HOW TO EAT AND LOSE WEIGHT:

A SECOND LOOK

by

Sue Dunham

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Bioinformatics and Computational Biology

Summer 2014

HOW TO EAT AND LOSE WEIGHT:

A SECOND LOOK

by

Sue Dunham

Approved: _____

Eleftherios T. Papoutsakis, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____

Fats Domino, Ph.D.
Chair of the Department of Nutrition

Approved: _____

Minnesota Fats, Ph.D.
Dean of the College of Hard Knockwurst

Approved: _____

James G. Richards, Ph.D.
Vice Provost for Graduate and Professional Education

ACKNOWLEDGMENTS

I write this paper with unending thanks for my family, friends, the love, support and encouragement that they give, and the lessons they have taught me. With special thanks for my mother Donna, father Thomas, and sister Allison. With special thanks to my mother; her character and duty towards others is inspirational for this work's focus on sustainability and climate change. With special thanks to my Father, for inspiration through his work ethic, leadership, and open mind. With special thanks to my sister for her support of my education, curiosity, and maturity; I would not be where I am without her encouragement and acceptance. I write this with gratitude to my family for the life they have given me, the sacrifices they have made on my behalf, and most importantly their love. With special thanks for my grandfather George inspiring my pursuit of science and chemistry. With special thanks for my grandmother Winnie for the inspiration of her love and optimism which bring me through each challenge. With special thanks for my uncle Kevin for his curiosity, friendship, optimism, and support. With special thanks for my brother-in-law Matt for his friendship and encouragement. With special thanks for my nieces Violet and Ruby for their love, for the lessons that they teach me, and their infectious energy and optimism. Many thanks for Madeline for her love, open ear, curiosity, support, and encouragement throughout the years. Many thanks to my best friend Andrew for his friendship, curiosity, support, and encouragement. With many thanks to the rest of my wonderful and supportive family and friends for their unconditional love and support.

Many thanks to Karol Miaskiewicz for his consistent and wonderful friendship throughout this project. Many thanks to all of the members of the Bioinformatics program. Particularly, I'd like to thank Erin Crowgey for her mentorship and support of my efforts to learn NGS bioinformatic analyses and Ryan Moore for tossing ideas

around together. I'd like to thank Shawn Polson for his open ear and perspective. I'd also like to thank Dr. Wu for her support and encouragement throughout the program and the many members of the Wu group for their support as well.

Many thanks for my mentors, Drs. Keerthi Venkataramanan and Terry Papoutsakis, for their mentorship, support, critique, and understanding throughout this project. The success that we've seen in this effort I owe to Keerthi's guidance and training. Many thanks for each of the the many members of the Papoutsakis lab for their friendship, support, and critique. Many thanks to Bruce, Olga, and Summer for their work in the Sequencing and Genotyping Center in support of this project.

Ad maiorem dei gloriam.

TABLE OF CONTENTS

LIST OF TABLES . . . . . **vi**
LIST OF FIGURES . . . . . **vii**
ABSTRACT . . . . . **viii**

Chapter

1 METHODS **1**

1.1 Culture 1
1.2 RNA preparation 1
1.3 RNA concentration and RNA-seq library preparation 2
1.4 Data Analysis 2
1.5 Molecular Methods 3

2 RESULTS **4**

3 DISCUSSION **5**

4 CONCLUSIONS **6**

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Chapter 1

METHODS

1.1 Culture

Wild type *Clostridium acetobutylicum* ATCC 824 was cultured anaerobically in 4L New Brunswick Scientific BioFlo 310 bioreactors at 37 °C, pH 5.0, 200 mL min^{-1} N_2 and 200rpm agitation in a defined *Clostridia* growth medium, as described previously (Venkataramanan 2013). When the cultures were grown to A_{600}=1, the N_2 flow rate was decreased to 50 mL min^{-1} and cultures were stressed to a final concentration of 90 mM *n*-butanol, 48 mM potassium butyrate, or left unstressed. 15 mL samples were acquired at 15, 75, 150, and 270 minutes after treatment and centrifuged at 8,000rpm, 4 °C for 20 minutes. After discarding the supernatant, cell pellets were then immediately frozen at −85 °C.

