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. Culturing and Harvesting the Mouse Embryonic Fibroblasts (MEF)

E13.5 timed pregnant mice were ordered from Charles River, Cambridge, MA. The uterus (containing the embryos) was cut free from the mouse and placed in a sterile petri dish. Embryos were removed from the uterus one at a time in sterile PBS in the tissue culture hood. Head and all of the soft tissues from each embryo were removed under a dissecting scope so that only the carcasses are left. Embryo carcasses were minced using small scissors, and then washed with PBS 2-3 times in the 15ml cornical tubes. Enough trypsin-EDTA solution was added to just cover each minced carcasses. The mixture was then incubated at 37°C for 30 minutes. The MEF media (10 mL) was then added to the cornical tubes, and the tissue was dissociated by vigorous pipetting. By this point, most of tissue should have been dissociated into single cells. The digested tissue was passed through a 70μM cell strainer to get rid of un-dissociated tissue clumps. The cells that flowed through the cell strainer were washed once with fresh MEF media, and cells from each embryo were plated to individual 175cm flasks. MEF cells were passaged every 3-4 days or until they were confluent. DNA samples were collected from discarded head or soft tissue for genotyping to determine the sex of each embryo.

MEF culture media: to make 500ml, add 450ml of DMEM with high glucose (4.5g/L), 50ml of FBS, 5ml of Penstrep, and filter sterile.

Trypsin-EDTA: 0.25%

Crosslinked buffer: 0.1M NaCl, 1mMEDTA, 0.5mM EGTA, 50mM Hepes (pH 8.0) and add fresh 37% formaldehyde to a final concentration of 11% before use.

For harvesting MEF cells, chromatin samples were harvested from early passaged MEF cells (e.g. passage P3). 1/10 volume crosslinked buffer was added to the culture dish to fix the cells at room temperature for 15 minutes. Then 1/20 volume of 2.5M Glycine was added to quench crosslinking by incubating the cells at room temperature for 5 minutes. Cells were then washed with cold PBS twice in the culture dish. Cold PBS was added to the dishes again for scraping the cells off the dishes, and transferring to a cornical tube. Cells collected in the cornical tubes were centrifuged at 3,000 RPM for 10 minutes at 4°C. Cell pellets were snap frozen in liquid nitrogen and stored at -80° C until further processing.

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.