Meet the HGCB Teams

Since May 2002 the HGCB at ECACC has been headed up by Dr Pippa Bracegirdle. Pippa comes to ECACC after spending 10 months in the Foot and Mouth Disease Virus Sero-surveillance Unit at CAMR where she was involved in the processing of over half a million blood samples as part of the National effort to combat the outbreak.

Pippa is supported by Ros Packer and Alex Hiscott. Ros, who is well known to many of you and has 10 years experience at ECACC, is responsible for sample reception, liaison with external sites and processing of blood samples to the PBL stage. Alex has also been with ECACC for many years and is responsible for supervising the EBV transformation team.

The HGCB has administrative support from Carol Phelps, another longstanding member of the HGCB team whom many of you will have had contact with in the past concerning enquiries into the status of samples deposited.

Many of the process improvements mentioned would not have been possible without the support of ECACC's Development Group, headed up by Jim Cooper, and the IT group consisting of Anna Rance and Roger Kirkam.

Dr. Pippa Bracegirdle.

Ros (back, left) and her team.

Alex and his team.

Obtaining Cell Lines from the ECACC Human Genetic Collection

The cell lines available for research purposes can be found either on ECACC's website www.ecacc.org.uk or by using the CD catalogue available free of charge on request. Once you have found the cell line you wish to order you should check the distribution status of the sample you have chosen, as over 50% of the specimens are held as cryopreserved, untransformed lymphocytes. This means there could be a delay of at least 10 weeks before a cell line can be established and dispatched. There is a small risk that a cell line will not be successfully established so it is important to consider these facts when choosing your samples. You will be advised of estimated due dates at the time of placing your order. The costs of supplying cell lines are provided on our website.

ECACC is able to provide extracted DNA, if preferred, but there will be an additional cost which is again provided on the published price list. In addition ECACC is also able to provide a bulk cell production service whereby cell lines can be grown up to provide cell pellets of varying sizes for DNA extraction either here at ECACC or for the customer to extract themselves. Custom quotations for this service can be provided upon request.

Do You Require Control DNA?

Recently ECACC has developed a series of 5 Human Random Control (HRC) DNA panels (5x96) representing 480 healthy randomly selected blood donors. The HRC DNA in the panels has been extracted from lymphoblastoid cell lines derived by EBV transformation of peripheral blood lymphocytes from single donor blood samples. The donors are ethnically matched as UK Caucasian and characterised by gender and age at venesection. Informed consent has been obtained for their use in research. All five panels are available immediately in 10ug and 50ug amounts in standard tube format at a concentration of 200ng/ul.

In addition DNA panels HRC-1 and 2 are available in 96 well array format at a concentration ready to use for PCR (8ng/ul). See our website for further details or contact ECACC to receive our comprehensive DNA brochure.

Human Genetic Cell Bank Open Day - 23 October 2003

Take the opportunity to visit ECACC and find out what really happens to the blood samples you package up and send off to Porton Down. The day will start at 11.00 and close at 4.00 and will consist of a series of short talks with escorted tours around the facility. There will be opportunities for meeting and discussing your current or future projects with the ECACC teams mentioned in this newsletter. Refreshments and lunch will be provided but you will need to cover your own travel expenses. A more detailed programme will be published on our website nearer the time. If you are interested in attending send an email to ecacc@hpa.org.uk marking the subject HGCB Open Day.

DNA control panels

Contact Details

European Collection of Cell Cultures
Health Protection Agency
Porton Down
Salisbury, SP4 0JG, United Kingdom.
Telephone: +44 (0)1980 612512, Fax: +44 (0)1980 611315,
E-mail: ecacc@hpa.org.uk, Website: ecacc.org.uk

Annual Update

September 2003

Human Genetic Cell Bank (HGCB)

Welcome to the first dedicated newsletter from ECACC's Human Genetic Cell Bank. This update is intended to describe some of the recent changes to the service offered to our HGCB customers and collaborators, and to raise awareness of the service levels that ECACC can now offer to genetic research in the UK. We aim to produce this newsletter annually and will also make it available on our website.

ECACC has provided an EBV lymphocyte immortalisation and cell banking service to human genetic research in the UK since 1986. Long-term programmes supported by ECACC have included major studies of diabetes funded by the British Diabetes Association (now Diabetes UK), the International Histocompatibility Workshops and the UK Human Genome Mapping Project (HGMP) largely supported by the MRC.

During this period ECACC has provided support to hundreds of research groups and amassed cell banks representing up to 100,000 donor subjects from over 800 different genetic disorders. ECACC's Human Genetic Cell Bank (HGCB) is now one of the largest collections of its type in the world.

Find out what is available by searching our on line catalogue at www.ecacc.org.uk or by requesting a CD catalogue.

Completion of the human genomic sequence has provided the foundation for a world-wide expansion of human genetic research particularly in the area of functional genomics. Coincident with the onset of this new era in human genetic research, demand for the services of ECACC's HGCB has increased dramatically and ECACC has had to respond to this demand by fundamentally upgrading the scale and design of its operations.

