Answer Key Problem Set 5

1. a) Genetic properties of gln2- and gln 3-:

Both are **uninducible**, as they give decreased glutamine synthetase (GS) activity.

Both are **recessive**, as mating them with wildtype produces normal GS activity.

Both are **trans-acting**, as when either mutation is crossed to gln1-, complementation takes place; if either gln2- or gln3- were a cis-acting element regulator of gln1, we would not see complementation, as the two mutations would be in the same gene. (i.e. the promoter is considered to be part of the gene it acts on.) Since we see complementation in both cases, we know that both mutations must be trans-acting.

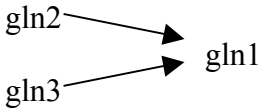
Therefore both gln2- and gln3- are mutations in genes for **positive regulators**.

b) Linear models:

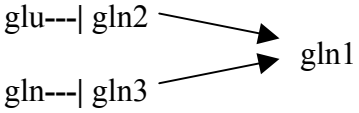
gln2→gln3→gln1

gln3→gln2→gln1

Parallel model:



c) The **parallel** model from part b) best fits the experimental results, as we are told that the addition of glutamate (glu) and glutamine (gln) independently regulate the GS activity (i.e. the results of the addition of each is different from the addition of both).



d)We would expect the gln2-gln3- double mutant to be **uninducible**.

e) The cis-acting elements in the promoter are:

- **Deletion1** (-300 to –250): **GLN2 function** (activity pattern looks like gln2- when deleted), **UAS** (Upstream Activation Sequence -involved in activation)
- **Deletions 3, 4** (-200 to –100) **GLN3 function** (activity pattern looks like gln3- when deleted), **UAS** (Upstream Activation Sequence -involved in activation)
- **Deletion 6** (-50 to 0) TATA sequence

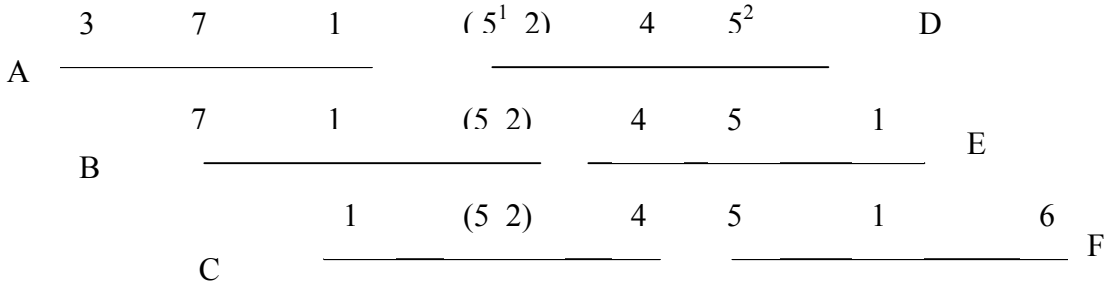
f)We would expect to see no **change in beta-galactosidase expression in Deletion 1** in a gln2 mutant, as, in e), we determined that the region deleted in 1 is necessary for GLN2 function. (i.e. deleting both the cis-acting element and gln2will look the same as deleting either one.)

We would expect **0 units of betagalactose from Deletion 4** (or low baseline level), as deletion 4 is necessary for GLN3 function, and we know that when both the gln2 and gln3 pathways for activating GLN1 are mutated neither pathway can activate GLN1.

