University of Otago
Te Whare Wananga o Otago

Centre for Gene Research

NEWSLETTER

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Greetings

This will be our fourth and last Newsletter for the year and, as Director of the Centre, it is my pleasure and privilege to write thanking all those who have supported the Centre's activities over the past year. For many people our major activity is running the DNA sequencer. Here we are very fortunate to have Alan Crawford as our technical advisor and, of course, Tracee Masson-Lawrence and Janet Dewdney to run the service. The year has seen an upgrade of our sequencer (funded by Lottery Health) and we are currently putting through on average four gels a week - that is something like 120 samples. Alan has a section in the Newsletter about the sequencer. However, we are always keen to get feedback, both positive and negative about our service. This is so that we can maintain the best service we can. If you find the service is good and satisfies your requirements of it please let us know. This information is always the hardest to get. Alternatively, if you have problems, please check with Tracee in the first instance and then, if necessary, with Alan or myself. It would help to have documentation relating to any problems so that we can sort through them.

This year we have been pleased to sponsor a number of visitors to the campus who mostly have been contributing to meetings held locally but who each have given lectures to the Centre. It is unfortunate that these lectures have not necessarily been well supported by the members of the Centre. Let's try better next year.

A successful event held round the middle of the year was a hands-on course involving the use of the GCG computing programs. This ran over four successive mornings and was conducted by Mark Dalphin. With 20 people attending it was fully subscribed. Very positive feedback was obtained from those who attended. We are currently exploring the possibility of repeating this course next year. It would be useful to get an idea of the possible demand for this and if you wish to register an interest in attending please just send me a note (email: grigor@sanger.otago.ac.nz would be most useful but hardcopy OK).

One Saturday in October we held a very successful one day meeting on yeast technology in modern gene research. This was organised by Brian Monk and was focussed round two overseas visitors to our campus. We were also able to welcome people from Rotorua and Christchurch who came down for the meeting. Thanks to Brian for his work here. We would like to continue the concept of holding at least one such meeting/workshop a year and would like to canvas suggestions for suitable topics or fields. With sufficient notice we can probably build this round the visit of a suitable visitor from overseas and we look for support of people outside the campus and centre attending. At no charge for registration, these are the best value meetings in the country. Again, I would welcome any suggestions for this.

Earlier in November we held our Second Annual Poster Night. We had 46 posters displayed and the overall quality was quite outstanding. About half of these were entered in the student competition sponsored by Life Technologies and in the end our judges, David Christie from Auckland and Andrew Mercer awarded four prizes: to Sarah Jack, John Irvine, Stephanie Watson and Melanie McConnell. Congratulations to those four and also to all others who entered their posters. The judging was NOT easy.

Once again my thanks to the members of our committee who have assisted me in the operation of the Centre. David Jones has stood down as from our last meeting and I am pleased to report that his place has been taken by Andrew Mercer. Thanks also to Craig Marshall for assisting in editing the Newsletter. Finally thanks to SciTech and Life Technologies for support for the Yeast Meeting and Student Poster Competition respectively.

Murray Grigor

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Research Management Plan/Annual report for Centre

Many people on the Otago Campus will be aware the the University is establishing a research management plan where it is seeking major research themes within the university. A submission jointly prepared by Merv Smith and myself on "Gene structure and function" has been selected one such theme - indeed the number one such theme. It is uncertain how things will develop from here, but I

have been asked to submit to the registry a list of the major research programmes in this theme including the programme titles, programme leaders, number of effective full time workers and sources (not amounts) of funding. I have already sent email messages to those people I could identify as programme leaders and hard to copy to those without email addresses (get up to date you guys!). However, if I have missed any one, please accept my apologies - scanning a phone book is not the most useful was of identifying potential programme leaders, and I would be grateful if you could provide me with the necessary material by **11 December.**

I am expecting any day to receive a request from the Registry for the annual report of the Centre. As for last year's report, I would like to include a listing of the publications of members of the Centre for the year. Last year this ran to 12 pages! Please may I have authors, titles and journal reference for any paper published in 1995 by **10 January** next year? By the way are you aware that you can include the Centre for Gene Research as part of your address in any publications submitted?

