Two questions from Lecture 11 assigned as homework:

Consider the temperature-sensitive yeast strain that has a mutated *cdc7* allele. How could you identify a plasmid with the **wild type** *CDC7* gene? Give a complete flowchart—which strain you'd use to make the plasmid library, what you would transform, etc.

Make a plasmid library starting with:

- extract DNA from CDC+ yeast
- cut it partially with a restriction enzyme
- choose a vector able to allow growth in E. coli and yeast, cut it with the same enzyme
- mix, ligate, transform E. coli
- pool transformants → library

Extract plasmid DNA from the library; transform $cdc7^{ts}$ yeast, plate on complete plates and look for growth at 37°C.

Those cells should have a plasmid with the wild type CDC7 gene.

Toxoplasma, an opportunistic pathogen often found infecting immunocompromised individuals, can be treated with the drug pyrimethamine. A pyrimethamine-resistant strain of Toxoplasma has been found; resistance behaves as a dominant trait.

How could you clone the DNA sequence associated with resistance by "functional complementation"? Give a general outline, specifying the source of the DNA library and how you would isolate the clone(s) of interest. Assume: you have a suitable vector for Toxoplasma transformation.

Step 1: do partial digest of DNA from Toxoplasma dominant strain with a restriction enzyme; cut vector completely with the same enzyme.

Step 2: ligate Toxoplasma DNA into the vector.

Step 3: transfrom pyrimethamine-sensitive Toxoplamsa with the library, selecting for resistance to pyrimethamine