Supplemental Methods

0.1 Expression Array Processing

150 ng of the RNA was amplified with the 3' Express kit (Affymetrix). 10ug of the fragmented and labeled cRNA were hybridized to Drosophila 2.0 arrays overnight at 45 ° C, 60 rpm. The array was stained using Affymetrix Hybridization-Wash-Stain kit and Fluidics Script FS450_0002 on the Affymetrix 450 fluidics station. The arrays were scanned using an Affymetrix 3000 G7 scanner.

0.2 Hidden Markov Model

Activation marks such as RNA pol II, H3K4me3 and H3K36me3 are most often associated with focal regulatory regions such as promoters and enhancers. In order to examine this association more quantitatively we used a hidden Markov model (HMM) (Rabiner, Proc. IEEE 1989). A three state HMM with Gaussian emission probability was trained via the Viterbi algorithm using 5 random starting conditions on 3 test sets comprised of probesets from randomly chosen segments which included 10% of all probesets on each chromosome. Among all these runs, we selected the result that maximized the log likelihood function of the HMM. We applied this model to all probesets on the array for the three activation marks (RNA Pol II, H3K4me3 and H3K36me3). The three state HMM resulted in one high variance state, representing outliers, with a high probability of transition to a different state, and two low-variance states with a low probability of transition to a different state: one with a high median signal and one with low (Supplemental Figure 1). HMM analysis of all three activation marks indicates that these activation marks are almost exclusively found associated with the TSS or gene regions as expected. In fact, more than 90% of the high state probesets in all marks are within 750 nucleotides of a gene (Supplemental Table 1).

0.3 Clustering

Genes were assigned an activation mark score based on clustering analysis– assignment to a high-binding cluster counted as 1, and assignment to a low-binding cluster as 0. The score for each mark was tallied for each gene. To compare expression data to activation and repression marks, the cluster identification and expression data were merged on CG####-RX fields. The merged data set has

10547 transcripts. Box plot shows distribution of genes for each activation-mark group plotted against GCRMA expression score. Thick black line is median, top and bottom of box are 25th and 75th percentile, dashed lines show two standard deviation coverage (95%). For the repressive marks, average repressive mark signal in a +-750 basepair window of the transcription start site was divided into quartiles, giving each gene a score of 1-4.

0.4 ANOVA on Individual Replicates

The average trend of ChIP-chip enrichment over exonic regions from young and old ChIP-chip signals was compared using a two way ANOVA on the biological triplicates. For each biological sample, the average signal accross all genes was computed as in figure 1. A two way ANOVA, using age and distance from the transcription start site as factors, was run on the 6 vectors containing the average gene signal of the three young samples and three old samples. For each of the three activation marks, the pvalue resulting from the young versus old comparision is significant: polII=2e-9; H3K4=0; H3K36=1e-10. The pvalue resulting from the interaction term of the ANOVA is also significant: polII=0.00104; H3K4=0; H3K36=0.

0.5 Chromosome Painting Figure

Chromosome painting figures were created by calculating the mean of all probes with a positive signal in a sliding 50,000 window. The means were converted to a heatmap using the *image* function in MATLAB. The colocalization of HP1 and H3k9me3 was done the same way, with each factor on a different color channel. To quantitatively validate the sharp qualitative contrast in the image, we examined the heterochromatic and euchromatic signal from each individual replicate of the H3K9me3 and HP1 ChIPs, presented in Table 1 of this document. These means are calculated after the samples were quantile-normalized. When we use a t test on the means of the heterochromatic signals for triplicate young and old heterochromatic signals, we see statistical significance for the difference between young and old with p = 0.011 and p= 0.009 for HP1 and H3k9me3 respectively.

0.6 Fold change versus mark loss

This section describes how foldchanges between 10 and 40 day expression data sets are affected by selection of subpopulations of genes. The populations we are interested in are: Those that lose HP1 binding, those that lose H3K9me3 binding, and those that lose activation marks. For each population, we selected genes that were changing by at least c in either direction– a variable fold change cutoff represented on the xaxis. From those genes that meet the foldchange cutoff in either direction, we plot the percentage that have a higher expression value at 40 days than at 10 days. Pvalues at each fold change are calculated using the hypergometric distribution with the contigency tables Table 2 and Table 3 of this document. Here is an example of the pvalue calculation in R:

```
> k = 34
> m = 2807
> n = 108
> N = 10547
> N_m = N - m
> S = 0
> while (k <= min(n, m)) {
+     S = S + dhyper(k, m, N_m, n)
+     k = k + 1
+ }
> S

[1] 0.1491264
```

0.6.1 Repressive Marks

For the repressive marks, we used the mean signal +- 750 basepairs of the transcription start site to score the genes. Genes are given a score of 1-4 at 10 and 40 days based on which quartile the average signal falls. For HP1, we plotted genes that change in binding by at least two quartiles. For H3K9me3, we only looked at the genes that moved from the 4th quartile at day 10 to the first at day 40 (3 quartile change).

To calculate pvalue, we examine the number of genes that *increase* expression and lose repressive marks, k. We compare this number to the null population (plotted in black bars). The null population is the percentage of genes that go up ($exp40 - exp10 > c$) out of all of the of genes that change ($abs(exp40 - exp10) > c$).

0.6.2 Activation Marks

For the activation marks, each gene was scored based on the number of high binding clusters to which it was assigned. For example, a gene that was in cluster 1 of RNA polymerase at the TSS and cluster 1 of the H3K36 whole gene, but in clusters 2 for the other marks, would have a score of 2. We examined the popluation that lost at least two of the marks at 40 days.

The contingency table is slightly different for the activation marks than the repressive marks. For the activation marks, the pvalue is calculated using the number of genes that *decrease* in expression and lose activation marks as k. The plots, however, show the number of genes that *increase* with activation loss $m - k$ so that we can visually compare the population of interest to the null population plotted in black and the repressive marks plotted in red. See Table 2 and Table 3 of this document for clarification of the difference.

Table 1: Average Signal of Repressive Marks

Sample	Mean euchromatic signal	Mean heterochromatic signal
10d HP1 sample 1	-0.04733	0.9577
10d HP1 sample 2	-0.04089	1.364
10d HP1 sample 3	-0.1328	1.022
40d HP1 sample 1	-0.001783	0.6069
40d HP1 sample 2	0.01352	0.2572
40d HP1 sample 3	-0.03414	0.5974
10d H3K9me3 sample 1	0.01345	0.4324
10d H3k9me3 sample 2	0.04365	0.5388
10d H3k9me3 sample 3	-0.01443	0.6908
40d H3k9me3 sample 1	-0.002237	0.05789
40d H3k9me3 sample 2	0.0243	0.1324
40d H3k9me3 sample 3	-0.000243	0.2836

Table 2: Repressive Marks Test (LOSS)

	Exp UP	Exp DOWN	
Repression loss	k		m
No rep loss			N-m
	n	N-n	N

Table 3: Active Marks Test (LOSS)

	Exp UP	Exp DOWN	
Act mark loss	m-k	**k**	m
No Act mark loss			N-m
	N-n	**n**	N