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Simplified mammalian DNA isolation procedure

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The reverse genetics technologies that have recently been developed for mice have provided new tools to probe gene function *in vivo*. Unfortunately these powerful systems often require the analysis of large numbers of DNA samples. The gene-targeting technology requires screening of embryonic stem-cell clones and later of the mice themselves, the latter also being the case for standard transgenic technology. It is not always possible or desirable to rely on PCR analyses, necessitating the isolation of large numbers of DNA samples of sufficient quality for Southern blot analysis. We have simplified the standard mammalian DNA isolation procedure with the aim of minimizing the number of manipulations required for each sample. The basic procedure applied to cultured cells does not require any centrifugation steps or organic solvent extractions.

BASIC PROCEDURE

The lysis buffer has been adjusted to allow restriction digestion of the DNA without prior organic solvent extractions. Therefore, the procedure involves just three manipulations:

1. Addition of lysis buffer to the tissue or cells.
2. Addition of isopropanol.
3. Transfer of precipitate to TE.

1. *Lysis*: The lysis buffer (usually 0.5 ml) is added to the tissue or cells. Digestion is complete within several hours at 37°C (cells) or 55°C (tissues) with agitation.
Lysis buffer: 100 mM Tris.HCl pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 100 μg Proteinase K/ml.

2. *Isopropanol precipitation*: One volume of isopropanol is added to the lysate and the samples are mixed or swirled until precipitation is complete (viscosity completely gone).

3. *Recovery of precipitate*: The DNA is recovered by lifting the aggregated precipitate from the solution using a disposable yellow tip. Excess liquid is dabbed off and the DNA is dispersed in a prelabeled Eppendorf tube containing, depending on the size of the precipitate, 20 to 500 μl of 10 mM Tris.HCl, 0.1 mM EDTA, pH 7.5. Complete dissolution of the DNA may require several hours of agitation at 37°C or 55°C. It is important that the DNA is completely dissolved to ensure the reproducible removal of aliquots for analysis.

APPLICATIONS

Cultured cells: The complete DNA isolation can be performed in the same well used to culture the cells, since our procedure does not require centrifugation steps. As a particular ES-cell clone reaches confluency in a 24 well dish, its medium is replaced by 0.5 ml of lysis buffer and the culture of the whole 24-well dish is continued until each of the clones in the dish has been submerged in lysis buffer for at least several hours. The 24-well dishes containing the lysates are transferred to an automatic rocking or swirling table and agitated for 15 minutes. One volume of isopropanol (0.5 ml) is added to each well and the automatic agitation is continued until precipitation is complete.

Tail biopsies: The application of the basic procedure to tail biopsies does require one centrifugation step to remove hairs and tissue residue from the lysate. Tail biopsies of no longer than about one centimeter are transferred to 0.5 ml of lysis buffer immediately upon cutting. They are then transferred to a rotating tube rack or other means of continuous agitation at 55°C for several hours or overnight. The agitation is important to accomplish complete lysis. Failure to mix well during lysis may result in poor restriction digestion. Following complete lysis, the rack of tubes is shaken vigorously or the tubes are vortexed. The tubes are then spun in an Eppendorf centrifuge for as long as it takes to obtain a firm pellet (usually 10 minutes). The supernatants are then poured into prelabeled tubes, each containing 0.5 ml of isopropanol.

RESULTS AND DISCUSSION

We generally find the DNA yields to exceed those of isolates obtained using phenol/chloroform extractions and ethanol precipitation. In general the samples contain very little RNA. The DNA samples are of sufficient quality for Southern blot analysis using a variety of restriction enzymes, including those active only in low salt conditions. We have used this method to analyze fragments of up to 20 kb. We include 0.1 mg BSA/ml in the restriction enzyme digestion to absorb any residual SDS, proteases, or other inhibitory elements.

The procedure described above is now being used routinely in several mouse genetics laboratories. The reduced manual labor involved is perceived as an important benefit of the method. When problems were occasionally encountered, this was due to either too large a tissue sample, insufficient agitation during lysis, or insufficient agitation during dissolution of the DNA. If problems with restriction digestion are encountered, the option of a phenol/chloroform extraction followed by a reprecipitation is still open.

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