Transfusion Medicine, 2003, 13, 77–81

ORIGINAL ARTICLE

Used leucodepletion filters as a source of large quantities of DNA suitable for the study of genetic variations in human populations

M. A. Cook,*†‡ S. E. Jobson,* D. C. Atkinson,* D. P. Lowe,* S. L. Farmer,* W. J. Alvi-Ali,* N. A. Smith* and D. C. Briggs* *H&I. National Blood Service and †Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, UK

Received 10 September 2002; accepted for publication 20 January 2003

SUMMARY. A simple technique for developing large control panels with large quantities of DNA suitable for studies in population genetics was established.

Background and objectives: Both a lack of suitable controls and insufficient quantities of DNA for repeated analysis of the same control group often hamper the investigation of genetic markers for disease.

Materials and methods: Using a waste product from routine blood donation, we describe a simple method that allows the investigator to extract large amounts of DNA.

Results: A mean of 1520 μg of DNA per sample was obtained. The DNA obtained remains suitable for polymerase chain reaction and sequencing techniques after 2 years of storage at both 4 °C and −40 °C.

Conclusion: This technique allows the development of a large panel of controls with sufficient quantities of genomic DNA for thousands of tests.

Key words: control samples, DNA extraction, leucodepletion filter.

Molecular genetic techniques such as the polymerase chain reaction (PCR) and DNA sequencing have stimulated an explosion in the search to find molecular genetic markers for common diseases such as asthma (Hakonarson & Wjst, 2001) and hypertension (Doris, 2001).

Reliable assessment of disease markers requires knowledge of the distribution of those markers within the population. If a particular marker is present at a significantly higher frequency in a patient population than in a comparable nondisease/healthy control population, then the disease being studied may be associated with the genetic marker.

Studies can founder on the inability to obtain a normal control panel of sufficient size. Problems can also occur when new knowledge can stimulate the re-examination of past cases and their controls. If insufficient sample has been stored, then it may be impossible to return to the original donor of that sample, leading to a reduction in the size of the control panel. The breadth of genetic tests currently available can place high demands on a sample obtained from a single blood draw of 10–20 mL, which may yield 90–600 μg of genomic DNA using a commercial extraction kit. For example, investigation of highly polymorphic genes such as human leucocyte antigen (HLA) can use up to 40 μg of DNA when typed by PCR-sequence-specific priming (SSP) and cycle sequencing can use up to 500 ng of DNA per test. With some investigators utilizing several hundred reactions at a time, the modest yield seen with commercial kits may thus only generate enough DNA for a small number of projects. Some facilities have circumvented this problem by generating cell lines using transformation by Epstein–Barr virus. This is, however, expensive and very time-consuming. Utilizing current techniques, there are, therefore, limitations on the ability to develop a control panel that easily provides sufficient quantities of DNA to allow large

Correspondence: Dr Mark Cook, Tissue Typing, Birmingham Blood Centre, Vincent Drive, Edgbaston, Birmingham B15 2SG, UK. Tel: +44 (0) 121 253 4041; fax: +44 (0) 121 253 4110; e-mail: mark cook@nbs nhs uk

numbers of tests by different techniques such as PCR and DNA sequencing.

Currently in the UK, whole blood donations are leucodepleted at the blood centre where the donation is processed to reduce the theoretical risk of transmission of variant Creutzfeldt–Jakob disease. Leucodepletion filters are provided by Baxter and NBPI as part of an integrated system composed of the original bag into which the unit is taken, the leucodepletion filter and a receiving bag into which the filtered blood passes. After the filtration step, the original bag and filter are removed and, in the normal chain of events, discarded. The filter contains greater than 99% of the leucocytes that are in the original donation and therefore has the potential to provide large quantities of DNA. In this paper, we describe the use of a waste product from routine blood donations as the source of large quantities of DNA suitable for molecular genetic techniques.

MATERIALS AND METHODS

The source material was the leucodepletion filters removed from the bag assembly after filtration of a blood donation. Institutional ethical approval was obtained prior to collection of the filters. A total of 300 filters were collected. The donations used were selected from new donors only, who had passed a basic health screen. The use of new donors only removes the bias in donor selection that can occur if regular donors are used. The call-up of regular donors is often distorted by the ABO group requirements at the time, resulting in a higher collection of group O units compared with groups A, B and AB. No information identifying the donor was retained; the only records kept being those of age, sex and ethnic origin. All units were tested for human immunodeficiency virus (HIV)-1 and -2, hepatitis B, hepatitis C and syphilis by a combination of serology and nucleic amplification testing. All units collected were negative for these tests.