Changing Demand

The majority of human genetic cell lines stored by ECACC were deposited as part of the MRC HGMP initiative. ECACC is currently funded by the MRC to offer a subsidised EBV transformation and banking service to UK registered HGMP users. The number of samples being deposited under this subsidised scheme is now declining sharply as studies launched under this initiative draw to a close, and ECACC's current MRC grant which provides support for this subsidy will terminate on 30 September 2003. A new 5 year strategic project grant will replace the current award but there is no provision in this grant to provide subsidised services. In future, the full cost of the services requested must be built into each individual grant application, which is how all the projects listed in Table 1 have been funded.

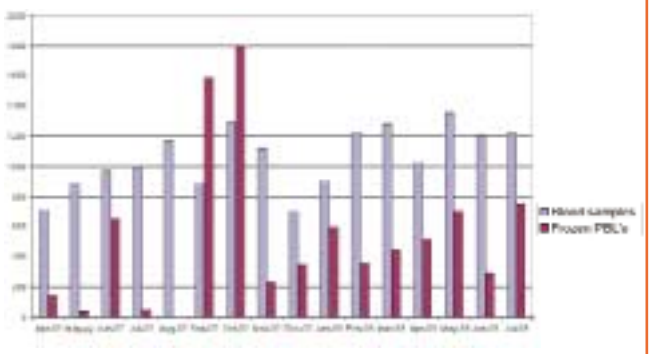

Figure 1. Number of samples received

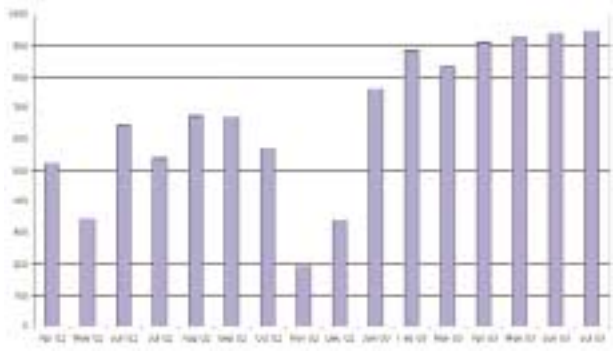

Figure 2. Number of transformations per month

Although the number of HGMP (subsidised) deposits is declining, the total number of specimens submitted to the ECACC HGCB has increased fourfold over the last 2 years, as a result of a number of large studies into common human genetic diseases (see Table 1). Thus the spectrum of demand has changed from multiple, small projects to a lesser number of much larger projects that might involve as many as 20,000 subjects. Figures 1 and 2 illustrate HGCB throughput over the last 18 months.

Table 1. Current major projects using ECACC's HGCB services

Disease	Depositor	Size of Study	Funding Body
Alzheimers (late onset)	J Williams, Cardiff	4000	MRC
Type 2 Diabetes Family Study	A Hattersley, Exeter	4000	MRC
Asthma/Eczema	W Cookson, Oxford	5000	MRC
Multiple Sclerosis	A Compston, Cambridge	2000	MRC
Hypertension	M Caulfield, London	6000	MRC
Bipolar	P McGuffin, London	4000	MRC
Family Heart Disease Study	A Hall, Leeds	6000	MRC
Nephropathy in Type 1 Diabetes	S Bain, Birmingham	2500	Diabetes UK/JDRFI
1958 Cohort study (pilot)	D Strachen, London	2000	Wellcome Trust

The Response of ECACC

The large increase in demand for the ECACC HGCB services illustrated in Table 1 has necessitated substantial operational and organisational changes. These changes have been instigated and driven by James Biggins, ECACC's Operations Manager who joined ECACC after many years of experience of industrial bioprocessing. These changes can be summarised as follows:

Staff

ECACC has established 4 separate teams specialising in:

- Blood processing and lymphocyte banking
- EBV transformation and cell banking
- Expansion of lymphoblastoid cell lines
- DNA extraction

Each team has an experienced Supervisor and Dr Pippa Bracegirdle was appointed as Head of the HGCB which comprises of the Blood processing and EBV transformation teams.

Information Technology

ECACC can have up to 2,000 lymphoblastoid cell lines at different stages of culture at any one time. Over the last 2 years we have developed barcode-driven electronic specimen tracking systems to manage this complex work (Figure 3). The same systems are used to generate regular status reports of work-in-progress for the Project Holders.

FIGURE 3: Use of the barcode tracking system.

FIGURE 5 – ECACC's new purpose built ultra-low temperature storage facility with capacity for up to 1 million cryovials.

Systems

Historically ECACC would often take more than one attempt to successfully transform a lymphocyte specimen. At current levels of throughput this is a luxury we need to avoid and so a substantial development effort has been invested towards establishing "right first time" regimens. Now an average of 95% of specimens are successfully transformed on the first attempt (Figure 4).

