**7.003 Spring 2022**
**Day 15 In-Lab Questions**

1) On Day 14 for the RNA isolation protocol, the WT and Mut yeast overnight cultures were diluted in YPD media and allowed to grow for several hours before treating them with $\alpha$-factor. Why did we dilute the cultures and not treat the overnight cultures directly with $\alpha$-factor?

2) What are the expected size(s) of any bands you predict you might see in your RNA sample lanes on your gel today? If there are any differences in the observed band sizes, what might be some potential explanations for that size difference?

3) Why is it important to use the same amount (ng) of RNA in each of your cDNA preparations today?

4) What is the purpose of each of the three different temperature incubations ($70^{o}$C, $42^{o}$C, $80^{o}$C) during the cDNA preparation procedure?

5) To synthesize cDNA, a primer is first annealed to an RNA sample, and then the primer is extended by a reverse transciptase (RNA-directed DNA polymerase).

A) Your labmate is trying to decide between using an Oligo-d$(T)_{23}$ primer or an Oligo-d$(T)_{23}$VN primer to make their cDNA. Which would you suggest and why?

B) Another labmate is trying to decide between using an Oligo-d(T)23VN primer or a random hexamer primer mix (a mixture of primers consisting of every possible combination/sequence of six nucleotides). In what different scenarios might someone want to use one primer type over the other for making cDNA?

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