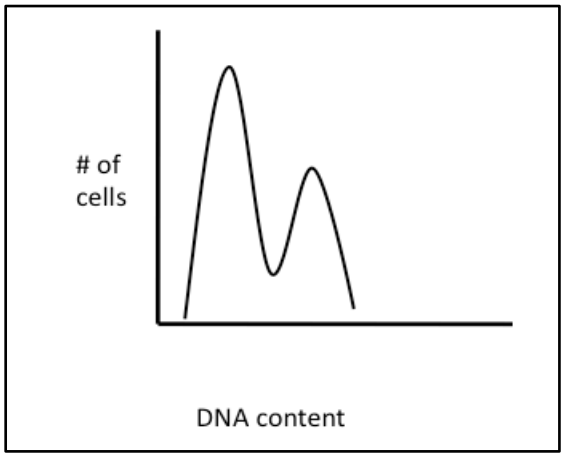
Problem Set 5

7.06 Spring 2015
Due April 3, 2015

1. You are interested in studying the cell cycle in budding yeast, *S. cerevisiae*. This organism is haploid.
A growing culture yields the following FACS plot:



A) **What would the two values be on the X axis for the DNA content?**

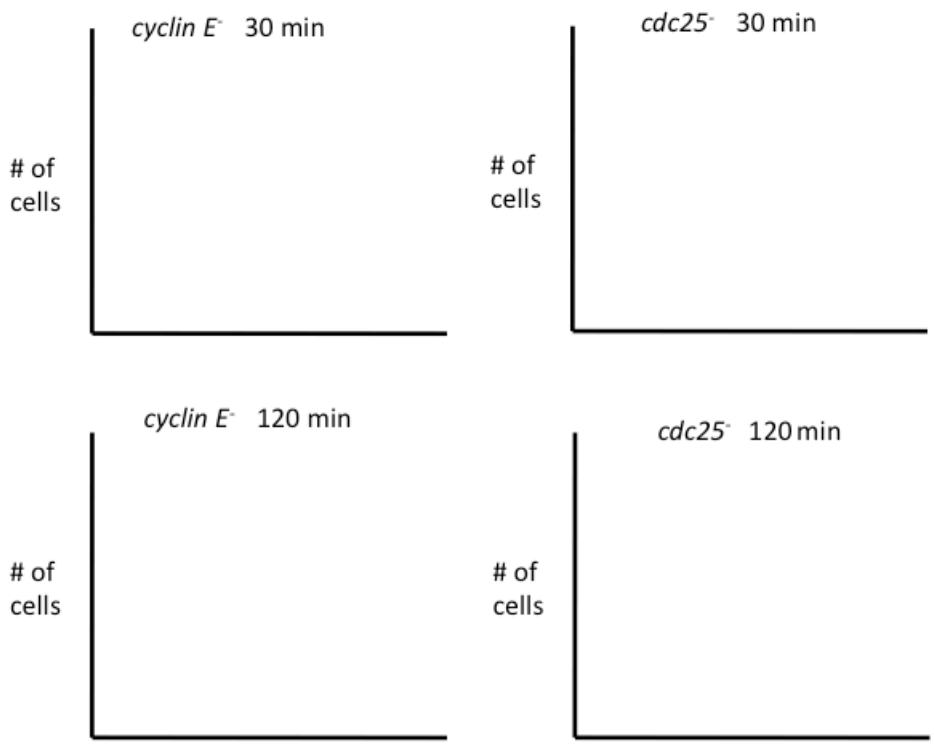
Write these on the axis in the figure.

B) You decide to isolate loss-of-function cell cycle mutants in budding yeast. **Why will these alleles need to be conditional?**

C) You isolate a conditional allele of:

1. The budding yeast equivalent of Cyclin E
2. The budding yeast equivalent of Cdc25

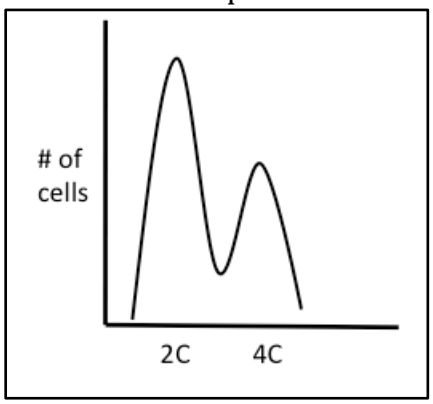
The cell cycle in budding yeast takes 90 minutes. **For each mutant draw what the FACS profile will look like for a culture after 30 minutes at the nonpermissive temperature and what it will look like 120 minutes after the shift to the nonpermissive temperature. Be sure to label the X axis.**



D) **Draw what the cells would look like for each of the two mutants after 120 minutes at the nonpermissive temperature.**

2. You decide to study the effects of drugs on the cell cycle in mammalian cell culture. Hydroxyurea (HU) inhibits ribonucleotide reductase, leading to a reduction in levels of dNTPs. Colchicine disrupts the microtubule cytoskeleton that is necessary for chromosome segregation in mitosis. The cell cycle takes about 24 hours in these cells. 10 hours of this is taken up by S phase and 1 hour by mitosis.

This is the FACS profile for untreated cells.

