Centre for Gene Research

CGR Newsletter for 1997

From your Director

While you were enjoying your summer holiday (sic), some of us were labouring on your behalf in preparing the final Wellcome Major Equipment Grant for an upgrade of the DNA sequencing facility and robotics — it was due on Jan 24th. A special thanks to Diana Hill and her team who managed to cope with the 20-page application form and to the secretarial staff of the Biochemistry Department and also to Andy Mercer, Howard Jenkinson and Warren Tate who provided supporting material. If successful, Research & International (Ian Smith) will provide the salary of a Research Fellow for the Centre to help with running the robotics of the expanded facility and for development of new methodologies. We are holding our breaths until some time in June. The next application to Wellcome will be for a quantitative PCR facility which Allan Crawford and Jean Fleming are organising on behalf of the Centre.

Summer is a good time to catch up on reading — like back issues of New Scientist and when you are really feeling those disparate withdrawal symptoms from your computer — those emails you filed away into the "Potentially Useless" mailbox. An email from Keith Ballard (Education) struck a chord with me when he talked about academics becoming "domesticated". A domesticated academic is "carefully timetabled, planned, frugal, protected, cautious and parsimonious rather than wild, extravagant and untamed" There are other interesting quotes from the book entitled "Academic Work" edited by John Smyth, 1995 (Open University Press). A "managed" University is one where the "curriculum [is] packaged into foolproof instruction kits, complete with clearly stated study methodologies, objectives and means of assessment." In Chapter 4 of this book there is an analysis made of the application of industrial models to university work. This is because "governments and employers want assurances that universities can boost the efficiency and quality of their enterprises." It is interesting to note that many

contemporary industrial organisations are moving away from the ‘goal setting’ of the 1950’s or the standardised mass production [Fordism] to a modern highly diversified market# place. The modern industrial models seek “to minimise hierarchy in favour of multi-skilling, teamwork and autonomy.” It seems that as we move into the 21st century, outmoded industrial models are being applied to us at this University.

A New Scientist article by Martin Brookes blames some of the poor image of science on beards. Scientists it seems have an inordinate fondness for beards (in my case it was my wife’s idea). The proportion of males with a full growth of facial hair is about one in fifty in the general population, but about one in five in science departments. The attraction of the beard to scientists goes inductively like this: Charles Darwin had a beard; Darwin was a genius; therefore people with beards are geniuses. According to Martin Brookes the scientific beard is redundant and should go the same way as the other appendages of the academic wardrobe, the tweed jacket and brown corduroy trousers.

I don’t know if many of you skimmed through the University of Otago Audit Report but there were some interesting points made. It was not a whitewash document couched in language that only the inner circle of administration could understand. It made two points which caught my attention:“OU’s Research Management Plan to 2000 is in embryonic form, and there is still some way to go in constructing a research policy, clarifying the concept and implications of identifying areas of excellence, identifying them in a flexible and useful way, developing effective and meaningful PIs, etc.” .... “This is a major challenge to the DVC (Research & International). Other challenges are to ensure that the system does not need an excessive amount of reporting, further encroaching on staff time, and that all major contributions to scholarship are appropriately recognised.” In another part of the Audit the role of the University as critic and conscience of society was mentioned. “The panel detected a view that OU’s support for the critic can be selective, and the fear was expressed that speaking out may prejudice research funding. Total commitment to a market-driven model would be inimical to the critic and conscience aspect of OU’s role, and senior bodies of OU may need to reaffirm strongly to staff that they will be supported if they find themselves needing to express well-founded professional criticism.” This is certainly food for thought and for serious discussion in other forums. Finally a quotable quote from Abraham Maslow on problem solving: “When the only tool you own is a hammer, every problem begins to resemble a nail.”

James Kalmakoff, Director
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Sequencer News

Report of the DNA Sequencing Service for 1997

Last year over 6000 templates were sequenced which is a 20% increase on the previous year. For the first time the charges for the service covered the costs of providing the service so we have no plans to increase the charges in 1997. The first two months of 1997 have been even busier with 150 templates sequenced each week for the first 6 weeks. This increasing custom gives us confidence that our plans to increase the size and scope of the service are warranted. To this end the Centre made a major application for an upgrade of the facility to the Wellcome Foundation. We await the outcome with interest. Trace Masson-Lawrence who runs the facility spent 3 days in the sequence facility at Genesis Corporation in Auckland comparing notes. In particular she brought back with her very stringent protocols for acrylamide gel preparation that have made a useful incremental improvement on the quality of the results we produce. We have also purchased a new computer to keep track of our administration and to ensure that customers are invoiced in a timely fashion. In summary, 1996 saw incremental improvement in the quality of our service. The number of templates sequenced was approximately 20% higher than in any previous year. All costs of running the service were recouped from the users. The DNA service is now poised for further expansion.
Allan Crawford
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Finding your way around the genome

The Lorne Genome Conference, February 1997, provided much food for thought or as Norman Pace, speaking on phylogeny trees, put it so succinctly: an opportunity for “mental masturbation”.