Thanks

Murray Grigor

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Electronic Bulletin Board

Earlier this year we established an electronic bulletin board for the Centre. About 30 people have subscribed and, on average, between 2 and 4 messages a week are being posted on the board. It is proving to be a very effective method for checking on aspects of technology, availability of materials etc. If you wish to sign up, please just send an email message to:

majordomo@stonebow.otago.ac.nz
saying: subscribe cgr-list your_email-address

By the way the Centre now has its own front page on the Universities WWW listing and notice of meetings and our latest newsletter is posted there. The address is:

http://biochem.otago.ac.nz:800/cgr_home.html

Meeting reports

"Hormones and the Brain"

In October Grant Montgomery and I had the pleasure of attending the third Prince Henry's Institute Annual Symposium, "Hormones and the Brain" at the Monash Medical Centre in Melbourne. Although focussed clearly on the neuroendocrinology of growth, stress and reproduction, this meeting was made more interesting by the diversity of the speakers' viewpoints, with clinicians, pathologists, psychiatrists, physiologists and even a few molecular biologists, contributing papers and discussion.

Roger Gorski (UCLA) talked about gender differences and sexual dimorphism in the rat brain, illustrated with what he described as a "rat porn video", showing how the administration of testosterone around day 4 to the neonatal female rat, can cause normal male sexual behaviour in the subsequent adult. Of interest was the fact that in rats testosterone is aromatised in the brain to oestradiol 17b, making it hard to define the effect of testosterone on sexual differentiation. Gorski is perhaps best known for his discovery of the sexually dimorphic nucleus (SDN) in the rat anterior hypothalamus. He presented evidence to show that one effect of testosterone is to inhibit neuronal apoptosis in developing rat brain SDN: thus this nucleus is larger in the adult male. The situation is, of course, much less clear in humans, where the SDN equivalent is a group of at least four interstitial nuclei of the anterior hypothalamus. Some, but by no means all studies of these nuclei reveal differences in the size of one of them in women, men and gay men. We weren't totally convinced.

Judith Cameron (Primate Research Laboratory, Pittsburgh) is an authority on the way that dietary intake regulates reproduction. Her paper discussed the effects of fasting on luteinising hormone pulsatility in monkeys and man. Fasting causes a reduced frequency of the LH pulses, whereas refeeding causes an increase in the LH pulse frequency. In order to rule out the effects of anticipation and stress on not being fed, clearly present in the monkeys studied, Cameron used "the more rational human male" and achieved the same result. A series of experiments refeeding

"food" with no nutrient value, or infusing various nutrients i.v., showed primarily that the monkeys knew perfectly well what food was fit to eat, but that either sugar, protein or fat infusion could stimulate the LH response and that the response had nothing to do with the stomach being full. Cameron also presented evidence that the decreased LH pulse frequency on fasting was not caused by an increase in circulating cortisol, nor was it a result of either increased growth hormone or insulin secretion. However fasting also led to a decrease in T3 secretion, which was restored by refeeding, and currently this appears to be the most likely mediator of the effects of fasting on reproduction.

Stafford Lightman (Bristol University) talked about the differential activation of CRF- and AVP-containing neurones in the paraventricular nucleus (PVN), during different types of stress. Lightman uses a model of chronic stress in rats, in which injection of mycobacterium tuberculosis adjuvant leads to arthritis in the paws. This treatment, which causes weight loss, paw swelling and an increase in adrenal weight, also causes amarked activation of POMC gene expression and ACTH and corticosterone secretion. In contrast CRF mRNA levels and CRF secretion in the portal blood system decrease. However AVP mRNA concentrations are higher in arthritic animals, especially after adrenalectomy (ADX). Studies in arthritic rats led to the conclusion that the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis may be proportional to the response of the animal to disease: the higher the corticosterone response, the better the resistance or recovery from the stress. Lightman also noted work recently published looking at effects in the adult rat following a single neonatal injection of endotoxin. Although the treatment only caused
the rat pups to be unwell for about 24 hours, the apparently normal adult rats had significantly higher CRF mRNA concentrations in the PVN and CRF peptide concentrations in the median eminence, suggesting that the early illness may have changed the adult HPA stress response. It's worth noting that the timing of the endotoxin injection was similar to the testosterone treatment used by Gorski.