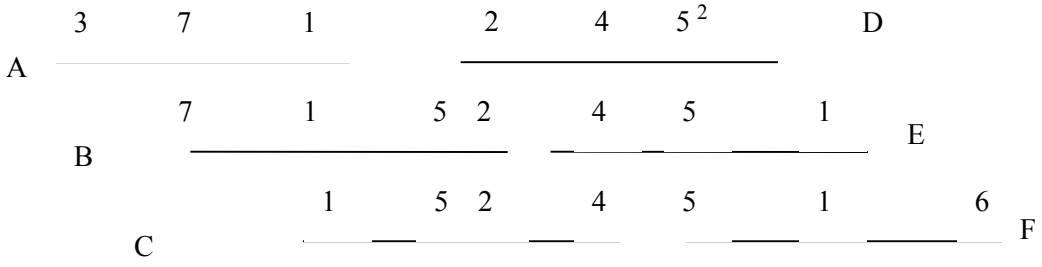
2.

a) We can use the information from the order of the BACs to figure out where each STS is. For instance, BAC A is positive for STS 3, which is not present in any other BAC; therefore it must be in the region of A that does not overlap with B or C. There are three ambiguities in the data, resulting in three possible maps. Most of the ambiguity lies in whether BAC D contains both STS 51 and STS 52, as PCR only provides the qualitative answer that STS 5 is present; we don't get quantitative data as to how many versions of STS5 are present on the BAC. Case1 displays the possibility that BAC D contains both STS5s. Given this case, we cannot determine the order of STS51 and STS 2 (thus the parenthesis). Case 2 displays the possibility that STS51 is not on BAC D. If this assumption is true, then we can determine the order of STS51 and STS2. Case 3 displays the possibility that BAC D contains only STS51. If this is the case, we cannot determine the relative order of STS52 and the second STS1.

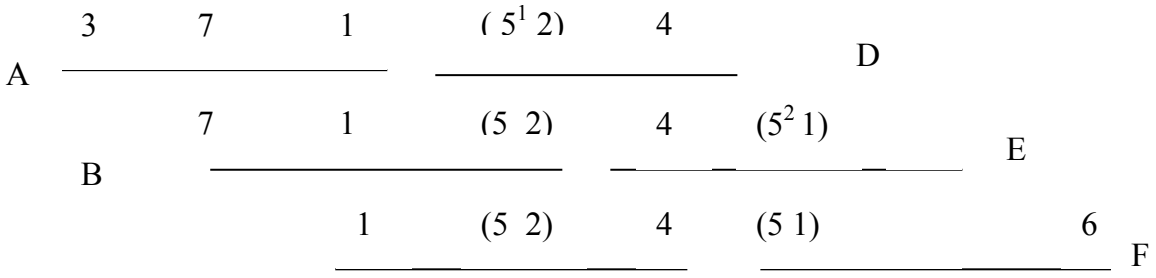
CASE1



CASE 2

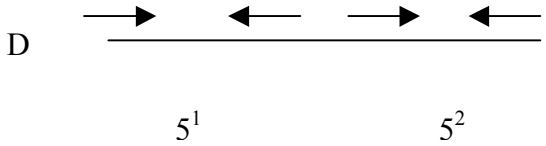


Case Three:



Therefore there are quite a few ambiguities in our map. The first is that we do not know the order of STS 51 relative to STS2. It is possible that STS 2 comes before STS 51 or STS 51 comes before STS 2. Also, we do not know if D is carrying STS 51 and 52 or if it is carrying just 52. Consider the possibility that D contains only STS 52; we would have to assume that STS 2 came after STS51, and the STS 5 we are detecting cames from STS 52. In addition, there is a third possibility, which is that the STS 5 contained on BACD is only STS51. If that is the case, then we don't know the order of STS5 with respect to the later STS1. We need an assay to distinguish between the possibilities.

b) . PCR just amplifies DNA to which primers bind. One could imagine that the PCR primers could anneal to both sites and amplify both sites. This would result in a heterogeneous mixture of STS51 and STS52.



