

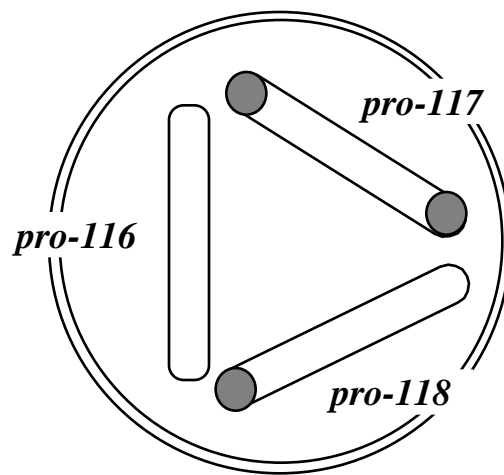
ONE-MIN WRITE 2/8/00

Several dozen new proline auxotrophs were isolated in *E. coli* and they all fell into three complementation groups.

- To determine the order of the biosynthetic steps affected in the *pro* mutants, a representative mutant from each complementation group was tested for crossfeeding. What medium would you use for this experiment?

ANSWER: Minimal medium without proline (or supplemented with a tiny concentration of proline). [If you used rich medium or added proline, all the mutants would grow so you would be unable to see if cross-feeding occurred.]

- The results obtained are shown below. Based upon these results, what is the order of the gene products in the proline biosynthetic pathway.



ANSWER: Note that a mutant defective later in the pathway will crossfeed mutants that affect earlier steps in the pathway. Thus the order is: 117 - 118 - 116.