At the end of a stimulating meeting the organiser, Iain Clarke provided this apt quote by Grant W Liddle to sum up his attitude to his work: "Some hormones are necessary for life, Others make life worth living."

Jean Fleming, Physiology

Biomedical Research in RS&T: 2010

Anyone who has had anything to do with the HRC recently will be more than aware of the current crisis in biomedical research funding in New Zealand. At a recent meeting organised by the NZ Association of Scientists, a remarkable group of people came together to consider the future of biomedical research and to discuss possible changes to the present funding structure. In the Treasury Conference Room, high above the Beehive and the Houses of Parliament, looking out over a wet and windy Wellington Harbour, research leaders from all of the country's Medical Schools sat down with members of the HRC, representatives from the Ministry of Health, leaders from MoRST, FRST, the NZAS and the Royal Society of New Zealand.

This was an historical meeting in many ways - from the calibre and diversity of the participants to the directness of their approach to fixing the crisis. Views expressed at the meeting repeatedly described the severity of the problem and the complete lack of career stability for young biomedical researchers. Officials from the Ministries of Health and RS&T made it quite clear that additional money would have to come from within the existing research allocations. The NZAS has subsequently produced a communique, based on the consensus view arrived at on the day, calling for structural changes in the funding of Biomedical and Clinical Research, primarily by including Biomedical Research in the MORST Science Envelope, funded through Vote RS&T, rather than Vote Health, but still administered through the Biomedical Research Committee of the HRC. The HRC would thus bid for funding from both Vote Health and Vote RS&T, which it could then distribute to HRC grant applicants (Biomedical, Clinical, Public Health, Maori and Pacific Island Health), roughly as it does now. I am happy to provide a copy of the communique to anyone interested.

Jean Fleming, Physiology
Associate Member of the NZAS Council

The First International Workshop on the Mapping of Human Chromosome 10

This workshop took place in Crete from September 30th to the 1st of October 1995, and, like other single chromosome workshops, organised to coordinate the mapping of a particular chromosomes, having an emphasis on mapping disease gene loci. The data generated at the meeting had direct implications for the human genome project, and there was, of course, a representative from GDB present to enter the data, and to provide online help. Like other inaugural single chromosome workshops there were only a handfull of participants, actually 22 from 8 different countries.

Chromosome 10 has been a relatively neglected chromosome until recently. However, loci involved in, amongst other diseases, prostate cancer and diabetes have now been localised to chromosome 10, so we now have enough interest to hold a whole workshop. In fact whole corporations (eg Genome Therapeutics Corporation, USA) are mapping the chromosome on literally an industrial scale. Presumably they can see gold at the end of it.

All participants at the meeting made a short oral presentation as well as a poster presentation of their work. This format took until afternoon coffee on the first day. Then the real work started! We then spent the remainder of the conference in subgroups, piecing together the information, like a jigsaw puzzle, and constructing maps. Sixteen new genes were mapped to chromosome 10, and new consensus genetic linkage and physical maps were generated at the meeting. In addition, two genes had a nomenclature change and four new genes were identified that are known to cause human diseases. There were two papers at the meeting that presented linkage of diabetes to chromosome 10 in the centromeric region. One of the papers talked about linkage of juvenile diabetes and the other paper talked about linkage of type 1 diabetes which was presented by Peter Reed from John Todd's lab at Oxford. Overall, the meeting very worthwhile because all participants were working on interesting genes, and a great deal of interaction between the participants occurred. In addition the contacts that I made will provide resources, including YACs, libraries and so on, for human gene mapping. The following passage was the report from the "Disease Gene subgroup" which I was part of. It is hoped that the full report of the meeting will be submitted and published in Cytogenetics and Cell Genetics.

Mike Eccles,
Cancer Genetics Laboratory

Disease Gene Map of 10q23-q26

Several disease genes have recently been localized to the chromosomal region 10q23-q26. Included amongst these are several tumour suppressor gene loci. It has previously been shown that loss of heterozygosity (LOH) occurred frequently on the long arm of chromosome 10 in prostate cancer, but it was not clear precisely which region was involved. Recently analysis of polymorphic markers in prostate cancers has shown that the highest frequency of allele loss, presumably due to the influence of LOH at a nearby putative prostate tumour suppressor gene, occurred at marker D10S541 (Gray *et al*,1995, in preparation) This marker corresponds approximately to the 10q23/q24 boundary (Gray *et al*, unpublished).

