# COVER PAGE

# TITLE PAGE

Possible titles:

* Investigation of long RNAs using a novel ligation-based approach
* Investigation of long RNAs using RNA templated DNA ligation
* Long RNAs: biology, technology, and perspective
* Examination of dynamic long RNAs

A Dissertation Presented

By

Christian Knauf Roy

Submitted to the Faculty of the

University of Massachusetts Graduate School of Biomedical Sciences, Worcester

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSPHY

(MONTH, DAY, YEAR)

BIOCHEMISTRY

# SIGNATURE PAGE

A Dissertation Presented

By

Christian Knauf Roy

The signatures of the Dissertation Defense Committee signify completion and approval as to style and content of the Dissertation

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The signature of the Chair of the Committee signifies that the written dissertation meets the requirements of the Dissertation Committee

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Chair of Committee The signature of the Dean of the Graduate School of Biomedical Sciences signifies that the student has met all graduation requirements of the school. (Signature)

Anthony Carruthers, Ph.D., Dean of the Graduate School of Biomedical Sciences Program (Typed)

Month, Day and Year (Typed)

# Front Matter

## Dedication

## Acknowledgements

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## Abstract

## List of Tables

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## Preface

# 

# Body Matter

## Introduction

## Research Chapters

### CHAPTER I : Introduction

1. What motivated my research
2. Topics that need to be discussed
3. Coordination in splicing
4. Introduction to piRNAs
5. Historical Review of the literature
   1. RNA Expression
   2. Splicing
   3. Alternative Splicing
   4. SR Proteins
   5. RNA-Processing
   6. mRNPs
6. piRNAs
   1. Small RNA
   2. Tissue specific RNAs
7. Technicalical limitations
   1. Sequencing history
   2. Deep sequencing history
   3. The Isoform problem
   4. lncRNAs
   5. Computaitonal tools
8. State of Current literature
   1. ENCODE
   2. Integration of different datasets for more complete transcirptonal pictures
      1. Mention lincRNAs
   3. Single Cell RNA-Seq
      1. 1. Shalek AK, Satija R, Adiconis X, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature*. 2013:1–5. doi:10.1038/nature12172.
9. Relationship between alternative splicing and protein domains
10. 1. Light, S. & Elofsson, A. The impact of splicing on protein domain architecture. *Current opinion in structural biology* 1–8 (2013).doi:10.1016/j.sbi.2013.02.013

### CHAPTER II : SeqZip as a methodology

1. Nature methods paper will do 90% of the work here
2. SeqZip Development
   1. Discovery of novel enzyme activity
3. Search for long and complicated RNAs
   1. CD45
   2. FN1
4. You should tie together the fact that Dscam ALSO has Fibronectin sections!
5. DSCAM

### CHAPTER III : SeqZip applied to HIV and piRNA precursors

1. piRNA precursors
2. HIV Transcript integrity

### CHAPTER IV : piRNA Chapter and Molecular Cell paper

### CHAPTER V : Perspective / Final Summary and Conclusions

Things to remember about this section:

1. Multiple people have stated that this can be highly speculative
2. This is my chance to show that I am a scientist
3. If I were to die tomorrow, what would be the experiments to conduct
4. Discuss long range RNA secondary structure and implecations of regulating alternative splicing (See Li, S., & Breaker, R. R. (2013). Eukaryotic TPP riboswitch regulation of alternative splicing involving long-distance base pairing. Nucleic acids research, 41(5), 3022–31. doi:10.1093/nar/gkt057) and Reg of AS by long RANGE SS folder in Mendeley
5. Full length analysis of mRNAs
6. Implications for discrination past one's DNA as it is the actual PRODUCT of the DNA and the actual biology (or at least closer to the functional biology) that is going on inside of every person
7. Catalogue of every possible mRNA isoform
8. Seperate the signal from the noise of transcription
9. For out there comments:
10. Most high cited pnas papers are methodology papers
11. www.pnas.org/reports/most-cited

# Back Matter

## Appendices

### Methods

#### SeqZip

#### Computational Scripts

#### Computational Approaches

##### Git

##### Perl

##### IGV

##### MySQL

#### ImageQuant

#### LOD of Radioactivity

### Oligo Database

### Important MySQL data tables

## Biblography