Genomic DNA Isolation System

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Restriction endonuclease digestion and Southern blot analysis of eukaryotes require the isolation of intact genomic DNA. Extra care must be taken during the purification to avoid shearing the high molecular weight DNA while removing tightly bound chromosomal proteins from the sample. In addition, consistent results are essential, since genomic DNA is often isolated from limited material. The ability to obtain genomic DNA samples that are acceptable substrates for subsequent analysis relies on the use of high quality reagents and a suitable protocol.

The new BRL Genomic DNA Isolation System, which includes the key reagents (table 1) for DNA preparation and a detailed manual, makes DNA purification rapid and reproducible. The simple protocol (figure 1) takes approximately 5 and 1/2 h to complete and contains several steps allowing flexible timing. DNA may be isolated from mammalian whole blood, tissue culture cells and nucleated erythrocytes. The protocol yields clean, high molecular weight DNA from thirty 5-ml whole blood samples or 1 x 10^7 cells that is efficiently digested using BRL restriction endonucleases and REact® Buffers according to the recommended conditions included in the manual (figure 2).

Product	Cat. No.	Size
Genomic DNA Isolation System	8350SA	30 assays

Table 1. Components of Genomic DNA Isolation System

Component	Amount
Lysis buffer	255 ml
0.64 M sucrose,	(3 x 85 ml)
0.02 M Tris-HCl (pH 7.8),	
0.01 M $MgCl_2$,	
2% (w/v) Triton X-100	
Saline-EDTA	60 ml
75 mM NaCl,	
24 mM EDTA	
Proteinase K	100 mg
20% (w/v) SDS	3 ml
2 M KCl	3 ml
TE buffer	5 ml
10 mM Tris-HCl,	
1 mM EDTA (pH 8.0)	

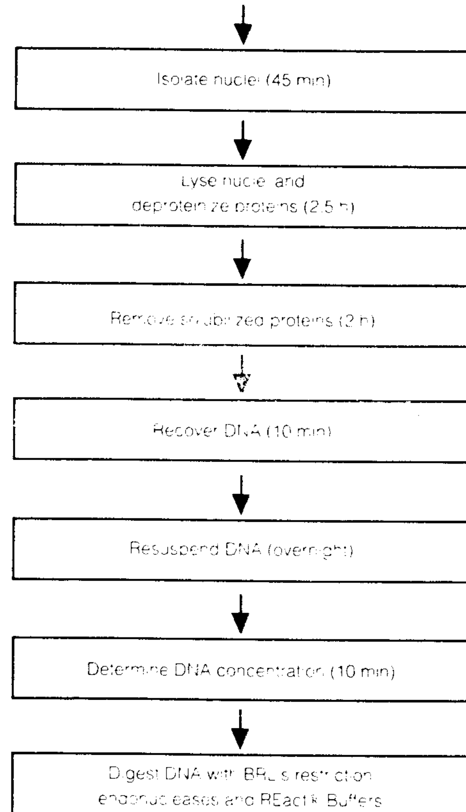

Figure 1. Genomic DNA Isolation System protocol flow chart.

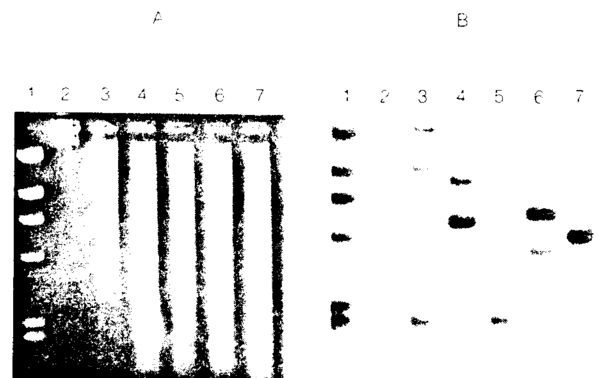

Figure 2. Southern blot hybridization of human genomic DNA. Human genomic DNA was prepared from whole blood using the Genomic DNA Isolation System. Samples containing 5 µg DNA were digested with several restriction endonucleases using the recommended conditions. Panel A shows the ethidium bromide staining pattern of DNA samples after electrophoresis on a 0.8% (w/v) agarose gel. The samples are *Hind* III-digested λ DNA molecular weight markers (lane 1), undigested DNA (lane 2), and DNA samples digested with the following restriction endonucleases: *BamH* I (lane 3), *Bgl* II (lane 4), *Cvn* I, *EcoR* I (lane 6), and *Pst* I (lane 7). The gel was transferred to a nylon membrane and hybridized with a pBR322 plasmid containing a 4.4-kb *Pst* I fragment of the human ß-globin gene labeled with [α-^{32}P]dCTP using BRL's Random Primers Labeling System. Panel B shows the autoradiograph resulting from a 24-h exposure with an intensifying screen.