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 Bone Marrow

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.

Using your hands, pull skin back over the legs, grabbing the quadriceps to pull the legs up through the skin, exposing them completely. The skin may also be cut away completely from the legs of needed. Using sharp scissors, poke through the leg perpendicularly just underneath the femur and open the scissors to widen the hole. Do the same thing just above the femur, and on either side. Cut away tissue around femur using these holes as starting points. Cut through femur as close as possible to the pelvis with scissors, removing the whole leg. Cut femur again right next to the knee and place in cold PBS on ice. Now, holding the foot, cut the tendons connecting the foot to the calf muscles all around the bone. Cut through the fibula near where it fuses with the tibia and pull this bone, along with the calf muscles now disconnected from the ankle, away from the foot, peeling it to the knee. Cut this tissue away with scissors. Cut the tibia right under the knee and right above the ankle. Place in cold PBS on ice. Repeat the same procedure for the other leg. Once this is done, while holding one of the bones with forceps, insert a syringe with a 27 gauge needle filled with PBS into one end of the bone. Squirt the PBS through the bone, which will push out the bone marrow to be collected into a petri dish. Repeat this until the bone appears almost completely white. Repeat this with all four bone fragments. Pipette up and down with an uncut pipette tip to break up the bone marrow before crosslinking.

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.