SfCRC Research Travel Grant 3.1: Queensland Institute of Medical Research (QIMR) Flow of Cytometry Methods Course and Workshop (Dr Melony Sellars: Student Andrew Foote)

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Project No. 2009/750

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PROJECT NO: 2009/750 **TITLE:** SfCRC Research Travel Grant 3.1: Queensland Institute of Medical Research (QIMR) Flow of Cytometry Methods Course and Workshop

PRINCIPAL INVESTIGATOR: Andrew Foote

ADDRESS: CSIRO Marine and Atmospheric Research, 233 Middle St, Cleveland, QLD.

OBJECTIVES OF RESEARCH TRAVEL GRANT: Develop knowledge and skills in flow cytometry to help advance the progress of my CRC project; advance my professional development; share my skills and knowledge with relevant CRC participants and projects.

NON TECHNICAL SUMMARY:

I have greatly developed and expanded my skills and knowledge using flow cytometry and can now competently use this device for a range of applications relevant to my project. By expanding the range of applications I can use this device for in various fields of research such as medical and cancer research, I have enhanced my professional development and future opportunities. I have discussed possible applications of this method of analysis to other CRC projects (2008756 & 2008/757).

OUTCOMES ACHIEVED TO DATE

OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT:

New skills and knowledge developed:

1. A greater understanding of how to interpret and manipulate the data output in assessing ploidy and a range of other applications– including identifying the quantity of cells at the various stages of the cell cycle. Alternative stains were also identified to use in assessing ploidy.

2. Different methods of tissue preservation depending on what information you are trying to obtain – eg intracellular staining verses live tissue analysis. How to overcome over-fixation problems associated with cross-linking agents (a problem in previous confocal laser-scanning microscopic analysis which used fluorescent stains).

3. A range of new applications I can use flow cytometry for were identified including: assessment of live, apoptotic, necrotic cells. How to run multiple colours and how to ‘compensate’ for spectral overlap during multi-colour analysis. These applications may assist in the gynogenesis section of my project where assessment of sperm viability and destruction of sperm DNA is needed.

BACKGROUND AND NEED

Flow cytometery is a very useful tool which can be used for almost any molecular application where fluorescent stains can be used. It has the advantage of fast and accurate processing of large quantities of data, including the analysis of several different components in each of the tens to hundreds of thousands of cells processed per sample. This technique is essential to my CRC project as it will allow me to determine the success of polyploidy experiments and also assist in analysis of DNA destruction in sperm.

RESULTS

I am now competent at using flow cytometry for analysis of DNA content for the polyploidy section of my project and relevant to CRC project 2008/757. Further, I have new knowledge of how to analyse interpret my data output. I have new knowledge about the range and function of various fluorescent stains to trial in work on determining if DNA is destroyed in sperm and if the sperm is still viable, relevant to my project and CRC project 2008/756.

EXTENSION ACTIVITIES

Build links with Greg Kent from Southern Shellfish Hatchery:
I have contacted Greg by phone and email and have discussed different methods of creating tetraploids and gynogens in oysters and prawns. We have discussed some of this issues, what has worked in oysters, and if these techniques would be transferrable to prawns. I also discussed the use of flow cytometry.

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

None to date

SUMMARY OF CHANGE IN INDUSTRY (WHAT IMMEDIATE CHANGES ARE EXPETED)?

No immediate changes.

WHAT FUTURE AND ONGOIN CHANGES ARE EXPECTED?

If successful tetraploidy techniques are developed (with the aid of flow cytometry), commercial production of all triploid progeny may be possible. This will also require substantial use of flow cytometry to determine ploidy status.

FURTHER ACTION REQUIRED IN REAGRDS TO COMMUNICATION?

Continued communication with Greg Kent to discuss ploidy and gynogenesis work in oysters and the possible transfer of techniques to prawns which is unpublished to date.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION? (IP PROTECTION, LICENSING, SALES, REVENUE ETC)

None

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS?

