Centre for Gene Research

April 1999

From your Director

Since the last Newsletter the Centre's DNA sequencing facility has moved into a newly refurbished laboratory on the 8th floor of the Microbiology Building. This has resulted in better facilities and a more spacious environment and an overall improvement of service to the members. The old Model 373 sequencer was recommissioned for genotyping (Genescan). However there may not be enough usage to justify the service contract on this instrument and plans are afoot to convert the Model 373 sequencer to the BigDye chemistry in order to take the sequencing overflow from the Model 377. This will mean that the Genescan runs will be carried out on a prebooking basis on the Model 377. The 7700 Taqman PCR machine is also housed in the Centre's facilities and currently several groups are investigating using SYBER Green for quantitative PCR (more about this elsewhere in the Newsletter).

Another change was the launching of the new CGR website (microbes.otago.ac.nz/cgr) and the move to an on-line DNA submission system. The website has been a pet project of mine, so you will have to bear with me if I rabbit on about it. The use of the on-line DNA submission requests has dramatically reduced the paper workload and the aggravation level in keeping track of samples and the accounts.We used to expend more time and energy in doing the paperwork than in carrying out the actual DNA sequencing itself. You can image the cost effectiveness of putting through a $6 DNA sequencing payment request through the entire University accounting system. Some of the invisible

benefits have been the development of direct electronic invoicing to the Registry Accounts by email. This not only cuts down the errors associate with data entry but Tracee is now able to send to departmental account managers a preview of the account charges prior to being sent to Registry. This allows correction of account codes etc, before submission for payment. In the past the University accounts system was used to pick up the errors and there was the inevitable bouncing backwards and forwards of account codes and a long paper trail . For instance we would get a DNA sequencing request from some graduate student (bless their little souls) who would scribble out some illegible, non-existent and/or long-since-expired account code (in a desperate bid to get some sequencing results). We would have carried out the sequencing (in good faith) and then some weeks later had the time to process the accounts by which time it would have bounced around the system and finally been picked up by some irate departmental account manager.

The other feature of the website is that it provides a 'virtual centre', a place where members can visit for news, for announcements, for information, for looking up archival material (such as this Newsletter) or searching for a profile on one of its members. In some ways a Newsletter such as this is less vital for keeping members informed as to the activities of the Centre. The CGR membership policy is that you need to rejoin by filling in the on-line membership form on the website. Your entry into the database is 'updatable' by using the update form and you can use embedded HTML codes to format your profile. You will need to remember your username and password since only you have access to your data entry. If you forget your password contact Tracee. A feature of a browser like Netscape is that once you are logged in you remain logged in until you "Quit" from Netscape &emdash; in other words the next computer user could cause mischief to your profile. This also applies to the DNA submission requests. Furthermore you may have to 'clear the caches' around the network by 'reloading' before changes actually appear on your computer screen.

There are plans to add other features to the website such as a 'chat forum' and other 'searchable' features. I would like you to feel that this is your website and that you can contribute to it on any subject you like. As an example see the presentation by Lynette and Claire: Marketing Yourself as a Successful Science Graduate under the 'Activities' section.

The website gets a significant number of 'hits' from overseas and I have to 'flick' people off who join from Japan, Canada and the USA since they are not eligible for membership unless they are associated with the University in some way.

As you are aware the Centre acts on behalf of the Research Theme: Gene Structure and Function. This year the Research Themes are being reviewed and will become part of the University Audit. This review is something we have to take seriously since funding priorities are established and based on these types of reviews. For instance, the funding for the 'microarray genomic initiative' (still to be announced!) is part of the University's strategic research planning based on the perception of research excellence in this area. Our review must be completed before August so as a PI you can be expected to be asked for some statistical information on papers published, postgraduate students, funding and other research 'outputs'. This will form part of the Theme's self-review which will then be followed by an assessment by an external reviewer.

The Centre is involved in organising the Queenstown Molecular Biology Meeting in August (see the website for details) and in the International Science Festival being held in Dunedin in June. The Science Festival theme is “The Global Village”. Join the Knowledge Revolution. So far our plans for the Festival are to have a demonstration of how computers are used in biomedical research and network communications. We hope to have the use of 10 iMacs for the occasion with the venue being

the Community Centre (on Princes Street: around the corner from the Octagon). This is a call for volunteers and ideas to 'raise the awareness of science to the public' and to promote the Centre.

