RIJKSUNIVERSITEIT GENT
INTER UTRUMQUE

LABORATORIUM
voor
MOLECULAIRE BIOLOGIE

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Dear Dan,

It was good to receive your letter last month with news of progress in the lab. Thanks for sending the preprint of the JMB article, which does look very nice.

Are the large-scale enzyme purifications faring well? Did you separate the two Hin enzymes by DEAE-cellulose chromatography alone? I have purified Hin 2 in an easy manner. The preparation of enzyme (blue tape; 5.5u/ml) that I used for partial digests was among the samples I brought here. After the trip the only activity remaining was that of Hin 2 (A, E, K plus 3 partials). Fortunately Ham's prep of 100u/ml suffered no apparent damage en route to Gent.

Has Ham found anything new about the methylases corresponding to Hin 1 and Hin 2? Hin 2 has the PuAC recognition sequence, does it not? Do you know whether Hin 1 makes a staggered or ~~a double~~ an even break?

The work with the defectives is exciting! Are the five fragments from P20 apparently equimolar? What were Bill's results on the kinetics of infection and on cloning attempts with P13? Have you heard from Winocour lately?

Enclosed is a release form for CSH. Would you please send a reprint with it for me if you haven't already sent one?

A new batch of fragments we recently prepared is providing considerable work for many here, since each transcription reaction yields on the order of 10^7 cpm of RNA. The T1 and pancreatic maps and partial digests for C → K look good. The maps for C and D are clearly different; so there's no trouble with contamination.

A ~~gd~~ trick to prevent leakage from the edges of slab gels is to be more generous with the vacuum grease. A syringe, sans needle, is handy for applying a "stream" of grease along each side of one plate, (over)

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about a cm from the edge. After the side spacers (~ 2 cm wide) are positioned, each receives a stream of grease down its center. Finally the second plate is laid on top and clamped into position. The bottom is sealed with Plasticine (no spacer). No leaks!

Recently I digested a sample of fd DNA RF I for Hans Schaller. Surprisingly the complete enzyme makes only one break per molecule; Hin 2 makes none. Another group, in Holland, found the same result for M13.

One other finding that might be of special interest is that MA 134 (A6MX, embryonic cell line from Microbiological Associates) yields virus stocks having titers of ~ 3.5×10^9 PFU/ml under the same conditions that BSC-1 stocks are 1.5×10^8 PFU/ml. The viral replication time (for 777) is considerably shorter in MA; e.g. the onset of DNA synthesis is around 12 hours in MA & around 19 in BSC-1 (Anderson & Gesteland, J. Vir. 9: 758–765, 1972). Soon I shall test labeled DNA prepared from the MA stock virus to be sure it's all SV40.

The life here is still quite pleasant. For the holidays last month I visited Madrid, Toledo, and Strasbourg (France). The Spanish architecture, landscape, and collections of art were lovely, and Madrid is the liveliest city I've yet to see. In February, I shall attend a Molecular Biology meeting and Tumor Immunology workshop in Cologne; since one major sequencing session is highlighted, I have been included in the list of invited speakers. If possible, would you send me some slides of the double digestion results: Hpa on Hin⁻ C, RI on Hin⁻ F and the total double digestion patterns of Hin + Hpa and Hin + RI. It would make my talk easier to present. Ghent is situated very nicely for short trips to spots of interest: Amsterdam is less than three hours by train and Paris, 2½. And the coast of Belgium will be particularly enjoyable when sailing season arrives in the spring.

Thank you for the copy of Strauss's letter about job openings for next year. Do you feel that it will be more difficult for me to find a job if I were to extend my stay here for 6–12 months? – You're right: it will probably be hard to leave here after one year, especially when the work is moving much faster. Right now, it's in the acceleration stage.

Beste wensen voor alles,

Kathy