

Chromatin Isolation by RNA Purification (ChIRP).

Journal:	J Vis Exp
Publication Year:	2012
Authors:	Ci Chu, Jeffrey Quinn, Howard Y Chang
PubMed link:	22472705
Funding Grants:	Noncoding RNAs in Cell Fate Determination

Public Summary:

Long noncoding RNAs are key regulators of chromatin states for important biological processes such as dosage compensation, imprinting, and developmental gene expression (1,2,3,4,5,6,7). The recent discovery of thousands of lncRNAs in association with specific chromatin modification complexes, such as Polycomb Repressive Complex 2 (PRC2) that mediates histone H3 lysine 27 trimethylation (H3K27me3), suggests broad roles for numerous lncRNAs in managing chromatin states in a gene-specific fashion (8,9). While some lncRNAs are thought to work in cis on neighboring genes, other lncRNAs work in trans to regulate distantly located genes. For instance, *Drosophila* lncRNAs roX1 and roX2 bind numerous regions on the X chromosome of male cells, and are critical for dosage compensation (10,11). However, the exact locations of their binding sites are not known at high resolution. Similarly, human lncRNA HOTAIR can affect PRC2 occupancy on hundreds of genes genome-wide (3,12,13), but how specificity is achieved is unclear. lncRNAs can also serve as modular scaffolds to recruit the assembly of multiple protein complexes. The classic trans-acting RNA scaffold is the TERC RNA that serves as the template and scaffold for the telomerase complex (14); HOTAIR can also serve as a scaffold for PRC2 and a H3K4 demethylase complex (13). Prior studies mapping RNA occupancy at chromatin have revealed substantial insights (15,16), but only at a single gene locus at a time. The occupancy sites of most lncRNAs are not known, and the roles of lncRNAs in chromatin regulation have been mostly inferred from the indirect effects of lncRNA perturbation. Just as chromatin immunoprecipitation followed by microarray or deep sequencing (ChIP-chip or ChIP-seq, respectively) has greatly improved our understanding of protein-DNA interactions on a genomic scale, here we illustrate a recently published strategy to map long RNA occupancy genome-wide at high resolution (17). This method, Chromatin Isolation by RNA Purification (ChIRP) (Figure 1), is based on affinity capture of target lncRNA:chromatin complex by tiling antisense-oligos, which then generates a map of genomic binding sites at a resolution of several hundred bases with high sensitivity and low background. ChIRP is applicable to many lncRNAs because the design of affinity-probes is straightforward given the RNA sequence and requires no knowledge of the RNA's structure or functional domains.

Scientific Abstract:

Long noncoding RNAs are key regulators of chromatin states for important biological processes such as dosage compensation, imprinting, and developmental gene expression (1,2,3,4,5,6,7). The recent discovery of thousands of lncRNAs in association with specific chromatin modification complexes, such as Polycomb Repressive Complex 2 (PRC2) that mediates histone H3 lysine 27 trimethylation (H3K27me3), suggests broad roles for numerous lncRNAs in managing chromatin states in a gene-specific fashion (8,9). While some lncRNAs are thought to work in cis on neighboring genes, other lncRNAs work in trans to regulate distantly located genes. For instance, *Drosophila* lncRNAs roX1 and roX2 bind numerous regions on the X chromosome of male cells, and are critical for dosage compensation (10,11). However, the exact locations of their binding sites are not known at high resolution. Similarly, human lncRNA HOTAIR can affect PRC2 occupancy on hundreds of genes genome-wide (3,12,13), but how specificity is achieved is unclear. lncRNAs can also serve as modular scaffolds to recruit the assembly of multiple protein complexes. The classic trans-acting RNA scaffold is the TERC RNA that serves as the template and scaffold for the telomerase complex (14); HOTAIR can also serve as a scaffold for PRC2 and a H3K4 demethylase complex (13). Prior studies mapping RNA occupancy at chromatin have revealed substantial insights (15,16), but only at a single gene locus at a time. The occupancy sites of most lncRNAs are not known, and the roles of lncRNAs in chromatin regulation have been mostly inferred from the indirect effects of lncRNA perturbation. Just as chromatin immunoprecipitation followed by microarray or deep sequencing (ChIP-chip or ChIP-seq, respectively) has greatly improved our understanding of protein-DNA interactions on a genomic scale, here we illustrate a recently published strategy to map long RNA occupancy genome-wide at high resolution (17). This method, Chromatin Isolation by RNA Purification (ChIRP) (Figure 1), is based on affinity capture of target lncRNA:chromatin complex by tiling antisense-oligos, which then generates a map of genomic binding sites at a resolution of several hundred bases with high sensitivity and low background. ChIRP is applicable to many lncRNAs because the design of affinity-probes is straightforward given the

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