This Week's Citation Classic ®

Holmes D S & Quiglev M. A rapid method for the preparation of plasmids. *Anal. Biochem.* 114:193-7, 1981. [Department of Biology, State University of New York. Albany. NY]

Plasmids can be isolated from cultures of *Eschehchia coli*cells by heating and precipitation. The plasmids can be used for further manipulations including restriction enzyme analysis, transformation into bacterial cells, and DNA sequence analysis. (The *SCI*® indicates that this paper has been cited in more than 2,325 publications.]

Rapid Preparation of Plasmids

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Plasmids are small independently replicating DNA molecules found in microorganisms. They became, and remain to this day, a central feature of many genetic engineering strategies. Almost every gene that has been sequenced and analyzed has been isolated and purified by cloning into one of the many plasmids now available.

Back in the late 1970s one of the popular ways for isolating plasmids involved a tedious procedure (lysis) for breaking open bacterial cells, followed by several additional steps, including a lengthy CsCl buoyant density centrifugation. The whole process could take two or more days.

The procedure worked well unless one became too greedy. The temptation was to grow the bacterial cells to a very high density to maximize the amount of plasmid that could be obtained. Problems occurred, however, because lysis could result in such a viscous solution that it was impossible to proceed any further. And this is where my story begins.

It was a dark and stormy night (honestly!), very late, after all the students had gone home. I was a new assistant professor at the State University of New York in Albany. I had left a bacterial culture growing far too long, and when I came to lyse the cells the resulting gelatinous mess, or goop, had the viscosity of a cold jellyfish. I couldn't even pour it into the centrifuge bottles. It was a relatively large culture, and I wanted the plasmid urgently, otherwise I might have discarded the mess and started again the next day.

Then I thought about warming it to decrease its viscosity. I decided 55° C would be OK. At this temperature enzymes that degrade DNA would be inactivated and yet the temperature would be too low to denature the DNA, perhaps ruining the plasmids. After a couple of minutes at 55° CI found the goop now behaved like a warm jellyfish but still couldn't be poured. I decided to heat the goop even further by placing it in a boiling water bath. I could hear the voice of my postdoc adviser, Norman Davidson, calculating the temperature at which the DNA would denature and concluding that I was *crazy,* but I also heard the voice of my graduate adviser, James Bonner, telling me to go ahead and do it and see what happened. Surprisingly the procedure worked, and it was very fast. Mike Quigley, who was working in my lab as a technical assistant, helped to check that the resulting plasmids could be used for subsequent genetic engineering manipulations. Mike went on to graduate school in neurobiology.

The paper was initially sent to *Nucleic Acids Research,* in which another popular rapid plasmid preparation procedure had recently been published.[1] However, they rejected our work on the grounds that they didn't want to publish another paper on the same subject. *Analytical Biochemistry* promptly accepted the paper and also published a later modification.[2] I also amended the procedure to make it more suitable for screening multiple samples. In this modification as many as 24 miniplasmid preparations could be processed easily by one person in under one hour.[3]

During the years from 1982 to about 1988 the original paper received a lot of citations. Then the procedure was incorporated into a very popular compendium of recipes for molecular biology that has recently been updated,[4] and now most researchers reference only this book. *Sic transit gloria mundi!*

Although my contribution contained no great intellectual leap forward I hope it has saved hours of boring work for countless students and researchers who, so freed, can use their time for better things.

1. **Birnboim H C *& Doly* J.** A rapid alkaJine extraction procedure for screening recombinanl plasmid DNA. *MucL Acid. Res.* 7:1513-23. 1979. (Cited 8.355 limes.) [See also: Bimboim H C. Citation Classic®. ***Current Contents*®*/Life Sciences*** **31**(45): 12. 7 November 1988.]
2. **Eisenberg A J & Holmes D S.** A note on the use of CsCl centrifugation to purify bactenal plasmids prepared by the rapid boiling method. *Anal. Biochem.* 127:429. 1987.
3. **Holmes D S.** Improved rapid heating technique for screening recombinant plasmids in E. *coli. Biotechniaues* 2:68-9. 1984.
4. **Sambrook J, Fritsch F *&* Maniatis T.** *Molecular cloning: a laboratory manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 1989. 3 vols. (Cited 13.555 times.)

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