A mini-screen technique for analyzing nuclear DNA from a single *Dictyostelium* colony

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The cellular *Dictyostelium* slime molds have been found to carry a number of different multi-copy, circular, nuclear plasmids (1,2,3). In addition, DNA-mediated transformation of *D. discoideum* is now well established. In order to facilitate the screening of wild isolates for endogenous plasmids, and of transformants for the presence of extrachromosomal or integrated vector sequences we have developed a rapid and reliable mini-screening technique which allows sufficient plasmid or total nuclear DNA to be obtained from a single 2 cm diameter colony of *Dictyostelium*, and eliminates many of the extraneous bands seen with lysis-in-the-gel procedures (1).

Isolation of plasmid DNA: Suspend a loopful of vegetative cells from a 2 cm colony in 1 ml of cold, sterile water and centrifuge for 1 s in a microcentrifuge. Resuspend the pellet in 1 ml of NP40 lysis buffer (10mM Mg acetate, 10mM NaCl, 30mM HEPES (pH 7.5), 10% sucrose, 2% nonidet P-40) and centrifuge for 30 s. Resuspend the nuclei in 150 μ l TE (pH 7.5) then add 200 μ l alkaline SDS (0.2 M NaOH, 1% SDS). Incubate for 15 min on ice, add 150 μ l 3M Na acetate (pH 4.8) then incubate for a further 15 min on ice. Centrifuge for 5 min, recover 450 μ l of the supernatant and precipitate this with ethanol.

Isolation of total nuclear DNA: Isolate nuclei as described above. After pouring off the NP40 lysis buffer and without draining the tube, add a fresh 25 μ l of NP40 lysis buffer and vortex. Add 375 μ l 0.2M EDTA, 2% Sarkosyl and incubate for 5 min at 65°C. Extract with phenol and chloroform, then precipitate with ethanol.

The precipitated DNA in both cases is resuspended in a small volume of TE buffer. Plasmid samples are loaded directly on an agarose gel. There is approximately 1 μ g of total nuclear DNA recovered, sufficient for a single digestion with restriction enzymes or for loading on a slot or dot blot. This work was supported by NIH grant R01 GM35168 and Utah Agricultural Experiment Station grant UTA 601C.

References

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