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Birnboim H C & Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acid. Res.* 7:1513-23, 1979.
[Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France]

A screening procedure for extracting plasmid DNA from bacterial cells is described. Chromosomal DNA is selectively denatured by alkali treatment while covalently closed circular plasmid DNA remains double-stranded; on neutralization, a heavy precipitate forms leaving partially purified plasmid DNA in solution. [The *SCI*® indicates that this paper has been cited in over 4,160 publications, making it the most-cited paper from this journal.]

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In 1978 I had arranged to take sabbatical leave from my position at the Atomic Energy of Canada laboratories in Chalk River to spend a year in Paris in the laboratory of Giorgio Bernardi. Several years earlier, Neil Straus, University of Toronto, and I had identified a class of repetitive sequences in the DNA of many higher eukaryotes that consisted of long polypyrimidine/polypurine tracts, up to 200 base pairs in length. As the recombinant DNA era was dawning, I chose to use my sabbatical period to clone some of these tracts in order to study them in more detail.

Necessity was the mother of invention, and the alkaline extraction procedure for screening plasmid DNAs grew out of a specific need related to this project. These tracts are resistant to acid treatment, which breaks the bulk of the DNA into fragments less than 20 nucleotides in length. The strategy I planned to use was to label individual clones from a mouse DNA library *in vivo* with $^{32}P_i$, extract plasmid DNA in fairly pure form, treat it with acid, and then separate the products on a sequencing gel. I expected that perhaps 1 in 100 clones might have a long pyrimidine tract, which should be detectable by autoradiography as a slow-moving band. Thus, the screening procedure had to be reasonably simple but also had to give fairly pure plasmid DNA largely free of chromosomal DNA, RNA, and other ^{32}P-containing material. In principle, the strategy worked; several clones of mouse DNA containing polypyrimidines were identified and one was eventually sequenced.[1]

Although it had been shown earlier that alkaline conditions could be used selectively to denature bacterial chromosomal DNA and not covalently closed circular DNA, it required rather careful adjustment of pH using a pH meter.[2] Obviously, this would not be practical for tiny volumes. A copy of the *Handbook of Biochemistry and Molecular Biology*[3] indicated that glucose could act as a buffer in the pH range 12-13, and then it was simply a matter of adjusting the ratio of alkali to glucose to give the proper final pH. On neutralization, chromosomal DNA forms a gel-like insoluble mass. High salt can precipitate high molecular weight RNA and protein-sodium dodecyl sulfate complexes. The final protocol combined these earlier findings into a single step.

I did not go to Paris armed with adequate fluency in the French language. This initially made it a little difficult for me to integrate into other projects in the lab, and I was thus able to concentrate on getting the alkaline extraction procedure to work. I used an old cylindrical electrophoresis system for running agarose gels because there was less competition for time on this piece of equipment. Janine Doly, a scientist in the lab at the time, befriended me and helped me set up some of the other techniques that were needed. In July 1979 the plasmid extraction method was introduced to a wider audience and tested for reliability when it was used as part of a European Molecular Biology Organization laboratory course on recombinant DNA held in Paris. It appeared to have passed the test. Further refinements and some applications to a preparative method were described subsequently, in 1983.[4]

The original publication continues to be widely cited as a basic tool of recombinant DNA technology because it has proved to be simple and reliable and produces fairly purified material. It led to no particular awards, but it is personally gratifying to have developed a procedure that has survived for nearly a decade.

1. **Dougan K V, Mitchell R E J & Birnboim H C.** Nucleotide sequence of polypyrimidines from cloned mouse DNA as determined by base-specific blockage of exonuclease action. *Anal. Biochem.* 129:88-97, 1983. (Cited 5 times.)
2. **Currier T C & Nester E W.** Isolation of covalently closed circular DNA of high molecular weight from bacteria. *Anal. Biochem.* 76:431-41, 1976. (Cited 315 times.)
3. **Fasman G D,** ed. *Handbook of biochemistry and molecular biology. Physical and chemical data. Volume 1.* Cleveland, OH: CRC Press, 1976. 552 p.
4. **Birnboim H C.** A rapid alkaline extraction method for the isolation of plasmid DNA. *Meth. Enzymology* 100:243-56, 1983. (Cited 145 times.)

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