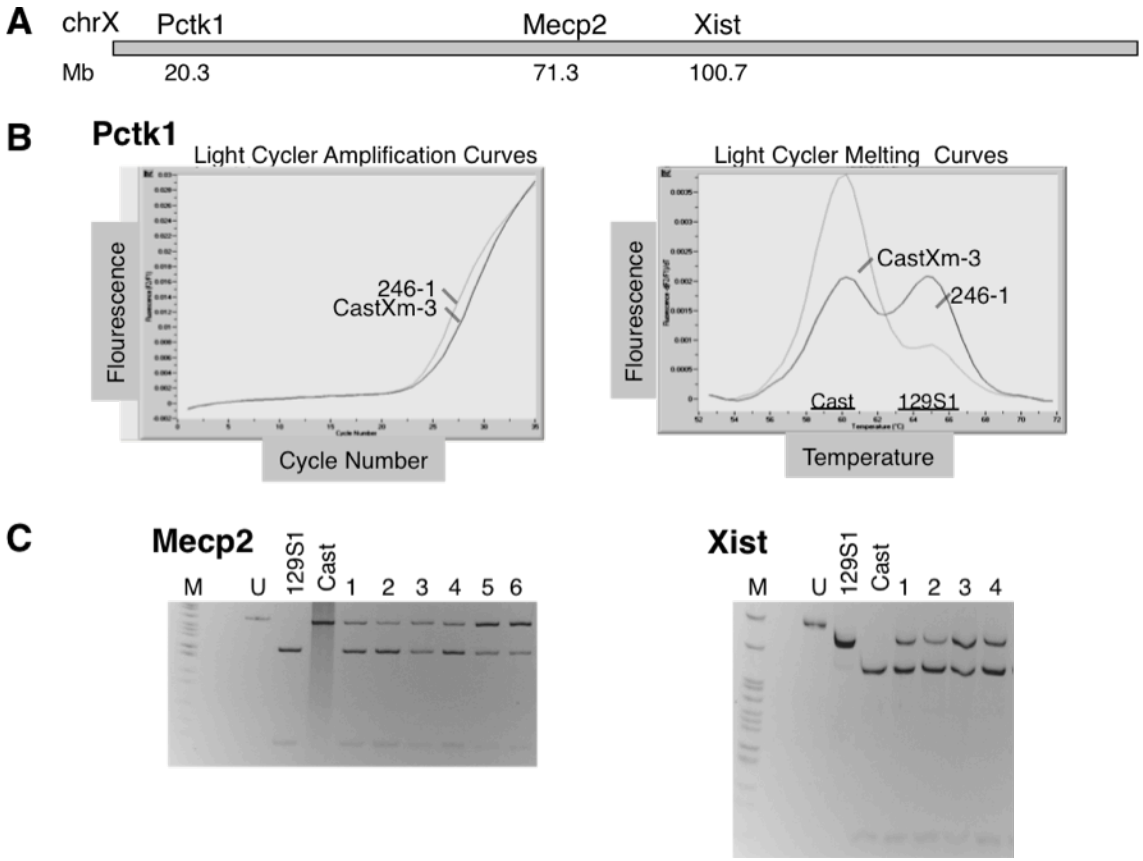


**Figure S1** Allele-specific Expression Assays to Measure the X Inactivation Ratio. **(A)** Location of genes on the X chromosome with assays for allele-specific expression. **(B)** *Pctk1* analysis using previously described Light Cycler Assay (PERCEC *et al.* 2002). The left panel shows the amplification curve of two control progeny samples: CastXm-3 is heterozygous for entire X chromosome; 246-1 is homozygous for 129S1 for the entire X chromosome except for the proximal end of the paternal X chromosome, which is Cast. The right panel

