Notice of the Final Oral Examination
for the Degree of Master of Science

of

STEPHANIE PEARCE

BSc (University of Victoria, 2013)

“Stability of Synthetic Temperature Inducible Lethal Genetic Circuits in
Escherichia coli”

Department of Biochemistry and Microbiology

Friday, August 19, 2016
10:00AM
Engineering and Computer Science Building
Room 130

Supervisory Committee:
Dr. Francis Nano, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Christopher Nelson, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Benjamin Koop, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Francis Choy, Department of Biology, UVic

Chair of Oral Examination:
Dr. Roderick Edwards, Department of Mathematics and Statistics, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies
Abstract

Temperature-sensitivity (TS) is often used as a way to attenuate microorganisms to convert them into live vaccines. Studies indicate that live vaccines are often necessary for the complete clearance of certain pathogenic organisms. In this work we explore the use of TS genetic circuits that express lethal genes for their potential utility as a widely applicable approach to TS attenuation. Here, we use restriction endonucleases as the lethal gene products. We tested different combinations of TS repressors and cognate promoters controlling the expression of genes encoding restriction endonucleases inserted at four different non-essential sites in the *Escherichia coli* chromosome. We found that the presence of the restriction endonuclease genes did not affect the viability of the host strains at the permissive temperature, but that expression of the genes at elevated temperatures killed the strains to varying extents. The location of the genetic circuit cassette in the chromosome was critical, and insertion at the *ycgH* site led to minimal cell death. Induction of the TS circuit in a growing culture led to a pre-mature leveling off of the optical density, and a shift in the number of cells that could exclude a dye that indicated cell viability. Incubation of cells initially grown at low temperature and then suspended in phosphate buffered saline at high temperature, led to about 100-fold loss of cell viability per day compared to minimal loss of viability for the parental strain. The Dual strain containing two different genetic circuits was found to have reduced escape frequency compared to single circuit strains. However, strains carrying either one or two TS lethal circuits could generate mutants that survived high temperature. These mutants included start codon deletions as well as upstream deletions of the TetRD1 encoding gene as well as complete deletions of the lethal gene circuits.