Supporting Information

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SI Materials and Methods

Antibody Validation. Pol II antibody that recognizes the C-terminal domain repeats of Pol II (8WG16, Covance) protein and a RNA Polymerase III antibody raised against RPC32 subunit (Santa Cruz Biotechnology) were used for this study. ChIP grade antibodies from Santa Cruz Biotechnologies were used for c-Myc, c-Fos, JunD, and c-Jun. Normal IgG control antibodies were purchased from Santa Cruz Biotechnology.

Antibodies specificity was analyzed by (*i*) immunoblot analysis of immunoprecipitated protein and (*ii*) mass spectrometry. Immunoprecipitations (IP) were performed using nuclear extracts of K562 and GM12878 cells for determining optimal concentrations of antibodies that yield maximal signal to noise in IP/Western blot assays. For the mass spectrometry experiments, the immunoprecipitated samples from two separate experiments were fractionated in a 4–15% polyacrylamide gels along with IgG control samples. Tryptic peptides were extracted from 12 to 15 gel pieces from each sample and analyzed by using an Orbitrap mass spectrometer. The shared subunits of polymerases were detected by both anti-polymerase antibodies; however, multiple subunits specific for each polymerase were detected only in the IP product of that polymerase. Likewise, c-Myc, c-Jun, JunD, and Fos antibodies immunoprecipiated their expected proteins but not other transcription factors.

Mapping Short Reads to Reference Genome. Briefly, short reads were first aligned to reference genome with SOAP program, allowing no gaps and up to 2 mismatches, to screen for reads of exonic origin. Second, the remaining reads were aligned to a library of junction sequences to screen for reads spanning exon splicing junctions. The junction library contain all possible splicing events computed based on AceView exons (1) is www.ncbi.nlm.nih.gov/IEB/Research/Acembly.

1. Thierry-Mieg D, Thierry-Mieg J (2006) AceView: a comprehensive cDNA-supported gene and transcripts annotation. *Genome Biol* 7(Suppl 1):S12, 1–14.

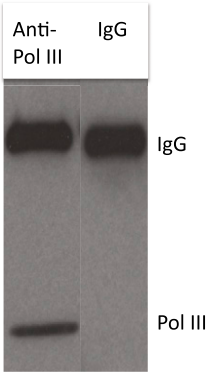

Fig. S1. Validation of Pol III antibody by IP/Western blot. Nuclear proteins extracted from K562 cells were immunoprecipitated by using either anti-Pol III antibody or normal IgG. Immunoprecipitated proteins were separated by SDS/PAGE and Pol III protein was detected by Western blot.

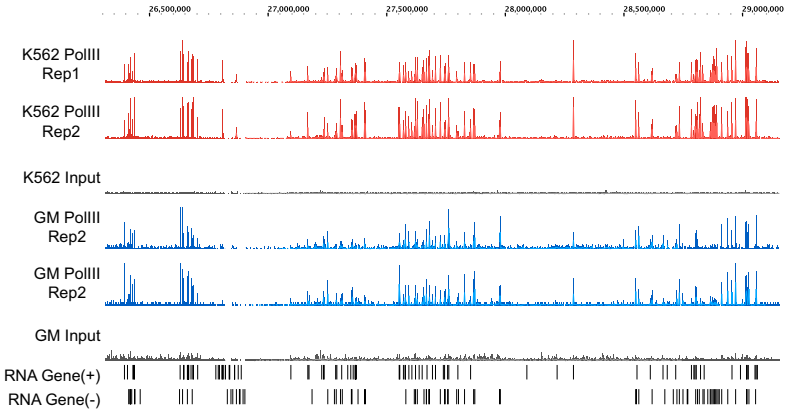

Fig. S2. Reproducibility of Pol III binding. Signal tracks show enrichment of Pol III ChIP DNA near tRNA genes in two biological replicates of K562 (red) and GM12878 (blue). Control Input tracks of K562 and GM12878 show no significant enrichment.

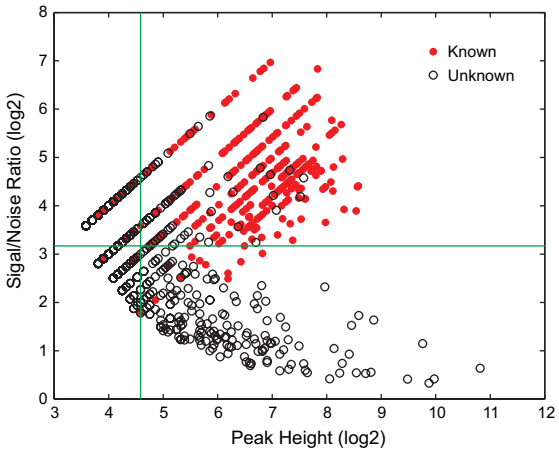

Fig. S3. Criteria for mapping Pol III binding. A stringent criteria that took into consideration peak width, peak height, and signal-to-noise ratio was used to map Pol III ChIP Seq reads. The stringent criteria was applied after peaks were scored by using PeakSeq scoring algorithm to keep false-negative discovery rate to <5%.

