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Reference

CHARETTE, Steve, COSSON, Pierre. Preparation of genomic DNA from Dictyostelium discoideum for PCR analysis. *BioTechniques*, 2004, vol. 36, no. 4, p. 574-5

PMID : 15088372

Preparation of genomic DNA from *Dictyostelium discoideum* for PCR analysis

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BioTechniques 36:574-575 (April 2004)

The soil amoeba *Dictyostelium discoideum* is a practical genetic system in which targeted gene disruption by homologous recombination allows the study of specific gene functions (1–3). The efficiency of homologous recombination, however, can be less than 1%, and the identification of the desired clones requires the analysis of the genotype of many individual clones. However, the standard protocols to prepare genomic DNA for genotyping require a large number of cells (>10^7) and also necessitate manipulation of organic solvents and ethanol precipitation (4). Commercial kits are expensive and highly inefficient for the isolation of *D. discoideum* genomic DNA. The easiest protocols known to us take at least a few hours for the preparation of genomic DNA.

Genotyping of the nematode *Caenorhabditis elegans*, another genetic model, is very simple and is based on the use of a lysis protocol employing detergent and proteinase K (PK) (5–7). Here we adapted this method for the isolation of *D. discoideum* genomic DNA, which is amenable to PCR analysis. Our optimized protocol is the following: *D. discoideum* DH1-10 cells (8) were resuspended in HL5 medium (8) or in water. One volume of cells was then mixed with four volumes of lysis buffer [LyB; 10 mM Tris, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Nonidet® P-40 (NP40), and 0.45% Tween® 20] containing PK (1 µL of 20 µg/µL of PK for every 25 µL of LyB). The activity of PK is so efficient that incubation at 65°C is not necessary (data not shown). The cell mixtures were placed at 95°C for 1 min to inactivate the PK. Each cell lysate (1 µL) was analyzed by PCR using the manufacturer's conditions for the DNA polymerase and

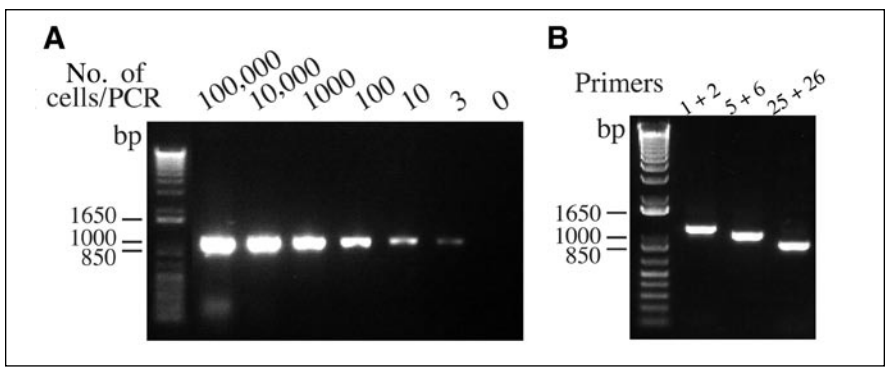

Figure 1. Optimization of the preparation of *Dictyostelium discoideum* genomic DNA using a standard lysis protocol. Different numbers of *D. discoideum* cells were resuspended in HL5 medium and mixed with lysis buffer (LyB) containing proteinase K (PK) (ratio HL5/LyB of 4:1) as indicated. The mixture was incubated at 95°C for 1 min. A 1-µL aliquot of each cell lysate, containing material extracted from (A) 0–100,000 cells/µL of lysate or from (B) 1000 cells/µL of lysate, was tested by PCR amplification using REDTaq™ DNA polymerase (Sigma) and primers OL5 and OL6 (5 + 6), in panels A and B, and also primers OL1 and OL2 (1 + 2) and OL25 and OL26 (25 + 26), in panel B. Five microliters of each PCR and one sample of a 1-kb DNA ladder (Invitrogen; first left lane) were subjected to electrophoresis on a 1% agarose gel.

