Cloning of Hepadnaviruses using microquantity serum

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The Hepadnaviruses includes the human hepatitis B virus (HBV) and duck hepatitis B virus (DHBV) which are enveloped and characterised by a circular partially double stranded DNA of 3 kb to 3.2 kb in length. The long complete strand is termed the negative strand, while the short, partially complete, strand covering 50–70% of the genome is the positive strand. The most frequently encountered problems in cloning of Hepadnaviruses are the availability of large quantities of serum/plasma and the purification of the virus. A simple and efficient method for cloning of viral DNA from micro-quantities of serum is described which circumvents these problems.

Virus positive serum/plasma (100 μl) was incubated with an equal volume of specific polyclonal antibody and Protein A Sepharose (200 μl) at 37°C for 2 hrs. The antigen-antibody-Protein A Sepharose complex was washed with twenty volumes Phosphate Buffered Saline (PBS, pH 7.2) by centrifugation (2000 rpm, 5 min, 4°C). The pellet was treated at 56°C for 2 hrs with Proteinase K (100 μg/ml) in 10 mM Tris–HCl pH 8. 0.5 mM EDTA, 0.5% SDS followed by a 5 minutes incubation at room temperature with a 10% NP 40–3% β-ME (1:1) solution. The Sepharose beads were pelleted down (6000 rpm, 1 min) and removed. The supernatant was treated with 1% Cetyltrimethyl-ammonium bromide (CTAB, Sigma, USA) and NaCl at a concentration greater than 0.7 M for 5 min, 37°C (75 μl supernatant 15 μl 5 M NaCl and 10 μl 10% CTAB). The advantage of using CTAB is its ability to complex with polysaccharides, denatured proteins and cell debris leaving the nucleic acid in solution clean for further use (1). This was followed by phenol/chloroform/isoamylalcohol (25:24:1) extraction. The supernatant was diluted with 3 volumes of Tris EDTA pH 8.0 and precipitated with ethanol in presence of yeast t-RNA (1 μg/ml). The precipitated nucleic acid was resuspended in T7 DNA polymerase buffer (400 mM Tris pH 7.5, 100 mm $MgCl_2$ 50 mM DTT, 500 mM NaCl, 500 μg/ml BSA, 2 mM of each dNTP), 5 units of T7 DNA polymerase (Amersham, UK) and incubated at 37°C for 40 minutes to fill up the single stranded gap and create a homogenous population of double stranded DNA (Figure 1A). The enzyme in the reaction mixture was heat inactivated at 65°C for 15 minutes. The DNA was digested with a suitable restriction enzyme (3 units of *Eco*RI for HBV/3 units of *Bam*HI for DHBV) in the same buffer, heat inactivated and ligated to compatible ends of dephosphorylated bluescript vector (pBS+) (Stratagene, USA) (2). The ligated mix was diluted five times and 10 μl of it was used for transformation of JM109 cells using polyethylene glycol (3) in order to prevent inhibition of transformation (4). The recombinants were screened by colony hybridisation (Figure 1B). The purified plasmid DNA from positive clones was confirmed by Southern hybridisation and polymerase chain reaction for the specific insert.

We find that the yield of DNA obtained is in sufficient quantity (50–200 ng approximately) for cloning. We obtained 200–300 transformants per test ligation used of which approximately 50% were recombinants by colony hybridisation (Figure 1B). This procedure has been used in our laboratory to clone both human hepatitis B virus and duck hepatitis B virus genome. It is simple, reliable and needs only 100 μl of serum which can be easily obtained.

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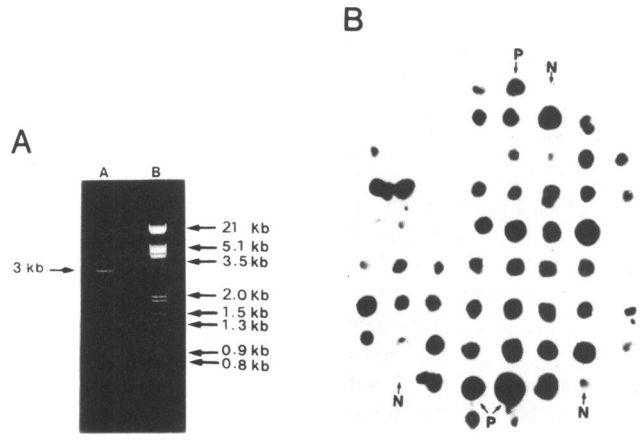

Figure 1. A. DNA isolated from 100 μl DHBV positive serum. Lane A—isolated DNA filled with T7 DNA polymerase and digested with *Bam*HI, Lane B— Lambda *Hin*dIII/*Eco*RI marker (Boerhinger mannheim,Gmbh). B. Plate showing number of positive recombinants clones by colony hybridisation for duck hepatitis B virus. Positive control (P) — pBR 322 containing DHBV, Negative control (N) — pBS^+ plasmid.