However, PCR alone does not help us distinguish between the three possibilities. This sequencing assay is our assay to distinguish between the three cases. Sequencing can tell us whether BACD contains both STS 51 and STS52, just STS51 or just STS52. Given these data, it looks as if BAC D is carrying both STS5s, which allows us to throw out cases two and three. There is a remaining ambiguity—we still do not know the order of STS51 relative to STS2.
c) depending on your answer it could confirm/refute. Most answers should be refined, as we can now distinguish between the three cases. There are some remaining ambiguities, but the map is significantly more refined after the sequencing assay of STS5.

d) You would expect both sequences to be present in the genome. BACs are derived from the mouse genome and are therefore a reflection of what is present in the genome. If we find to STS 5 in twice our BACS, there are probably at least two STS 5 on the mouse chromosome. Only BAC D contains both STS5s.
e) STS1 is present more than once; BACs A, B, C would have the same sequence E, F would have the same sequence. It is possible that the STS1 from BACs A, B,C have a different

sequence than those from STS1 from BACs E, F. Primers that distinguish between these two STS1 would be convenient as we would not have to be concerned about an ambiguity in our data in the future like there was with STS5 and BAC D.

f) Design primers that include the non-homologous sequence in STS51 and STS52. These primers should specifically amplify either STS51 or STS52.

g) Yes STS51 and STS52 should both be present in BAC D and the mouse genome{refer to (d)}

3. a. The integration of Pamylase-LacZ into the amylase gene is unlikely to occur when the construct is microinjected into the male pronucleus of a fertilized egg.

Integration of the construct into the endogenous amylase gene locus could occur via homologous recombination or by chance non-homologous insertion into the endogenous amylase gene. Homologous recombination requires homologous sequences to the targeted locus on both sides of the LacZ gene. The microinjected construct has homology on only one side of the LacZ transgene, thus, making homologous recombination an unlikely event in this case.

Although random insertion of a construct occurs with greater frequency than homologous recombination, the chance of a transgene inserting into a particular locus is exceedingly rare. The amylase gene is on the order of a couple of kilobases (kb), while the mouse genome roughly 3000 megabases (Mb). This makes the likelihood of randomly inserting into the amylase locus approximately 1 in 10^6.

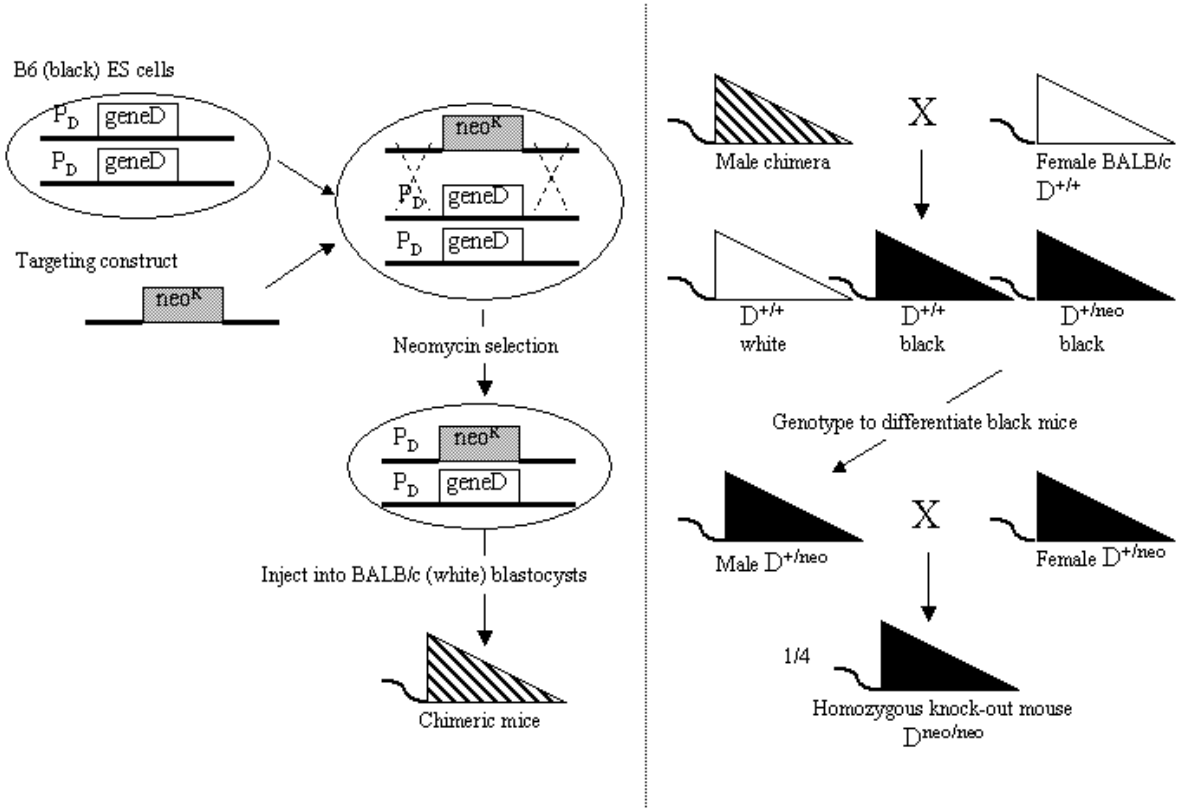
b. We would expect mice homozygous for the transgene to display LacZ expression in the pancreas as the heterozygous mouse does, assuming that the homozygote is a result of a cross between two heterozygous mice of the same transgenic line. In the homozygote the regulation of both copies of the transgene would most likely be equivalent to the regulation of the transgene in the heterozygote.