The opening paper by Eric Lander on the "Human Genome Project and Beyond" set the stage for the conference. The target date for the completion of the human genome sequencing project is 6 years. This corresponds to 500 Mb per year or ~2 Mb/day. To date, after a couple of years, 50 Mb have been sequenced. Yet the focus of Eric Lander's presentation was not that “we're running out of time”, but rather that researchers are excited about the mechanisation and new technology that is developing that should allow this target to be realised. In fact, 2–25% of the total genes have already been mapped by screening sequence tagged sites against YAC libraries and radiation hybrid panels. Third generation gene√tic maps, which include RFLPs (restriction fragment length polymorphism), SSLPs (simple sequence length polymorphism) and SNP (single nucleotide polymorphism), are under construction. Eric Lander gave an example of a reverse dot blot consisting of

a gridded template of 100,000 polynucleotides of 20–25 bases used to hybridise to DNA of interest to detect SNPs. The focus is already shifting from acquiring the data to how are we going to manage and interpret this data.
Eugene Stanley presented an “Analysis of noncoding DNA using methods of statistical physics”. He demonstrated that nucleotide sequence in noncoding DNA is power-law correlated in contrast to the coding sequence, which is not. Interestingly, the correlation exponent shows a systematic increase with evolution. While it's unclear what these observations mean, it does provide an algorithm for distinguishing coding and noncoding sequence.
Ian Humphrey Smith spoke on the need for “Proteome research: complementarity and limitations with respect to the DNA and RNA worlds”. A proteome is the total protein output encoded by a genome and Ian claims questions that need to be asked are:
• if and when a predicted gene product is translated
• the relative concentration of the gene product
• the extent of postranslational modification
• the effects of gene knock out/over expression ie the phenotype of the mutations.
The process of automated excision of protein spots from 2D electrophoresis gels onto PVDF blots and the derivation of proteomic contigs was described. Several electrophoresis gels can be used to construct a picture of protein expression within the cells.

We were then reminded by two papers, that information is encoded within the structural organisation of the primary DNA sequence itself. The first paper was Stephen Spiker's “Plant matrix attachment regions and transgene expression.” The nuclear matrix and interactions with the chromatin organise the DfiNA within the nucleus. Matrix attachment regions (MAR) on the DNA bind to nuclear matrix proteins and the nuclear DNA is then arranged into chromatin loop domains that can be actively transcribed. In the work Stephen Spiker described, a MAR from yeast was used to identify plant nuclear matrices from tobacco, which were in turn used to identify plant MARs. These were cloned to each end of a transgene, increasing the average levels of expression by more than 60 fold, in one case. In the second paper Sally Cross presented work on “DNA methylation and CpG islands”. Although the human genome is globally methylated, there is ~2% which is GC rich and consistently unmethylated. There are ~45,000 of these regions of 1–2 kb in length. These regions mark and overlap ~60% of the of the 5' ends of human genes. The distribution of the CpG islands has been mapped to chromosomes, these indicate areas of active transcription. Libraries have been constructed of CpG islands; using EST and cDNA data bases the full length transcript of many genes can be isolated. This research team have also identified two protein activities that bind to methylated DNA which could mediate indirect effects of methylation, one of these proteins encodes an

abundant chromatin protein which represses transcription from methylated promoters.

The relatively new field of bioinformatics has an important role to play in processing all the genomic sequence data. Greg Helt, who has just completed a PhD at Berkeley, presented an impressive “point and click” interface designed for manipulating and interpreting sequence information for the Drosophila genome.

Simon Easteal presented a paper; “The temporal dimension of macromolecules: Evolutionary information in genes and genomes”. The use of phylogeny trees in comparative analysis of related sequence for providing a quantitative estimate of evolutionary patterns was discussed. The actual times fêor divergence of a particular species could not necessarily be calculated , but the relative order within the tree can be accessed accurately. Norman Pace presented a revised evolution tree based on rRNA sequence which highlights the separateness of the Archae genus as a branch on the tree, ie Archae and Eucarya share the same ancestor. This has an important application as the study of Archae can help elucidate the basic requirements of eukaryotic cells.

