TRANSCRIPTIONAL GENOMICS

The DERC Transcriptional Genomics Core Facility provides three services; conventional gene expression analysis using commercial microarrays, ChIP-Chip analysis using the custom DERC 34K mouse promoter microarray, and bioinformatics support. An overview of the Transcriptional Genomics Core Facility is provided through the DERC website (http://www.derc.med.ucla.edu/core.htm). In this update, I provide background information and preliminary data that can be used in support of grant applications that propose to use the DERC 34K mouse promoter microarray. The original files can be obtained upon request at ckg@ucsd.edu.

BACKGROUND AND SIGNIFICANCE: GENOME-WIDE LOCATION ANALYSIS

Understanding how DNA binding proteins control gene expression at a global level requires knowledge of the chromosomal locations at which these proteins function. Currently, chromatin immunoprecipitation (ChIP) is used to determine whether a particular DNA binding protein is bound to the promoter or enhancer of a specific gene in living cells. In 2000, Richard Young’s laboratory demonstrated that it was possible to modify this assay to allow genome wide location analysis (GWLA) using yeast as an experimental organism (1). This methodology, generally referred to as ChIP-Chip, identified the promoters of all of the known genes for the yeast transcription factor Gal4, as well as several new genes that were confirmed by conventional ChIP assays and additional functional assays (1). This assay has subsequently been exploited to define a diverse range of transcriptional networks in yeast and to test specific hypothesis related to histone modifications and transcriptional control (1-3).

Despite the increased complexity of mammalian genomes, ChIP-Chip has recently been extended to explore histone modifications and the binding of transcription factors at specific chromosomal locations in human cells (4-8). To take advantage of the discovery potential of GWLA and the experimental power of mouse models of development and disease, the UCLA/UCSD/Cedar Sinai/Salk Institute DERC obtained supplemental funding through the NIH to develop a comprehensive mouse promoter microarray. The mouse promoter/genome microarray consists of 33,827 features (Mu34K array). The selection of promoters was based on the genes represented on Affymetrix, Codelink, Qiagen and Agilent expression microarrays. This strategy allows ChIP on chip data obtained from the Mu34K array...
to be compared to expression data obtained on any of the major, commercially 
platforms. Promoter sequences of 26,921 genes were extracted from the UCSC 
database, based on alignment of mRNA, Est sequences and location of CpG 
islands. The larger number of promoters than genes is accounted for by the 
presence of one or more alternative 
promoters for about 20% of the selected 
genes. Because this design does not 
allow for detection of events that occur 
at enhancer and silencer elements that 
are located far away from transcriptional 
start sites, 3015 additional features were 
designed to tile 48 genes of interest 
from –40 k b from the 
transcriptional 
start site to the end of the transcription 
unit. These features will provide insights 
into the extent to which important 
events are occurring beyond the 
proximal promoter.

PRELIMINARY STUDIES

An example of the enrichment of 
promoter sequences containing histone 
H3 modified by trimethylation at lysine 4 
in a single experiment is illustrated in 
Figure 1a. This histone mark is 
correlated with actively transcribed 
genes (9). Using a false discovery rate 
of 5% as a cutoff, the overlap in 
enriched genes identified in three 
independent experiments exceeded 
90%, indicating a high degree of 
reproducibility between experiments. 
Combining the complete data set led to 
identification of 6291 genes being 
consistently identified (Figure 1b). Of the subset of genes for which 
corresponding expression data was available, 96% of the promoters exhibiting 
the histone H3 trimethyl lysine 4 mark were found to be expressed. The 
overlap of promoters exhibiting trimethyl histoneH3K4, dimethyl histone H3K4 
and acetylated H3K9 in control RAW 264.7 macrophages is illustrated in Figure 
1b. Intriguingly, the AcH3K9 mark is almost completely dependent on the 
presence of the trimethyl H3K4 mark. Treatment of RAW 264.7 macrophages 
significantly increases the number of promoters that also contain the dimethyl 
H3K4 mark, resulting in an increase in promoters that exhibit all three marks 
(Figure 1c).