Finally a closing reminder: if you want to continue to be a member of the Centre for Gene Research, please fill out the on-line membership form (http://microbiology.otago.ac.nz/cgr) in the "Join" section.

James Kalmakoff

GMOs, Killer Tomatoes and Utopian Dreams

The last few weeks have seen a flurry of news media interest in genetically-modified organisms. Much of the comment has been along the lines of "Frankenstein Food May be Bad for Ozone Layer" and the tone has been generally against the idea of genetic manipulation of food. Indeed, the most vocal of these critics have concerns about the whole idea of genetic manipulation on any grounds. Those scientists that have spoken out have been forced into a defensive position and have been criticized for being apologists for such companies as Monsanto. One thing is clear; there has been little informed debate about the issue, but instead, the airing of many opinions.

But why has there been such an interest in genetically modified organisms? Part of the answer is probably related to the appearance of ERMA as a regulatory body. ERMA has made available a great deal of information about genetic modification in New Zealand. Opponents of such manipulation are able to find out what is happening in this area much more easily than they could in the past and they are appalled by what they have found.

Does this mean ERMA is a bad thing? I would say, on balance, that it is not. Although many researchers have been caught as minnows in a net set for much bigger fish, there can be little doubt that the release of genetically modified organisms to the environment is something not to be done lightly. It is less clear that the cloning of a favouritegene into E. coli is something that should be supervised so closely. Certainly there is little evidence for any danger associated with the routine cloning of most genes. Exposing large number of people to novel organisms, in theory at least, is potentially a much more serious issue.

It is here that the serious problem crops up. The distinction between the potential and actual risks is all the difference in the world. It is easy to imagine apocalyptic visions of mutant killer tomatoes. It is much harder to decide if the vision is plausible. To do so requires considerable knowledge of both theoretical and practical molecular biology. Too often we are left with spectacle of the technically-informed defending genetic manipulation against those whose concerns are of principle. Each side might be speaking a different language.

Perhaps what we are seeing is process by which Society forms an ethical view. Genetically modified food has been only a theoretical possibility until recently, but now we have to make a decision. Is such genetically modified food a good or a bad

thing? We are not in a position to put off making a decision; if we do nothing, the food will appear, but do we have good grounds for saying no? The difficulty with such decision making is that in principle each opinion is as good as another. To my mind however, informed and uninformed opinions are not of equal weight.

Much of the debate in the news media at present pits those who are opposed to genetic manipulation on principle against those who argue for it on practical grounds. Of course I don't mean to say that all opponents of genetically modified food know little of molecular biology, nor that all molecular biologists are in favour of it, but there is a tendency for both these things to be true.

What can be done to make sure that the debate is informed? Decisions will be made and, in the end, we will be faced with regulations or legislation designed to deal with the issue of genetically modified food. Sitting back and doing nothing in the hope that the problem will go away won't work. Actively supporting genetic manipulation is not to every molecular biologist's taste. Perhaps the most useful and important thing we can do is to try and keep the debate informed. We should offer information about molecular biology for those who are interested (perhaps through the CGR Web site). We should be prepared to counter those who claim ridiculous things. Genetically modified glucose in Easter Eggs is just plain silly.

Genetic manipulation offers neither utopia nor dystopia. The world will not be made an immeasurably better place where noone is hungry by the introduction of genetically modified food, but neither will civilization's end be hastened by eating transgenic plants and animals.

One thing has come out of the debate that seems, to me at least, incontrovertible. Genetically modified food, like all food, should be labelled for what it is for we should have the right to choose or avoid something for whatever reasons seem appropriate to us.

Craig Marshell

News from the IBSC

As you are no doubt all aware, the HSNO legislation requires that the development or importation of a genetically-modified organisms receives prior approval from ERMA (or for "Low-Risk" development, the UOO Institutional BiologicalSafety Committee acting under delegated authority from ERMA). Those of you with ACNGT approvals carried over to HSNO through publication in the NZ Gazette will no doubt have enjoyed your two seconds of fame as your name scrolled across the screen on "Holmes" a couple of weeks ago! There is no doubt that the use of recombinant DNA technology will continue to attract public scrutiny, which emphasises the importance of ensuring that your work is fully covered.

