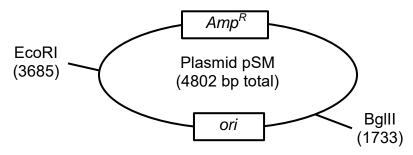
7.003 Spring 2022 Day 2 In-Lab Questions

1) Three labmates have each performed a "double digest" reaction to cut plasmid pSM with the restriction enzymes EcoRI and BgIII. (See plasmid diagram below – relevant restriction sites (with relative locations in basepairs) are indicated in the diagram.)



A) What extra considerations might you have to take into account when setting up a double-digest reaction (as opposed to just a single digest reaction)?

Your three labmates each run their digest sample on an agarose gel and see the following results below.

1-kb DNA Ladder	Peter Parker	Michelle Jones	Ned Leeds

B) What is the most likely explanation for each of your three labmate's digest reaction result? Explain what each band in each digest sample most likely represents.

2) Today, you are using the lithium acetate method to transform yeast. Comment on each of the key ingredients of your transformation mixture and their purpose:

Lithium acetate:

Polyethylene glycol (PEG):

Salmon sperm DNA:

3) You wish to use the 7.003 mTn3 transposon plasmid library to perform a mutagenesis screen in yeast to isolate mutants that are defective for synthesis of the amino acid tryptophan. You have the following six strains available below to use for your library transformation. Which strain is the best one to use? Explain why each of the other strains is not ideal.

Strain #1: MATa leu2 lys2 trp1 tyr1 ura3

Strain #2: MATa his3 his4 lys7 ura3

Strain #3: MATa ade1 his4 leu1 tyr2 ura4

Strain #4: *MAT*α *leu*2

Strain #5: MATa/MATα his3/his3 leu2/leu2 lys2/lys2 tyr1/tyr1

Strain #6: MATa ade1 arg4 his3 leu2 ura3

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