The leucodepletion filters were then kept at room temperature for up to 24 h after the filtration process.

Following cannulation of the sealed outflow tube with a 19G needle, 20 mL of sterile normal (0·9%) saline was flushed through the filter in the direction opposite to that of the original filtration. An equivalent volume of the filter contents was collected from the inflow tubing into a sterile container. The sterile container was centrifuged at $800 \times g$ for 10 min. The resultant buffy coat pellet was removed and placed in a new sterile container. A modified salting-out method for the extraction of DNA was used (Miller *et al*., 1988). Briefly, two rounds of red cell lysis were performed using 12 mL of lysis buffer (0·144 M NH_4Cl and 1 mM $NaHCO_3$). Overnight nucleus lysis of the resulting white cell pellet was performed at 57 °C following the addition of 4 mL of nucleus lysis buffer (10 mM Tris–HCl (pH 8·2), 400 mM NaCl, 2 mM Na_2EDTA (pH 8·0) and 5% SDS). After nucleus lysis, 2 mL of chloroform and 2 mL of 6 M NaCl were added to this solution. After 10 s of vigorous mixing, the mixture was centrifuged for 25 min at $1600 \times g$. The supernatant was decanted into 2·5 vols 100% ethanol. The precipitated DNA was then washed in 500 µL of 70% ethanol and dried by evaporation. Finally, the DNA was dissolved in ddH_2O. Storage was either at 4 °C or at −40 °C, for a period of up to 2 years.

Protein contamination was estimated using the ratio of absorbance at 260 nm and 280 nm (the 260/280 ratio) as measured by spectrophotometry, with a target ratio of 1·8.

The DNA was used in three different PCR-SSP systems: an HLA phenotyping system (Bunce *et al*., 1995), an in-house red cell genotype system and an in-house killer immunoglobulin receptor (KIR) genotype system. The HLA phenotyping and the red cell genotyping protocol use identical reaction conditions, yielding products up to 600 bp. The KIR system uses a different thermal cycler program, yielding products of up to 2 kbp.

Sequencing was also performed on the DNA. Analysis of exon 4 of the KIR gene *KIR2DL2* and of exon 2 of the Dombrock genes *Do*1 and *Do*2 was carried out on an ABI Prism 310 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

RESULTS

Elution of white cells from filters

Preliminary work showed that the eluate from the filters had a white cell count ranging from $20 \times 10^9\,L^{-1}$ up to $75 \times 10^9\,L^{-1}$, giving a total white cell content of $0{\cdot}4–1{\cdot}5 \times 10^9$ cells. A 20-mL blood draw from a person with a normal white count would be expected to yield $0{\cdot}08–0{\cdot}22 \times 10^9$ white cells assuming a white cell count of between 4 and $11 \times 10^9\,L^{-1}$.

Quantity and purity of DNA

The mean quantity of DNA obtained was 1520 µg, with the majority of filters yielding 1000–2000 µg of DNA (mean 1520 µg, range 450–3750 µg). The 260/280 ratios were between 1·60 and 1·85 (mean 1·69).

Usage of DNA

The DNA performed normally within all three of the PCR systems used (two shown in Fig 1), yielding products of the expected sizes up to 2 Kb. Sequencing of DNA is a more sensitive test of the quality of DNA. Clean reads of the sequences for *KIR2DL2* and *Do1*/*Do2* were obtained (one example shown in Fig 2).

DISCUSSION

We have demonstrated that it is possible to obtain a large quantity of leucocytes and subsequently genomic DNA suitable for use in molecular genetic techniques such as PCR and sequencing. The yield of leucocytes compares favourably with other investigators (Longley & Stewart, 1989; Vyas, 2001). Despite this, assuming a bleed of 450 mL and a normal white cell count, the filter should contain $1.8–4.9 \times 10^9$ cells, raising the potential of an even higher yield, if so desired.