The IBSC has just completed its assessment of the first round of applications (about 30) to develop genetically-modified organisms in containment under HSNO. The round was a learning experience for both applicants and the IBSC. Basically when assessing applications, the IBSC is acting under delegated authority from ERMA and the decisions have the same legal status (essentially district court level) as decisions made by ERMA. Hence the IBSC must give the application the same level of scrutiny as ERMA would. This means that all questions in the application form must be addressed and full information provided, including the glossary, and copies of all literature cited must be attached. Once the IBSC has made its decision, a copy of the decision form and the application are provided to ERMA. These are public documents and ERMA will provide a copy to anyone requesting one. (There is provision in the application form for confidential information to be so designated and kept confidential). This is one reason why the University of Otago has set the policy that all applications are in the name of the Institution and one reason why the location of the laboratory conducting the work is not listed. In fact, the actual laboratory is also not really relevant from ERMA's point of view - permission is given to develop a particular organism under a specified set of containment conditions. The location of the laboratory is relevant to the IBSC as one of the terms of reference of the IBSC is to ensure that the University is complying with containment controls - hence the IBSC requests that information on the cover sheet that is required with all applications.

Applications received in the first round varied considerably in the care that had been taken in their preparation and in the level of information provided, and hence in the number of times they were returned to applicants and in the amount of time the IBSC had to expend on them. Several people had put a lot of care and effort into their applications and this was much appreciated; others..... INDEED THE IBSC IS OF THE OPINION THAT THE LEVEL OF CARE REQUIRED IS SIMILAR TO THAT YOU WOULD TAKE WITH A GRANT APPLICATION. We will within the next couple of weeks place a "model" application on the network to provide guidance on the type and quantity of information required. This will be placed in the "Biological Safety" Folder on the shared disk "CGR Results", the same disk that Tracee places the sequencing results on. The Folder also contains application forms and two files that provide further information on the process to be followed: "IBSC pres. Feb 99 HO", and "GMO Information and Regulations".

As far as proposed new ERMA charges are concerned, ERMA have dropped their proposal to charge all applications $800 to cover the cost of public notification. To quote, "I would advise that on the basis of a review of the legal situation IBSC decisions are no longer notified and thus the notification fee does not apply". One small piece of good news! Hence there are no direct charges for "low-risk" development applications. I have not heard any further word on the proposal to charge an initial instalment fee of $5000 for importation applications. The IBSC sent in a strongly-worded submission on this as did other universities but I would not hold my breath, as ERMA are required by the Government to be self-funding by 2001. In an attempt to minimise the effect on U. Otago, the IBSC has submitted five importation applications to ERMA on behalf of University researchers, while the old fee of $750 plus GST still applies. If these are approved by ERMA, they will cover a broad range of transgenic E. coli, transgenic S. cerevisiae, transgenic gene knock-out mice, transgenic "over- expressing" mice, and transgenic murine cell-lines. Thanks to Iain Lamont and Chris Brown (Dept. Biochemistry) and Mat Walton (Malaghan Institute) for their efforts in preparing these applications. We will keep you informed of progress.

Approval of a GMO under HSNO makes it a restricted organism under the Biosecurity Act. This means the organism must be handled in a MAF-approved Containment Facility. The University is in the process of registering (hopefully) all labs involved in GMO work in Dunedin as part of a single Containment and Transitional Facility. The Facility comprises

eleven sectors with Sector Managers as follows: AgResearch Molecular Biology Unit, Department of Biochemistry (Dr Allan Crawford); Department of Anatomy and Structural Biology (Associate Professor Ian Mclennan); Department of Biochemistry (Dr Russell Poulter); Department of Botany (Dr Paul Guy); Department of Laboratory Animal Sciences (Dr John Schofield); Department of Microbiology (Dr Glenn Buchan); Department of Oral Sciences and Orthodontics (Dr Geoffrey Tompkins); Department of Pathology (Professor Antony Braithwaite); Department of Physiology (Mr Nairn Smith); Department of Zoology (Mrs Carleen Mitchell); Wellcome Research Building (Dr Paul Hessian). The Containment and Quarantine Manual has been approved by MAF, and the Facility has just been inspected by the MAF Supervisor. We expect approval next week. The MAF Supervisor was mainly concerned about security and access - labs need to be locked when there is nobody in them and freezers in public areas must be kept locked. In addition signs stating "Restricted Area - No Unauthorised Access" must be posted on lab doors. Microbiology also posts "BioHazard" signs. These precautions make sense to me and I think in the current environment we need to be very security-conscious. In operational terms,there is a need to maintain a paper trail such that uncleared biologicals can be tracked from import through to final disposal, and also a register of microorganisms (either imported or GMOs). Of course, people working in the MAF- approved facility will be expected to have read and to comply with the Containment/Quarantine Manual. Further information will be forthcoming from your Sector Manager in the next couple of weeks, who will also have a copy of the Manual. for those working with organisms other than microbes, transgenic mice need to be held in a Containment Facility for Vertebrate Laboratory Animals. We are in the process of registering the DLAS, with John Schofield as Manager. MAF have yet to release the Containment Standard for Plant, but when they do....

