Analysis of Long Noncoding RNA Chromatin Interactions by Chromatin Isolation by RNA Purification (ChIRP)

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ABSTRACT

Gene regulation plays a critical role in complex cellular processes such as development, differentiation, and cellular response to environmental changes. While the regulation of gene expression by transcription factors and epigenetic influences has been well studied over time, pervasive genomic transcription and the role of non-coding RNAs in this process is a rapidly evolving field that remains to be thoroughly explored.

Chromatin Isolation by RNA Purification (ChIRP) is a method that allows discovery of the sites of interaction of chromatin-associated RNAs (e.g. lncRNAs) with genomic DNA sequences by using probe-based hybridization to target RNA molecules in chromatin. DNA can be isolated from recovered chromatin and analyzed by quantitative PCR or next generation sequencing (ChIRP-seq). Alternatively, RNA may also be isolated to detect other RNA molecules that may be associated with the RNA of interest.

To enable the exploration of these RNA interactions in chromatin regulation, we have optimized the methods and developed ChIRP reagents. Using these reagents ChIRP experiments can be performed with reliable recovery of chromatin using lncRNA or other chromatin associated RNA as targets. Additionally, negative and positive control probe sets, and detection primers were developed to ensure first time success. We have performed ChIRP experiments with a HeLa cell lysate and capture oligos for the NEAT1 lncRNA. Isolated DNA was subjected to NGS library construction and sequenced on an Illumina HiSeq™ instrument. Sequences were aligned to the reference genome (hg19) and peaks were called. We successfully identified several NEAT1 binding sites in the genomic DNA sequence.

In summary the methods developed allow unbiased discovery of RNA-associated genomic DNA sequences, RNA sequences, and potentially proteins.

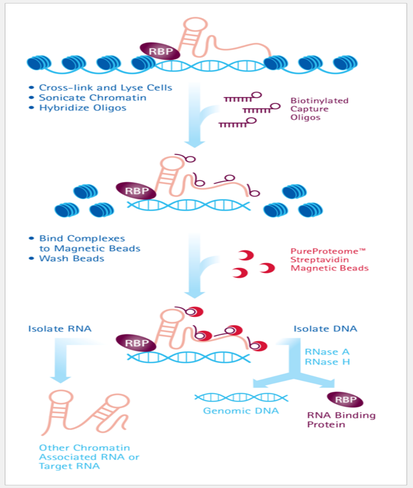
MATERIALS and METHODS

ChIRP
HeLa Cells were cross-linked with 1.0 % glutaraldehyde for 10 minutes then ChIRP was performed with following EMD Millipore kits and probe sets.
Magna ChIRP™ RNA Interactome Kits (Cat. No. 17-10494, 17-10495)
Magna ChIRP Negative Control Probe Set (Cat. No. 03-307)
Magna ChIRP NEAT1 lncRNA Probe Set (Cat. No. 03-308)
Magna ChIRP TERC Probe Set (Cat. No. 03-309)

qPCR and NGS Analysis
Percent input recovery was calculated by standard curve drawn with 1% or 10% input sample or delta delta Ct methods.

Sequencing
ChIRP DNA was column purified and sequencing libraries were prepared with PureGenome™ Low Input NGS Library Construction Kit (EMD Millipore Cat. # 17-10492). Sequencing was performed using HiSeq (Illumina)

Magna ChIRP Kits Components
PureProteome™ Streptavidin magnetic beads
10X Glycine
Lysis Buffer
Hybridization Buffer (without 15% formamide)
Wash Buffer
Proteinase K Buffer (for DNA)
Proteinase K Buffer (for RNA)
DNA Elution Buffer
0.5M EDTA
RNase Inhibitor
Protease Inhibitor Cocktail III, Animal Free **Contains DMSO
Proteinase K Solution, 600mAU/mL
DNase I RNase free
DNase I Reaction Buffer
RNase A (20 mg/mL)
RNase H (10U/ µL)
Magna ChIRP™ Negative Control Probe Set (LacZ) 50 µM
Components of 17-10495 Only
Magna ChIRP™ TERC lncRNA Probe Set (Even) 50 µM
Magna ChIRP™ TERC lncRNA Probe Set (Odd) 50 µM
RNA Positive Control Primers (TERC Gene)
RNA Negative Control Primers (GAPDH Gene)
Magna ChIRP™ Primers, WNT-1 precursor
ChIP Primers, GAPDH coding D2



