1. Description

Temporal and tissue-specific gene expression in mammals depends on complex interactions between transcriptional regulatory proteins and cis-elements such as promoters, enhancers and insulators.

Using the laboratory mouse as a model system, we are using chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) to conduct genome-wide analysis of active promoters, enhancers and insulator elements in mouse embryonic stem cells, embryonic fibroblasts, and a panel of embryonic and adult tissues. We will identify tissue specific promoters and enhancers, and characterize the regulatory mechanisms that control the gene expression programs in the specific tissues.

2. Harvesting Cortex Tissue

The head is cut off by slicing through cervical spinal cord. Using small scissors, the skull is cut through from the back of the head, entering through the hole where spinal cord meets the hindbrain. Cut along both sides laterally and, if preferred, along the dorsal midline as well. Peel back the bones with sturdy forceps. Remove the brain by sliding forceps under the brain and gently breaking the cranial nerves holding it in place. Lift from underneath and remove the whole brain in one piece.

Using fine tipped forceps, slip into the sulcus separating the cortical plate from the rest of the brain. Once separated, remove the meninges from the cortex surface carefully in cold PBS with fine tipped forceps under a dissecting microscope. Mince completely with a razor blade while on ice.

3. Enrichment and Library Preparation

Chromatin immunoprecipitation was performed according to
http://bioinformatics-renlab.ucsd.edu/RenLabChipProtocolV1.pdf
Library construction was performed according to
http://bioinformatics-renlab.ucsd.edu/RenLabLibraryProtocolV1.pdf

4. Sequencing and Analysis

Samples were sequenced on an Illumina Genome Analyzer GAII for 36 cycles. Image analysis, base calling and alignment to the mouse genome version mm9 were performed using Illumina's RTA and Genome Analyzer Pipeline software. Alignment to the mouse genome was performed using ELAND with a seed length of 25 and allowing up to two mismatches. Only the sequences that mapped to one location were used for further analysis. Of those sequences, clonal reads, defined as having the same start position on the same strand, were discarded. BED and wig files were created using custom perl scripts.