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

A Thesis Presented to the Faculty of

The Rockefeller Uniersity

in Partial Fullfillment of the Requirements for

the degree of Doctor of Philosophy

by

Tasos Gogakos

June 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 test2 (tEsT) 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

List of Figures

List of Tables

List of Abbreviations

tEsT test2.

tRNA transfer RNA.

Chapter 1

Introduction

1.1 New section

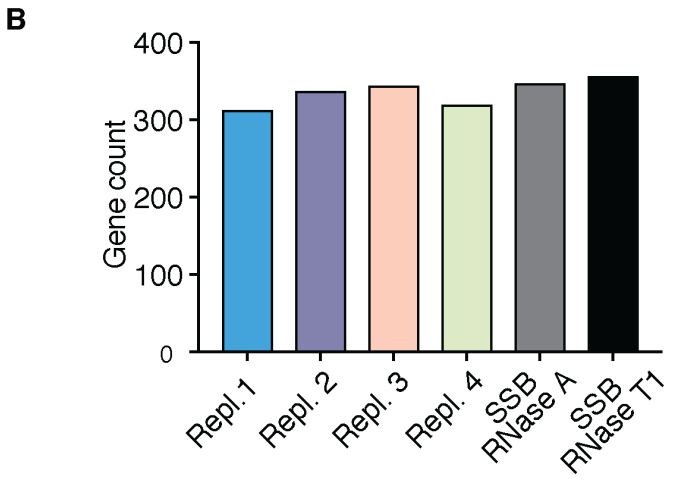

Figure 1.1: Venn Bars

[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.