Centre for Gene Research

September 1998

From your Director

"May you live in interesting times" was a curse that Chinese in the Middle Kingdom used for impending social and political changes. Today it seems more appropriate to quote Bob Dylan: "the times they are a changing..." It could be that time of the academic year but I am mentally exhausted with all the proposed changes, restructuring and being Foresighted to death. One of the changes that will directly affect the CGR is the move of the DNA sequencing facilities and ancillary activities to a new laboratory on the 8th floor of the Microbiology Building. This is likely to be completed by the end of October. This should provide for a more coordinated DNA sequencing service along with the recommissioning of the ABI 373 for GeneScan capabilities and a proper home for the quantitative PCR (Taqman) equipment. And who knows maybe the new CGR Website and the on-line DNA submission forms and accounting system will be ready by then as well (promises, promises..).

Some of you will be aware that we have gone through an exercise in strategic research planning for the University in regard to the purchasing of major pieces of equipment. As a follow up to the CGR Emerging Technologies Workshop held in August, there was a ground swell of support for some kind of "Bioinformatics, Genomics, Proteomics" initiative. A meeting was held in the Biochemistry Department attended by interested parties and a 'shopping list' (which totalled about $3 million) was produced and put forward to the 'higher authorities'. Rumour

has it that this list has received a favourable response and we are now awaiting some kind of announcement. Hopefully this will happen before Christmas!!!

In another arena, the CGR responded to Diana Twigden's document: Foresight Gene Technologies Submission. Briefly, Diana proposed the establishment of a National Gene Technology Centre (presumably in Auckland) where the cutting edge genomic research would be carried out. The view of the CGR Committee was that we did not favour a 'bricks and mortar' institute but preferred a National Genomic Network and that the money was better spent supporting 'working scientists' rather than buildings. An excerpt follows:

We agree that technologies associated with gene analysis are important to have and develop in New Zealand. There is no doubt that being part of such development is essential if NZ scientists are going to be internationally competitive in the coming years.

NZ is a small country and its expertise is widely spread over the main academic centres. For this reason, we favour the establishment of a National Genome Network rather than a single Gene Technology Centre. The different components of this network (nodes) could be located strategically throughout the country. Thus, different expertises would reside in different centres but linked in name and some notional coordinating administration. Obviously, each facility would also be linked by an electronic network.

[The CGR] has 213 members from within the University of Otago in Dunedin and the Christchurch and Wellington campuses. This membership represents some of the most creative and energetic scientists in New Zealand (the world?). In 1997 the members received about $20 million from external competitive granting agencies. One of strengths of the Centre [CGR] is its "grassroots" origins and the fact that it represents active research scientists.

The Centre [CGR] would clearly then be a useful member of any National Genome Network. Its already established network of scientists would clearly aid in the development of such a Network.

If anyone wants the full reply to Diana Twigden, I can email you a copy.

Finally, next year's Queenstown Molecular Biology Meeting is being organised by the CGR. It will be held August 15-20th. The planning committee consists of the 'A' team: Anthony (Reeve), Andy (Mercer), Antony (Braithwaite) and myself. We will be co-opting other people and are asking for suggestions of speakers and themes and sponsors and local participants. Contact any of the above members or me if you want to be part of the organising committee or have any suggestions.

James Kalmakoff

Emerging Technologies Workshop Report

The recent CGR workshop on emerging technologies was a great success, bringing together over one hundred researchers from around the University to discuss the latest developments in gene research technologies. The original mandate from James Kalmakoff was to host a meeting that would bring us up to date on quantitative PCR, microarray technologies, proteomics and bioinformatics. In addition to hearing about our own members' experiences and aspirations of these new developments currently taking the world of molecular biology by storm, we were also able to invite several leading scientists from Australia to give insights into their own particular fields of expertise.

The meeting kicked off on the Friday evening with a social gathering at the Staff Club where Professor George Petersen, in an after dinner speech, modestly re-lived some of his early years as a molecular biologist. His anecdotes of driving north from Oxford to attend the Edinburgh Festival only to be thwarted by a dodgy car engine and of his culinary skills when left alone for only a few days by his wife were delivered with alacrity. George's enthusiasm in visiting virtually every molecular biologist during a brief visit through the States in the 60s, combined with his unstinting commitment in setting up and leading a world-class biochemistry department back in Dunedin, are lessons for us all to take inspiration from.