depicts the corresponding melting curves: peak at 60°C corresponds to the Cast allele product; peak at 65°C corresponds to 129S1 allele product. Peak heights were used to calculate the X inactivation ratio (129S1 /(Cast + 129S1)). The ratio for CastXm-3 = 0.23; the ratio for 246-1= 0.50. **(C)** *Mecp2* and *Xist* assays using RFLPs. Lanes shown are pBR322 DNA-*Msp*I Digest (M), uncut PCR product (U) and cut PCR product (using *Tsp*509I for *Mecp2* and *Sml*I for *Xist*) for control and RX2 progeny samples. *Mecp2 Tsp*509I 129S1 digested fragment is 217 bp and Cast digested fragments are 155 bp and 62 bp. *Xist Sml*I 129S1 digested fragments are 279 bp, 82 bp and 24 bp, and the Cast digested fragments are 361 bp and 24 bp. Progeny tested in lanes 1-6 are CastXm-1, CastXm-7, 6443-1, 6443-3, 3695-1 and 3695-2, respectively. The ratio as measured by *Mecp2* for corresponding lanes are 0.31 (1), 0.22 (2), 0.40 (3), 0.28 (4), 0.58 (5) and 0.62 (6), and as measured by *Xist* for corresponding lanes are 0.31 (1), 0.21 (2), 0.46 (3) and 0.33 (4). Progeny tested in (**B**) and lanes 1-6 in (**C**) were from control or RX2-derived male mated with 129S1 female.

**Table S1 Genotype of X chromsome of progeny tested mice**

| | | | | Control Lines | | | | | | | RX1 Lines | | | | | RX2 Lines | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **X Marker/Gene** | **Mb** | **Xce1** [a] | **Xce2** [b] | **CastXf** | **2175f** | **CastXm** | **199m** | **88m** | **246m** | **246f** | **109m** | **217m** | **78m** | **228m** | **218m** | **137m** | **183m** | **2173m** | **3695m** | **6443m** | **2181m** | **5005m** | **6570m** | **830m** | **800m** | **1114m** |
| **DXMit53** | 16.5 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Pctk1** | 20.3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Hprt1** | 50.3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit73** | 57 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit144** | 61.2 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Mecp2** | 71.3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit62** | 90.1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit63** | 90.4 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit113** | 91.8/92.1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit114** | 95.3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit96** | 96.4 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Eda Exon8** | 97.59 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit229** | 97.9 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit41** | 98.3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit17** | 98.4 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit230** | 98.7 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit168** | 98.9 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Snp 846** | 99.135 | | | | | | | | | | | ND | | | | | | | | | | | | | | |
| **ss38408987** | 99.35 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit115** | 99.8 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit148** | 99.9 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit95** | 100.1/3 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit170** | 100.2 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXPas28** | 100.5 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Tsx** | 100.61/62 | ND | | | | | | | | | | | | | | | | | | | | | | | | |
| **Xite** | 100.63 | ND | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXPas29** | 100.63 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Tsix** | 100.63/68 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Xist** | 100.66/68 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Xist exon3** | 100.66 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Xlsnpg Ex1** | 100.67 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Xist Ex1** | 100.68 | | ND | | | | | | | | | | | | | | | | | | | | | | | |
| **ss38407822** | 100.69 | | ND | | | | | | | | | | | | | | | | | | | | | | | |
| **ss49779045** | 100.7 | | ND | | | | | | | | | | | | | | | | | | | | | | | |
| **DXPas31** | 100.8 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit18** | 100.83 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit171** | 101.05 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit40** | 101.35 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Abc7** | 101.5 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit64** | 103 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit97** | 116.47 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit234** | 138.5 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit152** | 144.1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Jarid1d1c** | 148.7 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **DXMit249/31** | 160.4 | | | | | | | | | | | | | | | | | | | | | | | | | |

