Centre for Gene Research

May 2000

From your Director

Recent public attention on genetic engineering means that the activities of molecular biologists and scientists in general will be occupying the hearts and minds of the nation. Congratulations to Jean Fleming for being named to the Royal Commission on Genetic Modification. Jean was a member of the CGR Committee and her absence will be missed. In this debate of the use of the public purse, intellectual capital, and making money from science I am reminded of something Jim Watson, from Genesis, said: "No one owes you a living as a scientist". He was talking about careers in Science, but it brought home to me the change in role of the Scientist over the years.

When I first entered Science (more than 30 years ago!) there was a perception (at least held by me) that a career in Science was more of a calling than a job, it was about pushing back the frontiers of knowledge. In those days the genetic code was being broken and there was a queue at the Science Library to reading the latest PNAS article to learn what the next three-letter code stood for. This was probably the heyday of intellectually driven investigative research; a far cry from the outputs and outcomes of today. In those days if you had a degree in molecular biology, the world was your oyster.

I have no problems with public accountability for the things I do as a scientist as long as they do not interfere with the way Science is conducted. The real challenge will be to make the public understand how Science is really carried out, that 90% of research projects do not

work (at least for me), that we make mistakes, that many popularly held hypotheses (guesses) have been proven to be wrong and that outside a particular scientific theory (paradigm) there are no such things as "facts". Furthermore you will have to tell them that many scientific breakthroughs were made by strong-willed individuals who flew in the face of the perceived scientific wisdom and that chance and serendipity have played a large part in many discoveries. Maybe a short course in the philosophy of science would benefit us all.

The end result of the public debate on genetic modifications may be that we will be prevented from doing certain kinds of experiments. This will be a "break of the faith" that the public had given to scientists to pursue the Holy Grail of Science, that is to seek out the truth by intellectually driven research. In the meantime I am getting ready to change the sign on the door from "Centre for Gene Research" to the "Centre for Things You Don't Have to Worry About".

James Kalmakoff

A Healthy Vision for Science and Technology

(first published in the Otago Institute's Newsletter)

The Otago Institute Lecture Programme for 2000 started off with a flourish with the Hutton Theatre packed to capacity to hear the Minister for Science and Technology speak about his vision for our scientific future. Mr. Pete Hodgson is no stranger to Dunedin and kept the audience, which consisted largely of scientists from "over the road", alert and interested with his lively speech. Amongst what could be the most critical of his electorate Mr. Hodgson was relaxed and spoke with convincing sincerity. He began by outlining his other seven portfolios and spoke of his initial difficulty in understanding science policy and his desire to do away with bureaucratic language, such as the dreaded "MoRST Code" associated with the Foresite Project , drawing appreciative chuckles from the audience.

He then went on to outline his vision for the future of New Zealand in 2010, and explained how he hoped that his party would achieve this transformation by remaining in power for most of the next 10 years. It went something like this: "By 2010 New Zealand will be spending 0.8%

of its GDP on research and development; private sector investment will have tripled from its present level; we will have become a world leader in marine science; strategic links will have been formed to other research initiatives around the world making us very much an integral part of global research; the Nobel prize will be won by a (female) Otago University scientist for her work done in the Dunedin "global cluster point" for that type of research; and science and technology will be embraced and supported by the general public!" Mr. Hodgson sees his and his government's role as that of the facilitators to help this to come about. He then went on to outline how they plan to do this. In order to increase the R& D share of the GDP to the desired 0.8% Mr. Hodgson proposed three main investment priorities, which he dealt with in order of importance.