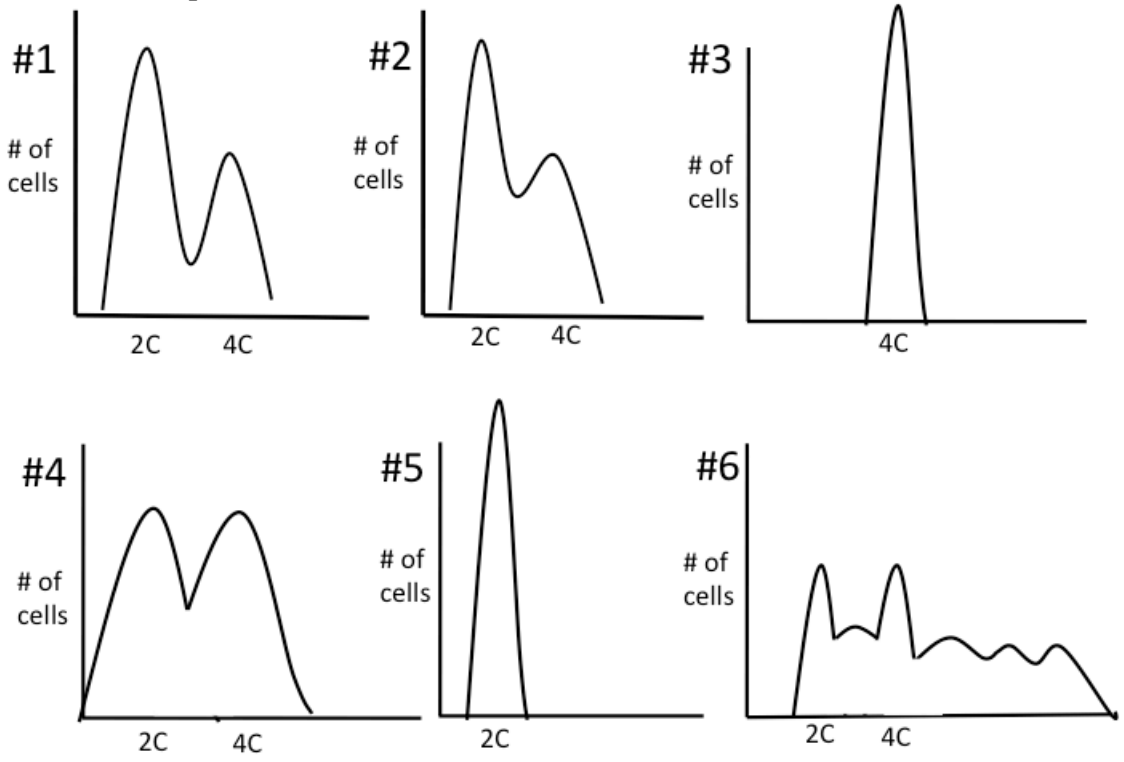


A) **Which of the FACS profiles below (#1-6) you would observe after treating the culture with HU for five hours?**

B) **Which would you observe after treating the cells with colchicine for five hours?**

C) **Which would you observe after treating the cells with colchicine for 30 hours?**

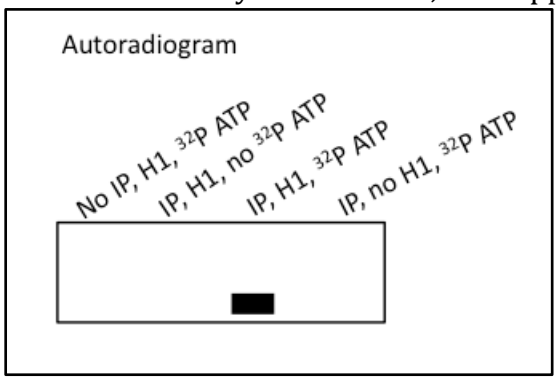
Possible FACS profiles



3. You want to assay the activity of CyclinB/Cdk1 during the cell cycle in mammalian cell culture.

A) **Will you need to synchronize your cell culture? Why or why not?**

B) All Cyclin/Cdk complexes readily phosphorylate histone H1, making this a good substrate to assay Cdk activity. These assays are done by immunoprecipitating (IP) the kinase using a bead coupled antibody. ATP with a radioactive gamma ^{32}P phosphate group, Mg^{+2} and histone H1 are added to the immunoprecipitate and incubated. The sample is boiled, run on an SDS protein gel, and visualized by autoradiography to detect phosphorylation of histone H1.
This is the result you would see, with appropriate controls.



If you want to look at Cyclin B/Cdk1 activity, does it matter whether you immunoprecipitate the Cyclin B or the Cdk1 subunit? Why or why not?

4. You examine Cyclin B/Cdk1 activity in the fission yeast cell cycle mutants below, comparing it to wild-type controls. **For each indicate whether the mutant:wild-type activity ratio would be equal to 1, less than 1, or greater than 1. Briefly explain why.**

A) wee1-

B) cdc25-

C) CAK-

D) wee1-, cdc25-

E) Cdk1 with threonine 161 changed to alanine

F) CAK-, Cdk1T161D (threonine 161 changed to aspartic acid)