Saturday dawned calm and sunny, but with the threat of an approaching southerly front to remind us that winter hadn't completely forsaken us this year. Our first speaker was Professor Tony Reeve who, with only three days' notice, had kindly agreed to step in following the unavoidable

withdrawal of Professor John Mattick from Brisbane. Tony's remit was to enthuse everyone about new genomic technologies. His introductory talk on the power of advanced molecular methods, particularly gene microarrays, to push back the frontiers of medical research ably demonstrated just what we wanted to achieve in hosting the workshop.

The next two speakers, separated by commercial rivalry as well as by morning tea, were both representatives from companies heavily involved in the development of PCR. Brant Bassam, from PE Applied Biosystems, gave an incisive talk on real- time PCR and its applications based on the TaqMan and SYBR Green chemistries, including quantitative PCR and allelic discrimination. This was particularly relevant and welcome in light of the CGR's recent acquisition of a GeneAmp 5700 system - the platform for these applications. Not to be outdone, John Mackay of Roche Diagnostics (formerly Boehringer Mannheim), followed up with a full and technical presentation of an alternative PCR platform on which to perform similar and additional applications - the Light Cycler.

Our first overseas invited speaker was Dr Marc Wilkins, from the Australian Proteome Analysis Facility at Macquarie University. Coining the term 'Proteome' in 1994, as a response and complement to the burgeoning interest in genomics and DNA, Dr Wilkins has been instrumental in setting up an original and highly resourced centre for comprehensive protein expression analysis. His talk, entitled "Proteomes and Proteomics: tools and techniques for the high-throughput analysis of proteins", was entertaining and not a little enlightening for the 'gene jockeys' amongst us, giving a timely reminder that genetic studies alone cannot be used to predict phenotypic outcomes - the subtleties of proteomics are another layer of molecular control altogether. Rest assured there'll still be lots to do once Venter has finished sequencing DNA...

Breaking for lunch back at the Staff Club those of us not way-laid by the media for comments on genetically modified food were given the opportunity to eat, drink and watch microarraying robots strutting their stuff. As well as trade displays from Roche and ABI, Stuart Elmes from the Cambridge- based company BioRobotics was also attending the workshop, showing a video of their MicroGrid instrument in action.

Suitably refreshed, it was time for the second of our overseas speakers - Dr Tim Littlejohn, the head of the Australian National Genomic Information Service. Speaking about "Integration, delivery and access to bioinformatics resources", Dr Littlejohn gave an excellent presentation on the downstream implications of all this genomic and proteomic data, namely how the hell do we set about analyzing it. Well, it seems the Australians have stolen the march and have developed a whole host of integrated tools for "database inter-operation, high performance methods for phylogenetic analysis and efficient methodologies for the delivery of biocomputing services". We must ensure that funding will be forthcoming to enable us to subscribe to these essential bioinformatics tools.

Afternoon tea was accompanied by the predicted southerly, heralding the final part of the workshop programme - microarray technology. This session was planned as an informal discussion, to briefly cover just what microarrays are being used for, dispel some of the hype and myths associated with the much vaunted "DNA chips" and to talk a little about what kinds of array experiments researchers at Otago might like to carry out. Introducing the session, I explained the highs and lows of expectation I'd experienced in talking to scientists in America and Europe who are developing microarray technology, more specifically for mutation detection. Dr Mike Sullivan, senior lecturer in paediatrics and a principal investigator in the Cancer Genetics Laboratory at the University of Otago, then eloquently described several experiments he could undertake immediately in order to answer questions about the coordinate expression of genes involved in childhood tumours, if only he could have access to microarray technology. Bren Collinson (from the company Molecular Dynamics, which manufactures some of the microarray-analysis equipment) gave us some insights into the pitfalls of this technology, though some of us thought he was being overly pessimistic (or perhaps just politic?). Watch this space.....

In summary, I think the workshop was both informative and very timely. It is clear that the University of Otago needs to embrace many of these new technologies if it is to compete successfully in the rapidly changing world of molecular biology. To take a leaf out of Professor Petersen's book, it wouldn't do us any harm if we were to actually take a lead in some of them too.

