

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

A Thesis Presented to the Faculty of  
The Rockefeller University  
in Partial Fulfillment of the Requirements for  
the degree of Doctor of Philosophy

by  
Tasos Gogakos

June 2017

©Copyright by Tasos Gogakos 2017

## **Abstract**

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

Tasos Gogakos, Ph.D.

The Rockefeller University 2017

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

# Table of Contents

<b>List of Figures</b>	<b>vi</b>
<b>List of Tables</b>	<b>vii</b>
<b>List of Abbreviations</b>	<b>viii</b>
<b>1 Sample Chapter</b>	<b>1</b>
1.1 New section . . . . .	1
<b>2 woohooo</b>	<b>4</b>
<b>References</b>	<b>6</b>

# List of Figures

1.1	Venn Bars . . . . .	2
-----	---------------------	---

# List of Tables



# List of Abbreviations

**tRNA** transfer RNA.

# Chapter 1

## Introduction

### 1.1 New section

Introduction Microprocessors are ubiquitously deployed in applications ranging from commodity devices to mission critical systems, Fig. 1.1 and while malfunctions in the former may cause no

harm other than inconvenience, the slightest malfunction in the latter may have catastrophic consequences.

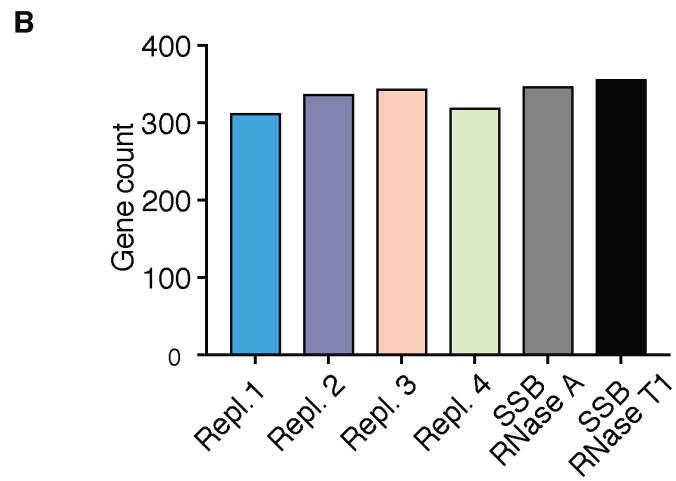


Figure 1.1: Venn Bars

[1]

# **Chapter 2**

**woohooo**

this is a new chapter

this is pager 2

# References

- [1] 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.