Moving in a telomeric direction, fourteen cM from D10S541 is a region which has been defined as containing the infantile onset spinocerebellar ataxia (IOSCA), and autosomal dominant progressive external ophthalmoplegia (adPEO) genes. These genes have been localized between D10S198 and D10S1268, and D10S198 and D10S562 respectively (Nikali *et al*,1995, and Suomalainen *et al*, 1995). These markers correspond approximately to chromosome band 10q24 (Nikali *et al*, unpublished).

Gray *et al* (unpublished) have described a YAC which was positive for D10S192 and D10S198, and which also contained the PAX2 gene. The co-localization of PAX2 with these markers is consistent with another report (Eccles *et al*, in preparation), in which PAX2 was localized less than 500Kb from HOX11 by interphase FISH mapping. HOX11 has previously been shown to be rearranged in T-cell acute lymphocytic leukaemias with t(10;14)(q24;q11) (Kennedy *et al*,1991, Hatano *et al*, 1991). Recently, a disease associated with mutations in PAX2 has been reported, called renal-coloboma syndrome (Sanyanusin et al, 1995), which involves optic nerve defects and renal anomalies. Previously PAX2 was suggested as being a candidate for Crouzon syndrome (now known to be caused by FGFR2 mutations, see below), but PAX2 was not detected in a YAC contig between D10S209 and

D10S587 (Ehrlich *et al*, unpublished).
Again moving in a telomeric direction, D10S221 is the centromeric boundary of a small (4cM) region of LOH in glioblastoma multiforme (GBM) (Albarossa *et al*, in preparation). The telomeric boundary of the GBM locus is defined by D10S209 in one case. However, an American group has found that the boundaries of LOH are between D10S587 and D10S216 in another group of gliomas. Ehrlich *et al*, (unpublished) have described a YAC contig containing the markers D10S187, D10S531, D10S100 and D10S221, and also the EMX2 and SLC18A2 genes. The FGFR2 gene, which is mutated in Crouzon (Reardon *et al*, 1994), Apert (Wilkie *et al*, 1995), Jackson-Weiss (Jabs *et al*, 1994) and Pfeiffer syndromes (Rutland *et al*, 1995), maps in 10q25, 6 cM telomeric to EMX2, between markers D10S209 and D10S587 (Ehrlich *et al*, unpublished). Ornithine aminotransferase (OAT) and an OAT pseudogene (OATL3) were mapped to a YAC containing D10S216, 6 cM telomeric to FGFR2 (Ehrlich *et al*, unpublished). The OAT gene has been mapped by in situ hybridization to 10q26 (Barrett *et al*, 1987).

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Research Grant

The AIDS virus, HIV-1, has to date infected more than 13 million people world wide.New information suggests that modified strains of the virus found in parts of Asia may be more readily transmitted heterosexually than the typical strains originating from the United States or Europe. Not surprisingly the search for new drugs to combat the virus remains a high priority.

There are steps in the biology of HIV which may be vulnerable targets for new drugs, because they are unique to the virus and are not used by human cells. HIV has one such unusual process in the way it produces its proteins for the production of new virus particles. The viral RNA fools the cell's protein synthesis machinery to become unfaithful at a specific point as it is reading the code for the viral proteins. This frameshifting event occurs at a frequency such that the viral proteins are made in just the right ratio. If this ratio is altered there is good evidence that viral reproduction is drastically affected.Surprisingly the step has received little attention as a site of potential therapeutic intervention, perhaps because it is an abnormal event and outside the processes normally studied by research groups interested in gene expression.

My research group has focussed on this step of HIV biology and the first phase of the work is now complete.Jules Horsfield who has just been awarded her doctoral degree, has discovered that the mechanism proposed in the 1980's to describe retroviral frameshifting could not explain her observations and she has derived a new ribosomal E/P site postranslocation slippage mechanism to explain how frameshifting occurs at the specific HIV site. Understanding the mechanism better means drugs which affect the ribosome can be selected for pilot studies to target the frameshifting step. Jules has completed these pilot studies with a small number of drugs using our safe in vitro model test system and shown the feasibility of this approach.