First was the necessity to leverage private sector involvement in R & D. At present, our level of private sector investment in R & D is only half that of other western countries. He proposed a dollar for dollar scheme, providing a leadership signal to the private sector. He spoke of schemes to lessen the costs of protecting intellectual property and technology, and of supporting small businesses. Instead of just start-up schemes he proposed schemes to help existing successful small businesses to expand, capitalise on their success and to look to the export market to help address our negative balance of payments. He gave the example of Swingthru International Ltd., a company who developed an ingenious system of container handling that has become sought after by large international companies. Pouring money in will not be the only answer but active industrial development is also needed. Regional clusters featured in his plans and his acknowledgement of the importance of human capital was well received. On this point he noted that presently our brightest people often do not do science and our skilled scientists are often forced to go overseas. To change this he proposed that we increase the number of science teachers by offering bonded scholarships to students undertaking to become science teachers. At the tertiary level, he said that we must cultivate centres of scientific excellence and provide money for postdocs.

Second on his list of investment priorities was the need to do basic science. He said that basic science underpins the progress of society. He said that the $11M given to the new NERF fund will increase over time and pointed out that some of the successful applicants in this round were from industry and that we must be aware that universities don't

have a franchise on wisdom. The fund was oversubscribed by ten times. He also said that he was also committed to increasing the funding available via the Marsden Fund over time. He used the fact that New Zealand at present contributes only about .013% of global R & D to illustrate the notion that we must intelligently tap into the global knowledge base and again stressed his commitment to an investment in human capital.

About his third investment priority, strategic research, he had this to say: that we need to improve our evaluation of the cost effectiveness of our strategic research. After this Mr. Hodgson picked out three major topics that he thinks we must focus on now. These were the Royal Commission on Genetically Modified Organisms, Biodiversity and the Science and Innovations Advisory Council (SIAC). The need for the first of these had arisen because the pace of scientific advances had outstripped the development of concomitant ethics and because the public don't trust technology. We need to manage the debate well and with tolerance. He gave an example of how we ought to choose GM technologies that will have high economic benefit and low risk, such as the production of pharmaceuticals in sheep. He stressed that Biodiversity must become one of his priorities.

The questions of our understanding of the state of the marine environment and biosecurity are also high on his list. He spoke briefly of the new Science and Innovations Advisory Committee, which he is setting up. This will be a group of seven "wise men" (eminent scientists?) who will meet with the Prime Minister quarterly to discuss important issues. They will also have the function of developing high level strategy and also engaging the public about the importance of science and technology. In his concluding remarks he said that interactivity is the prize of the future and reminded scientists that funding doesn't exist for them personally. In the future innovations system we must all be interactive players.

After his formal talk the Minister answered questions about education, the future of science centres such as discovery world and SIAC before being given an appreciative round of applause from the audience. The vote of thanks was given by Dr. Jean Fleming. The general mood of those leaving the hall was one of relief and cautious optimism, that the minister's heart was in the right place even if there was a note of familiarity about the "Big Think" ideas that he was proposing.

Michele Coleman

Update on the Microarray

The Otago Genomics Facility (OGF) is a University of Otago initiative that comprises two powerful technologies: laser capture microdissection (LCM), and gene expression microarray (GEM). This article provides an update on the status of the microarray laboratory of the OGF, and relates some recent events regarding the microarray set-up. DNA microarrays are fabricated by robots, generally on glass but sometimes on nylon substrates. There are two major types of information that can be investigated with DNA microarray technology. The first is the analysis of DNA sequence, and the second is the determination of gene expression levels by analysing the abundance of mRNA in a sample of interest. Two variants of DNA microarray technology are used to study either gene expression or DNA sequence, and the difference relates to the form of the nucleic acids used to generate the array. One method to study gene expression uses cDNA immobilised to a glass surface, and exposed to a set of labelled cDNA either separately or in a mixture. This method, traditionally called DNA microarray, is widely considered as being developed at Stanford University. However, a recent Trends in Biotechnology article provides some generally forgotten facts on the actual origin of this technology (1).

