Dear Josh,

This letter has been postponed more than is decent, particularly after your kind offer made in Stockholm! I want give you a list of reasons, but only apologize for the delay.

I am slowly getting used to the idea that I might settle in the US, but when one is in his forties, and with a family, things are no longer simple - with me at least. At least, I am interested enough to tell Harold, who wrote recently, that I wanted to hear of how the situation develops in Madison

The specimens you ask for are being sent to you today. Here is some information about them. Both come originally from Alexander's lab. RD is an influenza, Rd a parvovirus.

Growth media are the same for both, except for the special growth factors (DPN for both strains, Hemin for RD only). The stock-solutions of the factors are 250 μg/ml, added 1/100 (or a 10-30 times excess). Hemin is dissolved as follows:

25 μg into 12.6 ml of 1/2 Na₂HPO₄ - bring to a boil
+ 86 μl dist. water, + 1.6 ml of 1/2 K₂HPO₄. Autoclave.

DPN is dissolved in water, sterile filtered & kept cool.
The factor solutions are added to defibrinated media only. The solid medium is nutrient agar, supplemented with while heat with 1/50 vol of defibrinated rabbit blood - and needs no further addition.
The basal liquid medium for growth can be any kind of broth, pH 7.3-7.5. Periodically, growth may not start from a small inoculum, and I add therefore routinely 0.2% final of serum albumin (Bovine, fraction V from Armour).

The strains are kept lyophilized; growth from a plate of chocolate agar suspended in ca. 0.5 ml of horse serum → enough suspension for 3-5 lyophilized tubes. - kept thereafter at -10°C. - But when used routinely, the cultures are maintained on chocolate agar and stored at 37°C all the time, with one or two transfers a week.

Transformation experiments:

1) with Rs. The following medium gives reproducible frequencies (1/300 - 1/200) – For 1 liter, dist water:

<table>
<thead>
<tr>
<th>Defo Ractotryptone 20 gr</th>
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<td>Nodell 5 gr (to be traditional)</td>
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Bring pH to ca. 3.0 with conc HCl, Agitate with Norite (2 gr.)

Filter on paper, adjust pH to 7.5 & autoclave.
Completed before use with 1/20 vol of 4% albumin, pH 7.5 and factor.

Experiment.

Medium inoculated for 1 x 10^7 bact/ml from growing culture (convenient to have this latter inoculated the night before, in waterbath set to be turned on at 1 or 2 AM). Incubation unshaken. – You watch the optical density; when it is such that you have 3 x 10^8 bact/ml, competence is good (Doubling time ca. 30 min, but don't go by the time).

DNA added, contact 5-10 min.
Now, in order to have expression you can -
- either dilute ½ into fresh medium containing DNase (5-10%)
  incubate 90 min & plate on choc. agar + streptomycin (100 µg/ml)
  pass DNase to uninoculated culture,
- or take a sample into a tube with 3 ml of choc. agar
  pour onto choc. agar plate, incubate 2-3 hrs 37°C and
  then add 3 ml of melted nutrient agar + Sm 1000 µg/ml

If an accurate measure of frequency is required, a viable
  count is made after DNase was added.

My best frequency has been 1/30 - food gel may be as much as ½
  by aerating the culture, and then keeping it unshaken at 37°C
  for 90 min. Although I did get a rise in frequency deering culde
  ken incubation, I never got better than 1/200 at the end,
  as the initial frequency was so low.

8 with Fid

I used it as receptor only sporadically & found the optimal
  conditions for competence to be different at 6 months interval.
  I don't dare to recommend any particular medium or timing
  you must try for yourself. Trying means

a) try different complex media (+ all + fact) containing
  excess DNA (> 0.1 µg/ml), incubate at night & in the evening
  Culture given opportunity to express the next morning
  By either method described above, then challenged with Sm
  & with best medium, establish curve of competence against
time by submitting culture to successive exposures to DNA as
it grows — I never got better than $10^3$ with F12 — I have seen
confluence occur as late as 3 hrs after growth had stopped,
with this drug.

Hope I am not forgetting anything essential. Anyhow please
write if anything goes wrong.

Best regards,

Yours very sincerely

Pierre.