A Limitless Source of Genomic DNA or RNA

Studying a Cohort or a Few Individuals? – Would You Benefit From a Reproducible and Renewable Supply of Each Patient's Genomic DNA or RNA?

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The ultimate aim of the generation of a lymphoblastoid cell line from a patient's blood sample is to secure a renewable supply of that individual's genomic DNA. A cell line can be cryopreserved and resuscitated for cultivation and subsequent DNA and/or RNA extraction at any time. The Human Genetic Cell Bank (HGCB) Service enables clinical research groups to secure valuable case and control genomic material for genetic analyses. This removes the need to return to individuals for a second blood sample and the risk of exhausting unique DNA stocks.

Renewable Source

The Human Genetic Cell Bank Service is based on the preparation and Epstein-Barr Virus (EBV) immortalisation (also known as transformation) of human peripheral blood lymphocytes (PBLs) resulting in the production of lymphoblastoid cell lines (LCLs). These cell lines are subsequently banked, providing a renewable and expandable source of genomic DNA. Please see Figure 1 for an overview of the process.

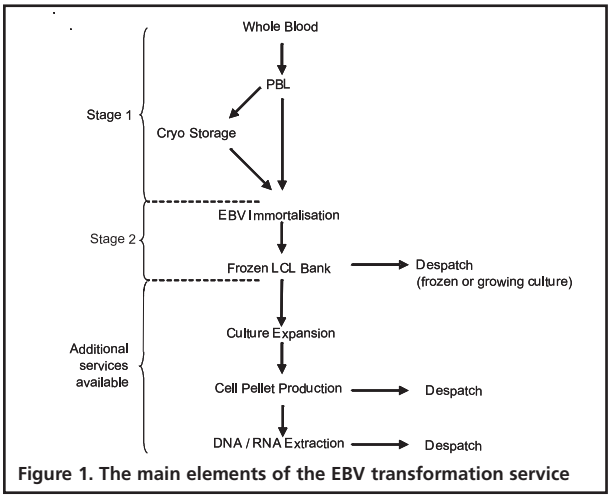

Figure 1. The main elements of the EBV transformation service

Application

With advances in molecular biology, a number of methods for whole genome amplification have become available and evidence is accumulating that it can give good results in genotyping (high call rates and concordance). This is considered a good method of stock replenishment where there is no alternative. However, whole genome amplified DNA cannot be used either in the study of DNA methylation or of very large genomic fragments (pulsed field gel electrophoresis). Moreover, it has not been validated for studies in gene amplification or repetitive gene sequences. It may also be unsuitable for study of telomeric repeats. By contrast, DNA from LCLs is suitable for all such studies. Furthermore, LCLs are suitable for studies on both transcriptional and translational products. ECACC is experiencing increasing demand for RNA extracted from LCLs from individuals with a range of genetic diseases such as asthma, diabetes and dementia.

Figure 2. Blood spot cards

Quality & Expertise

The HGCB has provided an EBV lymphocyte immortalisation service to human genetic research in the UK since 1986 and has amassed samples representing over 100,000 donor subjects. The HGCB currently initiates more than 1000 EBV transformations per month, the majority of which use PBLs resuscitated from liquid nitrogen storage. Normally ECACC achieves an average transformation success rate of >95% at first attempt. All procedures are managed in accordance with the quality standard BS:EN:ISO9001:2000. Quality control measures in place include mycoplasma screening and determination of cell numbers, percentage viability and sterility. Blood samples are retained on blood spot cards (see Figure 2) so that the identity of any derived lymphoblastoid cell line can be verified by comparison with the source material using DNA profiling techniques.

Current Users of the Service

The HGCB service has a diverse range of customers from researchers that deposit just a few blood samples a year from patients with rare genetic disorders to large studies submitting samples from thousands of individuals. These large studies have included Medical Research Council (MRC) funded investigations into the genetics of diseases such as Alzheimer's, Type 2 Diabetes, Multiple Sclerosis and Hypertension (see Table 1). The HGCB Service has been responsible for generating LCLs for two large control cohort collections – the MRC National Survey of Health and Development (NSHD) '1946 Cohort' and the National Child Development Study '1958 Cohort' in close collaboration with the Avon Longitudinal Study of Parents and Children (ALSPAC) for which the Wellcome Trust has provided funding. In addition, the HGCB has produced the cell lines from which human random control (HRC) genomic DNA is extracted for the HRC genomic DNA panels available from ECACC.