None

ACKNOWLEDGEMENTS

CSIRO covered transport and other costs associated with the course

APPENDIX (IF APPLICABLE): Email correspondence with Greg Kent:

From: gkent [mailto:Greg.Kent@utas.edu.au]
Sent: Thursday, 19 November 2009 8:18 PM
To: Foote, Andrew (CMAR, Cleveland)
Subject: Re: ploidy and gynogens

----- Original Message -----
From: Andrew.Foote@csiro.au
Date: Thursday, November 19, 2009 16:40
Subject: ploidy and gynogens
To: Greg.Kent@utas.edu.au

> Hi Greg,
>
> Thanks for the chat today and sorry for contacting you at a bad time; your mobile was the only contact information I could get!
Thats fine, good to talk to you.
>
> My PhD is 'Understanding penaeid prawn sex determination and developing monosex induction strategies for commercial application' - I have been investigating tetraploidy techniques other than heat which I found during my honours to only induce non-viable embryos due to problems with mitotic spindle regeneration. I have trialled cold which didn't produce any viable nauplii (hatched embryos) and trialled pressure with the same result; however, I feel there is more room for optimisation with pressure. It was interesting to note that some tetras were induced with PBI inductions in muscles - what method did they use to determine ploidy and did they grow to adult? I have only found literature on triploidy induction in oysters from the 1980's and nothing on tetraploids, do you know of any work that has been done since or is Debra Ballagh the person working on this?

Debra as far as i am aware is not looking at tetraploidy only triploidy. There are papers on tetraploid induction in the literature i will dig out some references for you. The primary commercial way tetraploids are induced in bivalves is to suppress polar body I from eggs from triploids. It is known as the 4Cs method and has a very shaky patent if used for commercial

production in some bivalves. I don't know what the fecundity of female triploid prawns is like, but despite the claims that triploid produces sterile animals, in oysters at least, it is possible to find some triploid females that will produce some eggs. These are stripped and fertilised with normal sperm from diploids and PB I is suppressed with CB. Result is a percentage of viable tetraploids (ranges from 15-80%). After you get tetraploids you can mate tetraplois with tetraploids to continue production. Triploid oysters are produced commercially by fertilising diploid females with sperm from tetraploid males. The reciprocal cross doesn't work.
>
> I am also looking at creating gynogens, though there are some large obstacles for the prawns as the female holds the sperm internally until she is ready to spawn and releases egg and sperm at once. I am planning on trying irradiated the sperm with gamma, uv and also use sperm from a related prawn to see if it activates the egg, then perform a PBII induction to hopefully restore diploidy. I'm not sure how transferable oyster methods would be to prawns, I haven't found any literature on gynos in oysters or any shellfish, so I'm guessing you are the first to look at it.

No, I am definitely not the first. There are a number of papers out there for oysters and scallops at least, but you will probably be the first for crustaceans. i will see if I can send you some references. Given the issues with prawns, sperm from a related species might be the best way to go. For freely released sperm, UV is excellent for denaturing the DNA but leaving the sperm structure intact and capable of activating an egg. However I'm not sure about the sperm package that is transferred from the males, Gamma maybe better in that circumstance.

One of the issues you will need to consider is determining success of gynogenesis in progeny. Microsatellites are the obvious choice but can be problematic especially if you have null amplifying alleles etc. I found using recessive phenotype females ie gold oysters enabled me to track parentage in progeny very quickly and cheaply. Also because I was looking at double haploid production (mitotic gynogens) having progeny that could display morphology based on parentage enabled a more targetted approach when using microsatellites. Do you have similar markers in some of your prawns?
>
> As I mentioned, I just completed a flow cytometry workshop at QIMR so if you plan on creating triploids for your hatchery and have some questions about flow cytometry analysis I may be able to help or can certainly give you the details of some of the world experts who were at the course and designed many of the techniques.

I used to work for a company that bought me my own flow for shellfish work so at least as far as bivalves go I have a series of protocols that work nicely, thanks.
>
> I hope I didn't bombard you with too much, but it was exciting to hear about someone who has done similar work in shellfish.
>
> I hope to stay in touch,

Cheers,

Greg
>
> Andrew
>
>
> Andrew Foote
>
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