Fine structure of the rII region in phage T4

The overall purpose of Benzer’s work was to measure map distances between very closely spaced point mutations. To add strength to his conclusions he chose to examine point mutations within a single gene. The ultimate results of this work appear in Figure 7.21(c) in your textbook. The purpose of Figure 7.21 and parts (a) and (b) of Figure 7.20 is to provide the experimental background so that the results of 7.20(c) can be understood. This material can be difficult to follow and I have therefore prepared this brief summary to provide an alternative approach as a supplement that will hopefully be helpful in understanding.

There are two general kinds of experiments that Benzer performed: complementation and recombination. The complementation tests were very straightforward and demonstrated that the rII region contained two genes, termed rIIA and rIIB. (Benzer called them “cistrons”, but we can quite safely replace that term with “gene”). These experiments are summarized in Figure 7.17(c.1) and will not be discussed in detail here although you are responsible for that material.

Figure 7.20 (d) presents the basic steps of the recombination procedure Benzer used. The application of this recombination technique is presented in Figure 7.21 (b) which shows the sequence of crosses that were used by Benzer. That figure has three parts (three unlabeled “panels”) which are the three stages of his experiments, performed in the sequence shown. His objective was to divide the strains with the point mutations into smaller groups so that he could be maximally efficient in measuring the very small map distances.

Panel a) Mating of different strains with deletions (all possible pairwise matings of the 145 different deletion mutants that he found)
Panel b) Mating of each deletion mutant with each of the 1612 point mutants that he had in his phage stocks.
Panel c) Mating of strains with point mutations with each other (all possible pairwise matings of point mutants that had been shown to lie in same subsection in panel b).

I believe the most confusing part of the figure is panel a, with some difficulties in panel b as well. Therefore, I offer the following two diagrams as replacements for panel a. This is the format Benzer used.
The 8 lines represent different, overlapping deletions. The full width from the start of deletion 1 to the end of deletion 8 is the rIIA gene. Note that this overlapping pattern leaves some regions which are covered by only one deletion. These regions are highlighted as follows, with dashed vertical lines added to the previous figure:

Since I used a different format in class for showing deletions, I have redrawn the above two figures in that format:
Please examine these figures to be sure that you see that they are presenting exactly the same information as the first two.

How does this relate to figure 7.21 (b)? Each of the regions I labeled with letters corresponds to one of the regions labeled as “subsections” in your book. For example, imagine that my region A corresponds in principle to subsection C in panel b. In their example, the results of crossing all the deletion strains with all the point mutation strains revealed that 5 point mutations (numbers 201, 155, 271, 279, and 240) were within subsection C. Once Benzer knew that there were only 5 point mutations in this narrow region, then he only had to do recombination experiments for just these 5 (10 different possible pairwise matings for these 5) to get the map distances shown in the bottom panel.

The value of Benzer’s stepwise approach is that he conceptually divided the rIIA gene into progressively smaller regions and then did matings between the point mutants in the smallest region. The alternative approach would have been to do all possible pairwise matings of the 1612 point mutations without knowing what subsections they were in. This would have taken 1,298,466 separate matings. Therefore, he achieved a tremendous efficiency by proceeding in stages as he did.

The final conclusion is that these map distances were superimposed on a physical map of the DNA for the rII genes as shown in Figure 7.21(c).