Developing the Service

At ECACC we are continuously seeking to improve all aspects of our service. Jim Cooper leads a Development Team which works alongside the HGCB and is dedicated to improving the existing HGCB service and developing new systems. 'We have a system that works well. Our aim is to further maximise the efficiency and capability while reducing costs using techniques such as miniaturisation and robotics. We are also investigating new technologies', explains Jim.

ECACC was awarded a Medical Research Council (MRC) grant in November 2002 for the development of the HGCB service as part of the MRC national DNA banking initiative. The key aims of this project are to improve the throughput of the system, identify ways to reduce costs and define alternative protocols for the transformation of PBLs.

A multiwell test platform has been generated for process development and reagent qualification which allows key parameters to be tested in a tightly controlled model. All steps of the process have been studied including: media volumes, cell concentration, EBV multiplicity of infection and serum concentration.

A key achievement of the project has been to secure a source of blood samples from the National Blood Service representing a "normal" population. This valuable resource allows new technologies to be tested using a standard set of samples, enabling direct comparison of results.

In general, approximately 2-7% of samples do not transform at the first attempt. There is a widespread belief that cells from certain individuals are resistant to EBV transformation. However, investigations suggest that failed transformation is most commonly due to poor blood sample quality - principally low numbers of viable PBLs. ECACC scientists are developing ways to assess the quality of samples according to defined criteria and so adjust protocols to enhance the potential for transformation success.

Alternative protocols are also being examined and developed. Significant steps have been made towards developing a protocol for whole blood transformation. This removes the need to separate peripheral blood lymphocytes prior to transformation, so reducing the number of process steps required.

Keeping the Service on Track

The early introduction of an electronic barcode-driven tracking system at the HGCB has had a major impact on sample throughput. The purpose built Laboratory Information Management System (LIMS) allows a paperless laboratory.

Study	Depositor	No. of samples
Alzheimer's Disease (late onset)	Prof. J Williams, University of Wales, College of Medicine, Cardiff	3500
Type 2 Diabetes Family	Prof. A Hattersley, Peninsula Medical School, Exeter	4000
Asthma/Eczema	Prof. W Cookson. Wellcome Trust Centre for Human Genetics, Oxford	2000
Multiple Sclerosis	Prof. A Compston, Addenbrookes Hospital, Cambridge	2000
Hypertension (BRIGHT)	Prof. M Caulfield, William Harvey Research Institute, London	4300
Unipolar Depression	Prof. P McGuffin, Institute of Psychiatry, London	1500
Family Heart Disease	Prof. A Hall, University of Leeds	4800
Glomerulonephritis	Prof. A Rees, University of Aberdeen	2200

Table 1. Medical Research Council DNA banking projects which have submitted blood samples and peripheral blood lymphocytes to the HGCB

Figure 3. Barcode-driven Tracking System

Hand-held, programmable, wireless scanners enable samples to be tracked and identified anywhere in the process (see Figure 3). A full audit trail history can be rapidly generated. A significant factor for the successful development of this tailor-made system is the dedicated IT personnel employed at ECACC. In addition to LIMS, the ECACC IT department has also developed 'ICAST' a robust electronic inventory control system.

Supply of DNA & RNA

The Wellcome Trust has supplied grant funding to ECACC, from autumn 2004, in order to expand the HGCB service to accommodate Wellcome Trust funded projects. This grant has enabled ECACC to invest in robotic technologies for the extraction, quantification, normalisation and generation of aliquots of DNA.

Advancements in the extraction of DNA and RNA from lymphoblastoid cell lines are on-going at ECACC. The Cell Products Team routinely generate very pure, high molecular weight genomic DNA suitable for a wide spectrum of genetic research applications. This will be expanded to include RNA in the future.

The human random control (HRC) genomic DNA panels, produced by the Cell Products Team, represent DNA from a control population of 480 individuals and are available for immediate use. In addition to DNA derived from LCLs, ECACC has also developed a process for the removal of white blood cells from leucocyte reduction filters for subsequent DNA extraction. Extraction of DNA from blood filters can enable very large control populations to be generated; potentially in excess of 1000 random control DNA samples can be produced upon request.

Many researchers appreciate the convenience of ordering DNA ready prepared from their cell lines of interest so that they are not required to maintain cell cultures themselves. Researchers need not be burdened with the recruitment and generation of control material. Rather they can purchase control DNA from ECACC and focus on the assays they wish to perform such as genotyping, linkage mapping and SNP analysis.

For information on using the HGCB service and/or the DNA/RNA products available from ECACC please consult the ECACC website **www.ecacc.org.uk** or contact us on **+44 (0) 1980 612512 or email: ecacc.technical@hpa.org.uk**