgtctggacttccaagaccctctatcccaacccaa
actccaccgtgctcttctctcagctgctcacaaaactccatggcaaggcaaagggaatgg

Eco #10x (20f)
cgaagttaggaatcttcttctcccctcccccactccctacccacacccccaccccca
cccctgctgcaggcaagacatgatggctattcaggggctgggggcagcagggctgaaaga
tggccanaaataaaggtggaatggccagcctgtagccaggagctgctcatagctgaccag
tcaggtcaaggccccaaagccgcccaggctggtggggtcagctagccttggctgagagat
cctcggggctcaaactgcttgatgaattcctcangacaaagatgggggtgcttgaggctg
tcacagagtccccttatgtcccaggccaagtgccccacgttgtcttggctgcaggtgagt
ttcccagccactgatacctgagggtagcctgctactgcactctgtctccaacagaggaga
ggactgggaccagccttgccccccaaatggagcacaggtctgtgttgagcccaGatcaaa
ctctgtggatctgttttcccctaGctgggaagaggagcaggtgggaagagatagcacagg
gtggtgtctgctgagtgctagccttgcctccagggctgtctggccc

Eco #11 (r22f) (43 b deletion in *Spretus* at 79 b upstream of the Eco RI site)
cctcctagaatgcaagaaggcatctatctaggtaaagccttgggccagggg
ccccactcagcccatttctgagctccatgatcaaggtcatccctgggaattagtatggtt
ggcaagcaggtggtgctgccacccctaatcccactgcccttccactggggaagaactgtt
ccgcatgcctggtaaggtccccctctaactggctccctttctttctcagcacagaacttt
ggccctatctaggaggggagatgggagaattcgaatcccacaagggttgtgggtagagct
ggtggccacattcaagggatgatgctgtaatcaaggtctttcagaatagccaataccaga
ggcaagaggacttctctgcctcagtattatctggggaggccggctggtgctactgggctc
attgaagacgtggtgtgaagttctcaaagtgctgctgttctgggccaagtatctcctatc
cctggtaacaagcctggagctaacccagggttctgggtagactctcagggtttttacagc
gccct

Eco #11x (r23f)
ggtcagcctcagagacccaactctctgcacagagc
taagtgaggacctggaggcttctggataggccaagtcagttcaacactggctctgaagtg
gggcaccaagtagaccagtgaaatagcccctggttgttactccaaagaatccaggcattg
gctgcgtttgtcaagggcctcgtgcctgaggatttagtaaaactgtaacggaagcttgat
aggtaatgtggcagtaatccctaaagaggcaatggctcctcatggccttcgcattgtttg
atggcacttgcttgccaggaattctccaaagggacagggaaatggaggccccacacctag
cacagctcactcaccactcgaagatggtgtctaaggaggtgctaggccaggaggtcagac
cattctcctgcctagagaccccaagtttactgtaccaggagaccctggtaacttagttca
cagcaatccatgctaagccagcatccatttcccaggtatccccatggcatttctaaatct
gaagccccttttgctctatgtcaagtgtgtgcccctggatattcatgtgggaactgagac
cacatgaaggtacttcttccagggtaggctccaattggactagtacccag

Eco #14 (14f)
actcctattcgtcctgcttctacctcccaagtgctaggcatgtcctccat
atcttgtttatgtagaaccaaggattgaattcaagacttggtacctgctaggcaagcatt
ctatcaactgagtcatatcctgattctatttttaaactttaaaacttagtgtccaggcaa
gatcaggcagctcccatagtacaacatgacctagttaacaactgttgttacgtatgtagc
ccatgcaggcaaagccacttggtgtggcctggctgctctgtacctcttggctttgagact
tccattccagatggggcatctccattcacaaagatttccataagtttacctggggtgcca
tggaagagtgcactagctagggacaccacaggaactctgtagatgagaacttagggtatg
gcagccttctgtgccagtaagagggtgttctggatgttggattcccaccaccagaacata
gaggcaaggcaaattggaatactggacacagtggataggcctctaataaaagggacttaa
gagataagtctggcataccagctccaccatgtacctcactgagttttccctctcctctac
agaagaggaaactgtctataggtccaaccatcctctgacatctgacactggtacaggtaa
ccagtggtccctaagcttcatagggcaaccaaaagatagttcctaggatcagctatggag
cattagagaaaaagaggaggaactttctagaaggtaaaatggtcccactgctcggatcag

