# Extraction of cellular DNA from crude cell lysate with glass

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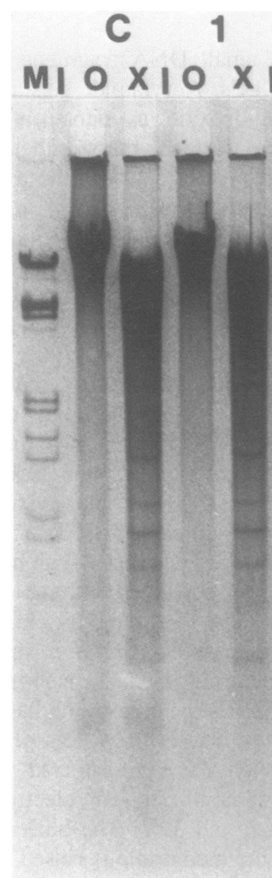


Extraction of DNA from animal cells or tissues with organic solvents yields only $1-2$ $\mu g/10^6$ cells and is labor intensive and time consuming, requiring several hours or days. The affinity of DNA for glass has been exploited to extract DNA from agarose (1) and plasmid from bacterial lysates (2), bt has not been successfully applied to animal DNA extraction because of DNA fragmentation and inefficiency of DNA binding in the presence of cell lysates. By using relatively large glass particles ($5-25$ $\mu$) and cells solubilized with guanidine thiocyanate, we have been able to develop a 45 min procedure which routinely yields $5-6$ $\mu g$ of highly pure DNA/$10^6$ cells from multiple samples. Pelleted cells were first lysed with 5M guanidine thiocyanate/0.1 M EDTA (pH8.0) to a concentration of $2\times10^7$ cells per ml. Lysate (100 $\mu l$) was then combined with 500 $\mu l$ of glass powder (Schleicher and Schuell, Keen, NH) suspended in binding buffer (50 mg glass/ml in 6 M sodium perchlorate, 50 mM Tris-Cl pH 8.0, 10 mM trans-1,2-diaminocyclohexane -N, N, N′, N′-tetracetic acid, CDTA), and the suspensions were mixed by rocking at room temperature for 20 min. The glass was pelleted by spinning in a microfuge for 30 sec, washed with 500 $\mu l$ of binding buffer, pelleted, then resuspended in 500 $\mu l$ of elution buffer (0.2 M sodium perchlorate, 50 mM Tris-Cl pH 8.0, 10 mM CDTA) and rocked at room temperature for 20 min. The glass was then pelleted, and supernatants containing the eluted DNA were transferred to fresh tubes. Two volumes (1 ml) of 100% ethanol were added, the contents were mixed by inverting, and the tubes were spun in a microfuge for 5 min at room temperature. DNA pellets were rinsed with 500 $\mu l$ of 90% ethanol, dried, then resuspended in restriction endonuclease buffer. In figure 1, lane 'M' contains 1 $\mu g$ of lambda DNA cleaved with *Hin*dIII and *Eco*RI (Promega). Lanes 'C' contain 5 $\mu g$ of DNA purified by conventional means from spleen tissue (3). Lanes '1′ contain DNA extracted from lysate of $10^6$ K562 cells. All DNAs were incubated for 16 h at 37°C in the absence (lanes '0') or presence (lanes 'X' of 25 units of *Xba*I (Promega). Alternatively, glass was washed once with 50% ethanol and DNA was eluted in any convenient buffer, but yields were reduced 40% (not shown). Bands in the cut lanes are cleavage products from repeat elements in the human genome (3).

## REFERENCES

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