1. (10 points) In class we discussed the functional cloning of the yeast LEU2, TRP1, HIS3 and URA3 genes. While cloning these genes Clark and Carbon fortuitously cloned autonomous replicating sequence 1 (ARS1), a yeast origin of DNA replication. Recall that ARS1 is located very near to the yeast TRP1 gene.

(A) What unique phenotype did the plasmid with TRP1 have that the other plasmids did not?

High efficiency transformation of the yeast trp⁻ mutant.

(B) What did Clark and Carbon discover when they did a Southern blot of total DNA isolated from the trp⁻ yeast transformed with the TRP1-containing plasmid? (Probe = plasmid DNA)

They found low molecular weight bands hybridizing to the probe, which represented supercoiled, relaxed circle and linear molecules.

(C) What did they find when they did a Southern blot of total DNA isolated from ura⁻ yeast transformed with the URA3-containing plasmid? (Probe = plasmid DNA)

They found a high molecular weight band hybridizing to the probe, which suggested that the plasmid had integrated by recombination into the chromosome.

(D) You decide you want to clone additional yeast ARS elements. You start by doing a partial EcoR1 digest of total yeast genomic DNA. You then ligate the fragments into the URA3-containing plasmid and transform E. coli selecting for ampicillin resistance. How would you determine what plasmids in the library have acquired an ARS?

Screen the library by colony hybridization using ARS1 as a probe. The probe will hybridize to ARS1 and to other ARS elements with similar DNA sequence. Screen the new ARS-containing plasmids for high high efficiency transformation of a yeast ura⁻ mutant.

(E) What control would you use for the experiment in part (D)?

Control is the parent URA3-containing plasmid that does not have an ARS.
2. (5 points) Shown below is the genetic pathway for adenine biosynthesis that we discussed in class.

\[ \text{ADE4} \rightarrow \text{ADE3} \rightarrow \text{ADE2} \rightarrow \text{ADE1} \rightarrow \text{adenine} \]

Gene R \[ \rightarrow \text{RED PIGMENT} \]

* Indicates a nonreversible step because it uses up 1 ATP

(A) Fill in this chart.

<table>
<thead>
<tr>
<th></th>
<th>Red or white color?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth without adenine?</td>
<td>N  W</td>
</tr>
<tr>
<td>ade3</td>
<td>N  W</td>
</tr>
<tr>
<td>ade1</td>
<td>N  R</td>
</tr>
<tr>
<td>r</td>
<td>Y  W</td>
</tr>
<tr>
<td>ade2</td>
<td>N  W</td>
</tr>
</tbody>
</table>

(B) ade4 and ade9 mutant are white. Why?

ade4 mutants are white because they do not make w, the substrate for ADE3. ade9 mutants are white because the conversion of y to z is nonreversible.

3. (5 points) Shown below are yeast cells patched onto plates. The plate on the left lacks adenine. The plate on the right contains adenine. Recall from lecture that ade2 is the mutant form of the normal gene, sup3 = wild type and SUP3 = mutant suppressor. (A) Shade the region on the plate on the left where you would expect to see growth. (B) Indicate on the plate on the right the regions where you expect to see white growth and red growth. (C) Indicate the region on the plates containing diploid cells.

\[ \text{Diploids} \]

( minus adenine)

(complete)

(D) Is the SUP3 dominant or recessive? Explain your answer.

SUP3 is dominant. The mutant tRNA will suppress both mutant ade2 alleles.