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Notes & Tips

Control of pH during plasmid preparation by alkaline lysis of *Escherichia coli*

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ABSTRACT

Alkaline lysis of *Escherichia coli* is usually the method of choice for plasmid preparation, but "ghost bands" of denatured supercoiled DNA can result if the pH is too high or the period of lysis is too long. By replacing the usual sodium hydroxide lysis solution with an arginine buffer prepared in the range of pH 11.4 to 12.0, we were able to stabilize the pH during lysis and obtain plasmid that is suitably pure for restriction digestion and DNA sequencing.

The extraction of plasmids from *Escherichia coli* is one of the most commonly used methods in molecular biology, and detergent lysis of cells in 0.1 M sodium hydroxide (NaOH,[2] final) is the most widespread approach [1,2]. The alkalinity denatures the chromosomal DNA, and the supercoiled plasmid DNA remains intact provided that the pH does not exceed a threshold of approximately 12.3 [3,4]. However, the pH of the lysis solution is approximately 12.8 in the absence of cells, and so it is important that biological macromolecules released into the crude lysate provide acid equivalents that partially neutralize and reduce the pH below the threshold. Without any external buffering during lysis, the reproducibility of the method depends on having enough cell mass to counteract a given volume of NaOH solution. "Ghost bands" representing irreversibly denatured plasmid have been reported in the literature [5–8] and in technical brochures [9] and appear to develop if the pH or length of exposure is excessive. We have investigated the effects of varying the pH during alkaline plasmid extraction using l-arginine as a buffer and found that a lysis solution buffered with arginine can replace the NaOH-based lysis solution of the usual Birnboim–Doly alkaline lysis method [1]. This modification allows comparable plasmid yield under reproducible pH conditions and prevents the generation of irreversibly denatured supercoiled DNA.

The optimal protocol is described below, with all steps being conducted at room temperature:

1. The cells are collected by centrifugation at 1100 g for 15 min and resuspended in 1/10 volume of 10 mM Tris–Cl and 10 mM ethylenediaminetetraacetic acid (EDTA, pH 8.0).
2. To each 1 ml of cell suspension, 1 ml of a lysis solution consisting of 1% (w/v) sodium dodecyl sulfate (SDS) and 0.5 M L-arginine (pH 11.7) is added, and the tube is capped and rocked briefly to mix. The lysate is allowed to sit undisturbed for 5 min.
3. The lysate is neutralized by the addition of 1 ml of 5 M potassium acetate (pH 4.75) for each 1 ml of original cell suspension and is rocked again to mix the solutions fully.
4. The lysate is subjected to centrifugation for 10 min at 20,000*g*, and the supernatant is transferred to a clean tube.
5. Isopropanol (0.8 volume) is mixed with the supernatant, which is subjected to centrifugation for 10 min at 20,000*g*.
6. The precipitate is washed once with 70% (v/v) ethanol, dried in vacuo, and resuspended in 10 μl of 10 mM Tris–Cl and 0.5 mM EDTA (pH 8.0) for each 1 ml of starting material.

Fig. 1 shows the DNA and RNA isolated by this procedure from *E. coli* cells (XL-1 blue strain, Stratagene) grown in Luria–Bertani (LB) medium with 0.05 mg/ml ampicillin and carrying the plasmid pGEM3Zf+ (Promega). In this experiment, the pH of the arginine-based lysis solution was varied between 11.4 and 13.5, and the plasmid is evident in Fig. 1 as supercoiled and nicked circular bands. The RNA is visible as a smear of staining material below the plasmid bands and is progressively hydrolyzed at higher pH and with longer incubation periods. The overall plasmid yield does not vary significantly with pH and is comparable to that obtained by the Birnboim–Doly method [1]; however, slight changes in pH have a strong effect on whether the plasmid is digestible with a restriction endonuclease (as discussed later).

Extending the lysis period from 5 min to 24 h does not eliminate the plasmid, but the gel shows a broad smear of staining material extending from the loading well to the plasmid band in samples incubated above pH 11.7. The extended period of hydrolysis at high pH may generate protein and peptidoglycan fragments that are precipitated by the isopropanol treatment, fouling the selective precipitation of nucleic acids and the gel electrophoresis. A lysis buffer with a pH of 11.4 allows for extended periods of

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[2] *Abbreviations used:* NaOH, sodium hydroxide; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; LB, Luria–Bertani.

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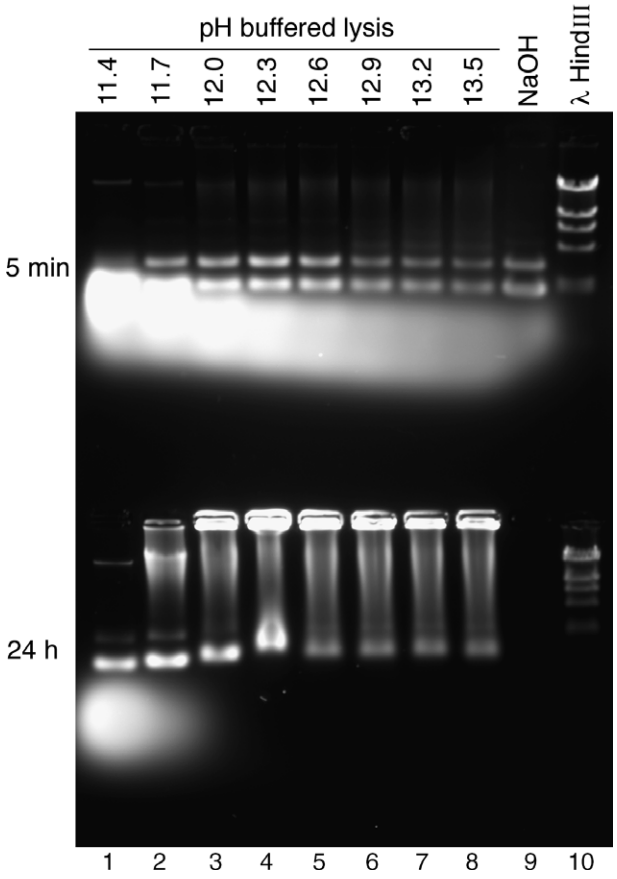

