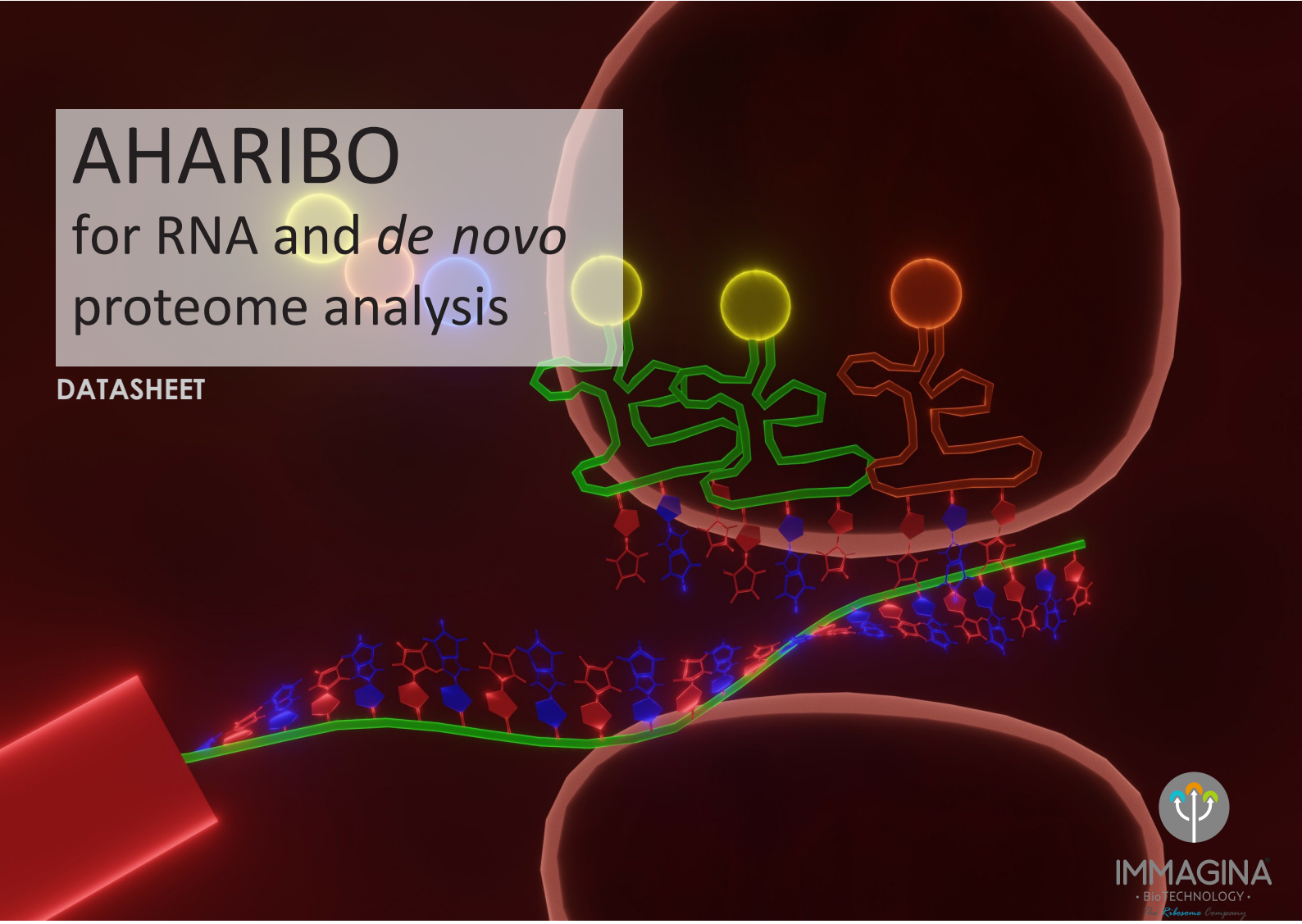
AHARIBO

for RNA and *de novo* proteome analysis

DATASHEET



Introduction

IMMAGINA BIOTECHNOLOGY provides an innovative solution for proteogenomic analysis with AHARIBO (*AHA-mediated RIBOsome isolation*).

