Arraystar DRIPc-seq

Profiling the lncRNA/mRNA/circRNA organized R-loops in gene regulation

Highlights

- Powerful profiling: To study R-loops as a new player in gene regulation in the genome.
- Strand specificity: To identify lncRNAs/mRNAs in the R-loops, precise locations, and strand directions in the reference genome.
- High reliability: Well established optimal experimental procedures to produce best possible results.
- Flexibility: For any species with a reference genome.
- Rigorous quality: The positive and negative controls ensure DRIPc-seq library quality.
- Results: Provided with rich annotation, genome browser tracks, and publication-ready graphics.

Introduction

R-loop is a RNA:DNA three-stranded hybrid structure formed between LncRNA/mRNA/CircRNA strand and the template DNA strand by base pairing, leaving the non-template DNA strand unpaired and displaced in the loop (Fig.1) [1]. R-loops are widely distributed, occurring in 5% of the mammalian genomes [2,3]. R-loops are often located in the CpG islands of the promoters or transcription stop sites. High GC skews (G enrichment over C in the downstream of TSS non-template strand), G-quadruplexes, DNA gaps and DNA/RNA modifications contribute the formation of R-loops [4]. R-loops have important biological functions in gene regulation, DNA replication, and DNA/histone modifications.

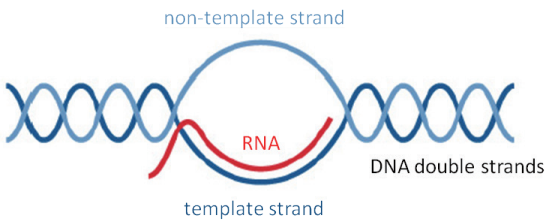


Fig 1. *R-loop structure[5].*

lncRNA organized R-loops impact transcription

R-loops can form between a lncRNA strand and the DNA in the loop. For example, TCF21 is a tumor suppressor in many cancers. TARID (TCF21 antisense RNA inducing demethylation) is a head-to-head antisense lncRNA of TCF21 gene and forms R-loop in the promoter region (Fig. 2). The R-loop is recognized by GADD45a, which recruits demethylase TET1, removes DNA methylation, increases TCF21 mRNA transcription, and regulates the cell cycles [6].

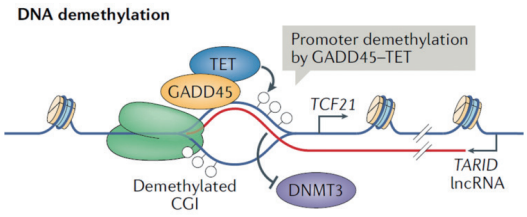


Fig 2. *Antisense lncRNA-TARID forms R-loop to regulate TCF21 promoter demethylation and TCF21 mRNA transcription [4,6].*

mRNA organized R-loops regulate DNA methylation transcription

Normally, the R-loop at BAMBI (a negative regulator of TGF) gene promoter facilitates more transcription. However in amyotrophic lateral sclerosis (ALS4), senataxin mutation reduces R-loop and increases the DNA methylation at the BAMBI promoter, leading to BAMBI transcription repression, TGF signal transduction upregulation, and ALS progression [7].

DRIPc-seq for R-loop profiling

Arraystar DRIPc-seq (DNA–RNA immunoprecipitation followed by cDNA conversion coupled to high-throughput sequencing) profiles lncRNA/mRNA organized R-loop distribution in the genome. Compared with traditional DRIP-seq that sequences the DNA fragments in the R-loops, the upgraded DRIPc-seq sequences the RNAs in the R-loops, which has the additional ability to determine the RNA strand directions, template/non-template DNA information, and semiquantification.

In the DRIPc-seq, S9.6 antibody is used to highly specifically immunoprecipitate the R-loops. The RNA strands in the R-loops are sequenced (Fig. 3). The DRIPc-seq data are bioinformatically analyzed to gain biological and functional insights (Fig. 4).

