RNA Immunoprecipitation (RIP)

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8. Solutions

Nuclear isolation buffer:

1.28 M sucrose
40 mM Tris-HCl pH 7.5
20 mM $MgCl_2$
4% Triton X-100

RIP buffer:

150 mM KCl
25 mM Tris pH 7.4
5 mM EDTA
0.5 mM DTT
0.5% NP40
100 U/ml RNAase inhibitor SUPERASin (add fresh each time)
Protease inhibitors (add fresh each time)

9. Further information

For a detailed protocol, please visit www.abcam.com/protocols, further information on the RIP protocol can be found at:

A. M. Khalila et al., "Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression." PNAS July 14 2009.

D. G. Hendrickson, D. J. Hogan, H. L. McCullough, J. W. Myers, D. Herschlag, J. E. Ferrell, and P. O. Brown, "Concordant Regulation of Translation and mRNA Abundance for Hundreds of Targets of a Human microRNA." PLoS Biology 2009.

D. G. Hendrickson, D. J. Hogan, D. Herschlag, J. E. Ferrell, and P. O. Brown, "Systematic Identification of mRNAs Recruited to Argonaute 2 by Specific microRNAs and Corresponding Changes in Transcript Abundance." PLoS One 2008.

J. L. Rinn, M. Kertesz, J. K. Wang, S. L. Squazzo, X. Xu, S. A. Brugmann, L. H. Goodnough, J. A. Helms, P. J. Farnham, E. Segal, and H. Y. Chang "Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs." Cell 129:1311–1323, 2007.

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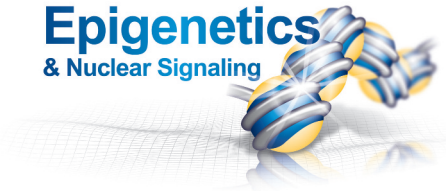

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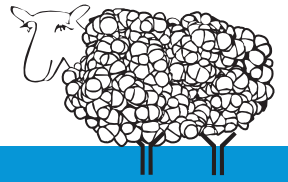
RIP is an antibody-based technique to map RNA–protein interactions *in vivo* by immunoprecipitating a specific RNA binding protein (RBP) and associated RNA that can be detected by real- time PCR, microarrays or e.g. sequencing.

This leaflet contains a brief summary of the RIP protocol adapted from Khalila et al. 2009, Hendrickson et al. 2009 and 2008, and Rinn et al. 2007.



