

File :

Wesselton

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Dear Lu,

Please pardon this long delay in answering your letter. I've been traveling since before Christmas. Last week I visited Washington University and was quite impressed with the present performance of Kornberg's system. Howard Schactman has been there and has found that the synthesized DNA resembles the primer in regard to sedimentation constant and (more significantly) intrinsic viscosity. When sonicated primer is used, the product DNA seems to be shortened accordingly although this last result is preliminary according to Howard. The system makes 10x increase in DNA over primer, but if T.P. is used as primer the transforming activity goes neither up nor down. However, if any one of the nucleotide triphosphates is omitted, the transforming activity is nearly completely eliminated in the experimental time period. They blame the inability to make net increase of T.P. on nucleases known to be present and are accordingly setting about the preparation of really pure enzyme from several hundred pounds of Coli! The system puts in deoxy-UTP as well as thymine TP and deoxy-inosine TP for GTP in accord with WC pairing expectations. Ribonucleotides won't go in. The base composition of DNA made with Coli primer resembles Coli DNA while T2 primer makes T2-like product DNA. The system seems to be well enough established to justify using heavy nitrogen DNA for primer and looking for half-heavy molecules in CsCl gradient. I'll make heavy T4 DNA for them and perhaps centrifuge it too if they don't prefer to do it there.

We have not done any new centrifuging (not even to repeat the transfer experiment) for two reasons. First, we're all out of CsCl

HMC?

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and our old supplier has vanished. (He does not answer letters and his 'phone in Phila. has been disconnected.) We have contracted with a local firm to make it for us but they won't have any for perhaps another month. The second difficulty Frank and I have had is that we have almost no time on the centrifuge schedules. We had tied up a machine for almost a year and now Dintzis, Vinograd, and Sinsheimer quite understandably want to get on with their own work. Our long equilibrium runs require a machine of our own. Accordingly Max has very quickly arranged for us to buy one to be kept in the phage group. It will be here in about a month. When we're going again, we'd be glad and interested to run P1 and the antigenicity mutants too. I hope we could be ready within two months..we will be if there are no snags in setting up the machine.

To make runs yourself, choose a CsCl solution of density equal to that of the phage under the assumption that the density of the phage is the arithmetic average of the protein(1.30) and the DNA(1.70) densities weighted according to the per cent DNA in a phage. Buffer at pH 7 with 0.01 M phosphate. In the first runs put in enough phage so the OD at 260A of the starting solution is about 0.4. With this much phage you will see which way they move in case the band is off scale. You can tell how much to shift the density of the starting solution ~~if you~~ to put the band in the middle with the aid of the approximate relation density ~~gradient~~ $= 8 \times 10^{-10} w^2 r$ with w in radians per second and r , the distance in cm from the center of rotation. I suggest a speed of 30-40 thousand rpm for exploratory runs. The CsCl equilibrium takes about 7 hours and with molecules as big as P1 two or three hours more will be enough to get quite near equilibrium at the above speeds. At lower speeds, the CsCl equilibrium takes still 7 hours but the macromolecules band much more slowly. The rate of band formation goes as $\log w^2$ over w^4 . The ratio of separation between two bands to their half-width is speed independent so resolution is the same at any speed. Good luck, *Matt*