1) (5 points) To look for mutants with altered levels of sectoring, you have transformed an ADE3-carrying minichromosome into the strain below, then mutagenized the transformed cell with the chemical EMS. EMS causes point mutations (single nucleotide substitutions) at random positions in DNA.

Screening through the mutagenized colonies, you have isolated two mutants: sec1 has increased sectoring compared to wild-type, and sec2 does not sector at all and has all red colonies. Recall from quiz section that the wild-type strain and minichromosome show a very low but detectable level of sectoring.

Which of following are possible molecular identities for sec1 and sec2? Place a yes or no in the columns to the right of the statements. Note that the statement may be correct for one of the mutants, both mutants, or neither mutant.

<table>
<thead>
<tr>
<th></th>
<th>sec1</th>
<th>sec2</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) A mutation in the CEN region of the minichromosome.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>b) A mutation in a gene encoding a kinetochore protein resulting in decrease (but not elimination) of binding of the protein to the centromere.</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>c) A mutation in a cell-cycle control gene that results in overreplication of the minichromosome.</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

d) Suggest one experiment to prove that the mutations you have isolated are on one of the regular yeast chromosomes or on the minichromosome.

Cure the cell of the minichromosome and retransform with a preparation of the starting minichromosome that has not been exposed to the mutagen.

Purify the minichromosome from the sec1 or sec2 mutant and transform into the starting yeast cell that has not been exposed to the mutagen.
2. I described in class how the Ty retrotransposon in yeast can be used as a mutagen. Shown below are the two components of the system: (1) The reverse transcriptase under the control of the galactose promoter (Pgal) (2) The mobile target of the reverse transcriptase containing two LTRs, a bacterial origin of replication (ori), the Amp\(^r\) gene, and a URA3 gene that has an intron inserted within its open reading frame in reverse orientation. Transcription of the target is also controlled by the galactose promoter (Pgal). Recall that the URA3 gene has its own promoter.

![Diagram of the retrotransposon system](image)

a) (5 points) In the absence of galactose is the cell URA\(^+\) or URA\(^-\)? WHY? Assume the normal URA3 gene on the chromosome has been deleted.

[URA\(^-\)] In the absence of galactose there is no transposition. Under those conditions the URA3 transcript made from its own promoter will have an intron in reverse orientation that cannot be spliced from the mRNA.

Starting with an \(\alpha/\alpha\) diploid, you add galactose and induce transcription of each component of the retrotransposon system. Drawn below is the structure of the LTR-containing target after it has transposed to a new locus.

![Structure of the LTR-containing target after transposition](image)

b) (5 points) How would you determine if the transposon has hopped into a gene involved in adenine biosynthesis? Assume that the mutation caused by the transposon is recessive. Recall that you are working in a diploid. Be specific with your answer.

Sporulate the diploids. Plate the spores on complete plates (everything should grow). Replica plate onto minus Ura plates (only the cells with the hopped transposon will grow). Replica plate onto minus adenine plates (if the transposon has hopped into a gene required for adenine biosynthesis the cells should not grow).

c) (5 points) Assume the transposon has hopped into a gene involved in adenine biosynthesis. Describe two different ways that you could determine if it is a new gene, or if it is one of the known ADE genes (e.g. ADE 1, ADE2, ADE3, ADE4 etc.).

1) Complementation test with each of the existing ade mutants (e.g ade1, ade2, ade3, ade4, etc.)

2) Clone the chromosomal DNA flanking the transposon (e.g. cut the genomic DNA with Hind III, ligate, and transform E. coli selecting for Amp\(^r\)), sequence the flanking DNA using a primer in the LTR, and BLAST the sequence against the yeast genome to determine if it matches any of the known ADE genes.