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Dr. S. E. Luria  
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University of Illinois  
Urbana, Illinois.

Dear Lu:

I have your letter mailed Sunday, and will answer it to the best of my ability (which is pretty small in this case, at least) immediately before the letter has a chance to get buried. Unfortunately you did not tell me very much about your experimental procedure, so I am guessing about it.

~~XXXXXXXXXX~~

We have not had any difficulty which seemed serious in connection with reduced burst size using T4rII's. I guess that your experiments are usually single infection experiments. Ours, of course are multiple infection. Also, we never do the experiment where no  $r^+$  is expected to appear in the cells. We usually expect recombinants in our rxr crosses (an exception noted in the data given below). To give you some idea about our experiments, I can list a few data:

<u>CROSS</u>	<u>Burst Size</u>	<u>Remarks</u>	<u><math>r^+</math> in yield</u>
$r_{145} \times r_{168}$	279	These are Benzer's rII's	0.001
$r_{205} r_{271} \times r_{320}$	249	" " " "	0.00027
$r_{67} \times r_{47}^{tu} 41$	340	These are T4D mutants	0.1
$r_{67} \times tu_{42b}$	404	" " " "	0.5
$r_{67} \times tu_{45}$	302	" " " "	0.5

If there is a significant difference between the first two and the last three experiments above, it could easily be that it is due to the differences between Benzer's T4 and ours. On the other hand, it doesn't seem enough to be alarming. ~~XXXXXXXXXX~~ Both of the Benzer rII crosses are with mutants exclusively in the A segment, so the parental particles should not have a complete functional unit as far as the r gene is concerned. This is the closest thing we have to your experiments, I suppose.

W.P. B?

If you are interested in the manner in which we do our experiments, the protocol follows: (everything is done at 30 C, except plates incubated at 37)  
Bacteria: We make 1:1000 dilution of overnight aerated B in broth (0.8% NB + 0.5% NaCl) and aerate at 30 C for 140 minutes. Concentrate 20x by cfn. Should be  $2 \times 10^8$ . Add CN to make 0.001M.  
Phage: Multiplicities are aimed at 6-7 of each parent. Loss of infect. ~~XXXXXXXXXX~~ bacteria is negligible under these conditions.

Adsorption permitted for 4 minutes. Then serum added to adsorption tube. The serum goes for 5 minutes, and then dilute to growth tubes. The rest is standard.

One thing that occurs to me which might be causing you some trouble is that you might be using rII phages which have been propagated on K-12s. In our experience, such phage stocks ~~\*\*\*\*\*~~ cause a great deal of loss of ~~\*\*\*\*\*~~ bacteria, so that assay of bacteria by colony count before infection is considerably lower than infected bacteria titer. This is true even at multiplicities as low as a total of 5 phage per cell, and possibly considerably lower. B-grown or S-grown stocks do not do this. ~~It~~ <sup>colicine</sup> seems possible that one might encounter lowered burst sizes from such a stock too.

If the difficulty is not due to one or the other of these differences, ~~it~~ I don't know what to suggest. If you continue to have trouble after checking these possibilities (broth composition, bacterial host culture, phage stocks) I would be glad to run a single infection experiment here to see whether we have your difficulties too, or whether we have just been luckier and can then investigate other differences.

Best regards to you and the others in Urbana. Also to Zella and Danny.

Sincerely,

  
A. H. Doermann