



**University  
of Victoria**

Graduate Studies

Notice of the Final Oral Examination  
for the Degree of Doctor of Philosophy

of

**RALPH MCWHINNIE**

BSc (University of Victoria, 2005)

**“Design of Temperature Inducible Transcription Factors and  
Cognate Promoters”**

Department of Biochemistry and Microbiology

Wednesday, May 11, 2016

10:00 A.M.

David Turpin Building

Room A144

Supervisory Committee:

Dr. Francis Nano, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)

Dr. Martin Boulanger, Department of Biochemistry and Microbiology, UVic (Member)

Dr. Christopher Nelson, Department of Biochemistry and Microbiology, UVic (Member)

Dr. Diana Varela, Department of Biology, UVic (Outside Member)

External Examiner:

Dr. David Weiss, Division of Infectious Disease, Emory University School of Medicine

Chair of Oral Examination:

Dr. Colin Bradley, Department of Mechanical Engineering, UVic

## **Abstract**

The ability to control expression of a gene of interest is an important tool of molecular biologists and genetic engineers. This allows the phenotype associated with the regulated gene or genetic pathway to be partially de-coupled from the genotype and expressed only under condition that lead to induction of the genetic control system employed. Such control is typically implemented through a repressor protein (Eg. TetR, LacI) which will repress transcription when bound to a promoter containing a binding site (operator) recognized specifically by that repressor. Many such repressors and their cognate promoters are well-defined and characterized in model genetic systems, such as *Escherichia coli*, and may function poorly in other bacterial species. A lack of genetic components that allow the controlled expression of heterologous genes in less well studied bacterial species may limit their bio-industrial potential and the sophistication of engineered phenotypes. The work presented here uses random mutagenesis and selection to isolate mutants of TetR that are inducible by increased culture temperature. Induction of protein expression by temperature change can have benefits over repressors that require small-molecule inducers in bio-industrial applications as reversal of induction and reuse of growth medium are possible. The host range of these, or any, repressor protein is limited by the host range in which its cognate promoter will function. To bypass this limitation and allow use of TetR in *Francisella novicida*, a method was developed by which TetR-responsive promoters that function in this host could be selected from random DNA sequence anking the TetR binding site (tetO). Many unique TetR-repressible promoters that function in *F. novicida* were recovered and tightly-regulated expression of both exogenous reporter genes and host virulence genes were demonstrated. This promoter selection technique was also applied to *E. coli*, which allowed comparison between *F. novicida*-selected promoters and those selected in an *E. coli* host. Adaption of this process for production of promoters responsive to transcription factors other than TetR would simply require the use of a different operator sequence, suggesting diverse applications for this technique. This success in promoter engineering should enable advances in synthetic biology and genetic engineering in non-model bacterial species.