While this work was being undertaken a human gene, ornthinine decarboxylase antizyme, was found which uses a frameshift in the opposite direction. This is the only one of the potentially 100, 000 human genes that has been discovered to use such a mechanism. However this was a potential disaster for our strategy to target the HIV site because this essential human function could also be affected. John Irvine, a medical student undertaking a Bachelor of Medical Sciences this year immediately began studying this gene to test whether it was susceptible to the same drugs as the HIV event. Fortunately the two sites are affected in different ways; some drugs affect the human gene but not HIV, and others affect HIV and not the human gene (the desired affect of a drug). Although these studies are only the first phase of the development of potentially useful drugs they clearly demonstrated the feasibility of the strategy.

As a result of our phase 1 studies we have been invited to be part of an International consortium of research groups to target the frameshift event in HIV. It is headed by Professor Yoshikazu Nakamura, at the Institute of Medical Sciences, University of Tokyo, with the other participants being 5 Japanese groups, and 1 from the United States in a major new initiative by the Human Science Foundation, a branch of the Japanese Ministry of Health. This is the first international effort they have sponsored and it aims at developing test systems for mass screening of potential anti AIDS drugs being developed by the research wing of a Japanese Pharmaceutical Company, which is part of the consortium. Initially funded at an equivalent of about NZ$1 million dollars for a three year programme to develop a number of test systems for the drugs, if successful it is anticipated that the funding will continue for an extended period.

Warren Tate

DNA Sequencer News

Quality Control

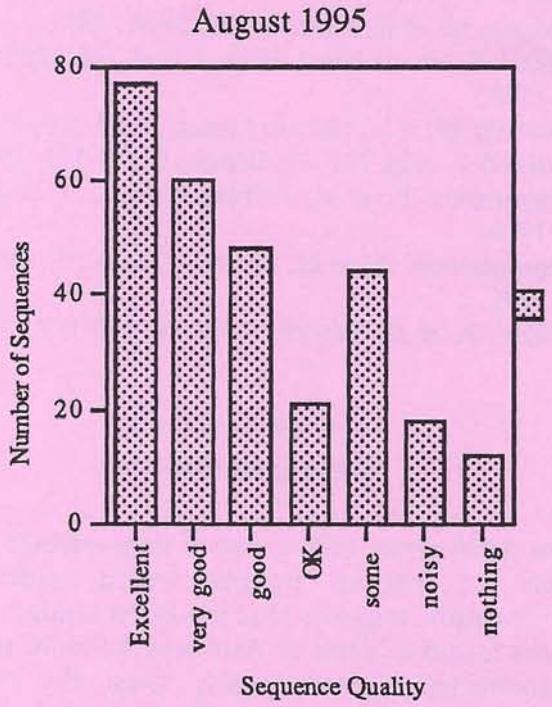
The teething problems that we had when introducing the stretch upgrade highlighted the fact that we have not publicised the quality control procedures that are undertaken with each gel that is run by the DNA sequencing service. With every gel we run between one and three control reactions. If the gel is all dye terminator reactions that is the only contol we run. However if there are reverse or forward dye primer reactions on the gel these controls are also run. These act as both a check on the reaction conditions, the condition of the kits and whether the gel itself has run correctly.

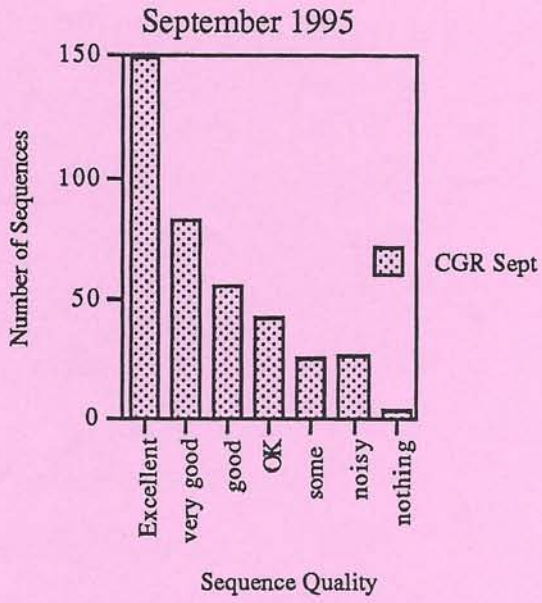
The results are posted in a dated folder on the bulletin board CF= Control Forward; CR = Control Reverse; C.term = Control termination. As template we use either the control template (pGEM) provided with the kits or when that runs out we use CsCl purified pBluescript M13+. The controls are routinely excellent.