Using what is normally a waste product from routine blood donation and processing can yield milligram quantities of DNA. We used a modified salting-out technique, a method that is less efficient than the current commercial DNA extraction kits but capable of handling higher cell loads and thus yielding larger quantities of DNA. This provides the opportunity to construct DNA panels suitable for controls in multiple studies examining genetic markers. With the large yield, a 'once-only' sample is required, obviating the need to return to a donor to replenish diminishing amounts of DNA. Previously, population studies for genetic variation have used varied methods, such as Guthrie cards (Hamvas *et al.*, 2001), buccal samples (Feigelson *et al.*, 2001; Garcia-Closas *et al.*, 2001) and blood samples (O'Dell *et al.*, 1995), none of which can provide a large enough sample for recurrent large-scale testing.

The donors used were all first-time donors. Most control populations will possess an inherent bias, e.g. cadaveric donors have a high percentage of donors who have suffered from intracranial events (Gralnek *et al.*, 1999; Ojo *et al.*, 1999). It can be argued that first-time blood donors are themselves a biased population, as those motivated to donate may be at a higher or lower risk of particular diseases under genetic control, although this has not been demonstrated epidemiologically. In addition, we have tested for certain viral markers. Exclusion of those donors positive for these markers introduces a bias. We have not, as yet, had a positive test for any of the donors used. Statistically, viral positivity is higher in first-time blood donors (most recently estimated as a two-fold risk when compared with repeat donors (Dodd *et al.*, 2002)), but still a relatively rare event in the UK. First-time blood donors represent a pragmatic balance between the requirement for a completely random population and the ability to easily access

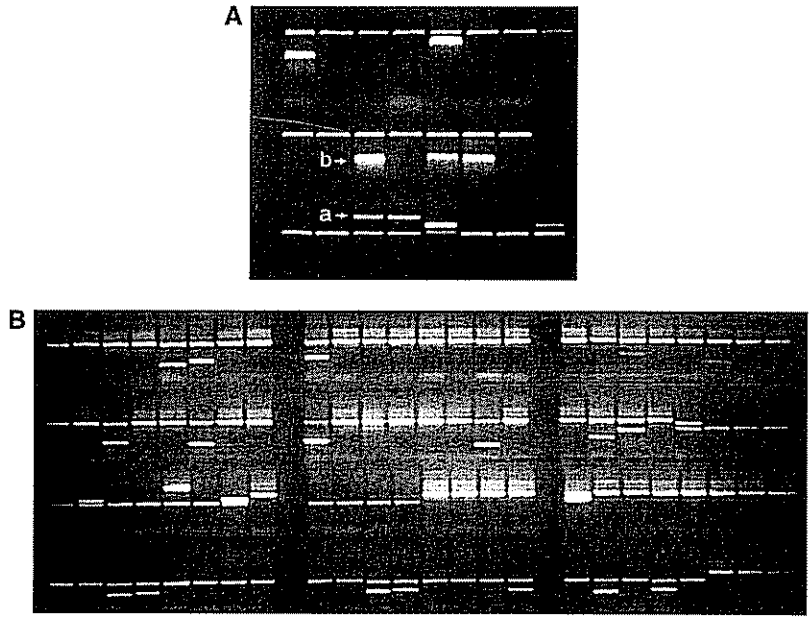

Fig. 1. (A) Gel-image of polymerase chain reaction-sequence-specific priming (PCR-SSP) typing for killer immunoglobulin receptor (KIR) genes, demonstrating the amplification of bands from 125 bp (a) to 2 kbp (b) (frozen DNA stored at −40 °C for 2 years) and (B) Gel-image of PCR-SSP typing for human leucocyte antigen (HLA) class II (DNA stored at 4 °C for 2 years).

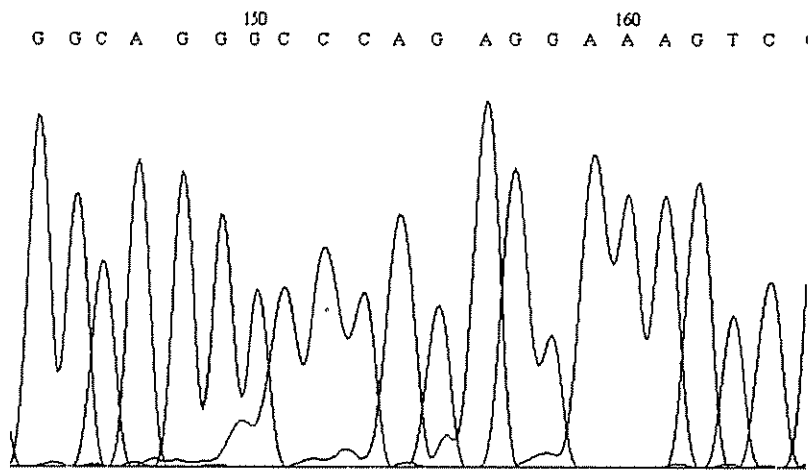

Fig. 2. Part of the sequence of exon 5 of the gene *KIR2DL2* obtained by sequencing genomic DNA that had been stored frozen for 2 years.

the population to be tested. An important part of our process was the 'unlinking' of donor and sample. Retention of donor identifiers may have future legal and financial implications for any institution embarking on a programme of DNA banking. In addition, it is desirable to obtain explicit donor consent at the outset, with an emphasis on the anonymity of the samples collected.

