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 Embryonic Limb Tissues

While the embryos are still on ice, use fine tip forceps to remove all four limbs by pinching close to where the limbs join with the torso. After collecting from all embryos, use fine tipped forceps to carefully mince limbs in a small amount of cold PBS.

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.