No workshop is successful without the input of many people. In addition to all the speakers, I would especially like to thank the rest of the organising committee: Chris Brown, Glen Buchan, Bronwyn Carlisle, Mark Dalphin, James Kalmakoff, Mike Hubbard and Craig Marshall. Thanks too to the graduate students who worked the projectors and to the University who subsidised the meeting through the CGRs 'Gene Structure and Function' theme.

Scott Tebbutt

Notice

On Monday 2nd November, Jan Luton from Genomic Solutions will be in Dunedin to talk about her company's products, including microarraying robotics and all downstream analysis tools. Genomic Solutions also has products in development relating to 'Proteomics'. A Special Seminar will be advertised closer to the date, but if anyone would like to meet with Jan, please contact Scott Tebbutt (tebbutts@sanger.otago.ac.nz).

The new ABI 7700 Sequence Detection System (Real time PCR)

In May this year the Center for Gene Research purchased the ABI 7700 sequence detection system. It has been installed on the 8th floor of the Microbiology department. This system enables fully- automated real-time detection of specific PCR products possible. The system integrates four major elements : 1. fluorogenic chemistry for target- specific oligonucleotide probes 2. exploitation of the polymerisation-dependant 5" nuclease activity of the DNA polymerase 3. instrumentation to measure fluorescence signal within a closed PCR reaction tube 4. software to process and analyse the data. The 96 well format allows high-throughput particularly important in genetic screening studies. There are several applications for this technology : pathogen detection (several kits are available from the company), allelic discrimination and quantitative PCR. This technology has specific guidelines for PCR primer and probe design and dedicated software has been supplied. The latest updated version has now been installed and a manual is available. The company is looking at producing a start up kit in order to reduce initial costs. This will include a Taqman core kit (enzyme, reagents, Rox

dye for standardising the background & one probe) and specialised tubes.

To date it has successfully been used to genotype over 200 animals and RT-PCR quantitative analysis. Below are comments from the two current users.

I was looking forward to the arrival of this system as for the past two years I have done semi-quantitative RT-PCR the "old way" ie blotting, probing & scanning. The advantage of the ABI system is that it is quantitative & fast with no further manipulations after the PCR run. After an initial change in my thinking for optimisation, things fell into place. Both primer & probe concentrations need to be optimised, in order to save money finding the lowest concentration of probe required is beneficial. The results from my first run looked nothing like the test run we did with the technician during the installation. Fortunately they have an 0800 help number to their technician, the number has been well used. At last I could run some "real" samples. It was great to finally be able to put a quantitative value on the amount of message I had previously been seeing. I did a run with the ABI machine & a run the traditional way (gel & scanning). The ABI results were completed by the end of the morning while it took the rest of the day to complete the other, & considerably more "hands on" work. The results are reproducible & the amount of starting material calculated for you, what could be easier. The biggest delay has been waiting for probes to arrive. The probe design programme is user friendly & to date has created probes that work well. I have not worked out my current costs but the convenience of no down-stream manipulations makes it well worth it.

Lynn Slobbe

The greatest interest in the ABI PCR machine at present seems to be its use for quantitative PCR. Its other main use is for allelic discrimination. My work using this machine has been looking at two single nucleotide polymorphisms in sheep situated 8bp apart. By using probes specific for each allele (labeled with different fluorescent reporter dyes) in the PCR assay it is possible to determine whether an animal is homozygous (and if so for allele 1 or for allele 2) or heterozygous. This process is efficient and allows high throughput. As no post PCR analysis (gel running etc.) other than ~15min of computer is required, time involved is minimal. I find it takes me about 25 minutes to set up the PCR for 96 animals, 2

hours to run the PCR and about 15 minutes to analyze my results to completion. I have found the machine to be user friendly and results so far have been excellent (reproducible, consistent and clear). If you're wondering about the cost, my PCR assay costs about $2 per sample.

Korena Paterson

I will keep you up to date with developments. If you have any questions please contact me.

Lynn Slobbe

Marsden Fund

The following members of the CGR were successful in the latest round of Marsden Fund grants:

David J Galloway, Biochemistry Department; Principal Investigator
Do New Zealand's native grasslands and forests like lichens?

The project will use large, fast-growing lichens in the genus Pseudocyphellaria, from tussock grassland and rainforest biomes in New Zealand, to find out how these unique symbiotic systems can exploit both low-light and high-light levels for photosynthesis and nitrogen-fixation in biomes that are naturally nitrogen-limited. The investigators' skills in lichen systematics, nitrogen fixation and in cyanobacterial photosynthesis, will be strongly linked in this novel interdisciplinary study which will attempt to explain, in biochemical terms, the contributions that diazotrophic lichens make as "biological fertilisers" to the maintenance of biodiversity in our grasslands and forests.

