Genomics Data 2 (2014) 82–84

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

CTCF regulates positioning of the human cystic fibrosis gene in association with a histone deacetylase

Joscha Muck *

Institute of Bioengineering and Nanotechnology (IBN), Department of Cell and Tissue Engineering, 31 Biopolis Way, The Nanos, 138669, Singapore

ARTICLE INFO

Article history:
Received 12 April 2014
Accepted 2 May 2014
Available online 10 May 2014

Keywords:
Trichostatin A
Histone acetylation
ChIP–chip
Agilent
HeLa

ABSTRACT

The nuclear positioning of mammalian genes often correlates with their functional state. For instance, the human cystic fibrosis transmembrane conductance regulator (CFTR) gene associates with the nuclear periphery in its inactive state, but occupies interior positions when active. Treatment with the histone deacetylase inhibitor trichostatin a (TSA) changes the radial positioning of the CFTR gene in HeLa S3 cells. The gene relocates from the nuclear periphery to the nuclear interior. In Calu-3 cells the gene is located in the nuclear interior. To identify potential regulatory elements for the positioning of CFTR, the histone H3 and H4 acetylation patterns of untreated and TSA-treated HeLa S3 and untreated Calu-3 cells were determined by ChIP–chip. Here is a detailed description of the datasets associated with the study by Muck et al. published in the Journal of Cellular Biochemistry in 2012.

© 2014 The Author. Published by Elsevier Inc. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

Specifications	
Organism/cell line/tissue	*Homo sapiens*/HeLa S3, Calu-3/cervix adenocarcinoma, lung adenocarcinoma
Sex	HeLa S3 (female) Calu-3 (male)
Sequencer or array type	Agilent 4x44K DNA-chips
Data format	Raw data: GPR files, processed data: SOFT, MINIML, TXT and RData
Experimental factors	Histone modification, TSA treatment, celltype
Consent	n/a

Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29360.

Experimental design, materials and methods

Cell culture

HeLa S3 and Calu-3 cells were obtained from the American Type Culture Collection (Manassas, VA). HeLa cells were cultivated with DMEM containing 10% fetal bovine serum. Calu-3 cells were cultivated as recommended. TSA (Sigma-Aldrich, Singapore) was used at a concentration of 10 ng/ml.

Antibodies

The rabbit anti-histone H3 (ab 1791) antibody was purchased from Abcam (Cambridge, UK).

The rabbit anti-acetyl histone H3 (06-599) and anti-acetyl histone H4 (6-866) antibodies were purchased from Millipore (Billerica, MA).

Chromatin immunoprecipitation (ChIP)

For cross-linking chromatin immunoprecipitation (X-ChIP) the Abcam protocol (http://www.abcam.com/index.html?pageconfig=resource&rid=11698) was applied with the following modifications: cross-linking was performed with 1.7% formaldehyde for 6 min. Cells were lysed in RIPA buffer (150 mM NaCl, 0.25% deoxycholate, 1% Triton X-100, 50mMTris–HCl, pH 8.0) with 2 mM EDTA and freshly added protease inhibitor set (complete mini, Roche, Basel, Switzerland). Chromatin was sonicated with a HTU Soni 130 ultrasonic homogenizer (G. Heinemann, Schwäbisch Gmünd, Germany). Gelelectrophoresis and densitometric analysis revealed that ~60% of the fragments had a length of <200 bp (~80% <1,000 bp). Immunoprecipitations were performed overnight at 4 °C using 5 µl of the respective antibody, bound to protein A/G Dynabeads (Invitrogen). The A/G Dynabeads were washed 3 times using RIPA buffer, resuspended in 100 ml PBS with 10 ml 5 M NaCl-solution, and incubated for 6 h (or overnight) at 65 °C to reverse cross-links. DNA was purified using the Qiaquick PCR Purification kit (Qiagen, Hilden, Germany). Amplification of the ChIP–DNA was

* Umeå Centre for Molecular Medicine, Umeå University, SE-901 87 Umeå, Sweden. Tel.: +46 7205055411.
E-mail address: joscha.muck@umu.se.

http://dx.doi.org/10.1016/j.gdata.2014.05.002
2213-5960/© 2014 The Author. Published by Elsevier Inc. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

Table 1
Data series and description ((*) indicates reverse reference).