1.2 RNA preparation

RNA was extracted by first washing the cell pellets in 1mL of RNase-free SET buffer (25% sucrose, 50 mM EDTA [pH 8.0], 50 mM Tris-HCl [pH 8.0]) before resuspending cells in a 220 mL solution of RNase-free SET buffer containing 4.55 U mL^{-1} proteinase K and 20 mg mL^{-1} lysozyme and incubating for 6 minutes. Resuspended cells were vortexed with 40mg of RNase-free glass beads ($\leq$106 µm) at maximum speed and room temperature for 4 minutes. Then each sample was mixed with 1 mL of ice-cold QIAzol (Qiagen, Valencia, CA, USA) and then 200 µL of ice-cold chloroform. After a 3 minute room temperature incubation, samples were centrifuged at 11,000rpm for 15 minutes at 4 °C. The aqueous phase was then mixed with 1.3 mL of ice-cold ethanol before transfering to a miRNeasy Mini spin-column (Qiagen, Valencia, CA, USA), following the manufacturers instructions. Samples were then precipitated in 0.3M sodium

acetate and 75% ethanol overnight, centrifuged at 14,000 rpm for 30 minutes, washed with ice-cold 70% ethanol, and rehydrated in RNase-free water. Next, samples were treated with the Turbo DNA-free kit (Ambion, Austin, TX, USA) according to manufacturers instructions. Samples were then precipitated, washed, and quantified with a Nanodrop ND-1000, aliquoted for quality analysis with the BioAnalyzer platform (Agilent, Wilmington, DE, USA), and 10 µg aliquots in 10 µL samples were stored at −85 °C.

1.3 RNA concentration and RNA-seq library preparation

Ribosomal RNA was removed with the MicrobExpress kit (Ambion, Austin, TX, USA). Enriched RNA was assessed for quality with the BioAnalyzer platform (Agilent, Wilmington, DE, USA). High quality samples were used to prepare RNA-seq libraries with the ScriptSeq v2 library preparation kit and indexed PCR primers (Epicentre, Madison, WI, USA). Libraries were multiplexed and sequenced for 101 cycles over two lanes of an Illumina HiSeq 2500 at the University of Delaware Sequencing and Genotyping Center (Newark, DE, USA).

1.4 Data Analysis

Paired-end sequencing resulted in pairs of 100bp reads which are deposited in the Sequence Read Archive ( ). Summary statistics for the libraries are shown in table/appendix ( ). The basic bioinformatic processing pipeline is described on Github. In brief, after removing adapters and low quality bases with Trimmomatic, reads were first mapped to the ribosomal RNA sequences before mapping to the *C. acetobutylicum* genome both with Bowtie 2.1.0 [parameters:]. The resulting alignment was then cleaned, validated, sorted, and indexed with SAMtools and Picard. Unmapped and duplicate reads were flagged for omission from further analysis. Reference transcriptome assembly was performed with the Cufflinks suite (v. 2.2.0) or Trinity. The coverage vectors for each strand was calculated by assembling proper pairs into fragments in a BED file, and calculating coverage with BEDtools. Due to the dense nature

of the *C. acetobutylicum* transcriptome, the resulting transcriptome assemblies were manually curated to correct for unreasonably large transcripts. Transcriptional start sites were identified with with two peak-finding algorithms TSSi and MACS. The fully merged transcriptome assembly were used to determine digital gene expression for visualization and statistical analyses in R and Circos. Clustering was done with and promoter predictions were performed with MEME, and RSAT.

1.5 Molecular Methods

Fold changes were confirmed for randomly selected genes by qRT-PCR. Transcriptional start sites were verified by 5′RACE for randomly selected genes. Small RNAs were confirmed by RT-PCR and Northern blot.

Chapter 2

RESULTS

Chapter 3

DISCUSSION

Chapter 4

CONCLUSIONS