Finally, there is a need for a rapid avenue for information dissemination. The IBSC is distributing information via the CGR email list-server but it is apparent this has been missing a lot of relevant people. If you are not registered, you will miss out. Joining the CGR does not automatically subscribe you to the list - you need to register separately. Tell your friends!

Clive Ronson

Real-time PCR News

The ABI 7700 has now been in operation for almost 1 year. To date all the users are pleased with the results. It is good to see another group using the technology. The SYBR green dye profile has been installed so there is no longer the need to buy probes. While this technology has not yet been used on the machine two groups are about to give it ago. The Primer Express software has been used by many people and is available to anyone to design primers (& probes).

After discussions with Applied Biosystems and based on nearly 12 months usage they have offered a discount of 5% on consumables required to run the machine, including the 40nM & 0.2uM scale fluorogenic probes. If we increase our purchases/usage then the discount will be greater, likewise if if drops we loose the discount. There is still the offer of a special discount (approx. 20%) for new users which can be arranged through the NZ rep Stacey Nelson. The CGR has purchased tubes/caps/retainers for selling to users. This allows the purchase of small numbers, 100-200 tubes rather than 20000. This service is merely to help people initially start & if you plan long term usage then buy your own tubes. They will NOT operate as a store and if abused the service will be with drawn.

Please contact me for details on the discount or any other matters on use of the ABI 7700. Look forward to hearing from you & seeing greater usage of the technology.

Lynn Slobbe

Vascular Biology - Looking for a Research Portfolio

The Department of Surgery invites all interested parties to asymposium on vascular biology in the Sayers Common Room on the 29 April 1999 from 10am to 4pm. The aim of this symposium is to identify and explore avenues for collaborative research in vascular biology and examine the possibilities and initiate research portfolio(s) in the area of vascular biology and disease.

The keynote speaker and facilitator is the William Evans Fellow, Professor M David Tilson, of the Columbia UniversityDepartment of Surgery. He is well known for his wide-ranging research on the etiology of abdominal aortic aneurysms (AAA), and was one of the first researchers to draw attention to the strong genetic component in AAA.

Previously, Professor Tilson has done research on the types and expression of elastases and proteases involved in the formation of abdominal aortic aneurysms. More recently, his work has focused on relationships between the immune system and AAA. IgG from the abdominal aortas of AAA patients undergoing elective repair was used to identify an 80 kd protein in the vascular wall found in the majority of AAA patients. The autoantigen is a microfibril- associated glycoprotein with homologies to the tenascin superfamily. An expression library was made with mRNA from the adventitia of an AAA patient and several clones immunoreactive with IgG from AAA patients have been partially characterized. These clones share motifs with vitronectin, fibrinogen and IgK. In collaboration with the Vascular Biology Group at Otago one was mapped by FISH to chromosome 2 near the Ig kappa cluster.

Professor Tilson using serology and Cornelia Weyand of the Mayo Clinic using molecular methods have found an association between HLA-DRB1 alleles and AAA. To test this association in our AAA patients the Vascular Biology Group is using a molecular method to type our large database of AAA patient samples for HLA-DRB1 to a resolution equivalent to that obtained by serology.

Dr Greg Jones from the Department of Physiology, University of Melbourne will be speaking on his work using a rat model of vascular fragility. Other topics of discussion include vascular compliance, the clotting system, endothelial cell signaling, Apo- Lipoproteins, the genetics of vascular disease and the extracellular matrix.

The program will also focus on interactive sessions to explore possible portfolio development and giving impetus to substantive initiatives.