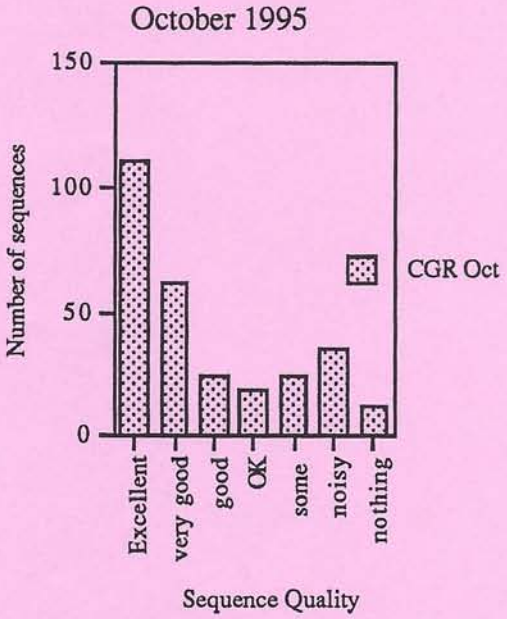
The gels are scored by Tracee to give us and the customer some idea of how successful the sequencing reaction has been. They are graded on a seven point scale .

Excellent: No background with good read length
Very Good: Similar to excellent but with slightly increased background.
Good: Some background with peaks not so well spaced.
OK: Considerable background with a large number of "N's".
Some: Mostly background noise but a few stretches of readable sequence.
Noisy: unreadable.
Nothing: No fluorescence detected.
We don't quantify the read length as many of our templates are now PCR products which are sequenced right to the end. Defining the endpoint of the sequence is quite subjective.

To give you some idea of the overall success of the sequence reactions I have included a graphical summary of the results achieved over the last few months. **If approximately 70% of your sequences are not good, very good or excellent you are doing worse than most.** This may be a reflection of the system you are using (eg a GC rich region of DNA to sequence) but if not perhaps you should consult with others getting better results and use their template preparation methods.







Sequencing GC rich DNA

From time to time we have mentioned methods others have found to give them good results. Andy Mercer of the Virus Research Unit has found that for very GC rich DNA they get better results with M13 single stranded templates than ds DNA plasmid templates so if you have a very GC rich region of DNA needing sequencing it may be worth preparing an ssDNA template. Many plasmid vectors now have the M13 origin of replication inserted and using helper phage it is relatively easy to generate ssDNA templates. They also find that dye termination gives them less "stops" and other secondary structure problems than dye primer reactions.

New Thermal Sequenase

From Monday 18th November we begun using the thermal sequenase kit for dye terminator reactions. Reaction to the results produced has been very favourable. The dye primer kits using this thermostable "sequenase-like" enzyme will not be available until the new year.

Closure over the Christmas Holidays:

The last day we are accepting templates for sequencing prior to Christmas is Friday 15th December at 5 pm. All results will be returned to clients by Thursday 22nd December. Janet will be returning for the week begining 8th January so she will be able to run up to 66 templates that week provided they are given to her by 9am Wednesday 10th January. The facility will close for the following week (Jan 15th to 19th) then begining Monday 21st January we shall be back to full service when both Tracee and Janet return.

Allan Crawford

STOP PRESS
This week both our dye primer kits from ABI failed to give excellent sequence with our control DNA. We are sure the cause is due to loss of flourescence of the dye-labelled primer and until this problem is resolved by ABI we shall be sequencing all dye primer requests using unlabelled reverse and forward primers and the dye termination reaction.

MERRY CHRISTMAS FROM THE DNA SEQUENCING STAFF