The quality of the DNA is good enough to perform well in three different PCR systems producing product sizes of 200 bp to 2 kbp. In addition, successful sequencing has been performed on this DNA, a process that is highly dependent on the quality of DNA. The DNA extracted in this way has remained stable for 2 years at both 4 °C and −40 °C, performing well in both PCR reactions and sequencing after prolonged storage at both temperatures.

By demonstrating the feasibility of extracting DNA from leucodepletion filters, the possibility of establishing a large control panel is raised. In the West Midlands region of the UK alone, there are 250 000 donations per year, approximately 10% of which are from new donors

In addition, it may be possible to manipulate the extraction process, increasing the yield of white cells from the filter, e.g. by altering the pH of the flushing solution. At present, there are no commercial kits available that can cope with the cellular loads required for more than 1 mg of DNA, which is why we used a modified salting-out method. There remains the possibility that it may become possible to adapt commercial kits to accommodate the large cellular load that we have used, raising the possibility of semi-automation and even higher yields than we have achieved.

REFERENCES

Bunce, M., O'Neill, C.M., Barnardo, M.C., Krausa, P., Browning, M.J., Morris, P.J. & Welsh, K.I. (1995) Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens*, **46** (5), 355–367.

Dodd, R.Y., Notari, E.P. IV & Stramer, S.L. (2002) Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion*, **42** (8), 966–972.

Doris, P.A. (2001) Hypertension genetics, single nucleotide polymorphisms, and the common disease: common variant hypothesis. *Hypertension*, **39** (2 Part 2), 323–331.

Feigelson, H.S., Rodriguez, C., Robertson, A.S., Jacobs, E.J., Calle, E.E., Reid, Y.A. & Thun, M.J. (2001) Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. *Cancer Epidemiology, Biomarkers & Prevention*, **10**, 1005–1008.

Garcia-Closas, M., Egan, K.M., Abruzzo, J., Newcomb, P.A., Titus-Ernstoff, L., Franklin, T., Bender, P.K., Beck, J.C., Le Marchand, L., Lum, A., Alavanja, M., Hayes, R.B., Rutter, J., Buetow, K., Brinton, L.A. & Rothman, N. (2001) Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiology, Biomarkers & Prevention*, **10**, 687–696.

Gralnek, I.M., Liu, H., Shapiro, M.F. & Martin, P. (1999) The United States liver donor population in the 1990s: a descriptive, population-based comparative study. *Transplantation*, **67** (7), 1019–1023.

Hakonarson, H. & Wjst, M. (2001) Current concepts on the genetics of asthma. *Current Opinions in Pediatrics*, **13** (3), 267–277.

Hamvas, A., Trusgnich, M., Brice, H., Baumgartner, J., Hong, Y., Nogee, L.M. & Cole, F.S. (2001) Population-based screening for rare mutations: high-throughput DNA extraction and molecular amplification from Guthrie cards. *Pediatrics Research*, **50**, 666–668

Longley, R.E. & Stewart, D. (1989) Recovery of functional human leucocytes from Leukotrap filters. *Journal of Immunological Methods*, **44** (1), 93–102.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, **16**, 1215.

O'Dell, S.D., Humphries, S.E. & Day, I.N. (1995) Rapid methods for population-scale analysis for gene polymorphisms: the ACE gene as an example. *British Heart Journal*, **73**, 368–371.

Ojo, A.O., Wolfe, R.A., Leichtman, A.B., Dickinson, D.M., Port, F.K. & Young, E.W. (1999) A practical approach to evaluate the potential donor pool and trends in cadaveric kidney donation. *Transplantation*, **67** (4), 548–556.

Vyas, G.N. (2001) Human peripheral blood mononuclear cell substrate for propagating wild type HIV-1. *Developmental Biology (Basel)*, **106**, 345–356.