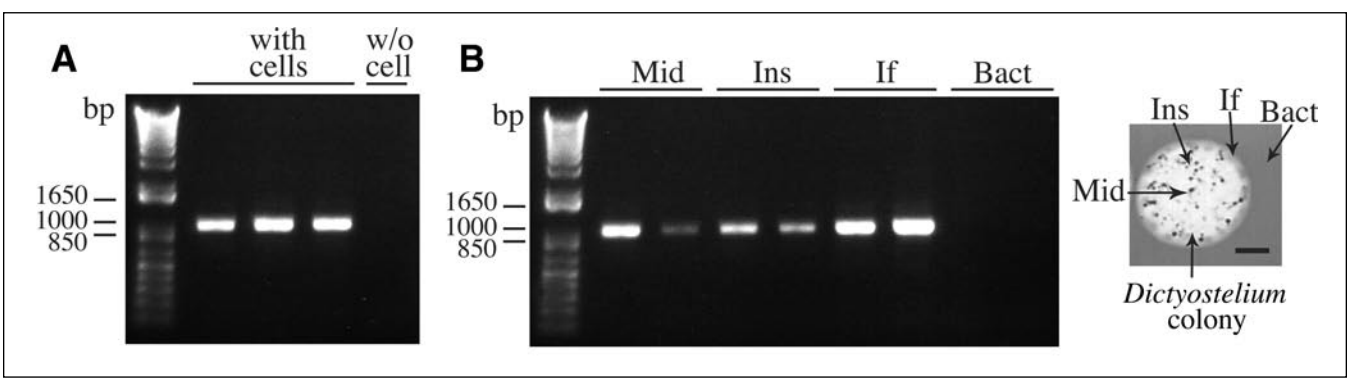

Figure 2. Lysis of *Dictyostelium discoideum* clones. (A) Genotyping of clones grown in a 96-wells plate. *D. discoideum* cells were cloned by limiting dilution in a 96-wells plate. After 2 weeks of culture, cells were gently resuspended in the well by pipeting. Aliquots from three wells containing variable numbers of cells and one without cells (negative control) were mixed with four volumes of lysis buffer (LyB) containing proteinase K (PK) and incubated for 1 min at 95°C. (B) Genotyping of *D. discoideum* clones grown on a bacterial lawn. *D. discoideum* cells were plated on an SM plate [10 g/L peptone, 1 g/L yeast extract, 16.2 mM KH_2PO_4, 5.7 mM K_2HPO_4, 4 mM $MgSO_4$, 1% glucose, and 20 g/L bacto-agar (Brunschwig DB Difco, Basel, Switzerland)] together with *Klebsiella aerogenes* bacteria. After 1 week, cells were picked with a toothpick from different areas of one colony (two different samples per area): in the middle (Mid), 1 mm inside (Ins) the border between *D. discoideum* and bacteria, and at the bacteria/*D. discoideum* interface (If) as illustrated on the colony picture (scale bar, 2 mm). Samples from an area with only bacteria (Bact) were also picked as a negative control. Cells and bacteria on the toothpick were resuspended in water (10 µL) and then mixed with four volumes of LyB and incubated at 95°C for 1 min. One microliter of each cell lysate (from A and B) was tested by PCR amplification using REDTaq DNA polymerase and primers OL5 and OL6. After PCR, 5 µL of each PCR were subjected to electrophoresis. w/o, without.

