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 Liver

Secure mouse to a piece of Styrofoam or a dissecting pad with pins through its hands and feet, stretched out completely, but not too tight, to each corner. Grab skin below the center of the belly with forceps and cut through the skin only, not the underlying abdominal cavity. Cut along the ventral midline of the mouse stopping when even with the shoulders. Gently pull at the skin to separate it from the underlying abdominal and thoracic cavities.

Cut into the abdominal cavity following the same line you cut along through the skin. Remove each lobe of the liver by lifting up with forceps and cutting through where it attaches to the abdominal cavity with scissors. Wash with cold PBS and 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.