Parry Guilford, Biochemistry Department; Principal Investigator.
E-cadherin: An evolutionary link between bacterial immunity and cancer susceptibility.

We have recently shown that mutation of the E- cadherin gene is responsible for inherited predisposition to stomach cancer in several extended Maori families. It is likely that mutations in this gene are common in the Maori population and may have provided a survival advantage which outweighs the increased risk of cancer. We are going to test the hypothesis that variation in this gene leads to resistance to

the bacteria which cause Listeria. This knowledge will lead to a better understanding of the causes of cancer and may provide innovative strategies for treating bacterial infections.

Sally P A McCormick, Biochemistry Department; Principal Investigator.

Designer peptides to inhibit atherosclerosis
Heart disease is a leading cause of early death in Westernised countries including New Zealand where it claims over 6500 lives per year. A major risk factor for developing heart disease is high blood levels of lipoprotein(a), a cholesterol-rich lipoprotein formed in the blood by the binding of a low density lipoprotein to the apo(a) protein. There is currently no drug available to lower lipoprotein(a) levels. This research aims to develop a new strategy to prevent lipoprotein(a) formation and has the potential to provide an effective new therapy for people with high plasma lipoprotein(a) levels who are at high risk of developing heart disease.

Michael P Murphy, Biochemistry Department; Principal Investigator.
Modification of mitochondrial function within living cells.

We have developed a targeting system that uses the mitochondrial membrane potential to deliver novel, bioactive compounds to the mitochondrial compartment of animal cells. These compounds will be used to measure and modify crucial aspects of mitochondrial function within intact cells. This will lead to new insights into the role of mitochondria in the production of reactive oxygen species and programmed cell death. This work will also lead to new methods to modify the expression and replication of mitochondrial DNA.

Russell T M Poulter and John F Cutfield, Biochemistry Department; Principal Investigators.
The origin of vertebrate retroviruses: Horizontal transmission from fungi?

Research by Russell T M Poulter at Otago has resulted in the discovery of the first vertebrate retrotransposon (termed sushi). This mobile genetic element was found in the genetic material of the fugu fish. This is a finding of major significance in the understanding of retroelements, the class of genetic elements that includes the vertebrate retroviruses such as HIV (the causative virus of AIDS). It is proposed to investigate

two phylogenetic questions: (1) Are the vertebrate retroviruses derived from this newly described class of vertebrate retrotransposon? (2) Can the origin of these vertebrate retrotransposons be traced to the horizontal transfer of a 'jumping-gene' from a fungus to an ancient vertebrate?

John Sullivan, Microbiology Department; Principal Investigator. Decoding the symbiotic island of Mesorhizobium loti The aim of this proposal is to define the genes contained on the 500 kb symbiosis island of Mesorhizobium loti by nucleotide sequence analysis. The symbiosis island is a large genetic element located on the chromosome that converts nonsymbiotic rhizobia to strains able to fix nitrogen with a legume host. It belongs to the same group of genetic elements as pathogenicity islands that confer virulence on otherwise benign bacteria. Sequencing will reveal the bacterial genes required for the Rhizobium-legume symbiosis, and will provide insight into the evolution and acquisition of these genetic elements that allow bacteria to interact with plants or animals.

Goodbye to Mark Dalphin

The rumours are true. Mark Dalphin is leaving the University of Otago and the Centre for Gene Research for a job with the drug company, Amgen, in California. While he vigorously denies the rumoured "six-figured" salary that is promised to "Bioinformatics Professionals", he isn't unhappy with what he has been offered.

Fortune Magazine in picking their "100 Best Companies to work for in America" (Jan 12, 1998) picked Amgen as number 74, saying:

Leader of biotech industry nearly quintupled work force this decade. Employees showered with benefits: stock options, subsidized on-site child-care center, free beer busts every Friday night, free refreshments, quarterly parties, on-site gym, 15 days' vacation in first year, host of on-site conveniences (film processing, flower shop).

At least he won't miss the Biochemistry Department's Happy Hour, though it will be hard to replace the Emerson's Weis Beir.