AHARIBO represents an effective tool to explore quantitative relationships between transcript and protein levels, offering a reliable and accurate approach for capturing active translation processes.

AHARIBO was developed to overcome the limits of classical methods such as polysome profiling or affinity purification-based techniques that are characterized by labor-intensive protocols and relatively poor correlations between mRNA and protein levels.

Highlights

Parallel RNA and protein analysis

- Parallel isolation and downstream analysis of translated RNAs (by RNA-seq or qPCR) and the associated newly synthesized proteins.

Enhanced and accurate proteogenomic representation

- High correlation between AHARIBO translatome and proteome;

Fast, simple and reliable protocol

- 4 hours from cell lysate to total RNA or protein;
- No ultracentrifugation required;

Low sample requirement

- From 500,000 cells.

Simple and fast protocol

This technique involves incubation of cell with l-azidohomoalanine (AHA) followed by sBlock treatment to stabilize AHA-labelled peptides on ribosomes. Active ribosomes and their interactome are purified by magnetic beads targeting the azido groups incoporated in the nascent chains (Fig. 1).

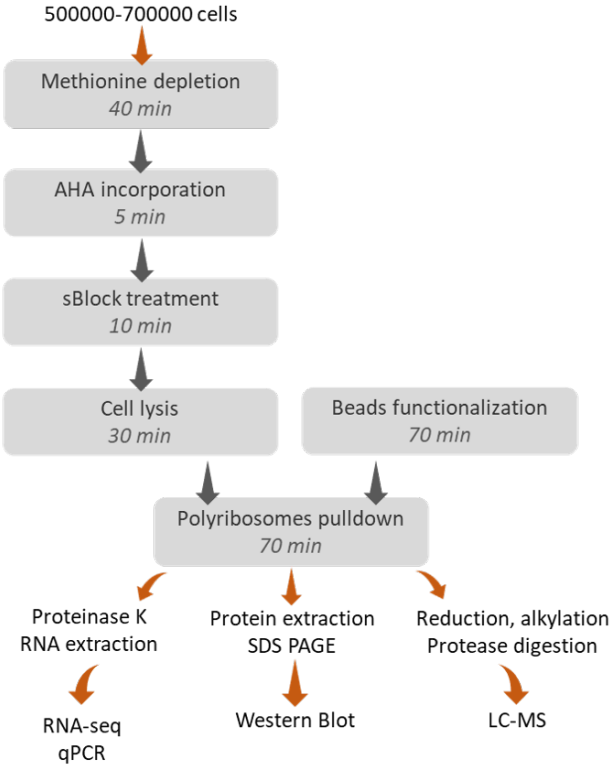


Fig. 1:
Schematic representation of AHARIBO workflow.

AHARIBO isolates simultaneously RNAs and nascent proteins associated with translationally active ribosomes

The ability of AHARIBO to capture proteins and RNAs associated with active ribosomes was first validated by mass spectrometry.

Indeed, the LC-MS analysis confirmed an enrichment of ribosomal proteins in AHARIBO samples in respect to the control (Fig. 2A).

Furthermore, we observed a reduction of rRNA captured by AHARIBO in samples with down-regulated protein synthesis (arsenite and heat shock treated) (Fig. 2B) demonstrating that AHARIBO is indeed able to capture *bona fide* active ribosomes.