Chromatin Fragmentation: Sheared DNA (100-500 bp in Length)

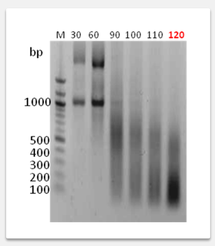


Figure 1. Chromatin Preparation
HeLa cells were cross-linked with 1.0% glutaraldehyde for 10 minutes.
Cells were lysed in Lysis Buffer. Time course experiments were performed with Q800R Sonicator (Qsonica). DNA was extracted by column after proteinase K digestion and analyzed by agarose gel. Cell lysate sonicated for 120 minutes (Lane 7) resulted in the best shearing for ChIRP experiments.

RESULTS

Example of Magna ChIRP Data Using TERC Probe Set

A. Successful retrieval of RNA by ChIRP with TERC probes

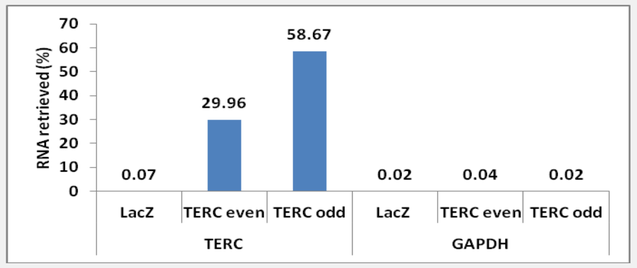


Figure 2.
ChIRP was performed using HeLa cell lysate and either Magna ChIRP TERC lncRNA Probe Set even odd or Magna ChIRP Negative Control Probe Set (LacZ). Purified RNA was then analyzed by qRT-PCR using RNA Positive Control Primers (TERC) and RNA Negative Control Primers (GAPDH).

B. Successful DNA binding by ChIRP with TERC probes

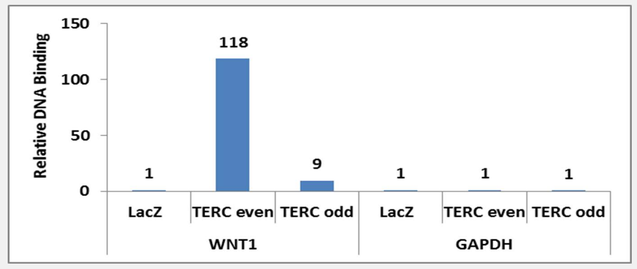


Figure 3.
ChIRP was performed using HeLa cell lysate and either Magna ChIRP™ TERC lncRNA Probe Set even odd or Magna ChIRP™ Negative Control Probe Set (LacZ). Purified DNA was then analyzed by qPCR using Magna ChIRP™ Primers, WNT-1 precursor (Positive target) and ChIRP Primers, GAPDH coding D2 (Negative Target).

Successful Detection of lncRNA NEAT1 Binding Sites by ChIRP-seq with lncRNA Probe Sets NEAT1

A. Successful retrieval of RNA by ChIRP with NEAT1 probes

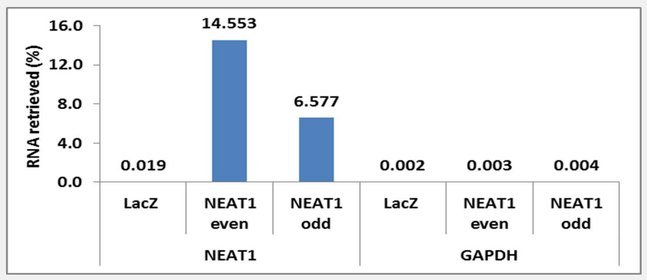


Figure 4.
ChIRP was performed using HeLa cell lysate and either Magna ChIRP NEAT1 lncRNA Probe Set even, odd or Magna ChIRP Negative Control Probe Set (LacZ). Purified RNA was then analyzed by qRT-PCR using Primers specific for NEAT1 (Positive target) and GAPDH (Negative Target).

