Previous quiz total = 100 points. By removing question 2B this quiz total = 120 points. The overall module grade % coming from quiz points will therefore be less than 25%.

1. (40 points) You have a haploid strain with a loss-of-function sad1-1 allele. You transform this strain with a genomic library in a high copy number vector to isolate suppressors of the loss of function phenotype. You also independently transform the strain with a genomic library in a ARS+CEN low copy number vector. The genomic DNA fragments that rescue your loss of function phenotype from the two different screens overlap in identity but some genomic DNA fragments were recovered in one screen and not the other.

**THIS QUESTION RELATES DIRECTLY TO WHY THERE ARE MULTIPLE TYPES OF ASSAYS FOR SUPPRESSION** – some genes will suppress only at low copy, others will suppress only at higher dosage

(A) **Why might a plasmid have been recovered by rescue at low copy but not high copy?**
- low copy library had the insert, but it was missing from the high copy library
- low expression level rescued phenotype but high expression level did not because the increased dose of the suppressor perturbed the balance of loss-of-function and suppressor function
- low expression level rescued phenotype but high expression level did not because higher expression level was generally toxic to viability (aberrant binding partners, aberrant localization, gain of function phenotype, etc.)
- etc.

(B) **Why might a plasmid have been recovered by rescue at high copy but not low copy?**
- high copy library had the insert, but it was missing from the low copy library
- high expression level rescued phenotype but low expression level did not because an atypically high level of protein was required for suppression (for example, to compensate for reduced interaction affinity with a mutant binding partner, or to protect a less well folded interaction partner against degradation)
- high expression level rescued phenotype but low expression level did not because high ratio of wild-type to mutant protein recovered function but equal ratio of wild-type and mutant did not due to co-dominance
- etc.

2. (20 points) Wild-type yeast grow at 30°C and also at 23°C. You isolate mutant strains with a cold-sensitive phenotype: normal growth at 30°C but no growth at 23°C. You use chemical mutagenesis to suppress the cold-sensitivity of growth. Assume a single additional mutation in each of your several isolated suppressor strains.

You mate one of the strains with a suppressor mutation to the initial cold-sensitive strain. Diploids are not cold-sensitive. How could you clone the mutant gene that provided suppression?

**YOU HAVE TO IDENTIFY THE MUTANT GENE.** The intended answer to this question rests on the given observation that diploids are not cold-sensitive, which means that the suppressor mutation is dominant. Thus, you can make a genomic library from the suppressor strain, transform the initial haploid cold-sensitive strain, and select for colonies that grow at 23°C.

[Additionally, although you did not need to state this for full credit, you could check whether the sequence of the gene on the plasmid is different than wild-type and maybe substitute the mutant gene for wild-type using an integration vector. Some of you suggested sequencing the entire genome, which isn’t really feasible but was given credit if you confirmed that the single mutant gene isolated had suppressor function.]
3. (60 points) You propose to test whether a protein encoded by the virus (Virus Protein 1, VP1) interacts with the RNA polymerase II C-terminal domain using a 2-hybrid approach.

(A) Describe the bait plasmid (indicate both parts of the expressed fusion protein and which gene you will use as the selectable marker).
Describe the prey plasmid (indicate both parts of the expressed fusion protein and which gene you will use as the selectable marker).

VP1 fused to the GAL4 DNA binding domain as the expressed fusion protein
URA3 as selectable marker (or other marker, as long as the reporter strain lacks the marker and you
aren’t using the same marker as a reporter below).

The RNA pol II CTD fused to a transcriptional activation domain as the expressed fusion protein.
TRP1 as selectable marker (or other marker, as long as the reporter strain lacks the marker and you
aren’t using the same marker as a reporter below; this also needs to be DIFFERENT than the
selectable marker on the other plasmid!).

Your fusion choices may have been flipped, with the same end result.
You could introduce both plasmids into a haploid cell, or you could mate haploid strains carrying
different plasmids, either works and wasn’t important here.

Describe the reporter strain (all essential features).

Has to be loss-of-function for both plasmid selectable markers, so that the presence of both plasmids
can be selected (ura3, trp1 in this example).
AND
Strain has to have A SELECTABLE REPORTER GENE ACTIVATED BY BINDING OF THE
GAL4 DNA BINDING DOMAIN. An example that could have been copied straight from your lab
manual and lab report is the Gal4 DNA binding site as a promoter upstream of lacZ and/or HIS3 (with
no HIS3 elsewhere in the genome, although I didn’t require this in the grading).

(B) How would you detect the positive result that the two proteins do interact? Use a FEW
SENTENCES only, please.

In my example above, the correct answer would state “plate on X-gal to measure activation of lacZ
expression: positive interaction indicated by blue color” AND/OR “plate on SC-His to determine
expression of HIS3: positive interaction indicated by robust growth.” NOTE THAT lacZ IS NOT A
SELECTABLE MARKER -- YOU CAN ONLY SCORE FOR IT.

(C) Describe TWO critical negative controls to make sure that your positive result is
meaningful. Use a FEW SENTENCES only, please.

Bait plasmid only (should see NO activation of lacZ expression, no growth on SC-His.)
Prey plasmid only (should see NO activation of lacZ expression, no growth on SC-His.)
Additional answers were fine as long as they were WELL STATED NEGATIVE CONTROLS.