Eco#15 (25f)
tgctggcctacttaggactggatagcttcctttacacaggaaa
ctagaggctagtgcctagagggaccaaaggggagaggggagagatcatgtctcctggagg
gaggtaaagggatcacaggaagacctagaggccatgattctccccagagatgaccagaga
gacaccactctacatagaagagcagaaagcaggactctgatcctctgtgaattcactcct
tcatattgttacgtaggccttttactttattgtgttgtgatatgtaacaaactaaaccac
acaagcacatagcttagtggtaataggtgtgagcccagtgctgggcagtgtctaagagtt
ttatcttcctctctggctgcagctccaaggcctcccaacttgcttgctcttctctctcag
ggcagagtggatgcagaggtggtcctgtgtggtgaagggactggagtctgtcatctcact
tcttaggcagatggattgagagccaggagaggcaggcaaagaaccaggccctgcagaacc
tagggctgtgtcctggagattgcacagcagagtaggccctgcaacctgacccatcccttc
cc

Eco#16 (26f)
tttgctgagcttcctcctgccccaatccagag
gttgttgggaactcctaggctgaagtgaaaggggtgggggtgggggtggagtgttctaca
acggcaagggccaagttctcactctcactcctaggagccagcctgggaagccctggttct
gggctatagcaggaaagaaagagacacagagtttgagaatttgccccactaacccccaac
aataccccaaagtgccccacccccaggacattcttttgaattctgtggacaggagtcaga
ccctcctagtgattttttgaattttcactgtccaacctcctaggttcaatgggcaaactc
ctataggtctgatccgtgctggagacccaatggtgcaccctaatttttcgggcttgacc
cactaaatcagcagttttcaacctgtgggtagcaatcatcaaaatgctcattgtttccaa
tgggtcttaggaactgagacacacttatagttatgatgtagcaataaaaatattatggtg
gggggtctcaaaaacataaggaactttattaaaatgtcatagcataaggaatgttgagaa
ctactgcac

Eco #27 (27f)
ctgaccatcctctaaatcaacacttgaagggtgctt
ttgctggccttcagcctcagccagcatacccccttctcccgcccgcccgcccgcccgcc
cgtgcactccagtgggggtggggctgagagcactgactccatctcagggggccccaggcc
ctgctcagaacccacagcccacagcagagctatgtcgcagagcaagaattccaaatccag
gcagtcaggcacgcagagacaggggcaagatgccacgtaggcacgagtggtaaggctggg
ctagtccacagcgctctgatgataccattactgctccccaaatctctaagcaaatttcct
ccttagaatcaaatttcacactgagacacactcataatcagaaacactggg

Eco # 30 (30f)
tagaaagttagctattgaactgtttaattgttgagatgtacagaaggcataattaaag
caggaaaaacctccacccgcctgtccagtgctggcgcaaggcaaggaacgtgaggaatgc
acccttaggctctgtgtggccccttggccctcacgtcccctcctctgccgggctccaggg
acccttcatctgaattccaccgtgttctgcaagtctcagcacataggataagttttttag
tttagtttaggtttgggcttctctttctttctttctttttctttttttctttttagcttct
ggggctcaaccccaggccttgagcatatgaagcaagctctttaccactgacccacatgca
gcccaacctatcccttaaatattaagtagttttaacttgtatggaggtgccttgctgctc
ttcttttgcatgctgccctcctgcatccagtatgtacctcctgccttggccttcgtgtct
gagagctggggtgcaggcgcttt

Eco # 31 (31f) (a 16-b containing Eco RI site is deleted in *Spretus*)
gatgagggtggctgagtgccttggtggatacaggaatgataccatctgcct
ggcacaacacaggacacacagatggataaataacacttgagaataggaagggtgtcaccc
taaaaatgtattggactgaattgaaacggtaggggcacacaggcatattaaatttatacc
agagaagccagaccaagaaaaaggtggacatccctcacccaaacttcctaaagaggtaga
gacctagaacagggatggatggaaatgtcatccaaccacctacccagtgtgtgcgtggtg
tgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgta

Eco #31 (31r)
caggaggagtgaggtgaagtgaagggcagggctgggtctagatgagccaatggatagata
gatgatggatagcaggtccagacaaaggaacagggggggggggncttacca

Eco # 33 (33f)
aagtcttcagagcgttggtcttctttgacccag
taattccagttctgggaatgtggcctaggcaaatagccctcaagatggaaaatgatttct
gcccagcgttatttatgattgtaaaatattggggaagcctgtgatcggggaatggttagg
aaaattatggtttgtccaattgatggaatattatgtggccattcaaaatatttaagaaga
ctatgtaaaaacatggaaaaatacttatgaatagtgctaaattttttaaaagcagaatt
caaggctatatagacagtcccgtgcttgaaaaaacatagtcaggatctttattttccaag
aacttcatgtagaaaaagaacaggaagaaataaagatcttttttcccctccctggtcctt
tccacagtctggctggtgggtgagtgggagaattatggatggattttttccccccttcatt
tgacttttatgtattttccaaattttcttaatgaggtgttttattttcatattgagaaca
taaataatttttctaaga

Note N3: Primer sequences

A set of 32 forward primers and one reverse primer (*) were used for 3C analysis. Each primer was marked as F (forward) or R (reverse) followed by the length (in nucleotide number) to the target EcoRI site. There were three insertion/deletions leading to polymorphic lengths (Eco #1, 7, and 11). In each case maternal allele was listed first, as in T21B-Eco#1-F90/112, forward maternal 90 bases, forward paternal 112 bases.