Fig. 1. Gel electrophoresis of pGEM3Zf+ plasmid extracted by alkaline lysis for 5 min (top half) or 24 h (bottom half). Lanes 1 to 8 are based on arginine-buffered lysis at the indicated pH. Lane 9 (top half only) is material prepared by the Birnboim–Doly method [1] using unbuffered NaOH. Each lane represents material extracted from 0.1 ml of *E. coli* culture. Lane 10 is λ DNA digested with *Hin*dIII. The gel was stained with 1 μg/ml ethidium bromide. The pH values of these lysis buffers were established by mixing a fixed amount of L-arginine with NaOH to establish zwitterion/anion molar ratios of 12.6 (pH 11.4), 6.31 (pH 11.7), 3.16 (pH 12.0), 1.58 (pH 12.3), 0.794 (pH 12.6), 0.398 (12.9), 0.20 (pH 13.2), and 0.10 (pH 13.5) based on a pK_a of 12.48 [10].

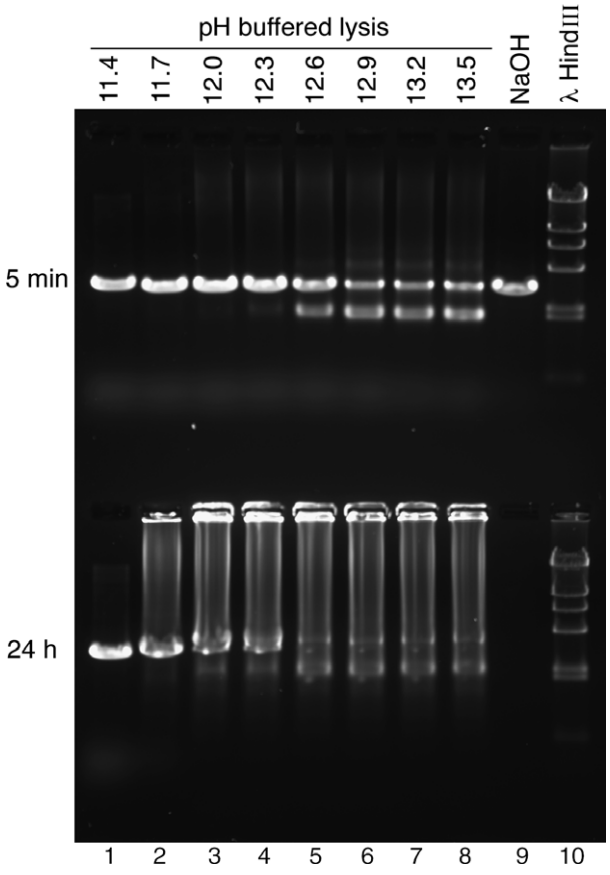

Fig. 2. Gel electrophoresis of pGEM3Zf+ plasmid extracted by alkaline lysis for 5 min (top half) or 24 h (bottom half) and digested with *Eco*RI and RNase A. Lanes 1 to 8 are based on arginine-buffered lysis at the indicated pH. Lane 9 (top half only) is material prepared by the Birnboim–Doly method [1] using unbuffered NaOH. Each lane represents material extracted from 0.1 ml of *E. coli* culture and digested with 0.15 U/μl of *Eco*RI and 0.05 mg/ml RNase A for 1 h. Lane 10 is λ DNA digested with *Hin*dIII. The gel was stained with 1 μg/ml ethidium bromide.

hydrolysis without this contamination (Fig. 1, lane 1), but this lower pH makes it slightly more difficult to separate the lysate supernatant from the pellet. Sodium acetate and lithium acetate solutions work comparably well to potassium acetate for the neutralization of the lysate provided they are at least 5 M and prepared as an equimolar mixture of acetate salt and acetic acid.

The plasmids prepared by lysis between pH 11.4 and pH 12.0 are readily digested with *Eco*RI (Fig. 2), and they are sufficiently pure to use in DNA sequencing, yielding more than 700 bases of readable sequence on an ABI 3130 automated DNA sequencer (Applied Biosystems) (data not shown). Plasmids collected by lysis at a pH greater than 12.3 are not completely digested by *Eco*RI, suggesting that the plasmid is either partially denatured or copurifying with inhibiting molecules released by hydrolysis.

Using arginine to control the pH during lysis provides important advantages. First, the extraction is made more reliable because the buffering compensates for variations in the amount of cell mass extracted per milliliter of lysis solution. Second, the method is less time sensitive because the plasmid is not exposed to a threshold pH that causes irreversible denaturation. The addition of arginine increases the cost of the lysis solution by less than 2 cents per milliliter and does not interfere with the use of silica-based DNA adsorbents to purify the DNA from the lysate (e.g., Wizard Plus MiniPreps DNA Purification System, Promega).

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