Opportunity 2021033

# TREX - A novel method for the isolation of RNA-binding proteins

TREX is a revolutionary RNA-centric method for the unbiased identification of proteins that directly interact with specific regions of RNA. This novel technique is more specific, efficient, and reproducible than current methods, is applicable to various types of RNA and enables the region-specific mapping of RNA-protein interactions.

## Background

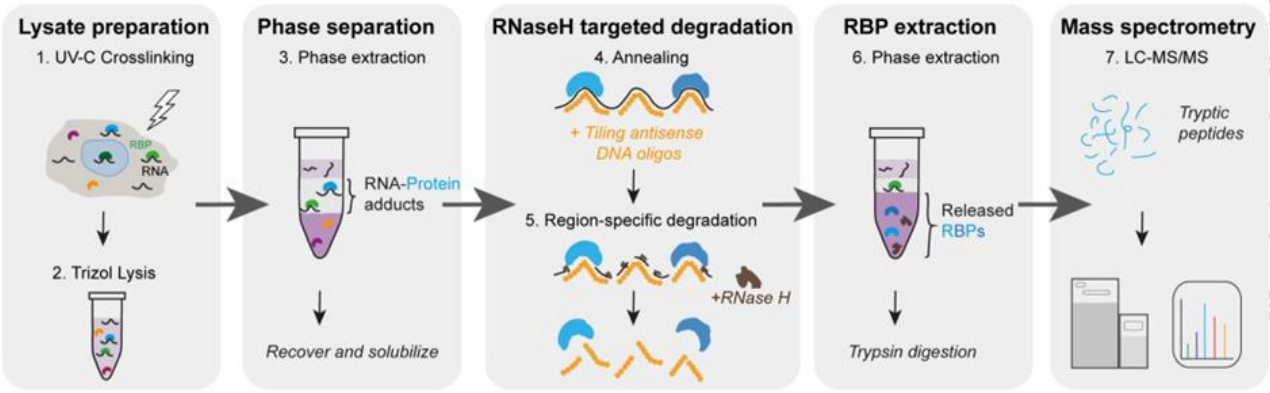
Regulation of an RNA molecule (e.g. mRNA of an oncogene, RNA genome of COVID-19 virus, etc.) is strictly dependent on its interaction with cellular proteins. These interactions are profoundly significant as they control gene expression, RNA processing, and post-transcriptional modifications, influencing the fate and function of RNA molecules. Protein- and RNA-centric approaches are used to investigate the intricate web of RNA-protein interactions, facilitating our understanding of gene regulation, disease mechanisms, and the development of targeted therapeutics.

## The Problem

RNA-binding proteins can be identified using either Protein-centric or RNA-centric approaches, however current methodologies have significant limitations. Protein-centric methods like RNA immunoprecipitation (RIP) and crosslinking and immunoprecipitation (CLIP) can be biased due to availability of suitable antibodies, thus significantly limiting their reach and applicability. Conversely, current RNA-centric approaches suffer from a low efficiency of RNA pull-down, a drastic lack of reproducibility between approaches, and significant off-target capture, reducing specificity. In addition, current approaches are often labour-intensive and require high starting material, limiting their widespread application and scalability.

## Invention: Benefits & Application

***Targeted RNase H-mediated Extraction of X-linked RBPs (TREX)*** combines UV-C crosslinking, organic phase separation, and RNase H mediated degradation, to extract and identify proteins that directly bind to a specific RNA sequence.





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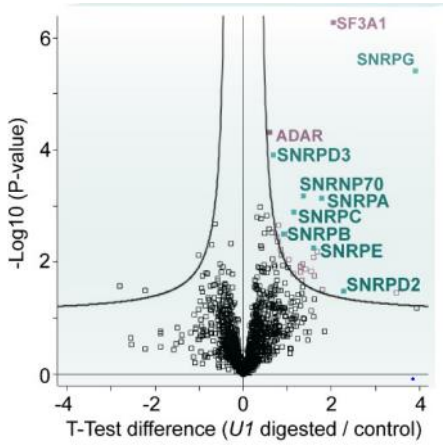
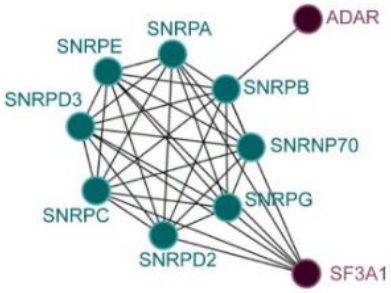
TREX is an innovative approach to the isolation and identification of RNA-binding proteins, that not only surpasses current methods in terms of efficiency, but it also introduces the ability to accurately estimate specificity, and to identify the region-specific interactors of any given segment of any RNA molecule in living cells.

Using U1 snRNA as an example, QMUL researchers identified 27 proteins as direct interactors of U1, including 8 of the 10 known U1 snRNP components, as well as 2 other established interactors. Importantly, TREX outperformed all previous studies and methods in identifying these interactors, while using 10x less starting material. In further experiments, the researchers also demonstrated the method can discover region-specific protein interactions. Using NORAD lncRNA ND4 domain, TREX identified 360 proteins as significant direct interacting partners of the region, with an overlap of up to 44% of hits with previous studies, as well as identifying novel hits, such as topoisomerase I (TOP1), consistent with a role in regulating assembly of the ribonucleoprotein complex.




**U1 snRNA-interacting proteins**

Volcano plot of the comparison of U1 digested vs undigested TREX samples, showing significant enrichment of the U1 SNRNP complex members, along with SF3A1 and ADAR

TREX also enables researchers to conduct comprehensive RNA interactome analyses on specific regions of a given transcript, including comparing process rRNA molecules. Using the 45S rRNA gene, which undergoes extensive processing to form the 18S, 5.8S, and 28S rRNA molecules, and included the analysis of the 5'ETS, ITS1 and ITS2 and 3'ETS spacer regions, QMUL researchers revealed many known and new binding proteins, most of which exhibited region-specific binding profiles, thereby expanding our knowledge of human ribosome biogenesis regulators.

## Patents

A provisional patent application has been filed in the UK.

## Lead Inventor

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## Publications

Dodel M, Guiducci G, Dermit M, Krishnamurthy S, Stojic L, Mardakheh FK (2023) *TREX reveals proteins that bind to specific RNA regions in living cells.* BioRxiv (https://doi.org/10.1101/2023.06.30.547259).



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