Table 1. Sequences of Primer Pairs Used for the Optimization of the Lysis Protocol for Genomic DNA Preparation from *Dictyostelium discoideum*

Genomic Region	Primer	Sequence	Product Size (bp)
ng8935	OL1	5′-GAAGATCTAAACTTAATCAAACAACAGAATGTCTC-3′	1200
	OL2	5′-GGGGTACCTGGAATATACACATGTGGCCAACTAAA-3′	
ng9670	OL5	5′-GAAGATCTTGTTTTATTTCAAATTTATAGT-3′	1050
	OL6	5′-GGGGTACCATTTACATTATTTTGATTTTGAG-3′	
ng1402	OL25	5′-GAAGATCTATGGCATCAGTGTCAAGCTCTTCAAGT-3′	900
	OL26	5′-GGGGTACCTTATACTGGTTCTTTGAGATGCTTGTT-3′	

These primer pairs amplify a specific segment of the indicated genomic regions. The size of the product is indicated for every primer pair.

0.7 µM of each primer (see Table 1 for the description of the primers) in a final volume of 40 µL. DNA polymerases of different sources [*Taq* DNA polymerase from Invitrogen (Carlsbad, CA, USA) and Sigma (St. Louis, MO,USA), *Pfu* DNA polymerase (Stratagene, La Jolla, CA, USA) or high-fidelity Platinum® DNA polymerase (Invitrogen)] were used with similar results (data not shown). The program used for the PCR was 95°C for 4 min, 40 cycles of 94°C for 30 s, 58°C for 30 s, and 65°C for 90 s, followed by 65°C for 10 min. As shown in Figure 1A, amplification of a portion of genomic DNA is observed using a wide range of cell concentration (from 100,000 to 3 cells per PCR). The HL5/LyB ratio can vary from 1:4 to 4:1 with no effect on PCR amplification (data not shown).

To establish the efficiency and the reproducibility of the lysis method, other pairs of primers were successfully tested (more than a dozen different genes) (data not shown). In Figure 1B, amplifications by two specific pairs, OL1 plus OL2 and OL25 plus OL26, are shown. These primer pairs allow generation of DNA fragments from genes other than the one amplified by OL5 plus OL6 primers (Figure 1A). Moreover, these primer pairs amplify genomic segments containing introns that are particularly AT-rich and thus more difficult to amplify. All the primer pairs used lead to similar quantities of PCR products, demonstrating that this method can be of general use for cell genotyping.

The final aim of this study was to test the robustness of this assay under conditions in which clones are routinely obtained (i.e., *D. discoideum* cells grown in HL5 in 96-wells plates or on a bacterial lawn). In both cases, the method allows amplification of the desired DNA fragment (Figure 2), demonstrating that it can be used effectively to directly screen *D. discoideum* clones without any additional culture or cell dilution. In the case of *D. discoideum* colonies growing on a bacterial lawn, stronger and more consistent results were obtained from bacteria taken from the borders of the colony.

The method proposed here consists of only three steps prior to PCR amplification: (*i*) resuspension of the cells in growth medium or water (resuspension volume can be as small as 1–2 µL, data not shown); (*ii*) mixing with an aliquot of lysis buffer; and (*iii*) incubation at 95°C for 1 min. This protocol has many advantages. It is simple, rapid, cheap, robust, and does not require specialized equipment or the use of organic solvents. It enables the use of very few cells, as low as 3 cells per PCR. As the whole procedure can be done in a single tube, it would be relatively easy to robotize for high-throughput applications. In addition, lysates generated by the method described here can be frozen and kept at -20°C and thawed several times to perform other PCR amplifications without any obvious loss in quality and quantity of the PCR products obtained (data not shown). It should facilitate enormously the isolation of specific knockout strains of *D. discoideum* and will thus allow a more systematic screening of the role of various gene products in this organism. It will prove particularly useful in situations where large numbers of clones must be tested to obtain a few specific knockout mutants.

ACKNOWLEDGMENTS

We thank Dr. Jillan Harrisson for her critical reading of the manuscript and her relevant comments. This work was supported by a grant of the Fonds National Suisse de la Recherche Scientifique. S.J.C. received a fellowship from the Human Frontier Science Program.

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Received 5 December 2003; accepted 15 January 2004.

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