

September 29, 1956

Dr. Francis Crick
Department of Physics
Cavendish Laboratory
University of Cambridge
Cambridge, England

Dear Francis:

I had delayed answering your letter of July 20 with a view to waiting until I could arrive at a completely satisfactory and reproducible method of shocking E. coli protoplasts. Actually, I am not completely satisfied yet, but we have a procedure which, at least in our hands, gives us excellent results four out of five times. The procedure is briefly described as follows.

Cells are grown up overnight in the Difco Pen-assay medium. In the morning they are diluted by a factor of three with fresh medium containing sufficient sucrose and penicillin to yield a final concentration of 18% for the former and 1000 units/ml for the latter. The culture is then reincubated with shaking, and within a period of 2 1/2 hours, complete conversion to protoplasts is achieved. For synthetic activity we find that it is not advisable to extend this period much longer. Thus, serious losses in enzyme-forming ability occur with an additional 3/4 of an hour incubation.

The protoplasts obtained are spun down and resuspended in our shocking medium, which is as follows: 18% sucrose, 0.6% HDP (Mg salt), 1% AA, $10^{-3}M$ $MgCl_2$, $10^{-4}M$ $MnCl_2$, and 1000 units/ml penicillin. To this is added 10 volumes of distilled water, suddenly with shaking. Within 1/2 minute, sufficient sucrose is added (in the form of a solution) to give a final concentration of 10%. The resulting material is then spun and the pellets recovered and washed with 10% sucrose. It is important to avoid the presence of inorganic phosphate at any stage subsequent to the production of the shockate. One may use Tris at a low level (.01M) as a suitable buffering agent.

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I am sending under separate cover a copy of my Johns Hopkins paper which I finally had mimeographed. I would appreciate hearing from you your opinion, particularly with respect to the first portion of the manuscript in which I say openly some obvious things that perhaps need be explicitly stated. I recognize, however, that it is symptomatic of an affliction unique to pedagogues, characterized by the compulsion to raise the obvious to heights of originality.

Sincerely yours,

S. Spiegelman
Professor of Bacteriology

SS:jb

P.S. Am writing to Sydney in the next day or so.