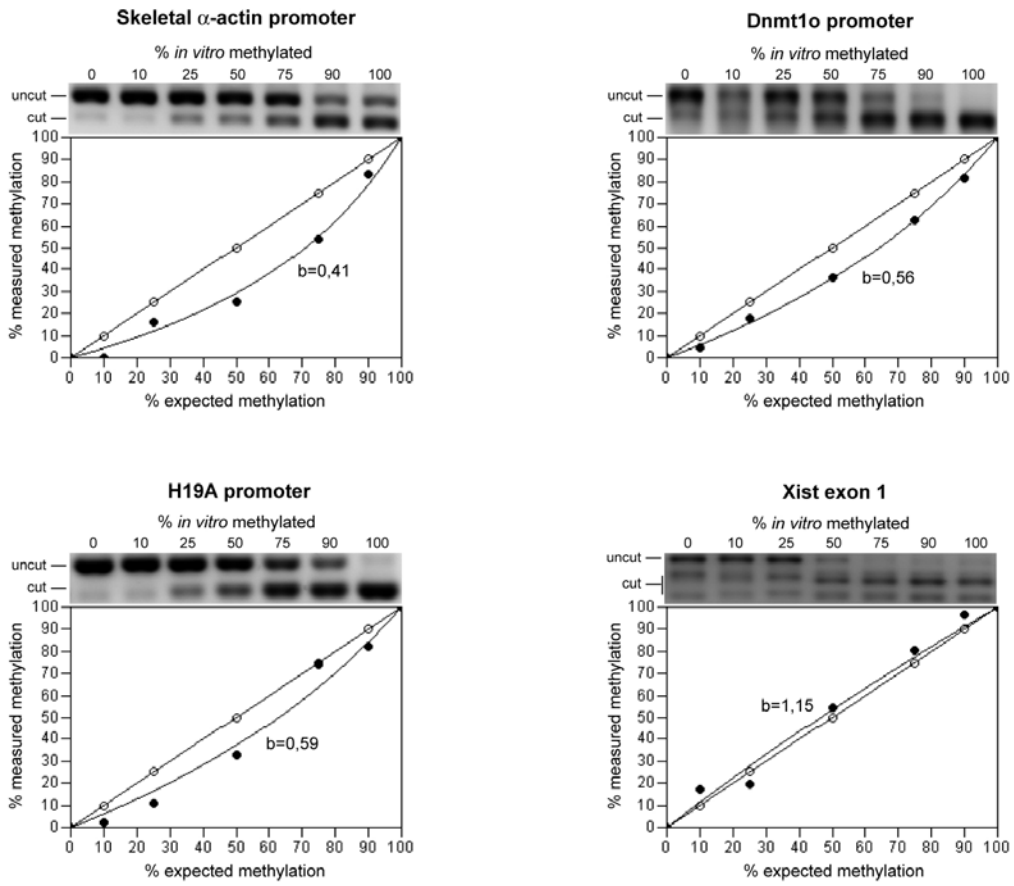

Supplementary Figure 6. Determination of PCR bias in COBRA assays. Genomic DNA from untransfected *Dnmt1*$^{-/-}$ J1 cells was methylated *in vitro* with recombinant SssI methyltransferase (New England BioLabs) and mixed with unmethylated DNA from the same cells in the proportions reported at the top of each lane. COBRA assays for the indicated sequences were performed on each sample as described in Materials and Methods. At the top of each panel the digests of bisulfite PCR fragments from these genomic DNA mixtures are shown. Uncut fragments, corresponding to unmethylated molecules, and restriction fragments (cut; only one out of two for *skeletal α-actin*, *Dnmt1o* and *H19A* promoters and two out of three for *Xist* exon 1), corresponding to methylated molecules, are shown. Measured methylation percentages were plotted against expected ones. Closed and open circles represent measured values and values expected in the absence of bias. Best fit curves and bias coefficients (b) were generated and calculated, respectively, using WinCurveFit (Kevin Raner Software) and according to the equation:

$$\frac{x}{100-x} \times b = \frac{y}{100-y}$$

where x is the expected value and y the measured one.