Alternatively, an array of oligonucleotides (20~25- mer) or peptide nucleic acids (PNAs) is synthesised either in situ (on- chip), or by conventional synthesis followed by on-chip immobilisation. The array is hybridised with labelled sample DNA and the identity/abundance of complementary sequences are determined. The method using oligonucleotides, historically called GeneChip(r) arrays or DNA chips, was first developed at Affymetrix, Inc. It is worth noting then, that the term GeneChip should be used in reference to Affymetrix oligonucleotide arrays, to distinguish from cDNA microarray. Spotted oligos may be used for both expression and sequence analysis, whereas cDNA microarrays cannot provide sequence information. The OGF microarray lab provides DNA microarray technology to allow researchers to determine gene expression levels using cDNAs arrayed on glass slides. The use of cDNA microarrays on glass slides provides a relatively simple means of obtaining large-scale expression data for thousands of individual genes

from a single experiment. But to do so requires (a) a robot to spot the cDNAs onto the slides, and (b) a means of reading the signals from the slides after hybridisation of fluorescently labelled test and reference cDNA species to the slides. The major items of microarray- related OGF equipment include a ScanArray 5000 microarray reader (GSI Lumonics, URL 1), a Molecular Imager FX (fluor - and phosphor imaging from BioRad, URL 2), a GeneTAC Hybridisation Station (Genomic Solutions, URL 3), a Biomek 2000 liquid handling robot (Beckman Coulter, URL 4), and an SDDC-2 (Semi- automated DNA Dispensing Cell Mk 2) arraying robot from Engineering Services Inc (ESI, URL 5).

Final installation of the Biomek 2000 liquid- handling robot is scheduled for May 8, 2000. All other components are operational and in use. The last and most crucial piece of equipment to arrive, the SDDC-2, was delivered and installed during early March. The SDDC-2 is capable of depositing greater than 64 000 cDNA spots (100 mm diameter) on a single microscope slide (75mm x 25 mm). This density is far greater than will initially be arrayed. For example, the OGF has purchased the 6K human known gene set of clones from Research Genetics. Duplicate spotting of the approximately 6000 cDNAs in this collection requires only about 12 000 spots. Preliminary arraying with the SDDC-2 in the OGF has been of 18 432 spots, with 180mm centres between adjacent spots. March 10th was the final day of SDDC-2 installation. I'm pleased to say that after a week of robot wrestling, Sasan, the engineer from ESI, had a grand days sightseeing involving Albatross colonies, a harbour cruise, a meal of lamb (a personal goal for his NZ trip), and an excursion on the Taieri Gorge train. I'm loath to admit it, but Sasan - a Toronto-domiciled Iranian - saw more of Dunedin and surrounds in one day than I have in 3 months (although, when pressed, perhaps many local residents could confess to the same?).

March 10th also signified the day of my departure from Dunedin bound for the Cancer Genetics Branch (CGB) of the National Human Genome Research Institute (NHGRI), at the National Institutes of Health (NIH) in Bethesda, Maryland (URL 6). Under the direction of Jeff Trent and Paul Meltzer, the CGB at the NHGRI is second only to the Brown Lab at Stanford in microarray experience, application, and publication. The purpose of this two-week visit was to gain hands- on experience with the NIH protocols relating to the fluorescent labelling of RNA and subsequent hybridisation to cDNA microarrays produced by the CGB. This lab was one of the first to embrace cDNA microarray technology,

and remains instrumental in the extension and enhancement of array analysis. Thus, the opportunity to meet and talk with researchers involved with microarray fabrication and analysis on a daily basis was very beneficial. In addition to rubbing shoulders with recognised luminaries of the microarray community, was the opportunity to gain an insight into the "NIH Experience". Research fuelled by large amounts of money certainly provides contrast to the situation in New Zealand. The annual budget for the NHGRI alone is in the order of USD 50 million. The NIH as a whole receives billions of dollars in federal funding annually. A trip to one of the on-campus consumables supermarkets exemplifies the amount of money being invested in research at the NIH, and was certainly a novel experience. Researchers commandeer shopping trolleys, and having perused the aisles and taken their fill of reagents, solutions, kits, and Gilsons, their trolley contents are scanned and packed at a checkout. The contented researcher then wanders back to the lab across the picturesque Bethesda campus, looking out for squirrels, and toting brown paper supermarket bags overflowing with goodies. Of course, this means that with no time to waste on making up solutions, or waiting for kits and reagents to arrive, there is no excuse for not getting that experimental work done for the next PNAS or Nature Genetics paper.

