July 23, 1946.

Dear Francis:

The culture labelled '679-680 double reversion' which you sent me some days ago has been scrutinized with the following results:

After preliminary tests indicated that I should expect to find what I did, I scraped most of the inoculum from the agar slant you sent, and inoculated complete medium with it, incubated with shaking and plated ca. 10^9 cells into minimal, leucine, and threonine agar. There were no prototrophs. Nor did any colonies appear on the threonine agar. In leucine, about 10^3 colonies appeared. The culture is thus predominantly the original double mutant with a few (10^{-6} of the population) threonine-reverted members. I cannot account for the original appearance of the colony. It is possible that it represented a contaminant which was heterotrophic, and supported by the other cells syntrophically, and has since died out, or that selection plays an important role in the mixed populations containing mutant and reverted cells. It will be important to compare ratios before and after growth of prepared mixtures of mutants and revertants.

Checking up on the triples that I sent you earlier (Y9 and Y10) the following comments are apropos: Y9, which is classified as Threonine-leucine-<u>methionine</u> does respond slightly to methionine, but much more markedly to some unidentified yeast extract component, which we hope to track down. Y10 - threonine-leucine-thiamin is what it purports to be except that there is a delayed growth in the absence of threonine, probably and adaptation, which I shall look into. I have gotten a double mutant in B/r (Arginine-methionine) by the detection method, which is working very beautifully in

that strain.

No new biochemical recombination types yet, mostly because I've been waiting to use twontriples together, and Y9 + Y 24 (BØC) did not work. However, from B-Ø-C-P+T+ and B+Ø+C+P-T-R (resistant to T-1) have gotten B-R and P- sens. which are recombination types, and support fusion vs. transformation.

Am working also to improve the 'mating' medium, and have found a hundred fold increase in proportion of prototrophs using a beef extract broth, or a supplement of acid hydrolysed E. coli . Other variations (pH, sugar, salt, temperature, shaking) had little or no effect. This looks like a good lead.

It's raining here now so I would have no immediate envy of your position but I hope you're having an invigorating vacation, so that there will be as productive a year next year as I think the last one was. If possible I will try to get up there towards the end of August.

Best regards to Betty and L.S.

Sincerely,

Josh.