#	Series	Ratio	Description
1	HeLa_H3ac_532_H3pan_635_control_REP_1	532/635	HeLa untreated, H3ac/H3pan
2	HeLa_H3ac_635_H3pan_532_control_REP_2	635/532	HeLa untreated, H3ac/H3pan
3	HeLa_H3ac_635_H3pan_532_control_REP_3	635/532	
4	HeLa_H3ac_635_H3pan_532_control_REP_4	635/532	
5	HeLa_H3ac_532_H3pan_635_TSA_REP_1	532/635	HeLa TSA-treated, H3ac/H3pan
6	HeLa_H3ac_635_H3pan_532_TSA_REP_2	635/532	HeLa TSA-treated, H3ac/H3pan
7	HeLa_H3ac_635_H3pan_532_TSA_REP_3	635/532	
8	HeLa_H3ac_635_H3pan_532_TSA_REP_4	635/532	
9	Calu_H3pan_635_HeLa_H4ac_control_532_REP_2	635(#12)/635	Calu-3, H3ac/H3pan
		532/532(#2)	HeLa untreated, H4ac/H3pan
10	Calu_H3pan_635_HeLa_H4ac_control_532_REP_3	635(#13)/635	Calu-3, H4ac/H3pan
		532(#3)/532	HeLa untreated, H4ac/H3pan
11	Calu_H3pan_635_HeLa_H4ac_control_532_REP_4	635(#14)/635	Calu-3 H4ac/H3pan
		532/532(#4)	HeLa untreated, H4ac/H3pan
12	Calu_H3ac_635_HeLa_H4ac_TSA_532_REP_2	635/635(#9)*	Calu-3, H3ac/H3pan
		532/532(#5)	HeLa TSA-treated, H4ac/H3pan
13	Calu_H4ac_635_HeLa_H4ac_TSA_532_REP_3	635/635(#10)*	Calu-3, H4ac/H3pan
		532/532(#6)	HeLa TSA-treated, H4ac/H3pan
14	Calu_H4ac_635_HeLa_H4ac_TSA_532_REP_4	635/635(#11)*	Calu-3, H4ac/H3pan
		532/532(#7)	HeLa TSA-treated, H4ac/H3pan

performed with the Genome-Plex Complete WGA Kit (WGA2) according to the manufacturer's instructions (Sigma-Aldrich). For labeling the Label IT mArray Labeling Kit Cy3/Cy5 (Mirus Bio LLC, Madison, WI) was applied.

DNA microarray design

The DNA microarrays were purchased from Agilent Technologies (Santa Clara, CA) and were designed with the software eArray (https://earray.chem.agilent.com/erray/). Validated isothermal probes within base pairs 116,200,000–117,400,000 (human genome assembly hg18) of human chromosome 7 were selected and a set of 9968 individual probes was obtained. Each chip contained four identical sectors and each sector contained four identical individual probe sets. In addition, each sector contained the (−) 3xSLv1 control set of probes and other controls. The design file can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL13608.

Hybridization was performed according to the manufacturer's instructions using the Stabilization and Drying kit (Agilent Technologies).

Experimental setup

ChIP of histone H3ac and H4ac and the reference ChIP of histone H3pan for each condition and cell type were performed in parallel, using the same chromatin. Amplified DNA samples derived from the same experiment were hybridized on the same 4x44K microarray (the microarray is indicated by the index "_REP_ ()" in the series record). In case of HeLa cells, DNA derived from the histone H3ac and H3pan ChIP was hybridized on the same sectors. DNA samples derived from the histone H4ac ChIP were hybridized on different sectors on the same array (using the same labeling dye as for their respective histone H3pan reference sample). For Calu-3 cells, DNA samples derived from the histone H3ac and H4ac ChIP were hybridized on separate sectors on the same array as their respective histone H3pan reference (using the same labeling dye in all cases).

