RNA pull-down assay

Long Noncoding RNA as Modular Scaffold of Histone Modification Complexes
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RNA pull-down assay using nuclear extract

1. Biotin-labeled RNAs were *in vitro* transcribed with the Biotin RNA Labeling Mix (Roche) and T7 RNA polymerase (Promega), treated with RNase-free DNase I (Promega) and purified with RNeasy Mini Kit (QIAGEN).
2. Biotin-HRP Northern blot was followed by manufacture's manual (NorthernMax kit, Ambion) to demonstrate that all the RNAs are biotinylated and transcribed at the right size.
3. Three micrograms of biotinylated RNA was heated to 90°C for 2 minutes, put on ice for 2 minutes, supplied with RNA structure buffer (10 mM Tris pH 7, 0.1 M KCl, 10 mM $MgCl_2$), and then shifted to room temperature (RT) for 20 minutes to allow proper secondary structure formation.
4. 10^7 HeLa cell pallets were resuspended in 2 ml PBS, 2 ml nuclear isolation buffer (1.28 M sucrose; 40 mM Tris-HCl pH 7.5; 20 mM MgCl2; 4% Triton X-100), and 6 ml water on ice for 20 min (with frequent mixing).
5. Nuclei were pelleted by centrifugation at 2,500 G for 15 min.
6. Nuclear pellet was resuspended in 1 ml RIP buffer (150 mM KCl, 25 mM Tris pH 7.4, 0.5 mM DTT, 0.5% NP40, 1 mM PMSF and protease Inhibitor (Roche Complete Protease Inhibitor Cocktail Tablets)).
7. Resuspended nuclei were mechanically sheared using a dounce homogenizer with 15–20 strokes.
8. Nuclear membrane and debris were pelleted by centrifugation at 13,000 RPM for 10 min.
9. Folded RNA was then mixed with 1mg of HeLa nuclear extract in RIP buffer and incubated at RT for one hour.
10. Sixty microliters washed Streptavidin agarose beads (Invitrogen) were added to each binding reaction and further incubated at RT for one hour.
11. Beads were washed briefly five times in Handee spin columns (Pierce) and boiled in SDS buffer, and the retrieved protein was detected by standard western blot technique.

RNA pull-down assay using recombinant proteins

PRC2 complex were obtained from BPS Bioscience (5m: 51004; 3m: 51003). LSD1 complex (LSD1.com) was purified by tandem affinity purification from HeLa S3 cells as previously described (*2*). The complex contained known components (LSD1, BHC80, CoREST, HDACs, and BRAF35) in nearly stoichiometric amounts. Recombinant GST-LSD1 and 6xHis- CoREST were purified as described (*2*). Recombinant Flag-LSD1 was purified from Sf9 insect cells using M2-agarose affinity chromatography as previously described (*5*).

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7. Resuspended nuclei were mechanically sheared using a dounce homogenizer with 15–20 strokes.
8. Nuclear membrane and debris were pelleted by centrifugation at 13,000 RPM for 10 min.
9. 0.1 microgram of biotinylated RNA was incubated with different amounts of indicated proteins (2.5 and 5 µg of PRC2-5m and PRC2-3m; 3 and 5 µl of LSD1.com; 0.4 and 4 µg of GST-LSD1; 1 and 5 µg of Flag-LSD1; 0.4 and 4 µg of His-CoREST).
13. RNA and proteins were added in 200 µl binding buffer (50 mM TrisCl 7.9, 10% Glycerol, 100 mM KCl, 5 mM MgCl2, 10 mM β-ME 0.1% NP- 40) and incubated at RT for one hour.
10. For Fig S3B experiment, 3 µl DNase I (Invitrogen, 18047- 019) was added incubated at 37 °C for 30 minutes, and then follow the IP process.
11. Twenty microliters washed Streptavidin agarose beads (Invitrogen) were added to each binding reaction and further incubated at RT for 30 minutes.
12. Beads were washed briefly five times in Handee spin columns (Pierce) and boiled in SDS buffer, and the retrieved protein was detected by standard Western blot technique.