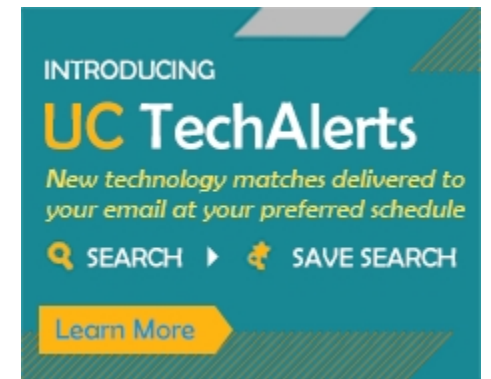


# (SD2020-306) Monitoring mRNA Translation by RNA Modifications -STAMP (Surveying Targets by APOBEC-Mediated Profiling)

Tech ID: 32403 / UC Case 2020-306-0

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## OTHER INFORMATION

### KEYWORDS

diagnostics, therapeutics, research

reagents, RNA Binding Proteins

### CATEGORIZED AS

- ▶ **Biotechnology**
  - ▶ Genomics
  - ▶ Proteomics
- ▶ **Research Tools**
  - ▶ Reagents

### RELATED CASES

2020-306-0

## BACKGROUND

RNA-binding proteins (RBPs) play essential roles in gene expression and other cellular functions. Thus their identification and the understanding of their mechanisms of action and regulation is key to unraveling physiology and disease.

To measure translation efficiency and different steps of ribosome recruitment, the state of the art is ribosome profiling (or Ribo-seq) and polysome profiling which uses millions of cells, sucrose gradients, centrifugation and often requires the removal of ribosomal RNA as part of the sequencing library preparation as it contaminates more than 50% of most ribosome/polysome libraries. Also, we cannot distinguish full length isoforms here, as the ribosome-fragments are short.

## TECHNOLOGY DESCRIPTION

Researchers from UC San Diego describe methodologies to fuse RNA modification enzymes to ribosome-associated subunits and proteins such as ribosome subunits at the entry site and ribosome release factors. By doing so our invention enables RNA modifications that can be detected by sequencing methods as a surrogate measure for ribosome initiation, scanning, elongation and release. As a proof-of concept, the inventors fused RPS3 (a protein part of the ribosome subunit) to the APOBEC1 enzyme that deaminates cytidine to uracil (C to U). The research team could quantify translation efficiency by RNA-seq to measure translation in full length transcripts (by long-read sequencing such as nanopore) or in single cells (using single cell RNA-seq in the 10X platform).

Antibody-based methodologies such as CLIP and RIP are staples used to identify RNA-binding sites and targets of RBPs. This new STAMP approach offers several advantages. First, CLIP is generally constrained by input requirements, frequently needing thousands to millions of cells. Here we demonstrate that STAMP can be used reliably at single-cell resolution to identify RNA targets, binding sites and even extract motifs from a few cells to a single cell. STAMP enables the combined identification of RBP binding sites and global measurement of gene expression, a long-standing goal for the gene expression, genomics and RNA communities. Second, CLIP requires fragmentation to separate bound and unbound RNA, but that precludes the discovery of isoform-dependent binding sites on mRNAs that may differ by an exon or translated regions.

## APPLICATIONS

Enables translation efficiency for genes to be measured genome-wide with potential for drug screens , characterization in single cells from complex cellular models and evaluation of drugs for safety.

## ADVANTAGES

- ▶ STAMP identifies RBP binding sites without immunoprecipitation.
- ▶ Long-read STAMP reveals isoform-specific binding profiles.
- ▶ Ribosome-subunit STAMP edits are enriched in highly translated coding sequences and are responsive to mTOR inhibition.
- ▶ Deconvolution of RBP-specific and cell type-specific RNA binding.
- ▶ The ability of STAMP to recover RBP-RNA targets in single cells suggests that targets of multiple RBPs can be simultaneously discovered from a single multiplexed experiment.

## STATE OF DEVELOPMENT

## INTELLECTUAL PROPERTY INFO

Patent-pending. UC San Diego is seeking partners to commercialize this technology.

The US Utility Patent has published and details what is claimed:

<https://patents.google.com/patent/US20230138328A1/en?q=US-2023-0138328-A>

## PATENT STATUS

## RELATED MATERIALS

► Brannan KW, Chaim IA, Marina RJ, Yee BA, Kofman ER, Lorenz DA, Jagannatha P, Dong KD, Madrigal AA, Underwood JG, Yeo GW.

Robust single-cell discovery of RNA targets of RNA-binding proteins and ribosomes. Nat Methods. 2021 May;18(5):507-519. doi:

10.1038/s41592-021-01128-0. Epub 2021 May 7. - 05/07/2021

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