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RIP Protocol

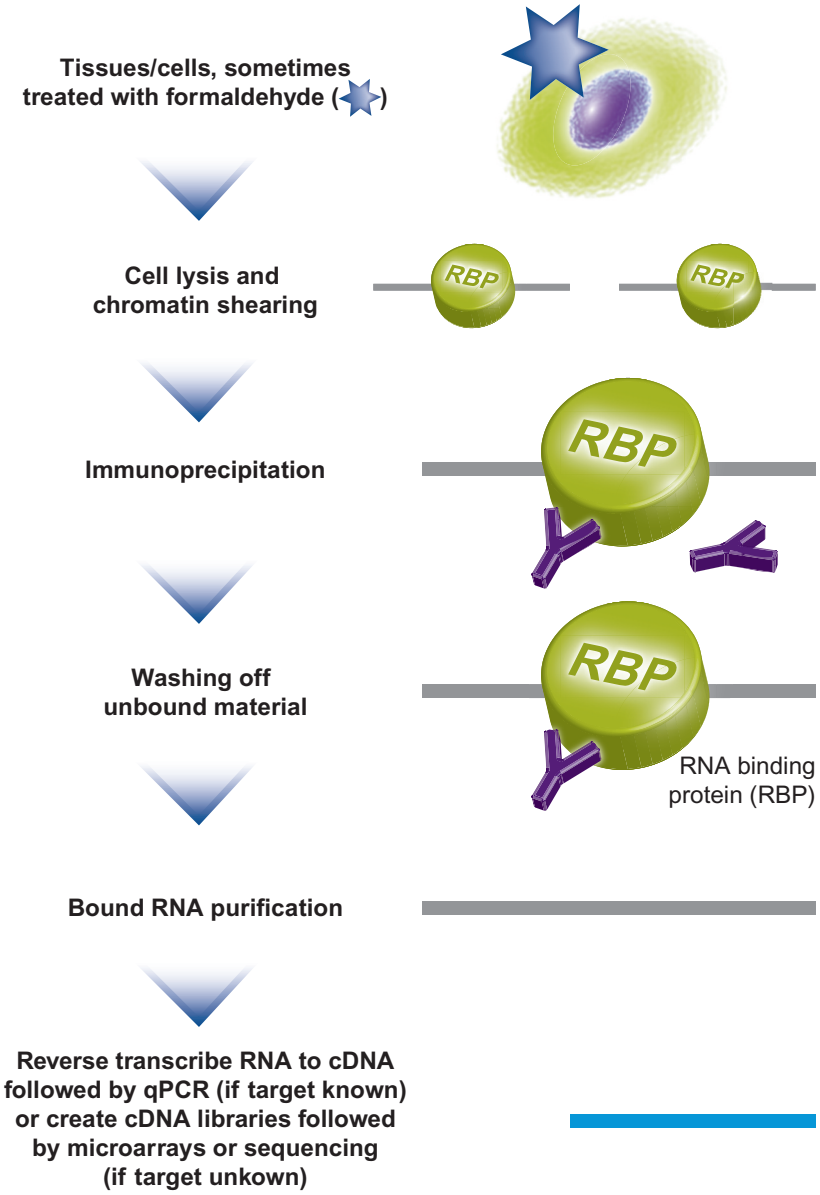

Figure 1: Schematic representation and summary of RIP protocol

1. Cell Harvesting

1.1. Harvest cells by trypsinization, resuspended in PBS (e.g. 10x7 cells in 2 ml), freshly prepared nuclear isolation buffer (2 ml) and water (6 ml), keep on ice (20 min, frequent mixing).

Tip: *One or more negative controls should be maintained throughout the experiment, e.g. no-antibody sample or immunoprecipitation from knockout cells or tissue.*

2. Nuclei isolation and nuclear pellets lysis

2.1. Pellet nuclei by centrifugation (2,500 G, 15 min).
2.2. Resuspend nuclear pellet in freshly prepared RIP buffer (1 ml).

Tip: *Avoid contamination using RNase-free reagents such as RNase-free tips, tubes and reagent bottles; also use ultraPURE distilled, DNase-free, RNase-free water to prepare buffers and solutions.*

3. Shearing of chromatin

3.1. Split resuspended nuclei into two fractions of 500 ml each (for Mock and IP).
3.2. Use a dounce homogenizer for shearing with 15–20 strokes.
3.3. Pellet nuclear membrane and debris by centrifugation (13,000 rpm, 10 min).

4. RNA Immunoprecipitation

4.1. Add antibody to protein of interest (2 to 10 µg) to supernatant (6 mg-10 mg), incubate (2 hr to overnight, 4°C, gentle rotation).
4.2. Add protein A/G beads (40 µl), incubate (1 hr, 4°C, gentle rotation).

Tip: *If an antibody is working in IP, this is a good indication that it will work in RIP.*

5. Washing off unbound material

5.1. Pellet beads (2,500 rpm, 30 s), remove supernatant, resuspend beads in RIP buffer (500 ml).
5.2. Repeat for a total of three RIP washes, followed by one wash in PBS.

Tip: *Optimization and stringent washing conditions are very important.*

6. Purification of RNA that was bound to immunoprecipitated RBP

6.1. Isolate coprecipitated RNAs by resuspending beads in TRIzol RNA extraction reagent (1 ml) according to manufacturer's instructions.
6.2. Elute RNA with nuclease-free water (e.g. 20 µl).
6.3. Protein isolated by the beads can be detected by western blot analysis.

7. Reverse transcription and analysis

7.1. Reverse transcribe DNAse treated RNA according to manufacturer's instructions.
7.2. If target is known use qPCR of cDNA; if target is not known create cDNA libraries, microarrays and sequencing can be used for analysis.

Tip: *The control experiments should give no detectable products after PCR amplification, and high-throughput sequencing of these control libraries should return very few unique sequences.*