The size of a specified 3C cross-linked product is the sum of the two EcoRI fragments plus 4 bases AATT (overhang EcoRI site). For example, the predicted Eco#3-Eco12A cross-linked product is F98 + F132 + 4 = 234 bases; and the Eco #3-Eco12B product is F98 + F129 + 4 = 231 bases.

T9C	-mLit1-F114	TCCAGCTAATGTCATCTATATCCCTTG
T11B	-mAsc-F100	GGATATCTTGGATGTTGGTCCTTCCCTT
T13B	-mTh-F97	GAGGATCTTAGGCCCCTGTGAGACCCT
T15B	-Ins-a-F129	GAAGGTTTTAAAGCCCAGTGACAGCCTT
T17B	-Ins-b-F104	ATACTGCAGGAGGATGTACCACAGGGCTT
T19B	-Ins-c-F98	GTGGAGGCTCTCTACCTGGTGTGTGG
T21B	-Eco#1-F90/112	CCTGCATTCTTCCCTTCCTAAACAATCAGT
T23B	-Eco#2-F92	TCACTATACATGCCATCTGCACCAGTAT
T25B	-Eco#3-F98	AGAGGCTGTGTCTGGTGGAATACTGGAGACT
T27B	-Eco#4-F111	TTGTTCTGTTCACAGGGAAAAGGTTTTGTTG
T29B	-Eco#5-F91	ATTAGAGGTGATTTTTCCCGGGTGTTGTA
T31B	-Eco#6-F37	CTCCCCTCGATCAGGACCAGATGT
T33B	-Eco#6x-F101	CATCTGGATGGGTCAAGAATCAGAGAGAT
T35B	-Eco#7-F182/160	GGGCCATGAAGAAGGACAGAACTTGGACT
T37B	-Eco#7x-F109	TCGAGTCTCAAGAAAAGAGCAGGTGGCA
T39B	-Eco#8-F105	AAGTAAGCAGGCTTGCATCATTGGGAGTT
T41B	-Eco#9-F105	ACTGTGAAGACTTTCACGGCTCCTCAG
T43B	-Eco#9x-F145	GGGGAGACTTCCTCGATTTATCTCTGCAGC
T45B	-Eco#10-F113	CCAGTTCTGCACATACAAACCCACTACT
T47B	-Eco#10x-F86	AGTCAGGTCAAGGCCCCAAAGCCG
T49B	-Eco#11-F171/128	TAATCCCACTGCCCTTCCACTGGGGAA
T51B	-Eco#11x-F90	GGAAGCTTGATAGGTAATGTGGCAGTAA
T53B	-Eco#12A-F132	CACAAGGAAAGAAAAAGGTTGGTGAGAAAAT
T55B	-Eco#12B-F129	CAAGGAGACCATGCCCTATTCTTGGAC
T57B	-Eco#13-F142	GTGAGGCTGTCTTTGGAGAATTTCAGGAC
T59B	-Eco#14-F46	TGCTAGGCATGTCCTCCATATCTTGTTT
*T60	-Eco#14-R119	TGTTGTACTATGGGAGCTGCCTGAT
T61B	-Eco#15-F91	GAAGACCTAGAGGCCATGATTCTCCCCA
T63B	-Eco#16-F69	AGTTTGAGAATTTGCCCCACTAACCCCCAA
T65B	-Eco#27-F83	TGAGAGCACTGACTCCATCTCAGGGGGC

T69B	-Eco#29-F78	ACATCTCCACCCCTTGATAAACCAAGAGT
T71B	-Eco#30-F80	AGGAATGCACCCTTAGGCTCTGTGTGGC
T73B	-Eco#33-F100	CAATTGATGGAATATTATGTGGCCATTCAAAAT

Note N4

We have observed predominantly maternal interactions from the E#3 target in fetal liver (Fig 4B) and in mouse embryonic fibroblasts (Fig. 3B). We also observed a few random interactions within the paternal allele in the vicinity of the *Igf2* E#3 target in embryonic stem cells SFG (Fig. 5B). More interactions were observed within each maternal and paternal allele as the chromosomes were compacted in cells treated with Nocodazole (Fig 5C). The compactness of higher order chromatin structure of the maternal allele versus the relaxed chromatin structure around the paternal *igf2* was reflected in the predominance of maternal interactions near the *Igf2* E#3 target site.

