PMB220
Bacterial genetics questions

1. You have mutagenized E. coli *metB1* (requires methionine, 43 min in *E. coli* map), *serA5* (requires serine) (63 min) and *hisA1* (requires histidine) (73 min) with Tn5 (encodes kanamycin resistance) and have isolated a mutant that is hypersensitive to oxidative stress. You wish to map the transposon. You cross the mutant strain with an Hfr strain (donor) that is streptomycin resistant and that transfers counterclockwise from 0 min and has a *strA7* mutation (at 90 min) (streptomycin resistant). After incubation, you plate the cells on minimal plates plus serine, histidine, streptomycin and kanamycin and pick 100 transconjugants.
    a. What does the number after each of the loci mean? (*hisA1, serA5).*

    b. Why did you plate the cells on this medium to select for transconjugants?

    c. After purifying 100 his+ transconjugants, you test them for other markers. 85 are Ser+ and 33 are his+ and 80 are kanamycin resistant. Where is the transposon inserted?

2. You wish to determine if the order of three markers is *metB1-argH5 rif8* or *argH5 metB1 rif-8* so you do a three factor cross. The donor for the transduction has the *rif-8* mutation (resistant to rifampycin) and the recipient has the *metB1* (requires methionine) and *argH5* (requires arginine) mutations. You plate on minimal plates plus methionine, purify 100 of the Arg+ transductants and test for the other markers. You find that 17 are Arg+ Met+ Rif-resistant, 20 are Arg+ Met+ Rif-sensitive, 60 are Arg+ Met- Rif-sensitive and 3 are Arg+ Met- Rif-resistant.
    a. Why was it necessary to plate the transductants on medium lacking arginine? Could you have used a different media, and if so, what?

    b. What is the order of the three markers deduced from the three factor cross data?

3. Are nonsense suppressors dominant or recessive? Why?

4. You have isolated a constitutive mutant in *Salmonella* that constitutively expresses genes in the tryptophan operon and inappropriately makes enzymes for tryptophan biosynthesis, even when tryptophan is included in the medium. When you introduce the wild-type *trpR* repressor gene on a plasmid into the mutant, expression of the tryptophan operon is still constitutive. Describe how you would determine if the mutant has a *cis*-acting operator mutation in the promoter of the tryptophan operon (the operator site is bound by the TrpR repressor) or if it has a dominant mutation ($TrpR^d$) in the *trpR* repressor gene (describe method other than mapping the mutations).