Long Noncoding RNA Reverse Transcription Associated Trap (lncRNA RAT Assay)

High Efficient RNA-Chromatin DNA interaction Analysis Novel Approach

Description

Long noncoding RNA (lncRNAs) are a large class of RNA molecules with a length of >200 bp without encoding proteins. It is now clear that lncRNAs have a wide range of critical functions in cellular and developmental processes. Exploring the functions of this RNA world is one of the most important challenges facing biology today. Currently, however, there are no reliable methods to delineate the mechanisms of lncRNAs in gene regulation, particularly at the level of transcription.

We have developed a novel method to determine the genome wide interaction targets by lncRNAs. In this method chromatin DNA associated with RNA can captured by Reverse Transcription Associated Trap (RAT) technology. The captured chromatin DNA can be used for PCR detection, chip-seq library preparation for DNA sequencing analysis. This method is *different from Capture Hybridization Analysis of RNA Targets (CHART), chromatin isolation by RNA purification (ChIRP).*

This highly effective capture strategy allows unbiased detection and discovery of RNA-associated genomic DNAs, RNA sequences, and proteins.

Kit Content for 10 Reactions

Name	Size	Store
2M Glycine	1.5 mL	4 °C
Cell Lysis Buffer	15mL	4 °C
RT Buffer	200ul	-20 °C
biotin-dNTPs	15 ul	-20 °C
RNase inhibitor	50 uL	-20 °C
Maxi Reverse Transcriptase	15 uL	-20 °C
0.5M EDTA	0.5 mL	4 °C
Nuclei Lysis Buffer	2.5 mL	-20 °C
10% SDS	1.5 mL	4 °C
SDS Remover	0.5 mL	4 °C
WB buffers	20 mL	4 °C
5M NaCI	1mL	4 °C
Elute buffer	3.0 mL	4 °C
DNA-RNA Reverse buffer	1.0 mL	-20 °C

Applications

lncRNA PCR detection
Chip-seq library preparation
DNA and RNA sequencing analysis

Procedures

Cell fixation:

1. Grow up target cells to confluence in a 6-well plate. Add 108µl of 37% formaldehyde into 2ml cultured medium (final concentration:2%) and fix cells for 10 min at room temperature with gentle agitation.
2. Add 125µl 2M glycine (final concentration: 0.125 M) to the medium and incubate at room temperature for 5 min with gentle agitation to quench the remaining formaldehyde. Spin cells at 3500 rpm 4 oC for 5 minutes.
3. Remove the medium, add 1ml PBS to wash the pellet, spin again for 5 min at 3,000 rpm, then discard the supernatant, and keep the pellet for use. Add 800ul Cell Lysis Buffer to the pellet, and keep in ice for 2-5 minutes. Samples were inverted a few times and spin at 3,000 rpm for 7 min.
4. Discard the supernatant, then add 500ul Cell Lysis buffer to wash the pellet twice for next step use.

***Gene-specific RNA reverse transcription(RT)*:**

5. Re-suspend the pellet in 16.5µl RT buffer mix, incubate at 65℃ 5minutes, add 3.5µl the pre-warmed Biotin Label cDNA Synthesis Mix (see Table2) at 65℃ to 20 µl final. incubate at 65℃ for 30 minutes (better on mixer 550/min).
6. Stop RT reaction by adding 4µl 0.5M EDTA. Spin at 3500rpm 4 ºC for 10 minutes. Wash the pellet twice with 400µl ice-cold PBS to remove free biotin-dNTPs.

Table 1. Biotin Label cDNA Synthesis Mix

Component	**Volume (µL)/each**
Gene-specific oligos primer complementary for RNA(5 µM)	1
Biotin-dNTPs	1
RNase inhibitor(20U/ µL)	0.5
Maxi Reverse Transcriptase	1

Nuclei lysis and sonication (Restriction enzyme digestion or Sonication)

7. Resuspend cell pellet in 200µl Nuclei Lysis Buffer containing protease inhibitors, add 3µl 10% SDS, incubate on ice for 10 minutes.
8. Add **18** µ**l** SDS Remover to sequester the SDS.
9. Sonicate chromatin on ice with a Branson sonicator with a 2-mm microtip to an average length of about 500 bp with setting: 10s on and 10s off, 40% output control and 90% duty cycle settings, for 15 min.
10. Spin down at 2500 rpm for 10 min (keep the supernatant and remove the residue).

Biotinylated DNA enrichment with magnetic beads

Note: 1). 10 µl magnetic beads used for 2µg Biotinylated DNA

2). Add 0.1% Tween-20 to the WB buffers to reduce non-specific binding.

1) Bead preparation

11. Re-suspend the magnetic beads in the original vial by rotation or vortex. Calculate the amount of beads required based on their binding capacity. Wash beads to remove preservatives with 0.5mL 1x WB Buffer.
12. Place the tube containing the beads on a magnet for 1-2 mins. Remove the supernatant by aspiration with a pipette while the tube is on the magnet. Remove the tube from the magnet.
13. Add 0.5mL 1xWB buffer along the inside of the tube where the beads are collected and resuspend. Repeat wash twice, for a total of 3 washes at least.

2). Immobilization of nucleic acids

14. Add 10µl the above-washed beads into 200µl sonicated sample, add 50µl 5M NaCl (final 1M NaCl for optimal binding).
15. Incubate at RT for 30 min with gentle rotation. Separate the Biotinylated DNA/RNA bound beads with a magnet for 2-3 mins. Wash 2-3 times with 0.5mL 1x WB buffer.

3). Release of immobilizated biotinylated molecules

16. Add 100 µl elute buffer to elude the Biotinylated DNA/RNA from magnet beads. Incubate 5min at 65℃. Repeat elution once. Combine two eludes and beads.
17. *Cross-link reverse*: Add 50 µl DNA-RNA Reverse buffer, and add H_2O up to 500µl, incubate at 65℃ for 2 hrs.

Chromatin DNA Purification

Note: The Chromatin DNA can be purified with Chromatin DNA purification Kit or tradition phono-chloroform method. This traditional method for DNA purification is described as below.

18. Add equal volume of phenol-chloroform-isoamyl alcohol, revert tubes several times, centrifuge at 4℃ and 12000 rpm for 20 min, transfer supernatant to a new tube, and add chloroform 1:1 to the tube, centrifuge at 4℃ and 12000 rpm for15 min.
19. Transfer supernatant to a new tube. Add 0.1 volume of 3M NaAc, 1.5µl glycogen, 0.6 volume of isopropanol, , mix, incubate at -40℃ 30 min, or -80℃ 10 min (or -20 C overnight). Spin the samples at 4℃ 12000 rpm for 20 min; wash by 0.5mL 75% ethanol, Centrifuge at 4℃ 12000 rpm for 5 min.
20. Short time spin and totally eliminate the residual ethanol. Open lid and dry at room temperature.
21. Add 20µl ddH2O, incubate at 37℃ for 10 min. This is the final chromatin DNA for further analysis. Measure the DNA concentration if you want. The sample keeps at -80℃ in long-term storage.
22. The DNA can be further used for PCR detection or library construction.