Suz12 ChIP-on-chip

Supplementary Data S1: Murine embryonic stem cells (mESC, clone CK35) were grown on a layer of mouse embryonic fibroblasts (MEF) in the presence of leukemia inhibitory factor (LIF) on gelatinised plates. mESC were crosslinked as previously described (Boyer *et al*., 2006) after separation from MEF in the presence of a final concentration of 1% formaldehyde for 10 minutes. $5x10^7$ cells were lysed as previously described (Boyer *et al*., 2006) and the chromatin was sonicated to an average size of 400bp using 10 cycles of 30 second bursts and 30 second resting on ice using a VibraCell (VibraCell, Danbury, USA) sonicator. For the replicate ChIP's, 50µl of ProtG-coated Dynalbeads (Invitrogen, USA) were incubated at 4˚C overnight with 5µg of anti-Suz12 antibody (Abcam, ab12073) as previously described (Boyer *et al*., 2006). Extracts corresponding to $2.5x10^7$ cells were added and incubated overnight at 4˚C. Beads were washed 5 times using RIPA buffer (50mM Hepes pH 7.6, 500mM LiCl, 10mM EDTA pH 8, 1% NP-40, 0.7% Na-Deoxycholate) and 1 time TE-NaCl (10mM Tris pH8, 1mM EDTA pH 8, 50mM NaCl). Two subsequent elutions at 65˚C were performed for 15 minutes using elution buffer (50mM Tris pH 8, 10mM EDTA pH 8, 1% SDS) and crosslinks were reversed by overnight incubation at 65˚C together with an input fraction. Samples were RNase and proteinase K digested and purified using two subsequent phenol:chloroform:isomaylalcohol extractions and a Qiaquick PCR purification column (Qiagen, Germany). DNA was eluted in water and known targets were validated by qPCR (Boyer *et al*., 2006). ChIP and input were amplified using a two-round T7 amplification protocol (van Bakel *et al*., 2008). The size of the RNA was validated on a bioanalyzer (Agilent, USA), indirectly labelled, 4.5µg of input and ChIP material hybridised to an Agilent mouse promoter array set and washed as previously described (van Bakel *et al*., 2008). The slides were washed and scanned in an ozone-free environment using an Agilent scanner and extracted using the Agilent Feature Extraction software.

This specific set of data is available at http://www.ciml.univ-mrs.fr/software/ferrier.htm.

References:

Boyer,L.A. et al. (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature,* 441, 349-353.

van Bakel,H., et al. (2008) Improved genome-wide localization by ChIP-chip using double-round T7 RNA polymerase-based amplification. *Nucleic Acids Res.,* 36, e21.