Facilities

A block of 6 newly refurbished laboratories has been dedicated to the HGCB operation. This permits each project to be segregated from other projects,

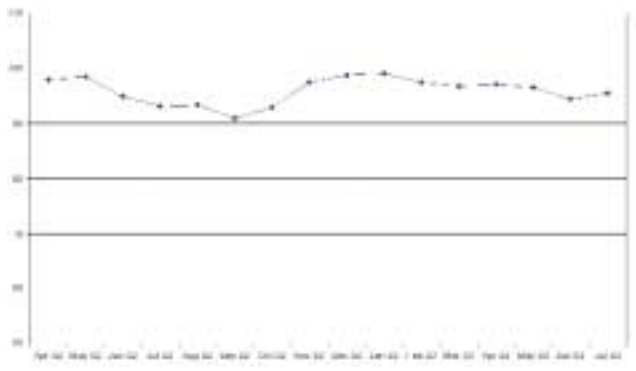

Figure 4. Transformation Success Rate (%)

a measure that has been found necessary as a precaution against the introduction of mycoplasma with incoming samples. Most significantly, a new, purpose-designed liquid nitrogen repository has been built, with state-of-the-art low temperature technology, to create the capacity for storing up to 1 million cryovials (Figure 5).

Lab procedures

The ability to work from frozen banks is an outstanding feature of the ECACC operation. Incoming blood samples are routinely processed to frozen peripheral blood lymphocytes (PBLs) on the day of receipt. Most EBV transformations are performed on peripheral blood lymphocytes recovered from liquid nitrogen storage. The ability to work from frozen PBLs means that ECACC can absorb large variations in the numbers of specimens received simultaneously and yet enter precisely regulated numbers of PBLs to the transformation process.

All has not always gone smoothly. Prototype systems were not initially successful and process efficiency declined for a brief period, to a point where operations were curtailed while we effected a recovery. Nevertheless, this prompted a concerted period of process improvement that has produced levels of efficiency that would not otherwise have been achieved.

The main process improvements can be summarised as follows:

- All blood samples are processed and frozen as untransformed PBLs.
- EBV transformations are carried out in batches of 50 samples from single projects, with each batch having a unique reference number. This allows problems to be detected early and if they are project specific this can be fed back to the depositor.
- Barcode driven work tracking system making it possible to tell where any cell line is in the process.
- Tracking system links directly into a sample history database.

In addition to the above the following quality assurance measures are in place:

- All procedures are managed in accordance with EN:ISO9001:2000.
- All inputs are controlled and all reagents are screened and validated before use.
- An assessment of the quality of the PBL is recorded, both at the time of preparation and at transformation.
- All cell lines are screened for mycoplasma by PCR
- Samples are segregated within the laboratory, by project. Transformation of PBLs prepared outside ECACC, which represent a potential source of mycoplasma contamination, is performed in a separate laboratory.
- 5% of cell lines are tested by ECACC QC Dept. for cell count and viability, sterility and mycoplasma (both Hoechst stain & culture).
- Test batches of transformations are performed for projects where only PBL storage is required.
- 5% of all samples transformed (10% for some projects) are authenticated by multiplex PCR for fidelity with source (Guthrie card).

Changes to the MRC HGMP Subsidy

The subsidised EBV transformation and banking service is being progressively phased out and all the new, larger projects (see Table 1) pay the full costs of the services they require. ECACC has agreed costs with the main funding bodies for its EBV immortalisation services. These costs take into account the economies of scale that are possible with large projects. The main funding bodies such as the MRC and the Wellcome Trust will expect these costs to be part of any grant application for a genetic research project involving the use of ECACC's EBV transformation service. The new, 5 year strategic project grant effective from 1 October 2003 has no provision to provide subsidised services. Should you wish to use ECACC's EBV immortalisation service then contact ECACC with details of your project and ECACC can advise on the costs involved.

Sending samples to ECACC

There is a comprehensive guide on ECACC's website which outlines in detail the requirements for the use of ECACC's EBV immortalisation service particularly emphasising the requirements for sending blood samples. Below are some of the key factors to consider before sending blood:

- Use ACD or CPDA blood tubes and ensure proper mixing by gentle inversion of the tubes.
- ACD tubes should be filled to the correct level for optimum function.
- Samples should arrive at ECACC as soon as possible after collecting, preferably next day but certainly within 72hrs.
- Store at ROOM TEMPERATURE before and during shipment. Do not refrigerate or freeze under any circumstances.
- Samples must be packaged according to IATA safety regulations – full details on website.
- If any blood samples have a known Hep B or Hep C or HIV risk then ECACC requires at least 1 weeks notice as special arangements have to be made to process such samples. Without this notice biohazardous samples will be destroyed on receipt.

If you are unsure at all contact Ros Packer on 01980 612611 or email ros.packer@hpa.org.uk