

**CHARACTERIZING HUMAN TRANSFER RNAS BY HYDRO-TRNASEQ AND
PAR-CLIP**

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Abstract

CHARACTERIZING HUMAN TRANSFER RNAS BY HYDRO-TRNASEQ AND PAR-CLIP

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The participation of transfer RNAs (tRNAs) in fundamental aspects of biology and disease necessitates an accurate, experimentally confirmed annotation of tRNA genes, and curation of precursor and mature tRNA sequences. This has been challenging, mainly because RNA secondary structure and nucleotide modifications, together with tRNA gene multiplicity, complicate sequencing and sequencing read mapping efforts. To address these issues, we developed hydro-tRNAseq, a method based on partial alkaline RNA hydrolysis that generates fragments amenable for sequencing. To identify transcribed tRNA genes, we further complemented this approach with Photoactivatable Crosslinking and Immunoprecipitation (PAR-CLIP) of SSB/La, a conserved protein involved in pre-tRNA processing. Our results show that approximately half of all predicted tRNA genes are transcribed in human cells. We also report predominant nucleotide modification sites, their order of introduction, and identify tRNA leader, trailer and intron sequences. By using complementary sequencing-based methodologies we present a human tRNA atlas, and determine expression levels of mature and processing intermediates of tRNAs in human cells.

Στους γονείς και τον αδερφό μου

Acknowledgments

First, I would like to thank my

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tRNA transfer RNA.

Chapter 1

Introduction

1.1 tRNA biology

tRNAs have been among the earliest studied non-coding RNA molecules [1, 2]. Yet, in recent years tRNAs received new attention in the context of codon-resolved translational control

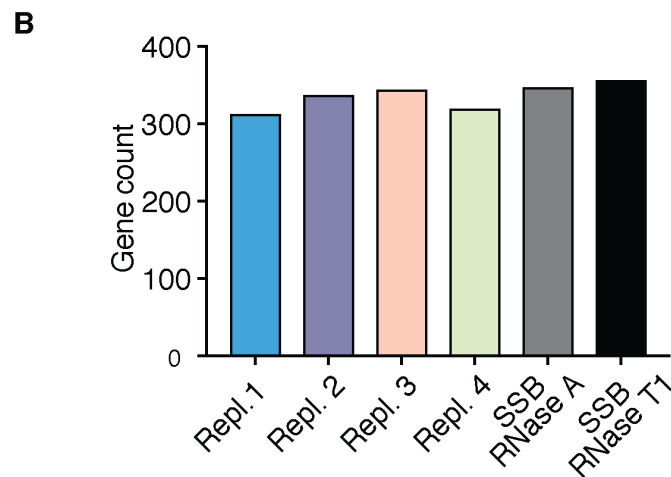


Figure 1.1: Venn Bars

[3, 2]

Chapter 2

woohooo

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References

- [1] C. Woese, *The Genetic Code. The Molecular basis for Genetic Expression*, 1st ed. Harper, 1967.
- [2] D. Soll and U. RajBhandary, *tRNA: Structure, Biosynthesis and Function*, 1st ed. ASM press, 1995.
- [3] A. G. Arimbasseri and R. J. Maraia, "RNA Polymerase III Advances: Structural and tRNA Functional Views," *Trends in Biochemical Sciences*, pp. 1–14, Apr. 2016.