The two weeks spent at the NIH were extremely valuable, and curtailed months of learning by trial and error. Heather Cunliffe and her husband Gerry hosted me during my stay. Heather completed a PhD with Ian Lamont in the Biochemistry Dept. at Otago University, before undertaking post-doctoral work with Mike Eccles in the Cancer Genetics Lab, also within the Biochemistry Dept. Currently, Heather is working on a breast cancer- related project under Paul Meltzer in the Cancer Genetics Lab at the NHGRI. Gerry has taken a mechanics position at a Ford dealership (and has become an ace Belt Way driver - no mean feat from the streets of Dunedin. With 4-6 lanes in either direction filled with congested, tailgating traffic moving at a speed in great excess of the speed limit, the I495 6 is not a place for the faint of heart!). Following my stint at the NHGRI, I spent a week in Toronto. The purpose of this visit was to spend time with ESI, the makers of the SDDC-2 arraying robot, and with researchers associated with the Ontario Cancer Institute (OCI; URL 7) within the Princess Margaret Hospital in down-town Toronto. A strong relationship exists between ESI and the OCI due to a collaborative effort between the two groups that resulted in the SDDC

line of arrayers. The staff at ESI are exclusively engineers and required a molecular biology testing facility to develop the SDDC. The OCI - 5 minutes walk from ESI - provided this. ESI is located on the University of Toronto campus, and leases space in the Canadian Mining Hall of Fame buildings (I am not making this up). The head of the company holds a chair in automation and robotics and still teaches at the university. There is at least one other Professor of engineering at the company, and post-graduate students working on projects within ESI further bolster the academic link. A small, focused company that hand-produce robust equipment with high quality components, we expect many hours of consistent and reliable spotting from the SDDC-2 within the OGF. Visiting the OCI also provided an insight into how a lab runs their microarray set- up on a day-to-day basis using the same equipment as the OGF - SDDC-2 arrayer and ScanArray slide readers. In the immediate future, the microarray lab within the OGF will be focusing on the following: running all the equipment "solo", re-amplifying the clone stocks for arraying (10 000 PCRs, all checked on agarose gels), and validating array and hybridisation quality e.g. are the pins spotting, has the DNA stuck to the slides, what are the best hybridisation conditions/ solution when using the GeneTAC hybridisation station. To quote from the Cold Spring Harbour Laboratory Microarray Course manual from 1999 "There are more tricks to this protocol than a dog could ever learn. Everybody does it differently". The OGF is currently evaluating what tricks work in Dunedin, and what tricks do not.

(1) R. Ekins and F.W. Chu. Microarrays: their origins and applications. Trends in Biotechnology, 1999, 17, 217-218
www.gsilumonics.com
www.bio-rad.com
www.esit.com
www.ocl.utoronto.ca/sevices/microarray

Aaron Jeffs

Real-time PCR News

In February this year a workshop on real time technology was held in the Microbiology department. It consisted of a series of lectures on the

latest technology and a "wet" hands on workshop. The comments back from both the organisers and participants indicated that it was very successful. Below are some of those comments. At PE Biosystems we were thrilled with the attendance at the University of Otago TaqMan workshop, both from the perspective of the amount of interest shown and the quality of questions and enquiries. The mix of technology presented by the invited speakers was well balanced and complementary giving an enjoyable introduction to some of the latest surges in biotechnology. During the wet workshop a total of 8 new TaqMan assays were trialed targeting a diverse range of amplicons truly reflecting the diverse science being undertaken on campus. All assay designs produced successful amplifications and provided wet workshop attendee's with plenty of material to discuss further. We think all attendee's left the workshop very well versed in the development and analysis of TaqMan data and are now well equipped to continue their experiments.