B. Successful DNA binding by ChIRP with NEAT1 probes

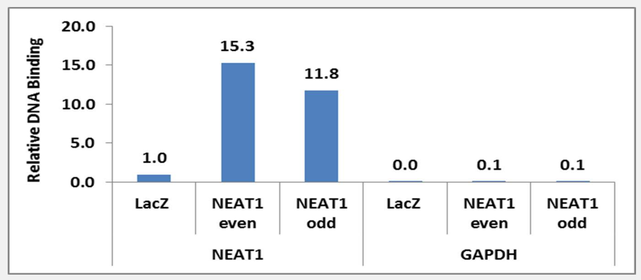


Figure 5.
ChIRP was performed using HeLa cell lysate and either Magna ChIRP NEAT1 lncRNA Probe Set even, odd or Magna ChIRP negative control probe set (LacZ). Purified DNA was then analyzed by qPCR using Primers specific for NEAT1 coding region and GAPDH coding D2 (Negative Target).

B. Successful DNA binding by ChIRP-seq with lncRNA probe sets NEAT1

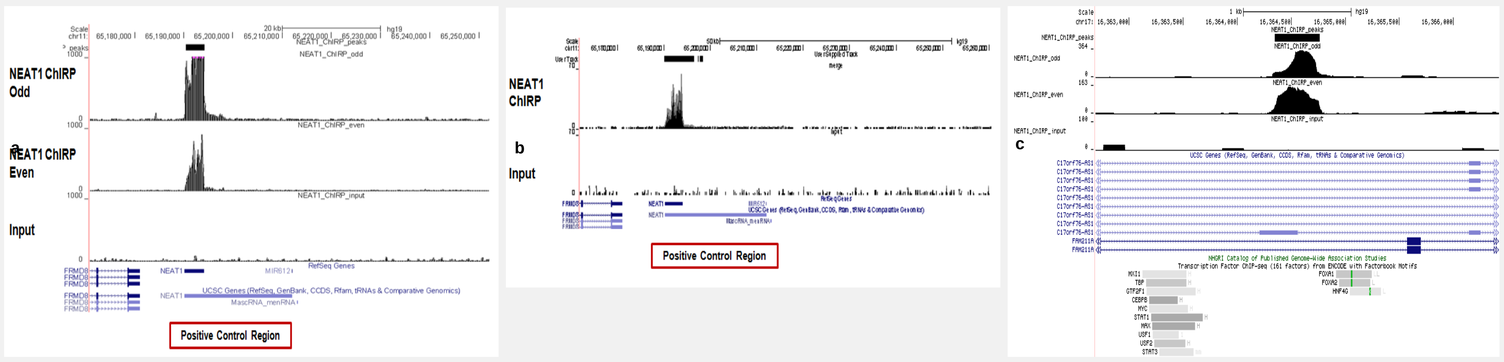


Figure 6.
ChIRP-seq was performed with lncRNA probe sets NEAT1. The sequence libraries were constructed with NGS Library Construction Kit (Cat. # 17-10492) and sequenced on HiSeq instrument (Illumina). The sequence reads were aligned to the reference genome (hg19) using Bowtie. (a) Peaks were called separately using data from even and odd probe sets. Algorithms such as MACS can be used for this purpose. Those in common were considered to be valid peaks. (b) A series of post-alignment processing and filtering steps were carried out using analysis software available from the laboratory of Howard Chang (http://changlab.stanford.edu/protocols.html). The data showing localization of NEAT1 mRNA to the NEAT1 gene region and another example region (c).

CONCLUSIONS

The Magna ChIRP RNA Interactome Kit

- Reliable recovery of chromatin using lncRNA or other chromatin associated RNAs as targets
- Readily identify sites of genomic interaction of chromatin associated RNA
- Allows robust pull down using included PureProteome™ Streptavidin magnetic beads
- Simplifies performance of ChIRP using integrated set of key buffers, enzymes, and reagents
- Enables successful ChIRP by providing negative control probe set and a detailed protocol with capture probe design guidelines
- Achieve first time success using the EZ-Magna ChIRP™ kits biotinylated positive control capture probe set and detection primers

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