

Resource Summary Report

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RNA-Stable Isotope Probing

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Type: Protocol

Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bsbxnapn>

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Group: SoWa RI Anaerobic and Molecular Microbiology (public)

Summary: The following protocol describes how to perform an RNA-Stable Isotope Probing experiment. The scope of this protocol only covers the parts involving separating labelled RNA from unlabelled RNA using ultracentrifugation in a caesium trifluoroacetate density gradient and downstream quantification to evaluate whether the labelling and separation of the RNA were successful. Total RNA should be extracted from an environmental sample or an enrichment culture that was incubated with an isotopically-labelled substrate. Labelling can be of the carbon, oxygen or nitrogen in the RNA (or any combination of the 3). For environmental samples, we recommend extracting RNA using our protocol Total Nucleic Acids Extraction from Soil and purifying it using the Purification of RNA from Crude NA Extract protocol. This protocol is based on the following papers: Whiteley et al. (2007); Dumont et al. (2011); Angel and Conrad (2013). For a comprehensive discussion on how to design a SIP experiment and how to analyse the resulting data, we recommend referring to the recent book on the subject: Stable Isotope Probing: Methods and Protocols, especially chapters: 1-3 and 9-18.

Affiliations: Soil and Water Research Infrastructure, Soil and Water Research Infrastructure, Soil and Water Research Infrastructure

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