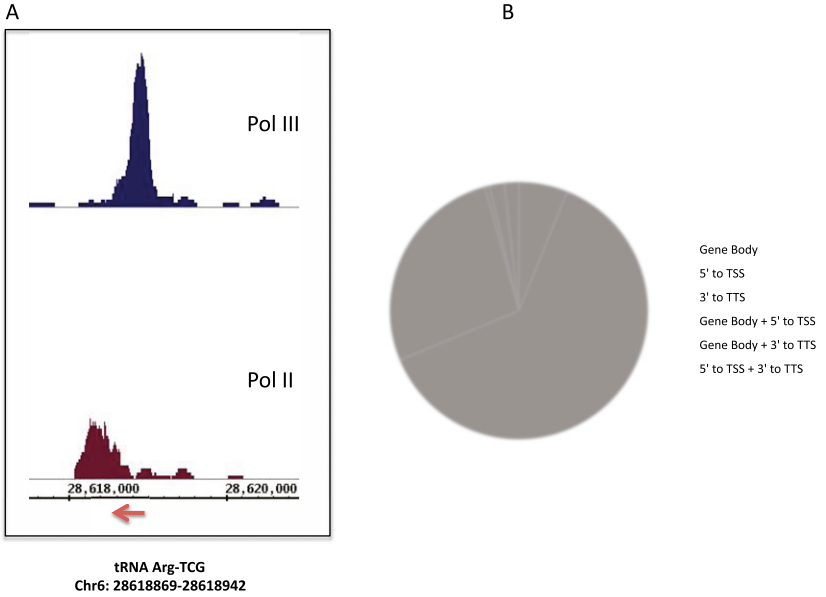

Fig. S4. Localization of Pol II binding sites mapped near Pol III genes. (*A*) Shows an example of a Pol II peak, which mapped downstream (near 3′ end) of a tRNA-Arg gene. (*B*) Shows distribution of all Pol II peaks mapped near Pol III genes. TSS and TTS indicate transcription start site and transcription termination site, respectively.

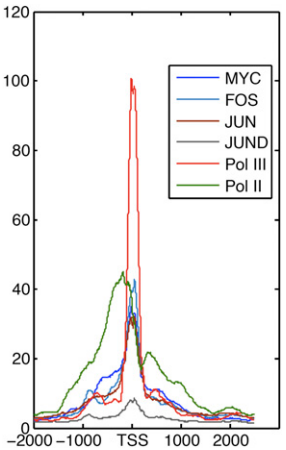

Fig. S5. Aggregate signal plot of Pol II, Pol III, and TF binding. ChIP Seq signal of Pol III, Pol II, c-Myc, c-Jun, c-Fos, and JunD were plotted over 2 kb upstream and downstream region of transcription start site of Pol III genes.

Other Supporting Information Files

Table S1. Mass spectrometry analysis of Pol II and Pol III protein complexes

Proteins immunoprecipitated with Pol II and Pol III antibodies from nuclear extracts were separated by PAGE and proteins were subjected to in-gel tryptic digestion. Eluted peptides were separated and analyzed by mass spectrometry (Orbitrap).

Table S1 (DOC)

Table S2. List of Pol III binding sites detected in K562 and GM12878 cells

Sequence reads from K562 and GM12878 Pol III ChIP DNAs were mapped by using ELAND software (2) to the latest build of human genome hg18 database (UCSC). A cutoff of 500 bases is used to assign a Pol III peak to a gene. Lists show Pol III binding to known and previously unidentified genes.

Table S2 (DOC)

Table S3. Differentially bound Pol III peaks in K562 and GM12878

Sequence reads from Pol III ChIP DNA of K562 and GM12878 were normalized with respect to total number of mapped reads from corresponding samples, and the log ratio was used to determine differentially bound Pol III peaks.

Table S3 (DOC)

Table S4. Expression of Pol III genes RNA sequence reads from untreated K562 cells generated by RNA Seq were mapped to coordinates of Pol III peaks

A cutoff of 10 uniquely mapped sequence reads was used to consider a Pol III binding site transcriptionally active.

Table S4 (DOC)

Table S5. Summary of ChIP Seq

Data summarizes number of total reads, uniquely mapped reads, and binding sites for Pol II, Pol III, c-Fos, c-Myc, c-Jun, and JunD obtained from ChIP Seq experiments.

Table S5 (DOC)

Table S6. Pol III genes down-regulated in α-amanitin–treated cells show fold decrease of expression of 22 Pol III genes in α-amanitin–treated cells

RNA sequence reads of untreated and α-amanitin–treated cells were normalized using 5.8S reads. Spike-in control RNA data indicated no significant change in 5.8S RNA level caused by α-amanitin treatment.

Table S6 (DOC)