Raw data extraction

DNA microarrays were scanned with an Axon Genepix 4200AL Scanner (Molecular Devices, Sunnyvale, CA) using the software Genepix Pro 6.0 at a resolution of 10 μm. Averaging was set to 2. For background estimation the (−) 3xSLv1 probes were used. For each probe the background was calculated by averaging the signal intensity of the 3 closest (−) 3xSLv1 probes (local background method).

Raw data files were deposited in the Gene Expression Omnibus database (GEO; [2]) and can be found here: http://www.ncbi.nlm.nih.gov/geo/download/?acc=GSE29360&format=file&file=GSE29360%5FRAW%5FGPR%5FFILES%2Etar%2Egz.

To calculate the enrichment levels, the extracted RAW data files were combined according to Table 1.

Data analysis

For quality control, probes that failed to meet the following criteria were "flagged" and excluded from further analysis: (1) Signal to noise ratio above 3 for both channels (Genepix Pro calculates noise as the standard deviation of the background pixels); (2) less than 10% of pixels saturated; (3) mean pixel intensity less than 1.2 times the median pixel intensity. The mean intensities of the four technical replicates (per array) of each probe were averaged.

ChIP–chip data were analyzed using the Microsoft Excel add-in Macro ChIPOTle [3]. The algorithm is based on a relatively simple model, assuming relatively few regions with relevant enrichment. Low levels of enrichment are modeled as "noise" and should follow a lognormal distribution. Quantile–quantile (Q–Q) plots were generated as described (http://lieblab.bio.unc.edu/ChIPOTle/ChIPOTle_Readme_v1.0.pdf) in order to determine whether the majority of data points display a lognormal distribution. Q–Q plots were analyzed by linear regression analysis and in all cases a lognormal distribution for data points with low enrichment levels was observed. For cross sector comparisons the datasets were normalized by generating histograms of log-transformed enrichment levels and setting the maximum of these histograms (center of the "noise") to zero. Datasets of the same kind were then averaged and processed data files were deposited in GEO under accession number GSE29360.

Statistics

Statistical significance testing was performed on the log-transformed datasets using the Microsoft Excel add-in Macro ChIPOTle. The following parameters were selected: sliding window of 1000 bp, step size of 250 bp, normal distribution and a P-value of 0.001.

qPCR validation

ChIP–chip data were validated at 63 positions using qPCR as described in [1].

Discussion

The datasets described were generated to analyze the relation between histone acetylation and radial gene positioning of the human CFTR locus. By monitoring local changes in histone acetylation during the process of gene repositioning, a critical binding site and consecutively a regulatory factor could be identified [1]. This is an example of how changes in a "generic" epigenetic modification, like histone acetylation, can be used to narrow down critical binding sites and potential regulatory factors.

References

[1] J.S. Muck, K. Kandasamy, A. Englmann, M. Günther, D. Zink, Perinuclear positioning of the inactive human cystic fibrosis gene depends on CTCF, A-type lamins and an active histone deacetylase. J. Cell. Biochem. 113 (8) (Aug 2012) 2607–2621, http://dx.doi.org/10.1002/jcb.24136.
[2] T. Barrett, T.O. Suzek, D.B. Troup, S.E. Wilhite, W.C. Ngau, P. Ledoux, D. Rudnev, A.E. Lash, W. Fujibuchi, R. Edgar, NCBI GEO: mining millions of expression profiles-database and tools. Nucleic Acids Res. 33 (Database issue) (Jan 1 2005) D562–D566.
[3] M.J. Buck, A.B. Nobel, J.D. Lieb, ChIPOTle: a user-friendly tool for the analysis of ChIP–chip data. Genome Biol. 6 (11) (2005) R97 (Epub 2005 Oct 19).