To extend the utilization of the Mouse 34K promoter microarray, we have 
performed GWLA experiments for the glucocorticoid receptor. The GR agonist 
dexamethasone (Dex) strongly in 
duces a large number of genes in 
macrophages in addition to repressing inflammatory response genes. To 
explore GR regulation of gene expression at a genome-wide level, we 
performed ChIP-Chip experiments in control and Dex-treated RAW264.7 cells. 
To optimize the ChIP assay, conventional ChIP experiments demonstrated that the 
MT1 promoter, which is Dex-responsive, was enriched following 
immunoprecipitation with a specific anti-GR antibody (Figure 2a). RT-PCR 
quantification of the immunoprecipitated product indicated a 7.5-fold increase in 
occupancy following dex treatment (Figure 2b). Using these conditions, GR was 
recruited to 1128 promoters represented on the Mouse 34 K promoter array in 
the presence of dexamethasone, including the MT1 promoter (Figure 2c). In 
addition to the glucocorticoid receptor, the Mouse 34 K array has been used to 
localize binding sites for PPARγ in adipocytes, PU.1 in myeloid progenitor cells, 
and the PGC1α coactivator in macrophages. The methodology currently in place 
is highly specific and reliably detects enriched promoters in a manner that is 
consistent with current concepts regarding the association of specific histone 
marks and transcriptional status.
PROCEDURE

Chromatin Immunoprecipitation (ChIP) assay

Cells are cross-linked with 1% formaldehyde at room temperature for 10 minutes and the cross-linking reaction is stopped by the addition of 125 mM Glycine. The chromatin is prepared and sonicated to produce DNA fragments between 150 and 500 bp in size. Immunoclearing is performed and supernatants are subjected to immunoprecipitation with 1-5 µg polyclonal antibodies overnight at 4°C. Then 45 µL protein A-Sepharose is added and the incubation is continued for 4 hours. Precipitates are washed and extracted with 1% SDS, 0.1 M NaHCO3 and heated at 65°C overnight to reverse the cross-linking. DNA fragments are purified on QIAquick Spin columns (Qiagen, Valencia, CA). One µL from a 50 µL extraction are amplified in duplicates by quantitative PCR on a 7300 Real Time PCR System from Applied Biosystem (Forster City, CA).

ChIP on chip

For ChIP on chip experiments, the immunoprecipitated and input samples are amplified by Ligation Mediated PCR (LM-PCR) as previously described and respectively labeled with Cy3 and Cy5 fluorescent dyes (Amersham). Two µg of labeled material were mixed with mouse Cot1 DNA (Invitrogen, Carlsbad, CA) and yeast tRNA (SIGMA, St Louis, MO) and hybridized on the Mu34K array according to the conditions previously described. Arrays are scanned using an Axon scanner (Axon Instruments, Foster City, CA) and the intensity of the spots determined using the GenePics software. A software program that performs a modified Lowess normalization, filters out the low intensity spots corresponding to negative controls and the flagged spots plots the data as an M.A. graph. Data from 3 independent hybridizations corresponding to 3 biological replicates are then analyzed together in order to provide a p value for each feature. Data from tiled regions are represented in the UCSC format.

References


DIRECTORS’ MESSAGE

Because of the NIH Roadmap and other changes, the NIDDK had a DERC Directors meeting in November, 2005. The new mission statement of the DERCs is as follows:

The prevalence of diabetes mellitus in the United States is reaching epidemic proportions and accounts for a huge national burden of morbidity, mortality, and health care expenditures. The mission of the Diabetes Centers is to serve as a key component of the NIH program to improve the health of Americans with diabetes and related endocrine and metabolic disorders. The Centers promote new discoveries and enhance scientific progress through support of cutting-edge basic and clinical research related to the etiology and complications of diabetes, with the goal of rapidly translating research findings into novel strategies for prevention, treatment and cure of diabetes and related conditions.

To accomplish this mission, the Diabetes Centers:

- Create an environment that supports important and innovative research
- Raise awareness and interest in diabetes research and clinical care at their institutions and locally, regionally, and nationally
- Enhance diabetes education and training opportunities for patients, students, scientists, and clinicians
- Attract and retain new and young investigators
- Provide core services that leverage funding and unique expertise
- Foster interdisciplinary collaborations, especially in the emerging areas of research, to catalyze new ideas and scientific approaches
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There was encouragement that the DERCs align themselves with the new Centers for Translational Science Awards (CTSAs) and that we develop a plan for enhancing translational research within our DERC. If you have any suggestions, please email Willa Hsueh or Jerrold Olefsky.

For more information visit our website!

http://www.derc.med.ucla.edu