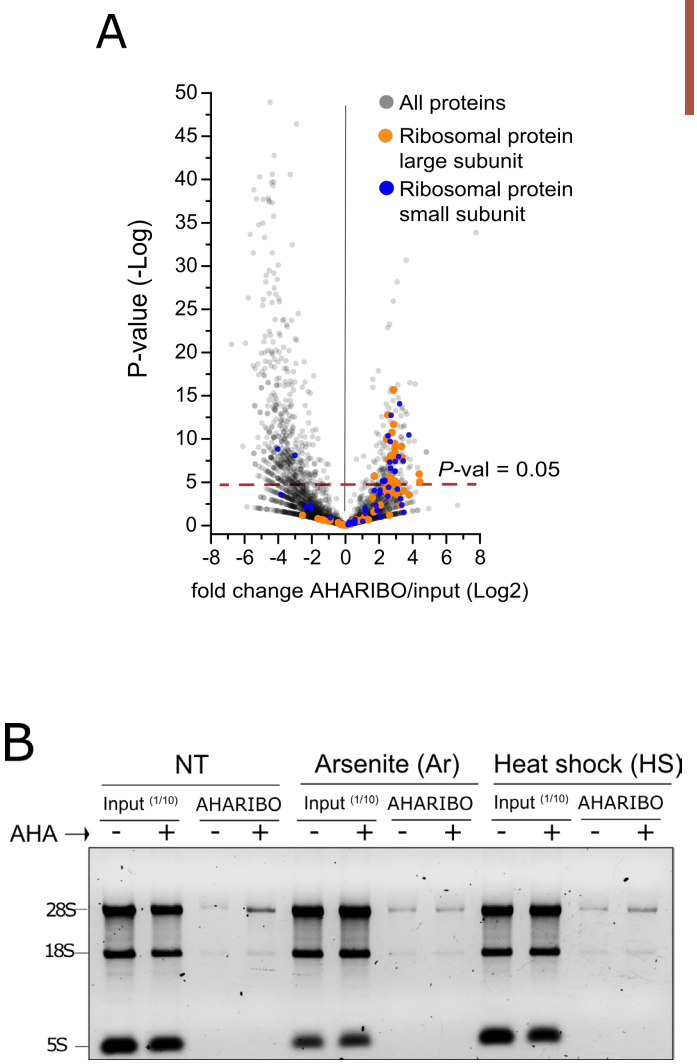


Fig. 2: **A)** Volcano plots showing the P-value (-Log) versus the relative abundance of AHARIBO-isolated proteins. Data are compared with input (starting AHA-containing lysate). Highlighted in orange and blue the ribosomal proteins belonging to the large and the small ribosome subunits, respectively. **B)** Agarose gel electrophoresis of total RNA extracted from input lysates and lysates subjected to AHARIBO pulldown, obtained from cells either not treated (NT) and treated with Arsenite or Heat shock.

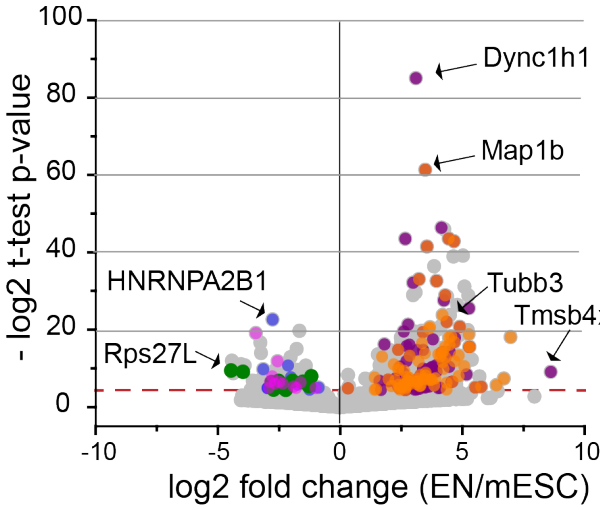


Fig. 3: Volcano plot displaying for each differentially expressed protein (EN/mESC) the AHARIBO proteome versus p-value (-log2). Orange and purple dots represent up-regulated proteins involved in cytoskeleton organization and neurogenesis respectively. Blue, green and magenta dots represent down-regulated proteins related to RNA processing, protein synthesis and mouse pluripotency respectively.

AHARIBO: genome-wide portrayal of the *de novo* synthesized proteome

We further tested the AHARIBO method on mouse embryonic stem cells (mESCs) and mESCs differentiated into early neurons (EN).

We found that proteins known to be expressed during the early stages of development of the nervous system (e.g. Map1b, Tubb3 and, Dync1h1) are enriched in EN cells, further confirming the reliability of AHARIBO in monitoring *de novo* protein expression.

Reproducible and reliable results

To evaluate the performance of AHARIBO kits, two replicates of both AHARIBO RNA and AHARIBO-protein pull-down on mESCs lysate were analyzed by RNA-seq and LC-MS, respectively. Both the methods revealed a good sample-to-sample reproducibility with an R^2 ≥0.98. These results demonstrate that AHARIBO-RNA and AHARIBO-Protein provide high accuracy data, providing the reliability of the method (Fig. 4).