Amgen is located in Thousand Oaks, about 1 hour's drive West-Northwest from Los Angeles (airport) on the US101 freeway. A further 45 minutes driving would bring one to Santa Barbara. A 30 minute drive

South on twisty roads thru the Santa Monica Mountains brings you to the Pacific Ocean, and to the North is Los Padres National Forest. The two neighboring towns, Simi Valley and Moorpark, along with Thousand Oaks, are consistently the "safest cities" (placed one, two and three on the list) in the USA. These are towns of about 100,000 people each: boring, middle-class suburban towns with large tracts of big family homes, mostly built from 1970 onwards. Median household income is about US$60,000 per year.

The climate looks nice, though a bit on the hot side: January average lows of 5šC and average highs of 15 with a mean of 10šC. July lows of 15, highs of 30 and mean of 20šC. And then there is the rain. Or rather, lack of rain: about 10 inches per year of which 7.5 inches fall in January. Gentle winds too with an average relative humidity of 50%. The place is dry!

The downside to all this is the high cost of living in the area. Two bedroom apartments cost between US$700 - US$1200 per month. The median cost of a three bedroom family home with two car garage, large section, etc is US$280,000! Food costs about the same as here; cars of similar vintage to cars here, cost about the same, but everyone drives newer cars. And then there is insurance! How about US$2,000 per year per car? And vehicle registration is 2% of the vehicle's sales price. At least the petrol costs about 1/2 what it does here.

Mark will be working in the new Genomics Group at Amgen, attempting to locate protein coding sequences in genomic DNA sequence prepared from some in house sequencing projects. He will be part of a team of 10 other scientists working on the same or similar projects. Novel coding sequences will be examined for potential as thereputic agents and promising candidates will be turned over to Molecular Biologists for cloning and creation of transgenic animals for testing the effect. Bad guesses are frowned upon.

Assuming the American Embassy provides the required visas in time, Mark will be leaving with his wife, Ruth, at the end of November.

GCG Course Will Not Be Held This Year

With Mark leaving Dunedin, the GCG Course will not be held this year. In addition, there is a strong possibility that our current GCG package

will be upgraded to something newer, which would require a re-write of the GCG Course material. The Centre for Gene Research expects to have a new, updated Sequence Analysis Course operating next year.

A Glimmering In the Far Distance

Amidst the doom and gloom that surrounds science in New Zealand just at present with scenarios of few scientific jobs for New Zealand and a general economic depression I was delighted to find something positive. Well, sort of delighted. The glimmering was to be found at the recent Protein Society meeting in San Diego. The nature of this meeting has changed markedly over the last 10 years. Early in the decade, those attending were almost all from academic institutions, but now the majority of attendees were from industry, primarily from the biotechnology industry.

I suppose this reflects the adoption by industry of protein engineering as a way of producing new products and making money. Perhaps what is surprising is the range of companies that have adopted this approach. The biggest companies and the small biotechnology startup companies were all represented at the conference.

From New Zealand it is hard to judge the significance of this development. However, consider that about a third of the newly rich in the USA acquired their wealth from biotechnology, mostly by creating a startup company and selling it when the product proved successful. Furthermore consider that there are more biotechnology laboratories and companies on one road in San Diego than there are in the whole of New Zealand.

Biotechnology is alive and well in the USA and making a great deal of money. Accordingly these companies are hiring talented people as fast as they can. On senior academic at the Protein Society commented that it was getting increasingly difficult to hire really good people because they were all going to much better paying jobs in industry. I found this a startling comment because for a number of years the post-doctoral job market has very much been slanted in favour of the hirer. Things, it seems, are changing.

So what was the glimmering in the distance? Post- doctoral positions in industry in the United States, particularly in California and around

Boston are abundant. Companies are hiring people with experience in "proteomics" and structural biology by the handful. Sadly, these jobs are in the United States and it seems likely that unless science (and biotechnology) is much better funded in New Zealand, those that leave New Zealand are even less likely to return.

Much could be said of the folly of allowing talented people to disappear like this. It seems blindingly obvious that this is not in the best interests of New Zealand. It is equally blindingly obvious that the best advice that can be given to a young scientist looking for a career past their PhD is to look to the far North East. Eldorado it might not be, but it is certainly better than scratching around looking for non-existent positions in an over-competitive environment in New Zealand.

Craig Marshell

A Grassroots Organisation of Active Research Scientists