In the case of newborn liver that demonstrated high levels of paternal *Igf2* transcription, such contrast is more extreme (Fig 4B). The frequency of random interactions within the paternal allele was much lower as compared to the frequency of interactions within the maternal allele. Such low levels of paternal random interactions presumably can be detected in some fetal livers with higher input 3C DNA and/or a greater number of PCR cycles. To verify that, we have performed independent 3C experiments on several fetal livers using more input 3C DNA (~800 ng) and more PCR cycles (23 cycles of second PCR). We could detect some random paternal interactions (paternal interactions from E#3 target to E#2, E#7x, E#9, E#9x, and E#12B) along with the predominant maternal interactions near *Igf2* E#3 target site (Supplement Fig. S1).

Note N5

One might question why the interaction from E#3 target forward primer to E#14 forward primer was detectable and of maternal origin (Fig 4B) while the interaction from E#14 target reverse primer to E#3 forward primer was undetectable (Fig 6AB).

Since 3C involves the ligation of two DNA fragments in the same nucleus vicinity after formaldehyde fixation and restriction digestion, switching from forward to reverse primer sets does not necessary yield similar abundance of the products. Forward and reverse primers at a specified restriction site are incorporated in two separate DNA fragments. Each of the two DNA fragments is 3C-ligated to another target DNA fragment with varying efficiency depending on the topology of the two 3C DNA fragments.

The interaction from E#3 target forward primer to E#14 forward primer (Fig 4B) represents the 3C interaction of the fragment E#2-3 and the fragment E#13-14. In contrast, the interaction from E#14 target reverse primer to E #3 forward primer (Fig 6AB) represents the 3C interaction of the fragment E#2-3 and the fragment E#14-15. Differential DNA-binding proteins in the E#13-14 chromosomal DNA and in the chromosomal E#14-15 DNA may affect the topology and therefore the abundance of the 3C products. The 3C interaction between the fragment E#2-3 and the fragment E#14-15 was low and not detectable in Fig. 6AB.

In Fig. 6AB, reverse (but not forward) E#14 target primer was chosen because of the presence of a polymorphic KpnI site near the reverse primer. Also note that the presence of both forward and reverse primers at the target location (Fig 6A column E#14) resulted in a local 3C re-ligation product (E#13-14 and E#14-15) that was unique at this target site and was derived from both parental alleles (Fig 6B column E#14).

Note N6

Kurikuti *et al* studied newborn liver and observed ICR-MAR3 interaction exclusively on the maternal allele using ICR reverse primer (E#12B) and MAR3 reverse primer (Eco RI site between E#6 and E#6x). We used E#12A forward primer and E#6 forward primer and performed 3C in newborn liver, MEF, and SFG1 cells. The target restriction sites in the two studies are close but not identical. Furthermore the primers in the two studies are in reversed orientations. In all three tissue/cells our results do not support close vicinity of ICR and MAR3 (depicted by E#12A forward – E#6 forward primer combination) on the maternal allele (MEF: Fig. 3B, E#12A Hpa II, column#6; Liver: Fig.4B, E#12A Hpa II, column#6; SFG1: Fig. 5B, E#12A Hpa II, column#6). Therefore, our proposed model (Fig. 7) differs substantially from the Kurikuti *et al* model, in that it does not illustrate a DMR1-MAR3-ICR looping on the maternal allele. We propose a knot looping with close vicinity of DMR1-ICR-Ig and Endo Enhancers on the maternal allele. On the paternal allele,the relaxed chromatin structure may result in local and random interactions from the ICR.

Note N7

We have shown that “switching target and forward primers” in all cases (except for some rare instances) resulted in consistent results. That was evident in many 3C panels across 24 target location sites (for examples, 12 panels in Fig 2B, 12 panels in Fig. 3A, 15 panels in Fig. 4A, and 12 panels in Fig. 5A). While analyzing hundreds of 3C samples in series of 24 panels we obtained >99 % consistency (for example, consistency in (24 x (12+12+15+12) = 1224 panel columns in Figs 2-4). There are some anomalies (< 1 %) that may appear in rare cases where PCR and/or gel loading are variable. The intensity of the 3C product after Hpa II digestion in Fig. 5B, E#3 (column 3, lower panel) was weaker than expected for the juxtaposition of DMR1 and ICR, probably because of variation in the handling/loading of this sample. In six other cases including the three tissue/cells studied, the combinations of DMR1 forward primer – ICR target forward primers (E#12A and E#12B) resulted in all major 3C products (Figs. 3A, 4A, and 5A; panels ICR-E#12A and ICR-E#12B; column 3), which is consistent with a close vicinity of DMR1 and ICR..