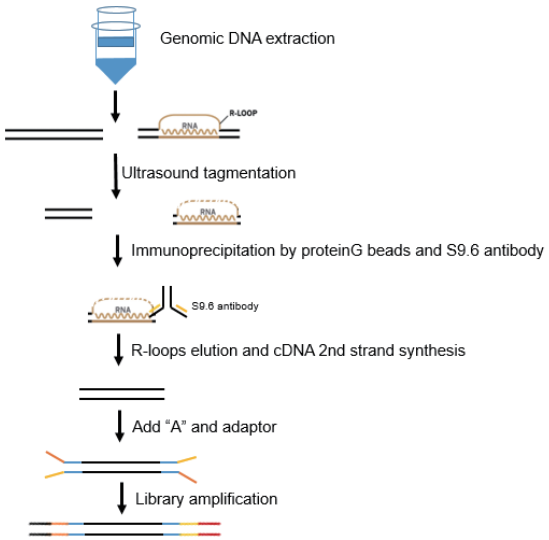
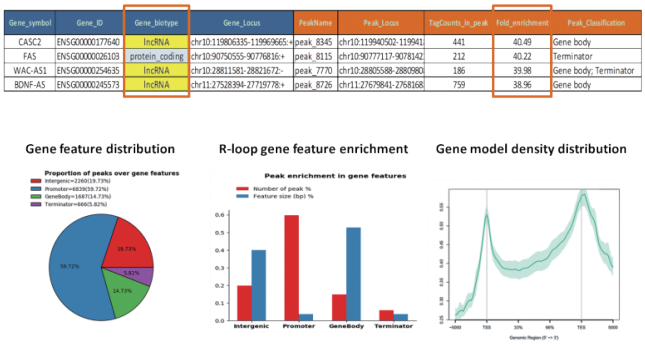


Fig 3. *DRIPc-Seq workflow.*



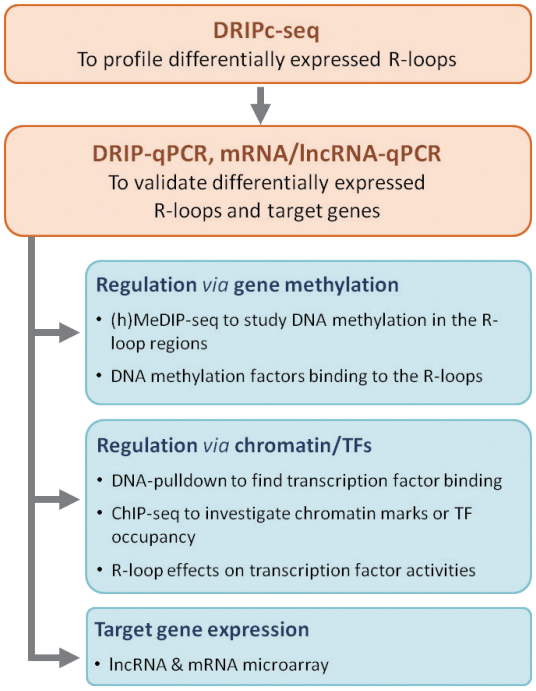
Gene_symbol	Gene_ID	Gene_biotype	Gene_Locus	PeakName	Peak_Locus	TagCounts_in_peak	Fold_enrichment	Peak_Classification
CASC2	ENSG00000177640	lncRNA	chr10:119806335-119969665:+	peak_8345	chr10:119940502-119941	441	40.49	Gene body
FAS	ENSG00000026103	protein_coding	chr10:90750555-90776816:+	peak_8115	chr10:90777117-907814	212	40.22	Terminator
WAC-AS1	ENSG00000254635	lncRNA	chr10:28811581-28821672:-	peak_7770	chr10:28805588-2880580	186	39.98	Gene body; Terminator
BDNF-AS	ENSG00000245573	lncRNA	chr11:27528394-27719778:+	peak_8726	chr11:27679841-2768168	759	38.96	Gene body

Fig 4. *DRIPc-seq R-loop peak analysis, annotation, and gene feature distribution.*

Along with LncRNA Array, MeDIP-seq, or ChIP-seq, DRIPc-seq provides valuable functional insights in epigenetic and transcriptional regulation by R-loops.

R-loop research roadmap ahead

With the wealth of information obtained from R-loop profiling by DRIPc-seq, the differentially expressed R-loops can be confirmed by DRIPc-qPCR, and the R-loop associated gene expression by mRNA/lncRNA-qPCR. As a research roadmap ahead, DNA methylation mediated by R-loops can be studied MeDIP-seq or chromatin changes or transcription factor bindings by ChIP-seq analysis, which can provide integrative view of R-loop regulatory effects by the epigenomic changes. Finally lncRNA/mRNA microarray can be used to measure the outcomes of target gene expression under the R-loop regulation.



References

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