Since the workshop we have had multiple productive contacts with new users of the TaqMan system and we encourage anyone with further issues to contac Richard Harrison for applications questions or Stacey Nelson.

The workshop was an excellent introduction to the Taqman technology. Having primers and probes designed by a PE Biosystems expert, an easy to follow protocol and plenty of guidance lead to results at the end of the day. During the workshop I ran a few samples to see if expression patterns and levels of the activin growth factors matched those I've been getting with the traditional "bands on gels" RT-PCR. Results were encouraging, giving the same expression patterns in similar quantities. Control experiments indicated that Taqman specificity was sufficient to ensure no RT minus amplification. The workshop has enabled us to confidently expand into Taqman technology.

Elspeth Gold

Please feel free to contact me for any information on the ABI 7700.

Lynn Slobbe

Protein Prattle

Something in our supposedly ultra-clean water? With the arrival of a second child imminent, Diana Carne recently resigned her position as Chief Operator of the Protein Microchemistry Facility to take extended parental leave. Thankfully, Diana managed to work almost right up to her "D day" and we hope that she will return to a part-time position next year. While the service operation suffered in Diana's absence, the Facility doors were kept open during April in large part due to earnest assistance from Jew Chung Kon and Mat Beyer who helped me process some urgent samples and do a major overhaul of the big instruments, the latter a tedious job that was much overdue. In the meantime, we are extremely fortunate to have secured the services of Joanna Mudford as our new full-time operator. With her previous experience operating the protein microsequencer and LC-mass spectrometer at Massey- Dairy Research Institute and more recent DNA studies in the Otago Cancer Genetics lab, Jo brings a particularly useful skill base to the job which increasingly involves interaction with gene jocks eager to learn something about their favourite (and perhaps only putative) gene product. Jo kicks off in mid-May and I am confident that she will be up to speed in a week or two, so please feel free to challenge us with your precious samples as from now.

Our other new baby is an LC-mass spectrometer which, while weighing a meagre 85 kilos, also exhibits a voracious appetite. Funded by the Otago University as a multiuser instrument, this "electrospray" MS will soon be made 8 available through the Protein Microchemistry Facility for routine investigations of peptides and proteins, complementing our widely-used MALDI-MS service. I intend to explore cutting-edge proteomic applications on my own and collaborative projects, and once established these procedures will be added to the Facility service operation. The instrument will also be available for small-molecule analyses (initially for on- campus users only from Pharmacy, Chemistry etc). Separate fluidpath components have been purchased to minimise the inherent cross- contamination problems. "Proteomics" is increasingly on people's minds, lips ..... and grant applications. For many, the desirability of getting into this high-profile area is overwhelmed by the steep learning curve and associated high costs. To help on-campus groups wanting to try their hand at 2-D gels, we are in the process of making specialist equipment (IPG focussing, large-format PAGE) and

supplies (IPG strips, chemicals) available through the Protein Microchemistry Facility. In-gel digests and high- sensitivity peptide separations will also be offered if the demand exists (currently these are available for collaborative projects only). Details are currently evolving, but those people wanting more information about this new initiative please do not hesitate to contact me.

The Protein Microchemistry Facility (Room 120) is located on the first floor (east end) of the Biochemistry Department. For general enquiries in 2000 please visit our website (enter through biochem.otago.ac.nz) or contact Jo Mudford (Chief Operator) by email (protein.microchemistry@stonebow.otago.ac.nz), phone (479 7542) or fax (479 7866). Alternatively Jew Chung Kon (Operator) might be tracked down in the Hubbard lab (phone 479 7938). Dr Mike Hubbard can be reached by phone (479 7831 or email (mike.hubbard@stonebow.otago.ac.nz).

Mike Hubbard

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