Isolation of plasmid DNA from mammalian cells with QIAprep

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A variety of studies of eukaryotic systems, including cDNA expression cloning (1), recombination (2) and extrachromosomal replication (3), require isolation of plasmid DNA from eukaryotic cells. This entails separation of a very small quantity of plasmid DNA from a much larger quantity of genomic DNA. Traditionally, the Hirt extraction method (4) has been used for this purpose. This method involves extensive precipitation, phenol and chloroform extraction, and 2–3 days of time.

In bacteria, the most common method for isolation of plasmid DNA involves specific precipitation of genomic DNA following alkaline lysis (5). Plasmid DNA, even in small quantities, can then be adsorbed from the supernatant onto glass or silica in the presence of chaotropic salts (6,7). This makes phenol/chloroform extraction and ethanol precipitation unnecessary. The QIAprep® Spin Plasmid Kit, which was designed for plasmid minipreparation from *E. coli*, employs these methods and requires only 30 minutes. We tested the QIAprep procedure for isolation of plasmid DNA from mouse cells transiently transfected with shuttle vectors, and compared it with the Hirt method.

Hirt extraction

Cell pellets were resuspended in 100 µl TE and lysed with 100 µl of 1.2% SDS by gentle mixing and incubating for 15 min at room temperature. Genomic DNA was precipitated by addition of 50 µl of 5 mM NaCl, incubation at 4°C for at least 8 h, and centrifugation at 15,000 x g for 30 min at 4°C. The supernatant was then recentrifuged for 45 min at 23,000 x g, and the supernatant was proteinase K digested (100 µg/ml final concentration) for 1 h at 65°C. Following phenol/chloroform extraction, the plasmid DNA in the aqueous supernatant was precipitated by addition of 0.5 µl of 20 mg/ml glycogen, 0.5 volumes of 7.5 M ammonium acetate and 2 volumes of ethanol. After incubation at -20°C for at least 12 h and centrifugation at 70,000 x g for 45 min at 4°C, the DNA pellet was resuspended in 50 µl TE.

QIAprep Spin Plasmid Kit procedure

The QIAprep Spin Plasmid Kit procedure was followed essentially as recommended for preparation of plasmid DNA from bacterial cells. Cell pellets were resuspended in 250 µl Buffer P1 (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A) and lysed by addition of 250 µl Buffer P2 (200 mM NaOH, 1% SDS) and incubation for 5 min at room temperature. Genomic DNA and cell debris were precipitated by addition of 350 µl Buffer N3 (containing potassium acetate and chaotropic salts), incubation on ice for 5 min and centrifugation at 10,000 x g for 10 min. Supernatants were loaded onto QIAprep spin columns and centrifuged for 1 min. After washing once with 500 µl Buffer PB (to remove residual endonucleases) and once with 750 µl Buffer PE (to remove residual salts), plasmid DNA was eluted with 50–150 µl TE by incubation for 5 min at 37°C and centrifugation for 1 min.

Results

Viable cells (6×10^4 – 6×10^6) of the murine pre-B cell line, 1881 A20, approximately 10% of which were transiently transfected with a derivative of the expression vector, pcDNA I*, were separated from dead cells by ▶▶ 12

* Invitrogen

▶▶ Isolation of plasmid DNA...

Experiment	Number of cells	Number of transformants Hirt	QIAprep
1	2.4×10^6	480	1200
2	2.0×10^6	530	100
3a	6.0×10^6	880	34,000
3b	6.0×10^5	210	620
3c	6.0×10^4	27	78

Table 1. Transformants (E. coli) obtained with plasmid DNA prepared from mouse cells using either the Hirt method or the QIAprep Spin Plasmid Kit. In experiment 3, DNA was prepared from serial 10-fold dilutions of the mouse cells.

Ficoll gradient centrifugation 16–20 h after electroporation. They were washed with PBS and divided into two portions. Plasmid DNA was then isolated using either the Hirt extraction or the QIAprep Spin Plasmid Kit. Yields were compared by transforming *E. coli* strain Sure® with 1/150 of each preparation (Table 1). On this basis, the yield was estimated to be 0.3–3.0 ng plasmid DNA from 1×10^5 cells for both procedures.

In further experiments, transfection was performed with pre-B cells and fibroblasts that express a viral T antigen and thus mediate replication of vectors carrying the viral origin of replication. Restriction digestion with the 6-methyl adenosine-dependent enzyme, *DpnI*, and Southern blotting (3) demonstrated that the DNA isolated represented replicated, intracellular plasmid DNA, and not residual input DNA remaining on the cell surface after transfection. Therefore, the QIAprep procedure yielded plasmid DNA in at least equivalent amounts and of equivalent quality to the Hirt method, but in 30 minutes rather than 2–3 days.

References

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