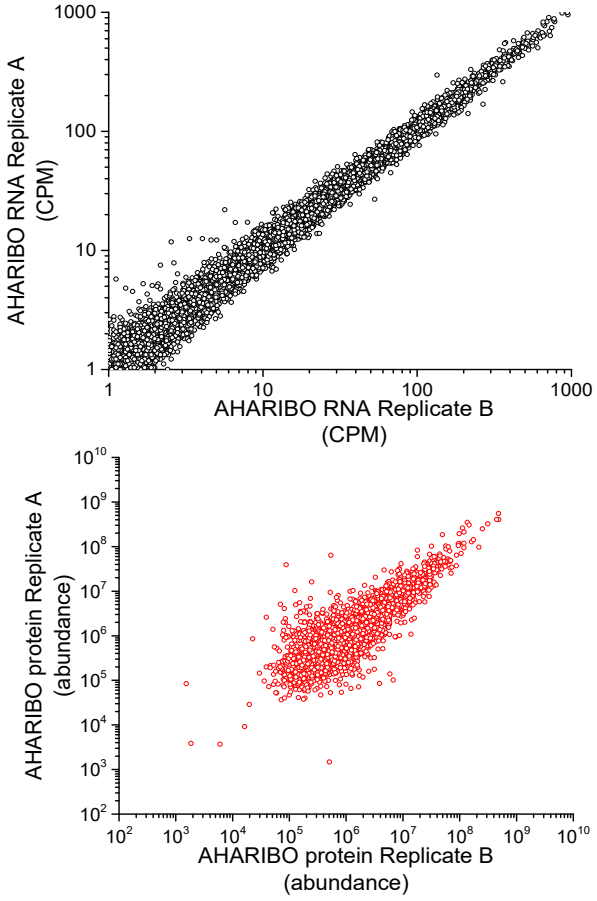


Fig. 4: Concordance plots demonstrating reproducibility of the integrated workflow using AHARIBO RNA (black dots) and AHARIBO (red dots). Results are representative of two independent replicates for each method.

AHARIBO-RNA: a better correlation with proteomic data

To demonstrate that the RNAs captured with AHARIBO-RNA kit (AHARIBO translatome) is indeed a better approximation of protein levels than classical translatome (obtained with sucrose cushion or polysomal profiling), we compared AHARIBO translatome and classical translatome to the respective proteomic data. Figure 5 clearly shows that AHARIBO translatome data are better correlated to protein expression than classical translatome levels.

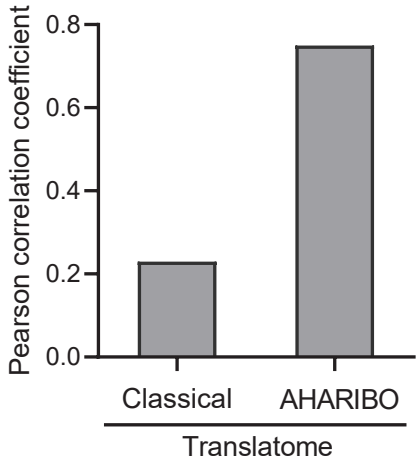


Fig. 5: Histogram showing Pearson's correlation analysis of classical translatome vs pSILAC fold change and AHARIBO-RNA vs AHARIBO-Protein fold change.

The benefits

AHARIBO translatome is **a BETTER PROXY OF PROTEIN LEVELS** than the classical translatome

FAST, SIMPLE and **RELIABLE PROTOCOL**, no nuclease digestion needed

LOW INPUT MATERIAL

SUITABLE FOR ALL THE MOST COMMON DOWNSTREAM ANALYSIS: RNAseq, qPCR, mass spec. and Western blot

Based on beads separation: possibility to run **MULTIPLE SAMPLES** in parallel for automated high-throughput (HT) experiments

DETECTION OF TRANSLATED or RIBOSOMES ASSOCIATED lncRNA

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Ordering information

Product name	Catalog no.	No. of reactions
AHARIBO RNA	#AHA003-R	6
AHARIBO protein	#AHA003-P	6
AHARIBO Western Blot*	#AHA003-W	6

For Research Use Only. Not for use in diagnostic procedures.

*product available only upon request

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