**7.003 Spring 2022**
**Day 9 In-Lab Questions**

1) Explain the purpose of each ingredient below in your yeast genomic DNA isolation procedure.

100% Isopropanol:

RNase A:

70% Ethanol:

2) Four labmates have isolated genomic DNA from yeast cells. They analyze their DNA samples using the spectrophotometer, and their results are shown below. Whose genomic DNA prep would be best to use for future experiments? Comment on each of their results and explain your answer.

| Labmate | DNA concentration | $A_{260}/A_{280}$ ratio |
|---|---|---|
| Bruce Wayne | 1400 μg/mL | 2.05 |
| Selina Kyle | 1000 μg/mL | 1.83 |
| Alfred Pennyworth | 16 μg/mL | 1.78 |
| James Gordon | 2000 μg/mL | 1.57 |

Bruce Wayne:

Selina Kyle:

Alfred Pennyworth:

James Gordon:

3) You are isolating genomic DNA from your three $\alpha$-factor resistant mutants and then you are setting up an EcoRI digest of your isolated genomic DNA today. Briefly explain why.

4) Open the mTn3 + pRSQ2 DNA construct file you previously created in SnapGene. The default Map and Sequence views currently only show "unique" restriction enzyme sites (i.e. restriction enzymes that only cut once in the entire construct). Go to the "Enzymes" menu at the top of the screen and click on "Choose enzymes…" Add EcoRI to the "Chosen Enzymes" selection. You can now use the Map or Enzymes tabs at the bottom of the screen to easily view where any EcoRI sites are located in your construct.

A) How many EcoRI sites are there in total in the entire mTn3 + pRSQ2 construct?

B) What would be the size of each DNA fragment if the mTn3 + pRSQ2 linear construct was digested with EcoRI?

C) Your instructor is concerned that the presence of multiple EcoRI sites in the mTn3 + pRSQ2 construct will be a problem for the remainder of your plasmid recovery procedure in 7.003. Do you agree? Explain why or why not.

# 7.003 Applied Molecular Biology Lab

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