This Week's Citation Classic ®

Marmur J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* 3:208-18, 1961.
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The isolation of purified DNA from bacteria is described in sufficient detail to make it applicable to most microorganisms. Previous experience in isolating biologically active *Streptococcus* transforming DNA was very useful in modifying the original procedure by Avery, MacLeod, and McCarty and applying it to various bacterial species. These DNA samples proved to be very useful in physical studies on DNA, in particular the denaturation and renaturation of nucleic acids. [The *SCI*® indicates that this paper has been cited in more than 6,700 publications.]

Isolating DNA

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In 1938, M.G. Sevag *et al.*[1] isolated nucleoproteins from *Streptococcus* by "intensive sonic vibrations" of cells and subsequent precipitation with 0.1 M HCl (procedures sure to denature, depurinate, and degrade DNA). DNA was then separated from protein by shaking with chloroform and isoamyl alcohol. In 1942, A.E. Mirsky and A.W. Pollister[2] isolated nucleoproteins from animal tissues by salt extraction. When trout nucleoprotein preparations made in this way were dialyzed against 1.0 M NaCl, one obtained "practically pure nucleic acid." The status of DNA isolation in the mid-1940s was described in a review by then current experts in the field of nucleic acids.[3]

My initial introduction to the isolation of high molecular weight DNA was in Rollin Hotchkiss's laboratory when I was a research associate at the Rockefeller Institute (now University) in 1952-1954. Hotchkiss was an ideal teacher: He took me through each of the steps for isolating biologically active transforming DNA from *Diplococcus* (now *Streptococcus*) *pneumoniae*, from growing the cells to sterilization of the purified DNA with ethanol. He lovingly described each stage of the preparation and transmitted to me the excitement of purifying its genetic material. The climax was always the precipitation of the DNA by ethanol and harvesting the DNA threads on a stirring rod. (One day, while isolating DNA, Hotchkiss introduced me to Oswald Avery who had come to visit the laboratory.)

There was a good deal to learn about DNA isolation from Hotchkiss's comments and by reading the classic paper by Avery *et al.*,[4] which provides the basis for most subsequent procedures for isolating DNA. DNA should not be dissolved in water lacking counter-ions (because it will denature and degrade), and DNA should not be dried. The isolation of transforming DNA was the first case of fractionating genetically active material by practices analogous to those used by enzymologists to purify enzymes—that is, by monitoring its specific biological activity. Other insights and useful information Hotchkiss provided were that: if the product doesn't dissolve readily in saline, it's not likely to be DNA; one should be aware that polysaccharides often co-precipitate with DNA and thereby mislead the investigator concerning the amount of DNA present in the alcohol-precipitated threads. Accordingly, a very useful modification of the original Avery *et al.*[4] procedure developed by Hotchkiss was to precipitate the DNA with less ethanol than originally prescribed, thereby reducing co-precipitation of other biological polymers.

Hotchkiss exercised great caution in the isolation procedure. The DNA was treated with great care: Deproteinization with chloroform was accomplished with gentle shaking, and the DNA was dissolved carefully after each precipitation step. The availability of the biological assay of transformation to monitor the quality of the DNA was particularly helpful to me in broadening the applicability of the isolation procedure to other organisms than pneumococcus. Thus, the higher the molecular weight of the DNA, the greater its specific transforming activity, especially the plateau level of the transformation-versus-DNA concentration response curve.

When I joined Paul Doty's laboratory at Harvard in 1956, I found myself in an ideal environment in which to study the physical characterization of DNA. At the time I arrived, the laboratory was principally investigating the macromolecular behavior of calf thymus and salmon sperm DNAs. Bacterial DNAs were not being studied, except for some *S. pneumoniae* DNA provided by Harriet Taylor. Having experience in microbiology and having already studied transforming DNA, I began isolating DNA from a variety of microorganisms for subsequent studies that would include the correlation of thermal stability and buoyant density in CsCl of the various DNAs with their base composition. Bacteria, with DNAs varying in their base composition from about 25 to 75 percent guanine plus cytosine (GC), provided a rich source of material to evaluate the physical and biochemical properties of their DNA. Most of the DNA samples were routinely characterized by their thermal denaturation profile, sedimentation coefficient, and intrinsic viscosity.

A critical initial step in DNA isolation is lysis of cells. The preparation of DNA from Gram-positive *S. pneumoniae* cells was relatively simple because those cells are readily disrupted by bile salts (e.g., sodium deoxycholate). The Gram-negative bacteria can be disrupted easily by other detergents such as sodium dodecylsulfate (SDS). It had been shown by J.S. Rowen and A. Norman[5] and S. Zamenhof *et al.*[6] that SDS and duponol, respectively, could lyse *Escherichia coli* cells.

Since DNA is thermally stable, I used a heating step to optimize cell lysis by detergents and to inactivate nucleases. Once the cells were disrupted, their DNA could then be isolated if nucleases were also inhibited by chelating agents, such as citrate or EDTA, that bind divalent ions.

Most Gram-positive bacteria can be converted to spheroplasts or protoplasts by exposure to lysozyme or other lytic reagents; these were tried and found effective. The same care that had been taken to isolate highly active transforming DNA was also followed in the isolation of DNA from various microorganisms. The isolation procedure was improved by an additional step using 1 M perchlorate to help dissociate the DNA from protein; and a step, consisting of adding 0.54 volumes of isopropanol, was introduced to differentially precipitate DNA from RNA. This step had been initially developed by Norman Simmons. By 1958-1959, I had developed a procedure for isolating DNA from bacteria for distribution in Doty's laboratory and circulated it among colleagues. A detailed procedure was submitted to the *Journal of Molecular Biology*.

The number of reprints distributed made it my overall best-seller; I must have sent out about 4,000 offsets. Why was the method popular? When I asked a colleague what he thought of the DNA isolation procedure he said, "I gave your paper to my technician who was successful in isolating DNA the first time!" The footnotes were helpful, detailing ways to overcome specific obstacles.

In addition to the studies on the relationship between the GC content of DNA and its CsCl buoyant density and thermal stability, bacterial DNA prepared in this way proved to be especially useful in the initial studies on strand separation and renaturation of duplex nucleic acids. Those investigations would have been much more difficult to perform with more complex mammalian DNAs.

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2. **Mirsky A E & Pollister A W.** Nucleoproteins of cell nuclei. *Proc. Nat. Acad. Sci. USA* 28:344-52, 1942. (Cited 225 times since 1945.)
3. **Gulland J M, Barker G R & Jordan D O.** The chemistry of the nucleic acids and nucleoproteins. *Annu. Rev. Biochem.* 14:175-206, 1945.
4. **Avery O T, MacLeod C M & McCarty M.** Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus Type III. *J. Exp. Med.* 79:137-59, 1944. (Cited 930 times since 1945.) [See also: **McCarty M.** Citation Classic. *Current Contents/Life Sciences* 28(50):26, 16 December 1985.]
5. **Rowen J S & Norman A.** The size of deoxyribonucleic acid molecules in *E. coli*. *Arch. Biochem. Biophys.* 51:524-8, 1954.
6. **Zamenhof S, Reiner B, DeGiovanni R & Rich K.** Introduction of unnatural pyrimidines into deoxyribonucleic acid of *Escherichia coli*. *J. Biol. Chem.* 219:165-73, 1956. (Cited 105 times.)

Received April 15, 1991

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