Expression of the LacZ transgene in the homozygote may also be observed in other organs. This can occur if expression from one copy of the transgene (as in the heterozygote) is just below the level of detection. Two transgenes in a single cell (as in the homozygote) could produce just enough LacZ to be detectable.

c. A possible explanation for the heart defect in mice homozygous for the transgene insertion is that the insertion disrupted a gene (let's call it gene D) that is haplosufficient. A single undisrupted copy of gene D could be sufficient to allow development of a normal heart, but when both copies are disrupted the mouse develops the heart defect.

Another possibility is the LacZ overexpression (with two copies of the transgene) causes the defect. When only a single LacZ transgene is present there is not enough expression to disrupt the normal development or the functioning of the heart.

d. We can test the possibility that disruption of both copies of a gene D causes the heart defect. First, we would want to clone the locus (gene D) into which the transgene integrated. This can be done by anchored PCR and sequencing using the sequence of the integrated transgene to design primers. Next, we can construct a targeting construct to knock-out gene D, in which we basically swap the coding region of gene D for a drug resistance gene (e.g. neomycin). This targeted deletion of gene D can selected for by the presence of the drug resistance gene after ES cells in culture are transfected with the targeting construct. The ES cells containing the correctly targeted locus can then be injected into blastocysts to generate chimeric mice. The chimeras can than be mated to wild-type mice to produce progeny that are heterozygous for the targeted deletion of gene D. (We can use a combination of coat color determination and PCR analysis to figure out the genotype of the mice.) We can generate mice homozygous for the deletion of gene D by breeding heterozygous mice. If our hypothesis is correct, then we would expect to see a heart defect in the homozygous knock-out mice and normal hearts in the heterozygous mice.



e. To use LacZ as a reporter for the expression of the endogenous amylase gene, we would want to put LacZ under the control of the same regulatory elements. Because it is difficult to determine the boundaries of promoter regions, we would want to insert LacZ in place of the amylase gene in the endogenous locus. To accomplish this, we would need a targeting construct that contains the LacZ gene flanked by sequences homologous

to amylase upstream and downstream sequences. The resulting heterozygous knock-out (knock-in) mice (after ES cells targeting, blastocyst injection to generate chimera, and mating of chimeras to wild-type mice) can be assayed to determine if LacZ is expressed in the pancreas. The expression of LacZ is likely to reflect amylase expression, but will not certainly do so.