Ask ECACC .... Focus on Epstein Barr Virus (EBV) lymphocyte transformation

ECACC has provided an EBV lymphocyte immortalisation and cell banking service to human genetic research in the UK since 1986. 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 orders. ECACC receives a diverse range of technical enquires and in this issue we have focused on EBV lymphocyte transformation.

1 How does EBV transformation work?

The virus binds to the CD21 receptor on the B cell and enters the cell. The virus DNA is then transferred to the nucleus where, by the action of various oncogenes the cell develops the capacity to reproduce indefinitely, thus a lymphoblastoid cell line (LCL) is generated.

2 Will EBV affect subsequent genetic analysis of genomic DNA from LCLs?

The effect of EBV transformation on genomic DNA is uncertain, although it is believed that the viral DNA remains episomal. DNA from LCLs is suitable for studies on both transcriptional and translational products. Unlike whole genome amplified DNA, DNA from LCLs can be used in the study of DNA methylation, very large DNA fragments, repetitive gene sequences and telomeric repeats.

3 Are all blood samples transformable?

This depends on the quality of the sample, the age of the individual, their disease status and treatment regimes. A small proportion of the population (1-2%) may also be inherently "un-transformable".

4 What transformation success rates can I expect?

The average success rate for EBV transformation at ECACC is currently >95% at first attempt. This will vary between specific projects and the quality of incoming blood specimens.

5 How can I ensure a good quality blood sample?

Blood should be sent to ECACC as soon as possible after collection, preferably within 48 hours. We recommend the use of Acid Citrate Dextrose (ACD) as an anticoagulant, and a minimum sample volume of 6ml. The blood should be kept at ambient temperature (20°C) after collection and during despatch. The blood tubes need to be packed correctly and safely and never refrigerated. Detailed information on how to send samples can be found at **www.ecacc.org.uk** under "Services".

6 Does the length of storage of frozen peripheral blood lymphocytes (PBLs) affect transformation success rate?

At ECACC, PBLs stored safely at a constant temperature in Liquid Nitrogen storage vessels for greater than 10 years have transformed with no apparent reduction in success rate.

7 Can I store PBLs that I have separated myself at –80°C? If so, for how long?

We do not recommend storing PBLs at –80°C. Long term storage of cell lines and PBLs can only be guaranteed by storage at liquid nitrogen temperatures. However, if there is no alternative, then –80°C storage should be employed only for weeks rather than months and never for years.

8 Do LCLs remain diploid (or genome remain stable) on long term culture?

LCLs may be polyploid rather than diploid (i.e. each cell will have more than two copies of each chromosome). Once polyploidy is established, cells will remain relatively genetically stable.

9 What safety level should LCLs be handled at?

Unless there is information to the contrary (e.g. the patient is known to be Hepatitis B or HIV positive) LCLs should be handled at UK ACDP containment level 2 (CL2) or equivalent, to contain any residual risk from potential adventitious agents that the cell line may be harbouring.

10 Can LCLs propagate HIV or Hepatitis B or C?

HIV primarily infects T cells and as far as we can determine from the literature, there is no cell line currently available that will propagate Hepatitis B and C viruses. ECACC is able to process blood samples and prepare LCLs from patients with HepB, HepC or HIV at ACDP containment level 3 by prior arrangement.

11 How can I be sure that the cell line DNA is identical to the DNA I have extracted direct from the patients blood?

Upon arrival at ECACC, before the blood sample is separated, a small quantity of blood from each patient is stored on a blood spot card. This allows for verification of the LCL against patient DNA using Single Tandem Repeat (STR) Multiplex PCR Profiling. As part of routine Quality Assurance, ECACC tests 5% of LCLs against source DNA. However, EBV transformation is a protracted, highly manual process, and as such, a small degree of error (usually < 1%) is possible.