For further details on the symposium, please contact Dr. Jeremy Rossaak at jeremy.rossaak@stonebow.otago.ac.nz. Anyone interested in contacting Professor Tilson whilst he is here, please contact the department secretary, Dawn Howe-Dennison at (33)8835 or dawn.howe-dennison@stonebow.otago.ac.nz.

Protein Prattle

With the increasing focus on gene products and a consequent upswing in use of the Protein Microchemistry Facility it is timely to briefly review the services available in 1999.

For those of you new to the protein game and perhaps wanting to check out a recombinant protein or just quantify it we endeavour to offer a simple 'one stop shop' where you can get the appropriate analyses done and (if you feel so inclined) learn something about the procedures involved. Recombinant protein expression offers several traps for the unlucky and the unaware (eg incorrect signal removal through to inadvertant expression of the wrong protein), most of which can be rapidly exposed by sequence or mass analysis.

Amino acid composition analysis remains the best way to quantify your protein, subject to it being reasonably pure. This is often the best starting point since accurate quantification can benefit the following more detailed (and expensive) analyses. For example, instead of trying to guess if there is enough protein on your blot for sequencing do a composition analysis and find out for sure. If no sequence is forthcoming then this also provides firm evidence that the terminus is blocked, as is common for natural proteins.

Mass spectrometry can substantially extend what you learn from SDS-PAGE. With over 100-fold greater mass accuracy than gels it is usually a simple job to establish whether your (whole) protein is of the expected size. Supplement this with peptide mass fingerprint analysis (eg compare actual with theoretical tryptic peptide masses computed from the cDNA sequence) or amino acid composition analysis and you will likely have solid evidence that you have what you desire.

Sequence analysis remains the most definitive way to confirm protein identity. On the down side it only tells you about the bits that you actually sequence (surprise surprise) so significant errors or modifications might go unnoticed. A complete sequence analysis can be an expensive and time consuming proposition but many shortcuts can be made using peptide mass fingerprint analysis, if the desired sequence is known.

To broaden our service repertoire we have established links with other groups in New Zealand and Australia who have instrument capabilities that complement or extend our own. Our access to automated C-terminal microsequencing is of particular relevance to those of you wanting to check out 'the far side' of your recombinant proteins definitively. We also have available a variety of supplies for small time users (eg sequence grade enzymes, sequencer-friendly PVDF membranes) and protocols.

Mike Hubbard

Angis/Encompass Bionode

The Otago University Bionode is now available (http://angis.otago.ac.nz).

The system is provided by Encompass Bioinformatics. Encompass grew out of the Australian National Genomic Information Service (ANGIS) which provides bioinformatics support for most of the Universities and Research institutes in Australia. This linkage has not previously been available outside Australia.

The service provides a range of bioinformatics tools via several interfaces running on the Sun Ultra computer 'angis'.

Easy access is available though the web interface, using Netscape v3.1 or higher. This interface allows you to:

1. Manage files remotely with file manager - WebFM;

2. Use the GCG package through a simplified Web interface - WAG. We recommend you try this. Most of the EGCG programs have also been installed in WAG. These include Phylip programs for phylogenetic analysis;

3. Search databases (including GenBank, EMBL, SwissProt) through SeqSearch - BLAST and FastA database sequence matching QueryDB - smooth database text searching These databases will

be updated weekly;

4. Browse Code - the quick way to obtain an entry if you know its database code or accession number;

5. Undertake Linkage analysis - WebLCP;

6. Convert sequence files from one format to another - ReadSeq. And undertake other useful data management tasks.

Most tasks will be able to be done via WebANGIS but for more specialised tasks A TelNet and XANGIS interface is available. Examples of these uses would be Sequence assembly using the Staden package, or ACEDB databases. An introductory booklet that focusses on the WebANGIS interface is available for download (angis.otago.ac.nz/Education/Materials/) or can be purchased for a nominal amount from the CGR.

A more complete set of four books 'The ANGIS Bioinformatics Handbook' (angis.otago.ac.nz/Education/Materials/book.html) is available in the Science Library. This will be revised in June, and may then be freely downloadable so don't rush out to buy it ($100 AUS).

More information on getting access OU Bionode is available here: http://biochem.otago.ac.nz:800/chrisb/bionode.html

Thanks to the School of Medical Sciences, CGR, and members of the Biochemistry Department for implementing it.

A Grassroots Organisation of Active Research Scientists