The question most frequently asked of me was “Why aren't there more New Zealanders at the conference?” There was only myself, Karen Nielson from Crop and Food in Levin (a recent immigrant from Australia) and Irene Rombel, who completed her PhD in the Biochemistry Department at Otago University and is now a Post-doc at Berkeley. Many people expressed the hope that we would be better represented next year; if you are interested, note that abstracts are usually required in early November.
Ruth Appleby
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Protein Prattle

The latest news from the Protein Microchemistry Facility is that Technician in Charge, Diana Carne, has just had her first child, a healthy wee lad who remains to be named (we could suggest Alanine, after father Alan, or perhaps Tyrosine, after that sticky aromatic residue he produced the other day…). Diana has parental leave for twelve months.

The Facility is still accepting Service Work in Diana's absence, although we cannot guarantee that things will be handled with the usual aplomb and efficiency. At present we have three part time ring-ins trying to fill Diana's shoes (not to suggest that three blokes are required to replace one woman!). First, on the amino acid analyser and MALDI-TOF mass spectrometer, we have Mr Jew Chung Kon. Kon has spent much of his summer vacation

learning the vagaries of high sensitivity amino acid analysis. He is already experienced in MALDI work as a result of his previous summer and stage 4 projects. His time helping out in the Facility will be squeezed around a busy PhD programme, which will involve some protein microchemistry amongst other things. Second, on office work and general gophering, we have Mr Mike McArthur, who is slaving away to complete his BSc in Biochemistry this year. Third, I will be looking after the protein sequencing, initially at least. Please contact Kon or myself if you are planning for, or require, service work this year.

It is obvious that the Facility is home to some rather sophisticated (read: expensive) equipment, and we all take delight when our results accrue with apparent ease. On the other hand, when problems surface they can be really good (read: expensive, time consuming) ones. And so it is timely to pay tribute to our Departmental electronic engineer, Murray Cockerill, and other members of the workshop staff who help us to keep the gear running. This past month has seen Murray sort out bugs (sp. complex) in the ultra-high vacuum system (10-7 torr) and the high voltage supply (20kV) of our mass spectrometer. It takes a special skill, and courage, to take this sort of work on, usually in the absence of adequate information about circuits etc. And I might add that it takes a certain amount of courage to be his assistant at times, as for example when the cover (resplendent with colourful warnings about potentially fatal voltages inside) was being unscrewed from the 20,000 volt controller and Murray asked me to “just hold this, I don't want it to fall on the floor…”. Fortunately, my initial shock passed uneventfully.

Mike Hubbard

The Protein Microchemistry Facility (Room 123) is located on the first floor (east end) of the Biochemistry Department. For general enquiries in 1997, contact us by phone (479 7542), fax (479 7866) or email (protchem@otago.ac.nz). Kon might be tracked down in the Hubbard lab, at phone 479 7938. Dr Mike Hubbard can be reached by phone (4797831), fax (479 7866) or email (mike.hubbard@stonebow.otago.ac.nz).
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New Products: seeDNA

This is a new product from Life Sciences (formerly Amersham) that we have been trying out. The idea is that if you are precipitating DNA or RNA, you add a µl of the red seeDNA solution, it co-precipitates with the nucleic acid giving a nice pink spot on the bottom of your Eppendorf. Handy if you don't have much material, how often have you looked at your tube from all angles trying to convince yourself that you have a pellet? We have tried it a couple of times

when precipitating DNA and it works fine; haven't tried it with RNA, Life Sciences suggest that it can act as a carrier (equivalent to the commonly used tRNA) if you are precipitating low amounts of material but we haven't tried that either. Cost is about $100, one batch gives you about enough for 200 precipitations. If nothing else, it gets my vote for the best product title for a while!
Iain Lamont.
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Computer Corner

The Centre for Gene Research Bulletin Board has been reincarnated with new software and in a new form. The board is now no longer moderated which means that messages are now posted automatically to the board without having to be approved by the moderator. This approach should speed up the posting of messages but does mean there is no "quality control" on the posting of messages. Please be careful about what you post. Most mispostings are mistakes but occasionally on boards like this an inappropriate posting is made. We have tried to minimize any such problems by limiting postings to those subscribed to the board. This is not always successful, viz the recent postings about pornographic Internet sites that appeared a few weeks ago. The best approach to such "spam" is to use the delete key and otherwise ignore it all.
When the new board was set up the old subscription list was transferred but we did find that one or two addresses would no longer work and these were removed from the list. This may explain why your subscription appears to have lapsed.

To subscribe to the new list, send the message:
subscribe cgr-list Fred Hippopotamus
to the email address:
listproc@stonebow.otago.ac.nz
(where Fred Hippopotamus is replaced by your own name). You should shortly thereafter receive a message confirming your subscription.
To post messages to the list, use the address:
cgr-list@stonebow.otago.ac.nz

When replying to the list please note that the default reply is to the whole list. This means that if you wish to respond to a particular individual you will need to specify their address when you reply. If you do not do this, your words of wisdom will be distributed to everyone on the list and you might have more company than expected at your intimate rendezvous.
Craig Marshall