[a]Simmler *et a*l. and see Figure 2A

[b]Chadwick *et al*. and see Figure 2A

| | |
|---|---|
| Not Determned | ND |
| Cast | |
| 129S1 | |

**Table S2 PCR primers and conditions**

| Gene or X Chr Location | Primer | Sequence 5' to 3' | NCBI SNP /Polymorphism | Product size /SNP location | Restriction site | Allele specific fragments (bp) | PCR onditions (Anneal Temp. /cycle number) |
|---|---|---|---|---|---|---|---|
| **Hprt1 cDNA** | Hprt F3 | TGCTGACCTGCTGGATTACA | ss46946097 | 303bp | SfaNI | 303-129S1 | 61°C |
| | Hprt R2 | GGCCTGTATCCAACACTTCG | A-129/G-Cast Exon6 | 201bp | | 192,111-Cast | 26-28 cycles |
| **Mecp2 cDNA** | Mecp2F3 | CCAGTTCCTGCTTTGATGTG | NA | 217bp | Tsp509I | 217-129S1 | 58°C |
| | Mecp2R3 | TTGTAGTGGCTCATGCTTGC | G-129/A-Cast | 157bp | | 155,62-Cast | 26-28 cycles |
| **Eda** | X97.59f | AGAGGCATTCTTGCTGCATT | ss38410803 | 156bp | StyI | 156-129S1 | 57°C |
| | X97.59r | TAGGCATGCATGTGGTCATT | G-129S1,C-Cast | 120bp | | 120,36-Cast | 35 cycles |
| **X99.35MB** | X99.35f | CGGTTGGCGAGTTAGAAAGA | ss38408987 | 250bp | Tsp45I | 93,157-129S1 | 57°C |
| | X99.35r | CTGGCCGAGAGTTACCTGAG | G-129S1,T-Cast | 96bp | | 250-Cast | 35 cycles |
| **Tsx** | Tsx g1f | ATCATTTATTTGGCCCCTGA | ss49779081 | 209pb | ApeKI | 129,80-129S1 | 57°C |
| | Tsx g2r | AGCTTGGCAAGTGTCCTCAT | T-129S1,C-Cast Exon4 | 131bp | | 209-Cast | 35 cycles |
| **Xist cDNA** | Xist E2F1 | TGGAGTCTGTTTTGTGCTCCTGCC | ss38407831 | 385bp | SmlI | 24,82,279-129S1 | 58°C |
| | Xist E4R1 | CCTTGCTGGGTTCAGGAAAGCGTC | G-129S1,A-Cast Exon3 | 106bp | | 24,361-Cast | 26-28 cycles |
| **Xist** | Xist IN2F1 | TCCGTTACTTGGTTGACTGAGA | ss38407831 | 245bp | SmlI | 168,77-129S1 | 57°C |
| | Xist E3R3 | TGTTCAGAGTAGCGAGGACTTG | G-129S1,A-Cast Exon3 | 168bp | | 245-Cast | 35 cycles |
| **Xist-LC Exon1** | XistF2 | CTCGTTTCCCGTGGATGTG | NA | 489bp | No site | NA | 57°C |
| | XistR2 | CCGATGGGCTAAGGAGAAG | A-129S1,T-Cast Exon1 | 172bp | | | 35 cycles |
| **XChr100.69MB** | X100.69f | ATATAGCGCCCGAGACTCAA | ss38407822 | 165bp | TaqαI | 165-129S1 | 57°C |
| | X100.69r | TCTCGTTGGGACCACACATA | C-129, T-Cast | 63bp | | 63,102-Cast | 35 cycles |
| **XChr100.7MB** | X100.7f | TTTCTCCTGTGTGATAGGGTCTT | ss49779045 | 158bp | BsrI | 60,98-129S1 | 57°C |
| | X100.7r | AGGAAGTACCCAGGCTCCTC | T-129, G-Cast | 64bp | | 158-Cast | 35 cycles |
| **Abcb7 cDNA** | Abc F4 | TTCGAAAAGCACAAGCATTC | NA | 219bp | Hsp92II | 51,158,10-129S1 | 58°C |
| | Abc R4 | TATCAATGGCCATGTCTGGA | G 129S1,C Cast Exon1 | 51bp | | 209,10-Cast | 26-28 cycles |
| **Jarid1c cDNA** | Jarid F5 | TTCCCGAGGAGATGAAGATG | ss38488639 | 291bp | Hpy188I | 292-129S1 | 58°C |
| | Jarid R2 | CCGCCAAAACTCCTTCTCTA | C-129S1,T-Cast Exon 8 | 94bp | | 96,196-Cast | 26-28 cycles |

NA Not Applicable