Recombinant DNA Techniques Course

Celeste Knowles

Project No. 2009/768

Copyright Australian Seafood CRC, CSIRO and Flinders University 2010

This work is copyright. Except as permitted under the Copyright Act 1968 (Cth), no part of this publication may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owners. Neither may information be stored electronically in any form whatsoever without such permission.

ISBN: 978-1-925982-18-3

PROJECT NO: 2009/768 Micromon recombinant DNA techniques course

PRINCIPAL INVESTIGATOR: Celeste Knowles

ADDRESS:
CSIRO Marine and Atmospheric Research
Castray Esplanade
Hobart, TAS 7000

OBJECTIVES OF RESEARCH TRAVEL GRANT

To participate in the Micromon recombinant DNA course conducted by Monash University in Melbourne, with the aim of learning new techniques as well as building upon previously learnt ones to assist me in conducting my PhD.

NON TECHNICAL SUMMARY:

The Monash Recombinant DNA techniques course layout consists of a series of lectures, tutorials and laboratory sessions. This is a very useful layout as it provides you with background and technical information prior to using them in the lab. The lab sessions were also very well set out having 1 tutor to 4 students, providing you with a lot of assistance. Tutors are also willing to provide you with assistance after the completion of the course.

OUTCOMES ACHIEVED TO DATE

In returning to Hobart in November after completing the course I have started using some of the techniques used and will use more in the near future. Recently I have been optimizing primers to prepare for the development of the real-time PCR assay. In order to optimize the primers I have been setting up standard PCR's, running gels, sequencing, extracting DNA and RNA, and turning RNA into cDNA. Other techniques I would expect to use during my PhD include cloning, ligation and real-time PCR.

BACKGROUND AND NEED

Amoebic gill disease (AGD) is a major problem affecting the production of Atlantic salmon in Tasmania. My project aims to develop a real-time PCR assay to identify *Neoparamoaba perurans*, which is responsible for AGD. This assay will then be tested on a variety of fish including vaccinated and non-vaccinated, freshwater bathed and non-bathed both before during and after the bathing process. This will provide information on the host-parasite relationship and lead to information on the infection process. The assay will then be applied to environmental samples to investigate if there are reservoirs for amoeba or if they can be transferred between sites.

In order to conduct these experiments a range molecular skills are required. Through participation in the recombinant DNA techniques course many of these techniques required will be covered both in lectures and through hands on laboratory work.

Some aspects of my PhD involving molecular techniques are as follows:

- Development of a real-time PCR method for identification of *N. perurans* which is responsible for amoebic gill disease (AGD). Firstly, standard PCR is to be conducted to test potential primers that could be used for real-time PCR. Additional techniques required are DNA and RNA extraction, gel electrophoresis, DNA extraction from gels, DNA and RNA purification, ligation, cloning and cDNA synthesis.
- Use of the real-time PCR method to quantitatively detect AGD in salmon. I will be applying the real-time PCR method on a variety of fish including vaccinated and non-vaccinated, freshwater bathed and non-bathed both before and after the freshwater bathing process. The PCR technique will then be employed to assess the relationship between pathogen load and resistance values in selectively bred salmon. I am also hoping to couple this parasite approach with transcriptome analysis of the host using microarrays. These techniques with involve cDNA labelling and hybridisation, scanning and bioinformatics.
- Water and other environmental samples will be tested to determine if there are reservoirs for the amoeba and if they can be transferred between sites. This again will involve the PCR method developed and will require the use of sophisticated RNA and DNA sampling procedures and extraction methods suitable for detecting trace amounts of amoebae nucleic acids in the environment.

RESULTS

Skills learnt from the course are currently being used in the laboratory and will continually be used in the duration of my PhD. At the moment primers are being tested in the lab through a series of PCRs. These primers will then be applied to real-time PCR to develop an assay that can be applied to samples collected over the summer.

In the end techniques learnt during the course will be used to conduct a large portion of my laboratory work and will result in conference presentations, published papers and a thesis.

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

The nature of this work does not pose an immediate change for the Atlantic salmon industry. However a real-time PCR assay will result in a more reliable method to quantify infection than the current gill scoring method. Information produced from my PhD will be used to help lead to a better understanding of host-parasite relationships. Experimental work will be conducted working with both vaccine project and selective breeding project fish.

ACKNOWLEDGEMENTS

I would like to acknowledge Seafood CRC, CSIRO and Flinders University for providing funding both my project and the costs associated with this course.