SUPPLEMENTAL DATA

Table S1. Gene expression levels in S2 cells.

Expression levels were determined both by Affymetrix gene-chip analysis and RMA normalization (keyed to FlyBase 3) and analysis on NimbleGen tiling arrays, using a program developed for this purpose (unpublished results) and keyed to FlyBase version 5.3. Only the NimbleGen data were used in the data presented, although very similar results were obtained using Affymetrix expression levels ($r = 0.88$).

Figure S1. Reproducibility of salt-extraction peaks.

Tracks are shown for the six 80mM salt fractions from independent experiments that were used to calculate the profiles shown in Fig. 3A. This is the same region displayed in Fig. 2.

Figure S2. Time-dependent variations in low-salt soluble chromatin recovery.

Comparisons between experiments using different extraction times at 4^{o}C.

(A) Salt extraction landscapes for a representative gene-rich region showing the results of successive 80mM (green track), 150mM (green track) and 600mM (black track) extractions from one experiment, successive 80mM (magenta track) and 150mM (80-150mM, magenta track) from a second independent experiment, and a single 150mM extraction from a third experiment. Although most features are reproducible between experiments, some peaks are differentially recovered in different fractions. (B) On average, 5' end profiles are very similar between independent 80mM extracts. (C) Average 5' end profiles reveal that longer extractions in 150mM NaCl extract only marginally more low-salt soluble chromatin.

Figure S3. Nucleosome solubility after EDTA and needle extraction.

After 5' and 10' MNase digestion, chromatin was lysed in EDTA and held on ice 30' to release the S1 fraction, then dispersed with a needle to release the S2 fraction (Jin and Felsenfeld 2007). The same gel image is shown at low and high gain. About 85% of the chromatin was released into the S1+S2 fractions, which indicates that this treatment is effective at solubilizing most of the chromatin. Note that mononucleosomes decrease in abundance from the pellet relative to oligonucleosomes with increasing time of MNase digestion.

Figure S4. Comparison of H3.3 and low-salt soluble chromatin at genic 3' ends.

See the legend to Figure 4A-B.

Figure S5. Comparison of insoluble chromatin and Pol II profiles.

Ends-analysis profiles of DNA from the insoluble chromatin pellet (average of 3 experiments, red curves) together with ChIP-chip profiles of Pol II Rpb3 subunit and Serine-2 phosphate (green) (Muse et al. 2007), for active (A) and inactive (B) genes. See the legend to Figure 4C-D.

Figure S6. Consistency of low-salt soluble chromatin recovery from different S2-derived cell lines.

Salt-extraction profiles from samples derived from two different S2-derived cell lines [biotin-H3.3 (green curves) and biotin-H3.3ΔN (red curves)]. Cell lines underwent severe bottlenecks during the puromycin selection process following transfection (Mito et al. 2005).

Aliquots of cells grown for several generation from these independent lines display very similar salt-fractionation profiles.

(A) 80mM salt-fractions.

(B) The corresponding 600mM salt-fractions.