Name:_____
Student #: _____

BCH4125 FINAL Exam 2013

Professor: Dr. Kristin Baetz

Total Marks: 50

Guidelines:

You must answer all questions directly on the exam paper in the space provided

You may not consult your class notes, textbooks or other materials

Calculators/cell phones etc. are not permitted.

You can write this exam in either English or French – BUT ONLY ONE LANGUAGE PLEASE!!

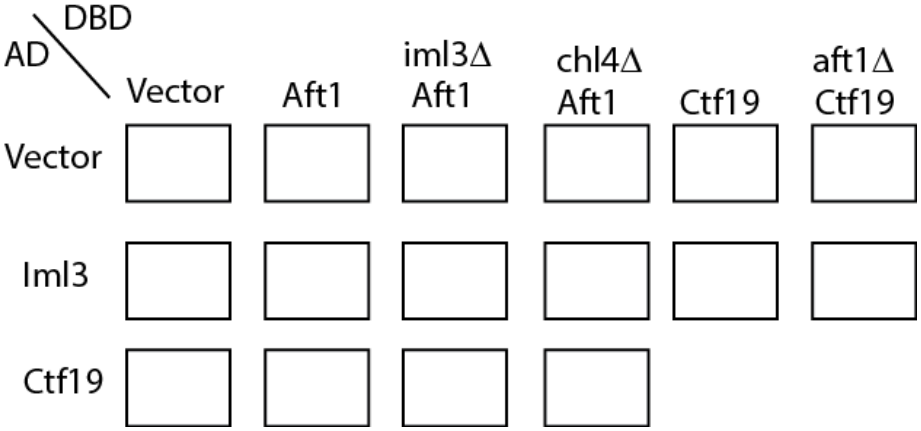
PLEASE WRITE YOUR NAME AND STUDENT # ON EVERYPAGE

Name:_____

Student #: _____

Question 1. Explain how a chromosome transmission fidelity (CTF) assay works. Hint – use schematics and explain your figure clearly (**4 marks**).

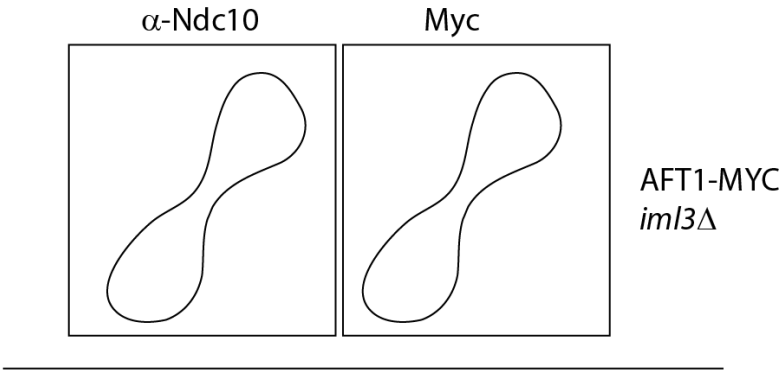
Question 2. As a secondary method to reconfirm our data in **Figures 3** and **Figure 4**, we performed a yeast two hybrid experiment. In the schematic below indicate where there should be growth (or interaction) by shading in the boxes. DBD = DNA biding Domain; AD = Activation Domain. It is important to note that DBD-Aft1 fusion does NOT activate transcription on its own. Where indicated above the columns, some of the two hybrid interactions were conducted in a deletion mutant background. (**6 marks**)

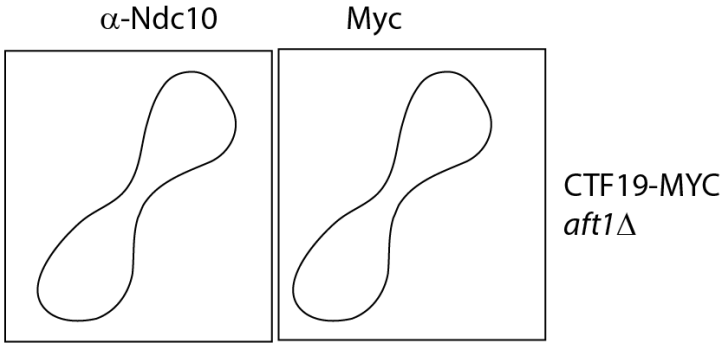


Name:_____

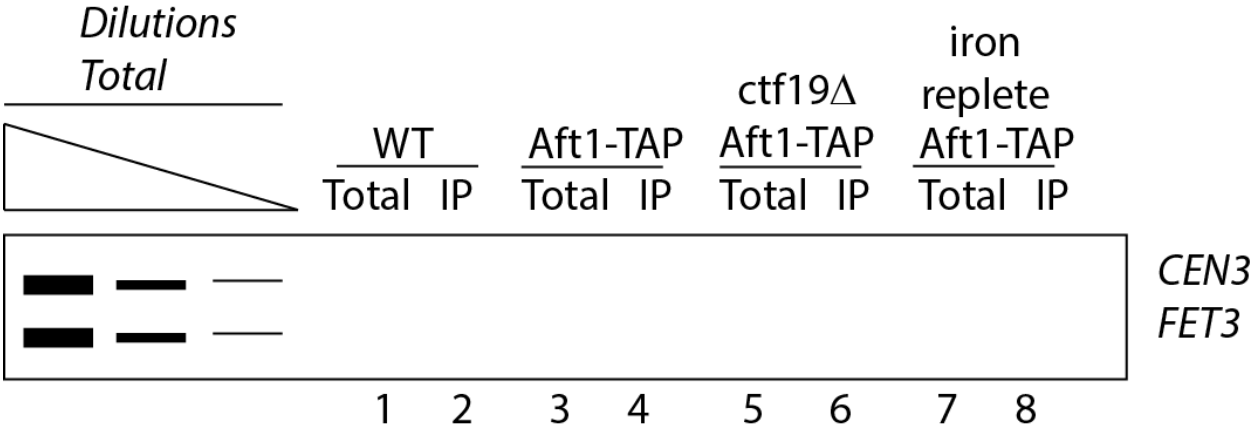
Student #: _____

Question 3: We performed additional chromosome spread experiments on the two strains listed to the right. Anti-bodies used in the spread were α-Ndc10 and α-Myc. In the DNA bilobes drawn below draw the anticipated results. In addition, for each strain explain your reasoning and do not forget to refer to the specific **Figures** and lanes to support your answer. (**4 marks**)





Question 4: Reviewers asked us to perform a chromatin Immunoprecipitation (ChIP) using Aft1-TAP in various backgrounds and conditions to determine if it localizes to *CEN3* or the promoter of *FET3.* Using information provided in the text and Figures, draw in the expected results in lanes 1-8 the ChIP blot drawn below. (**6 marks**)



Name:_____
Student #: _____

Question 5. While both *iml3Δ* and *smc3-42* (temperature sensitive point mutant) cells are viable at 25C, the *iml3Δsmc3-42* double mutant cells are synthetic lethal (inviable). For the following double mutants predict if think they will be viable or synthetic lethal and explain your prediction in 1 or two sentences MAXIMUM. (**4 points**).

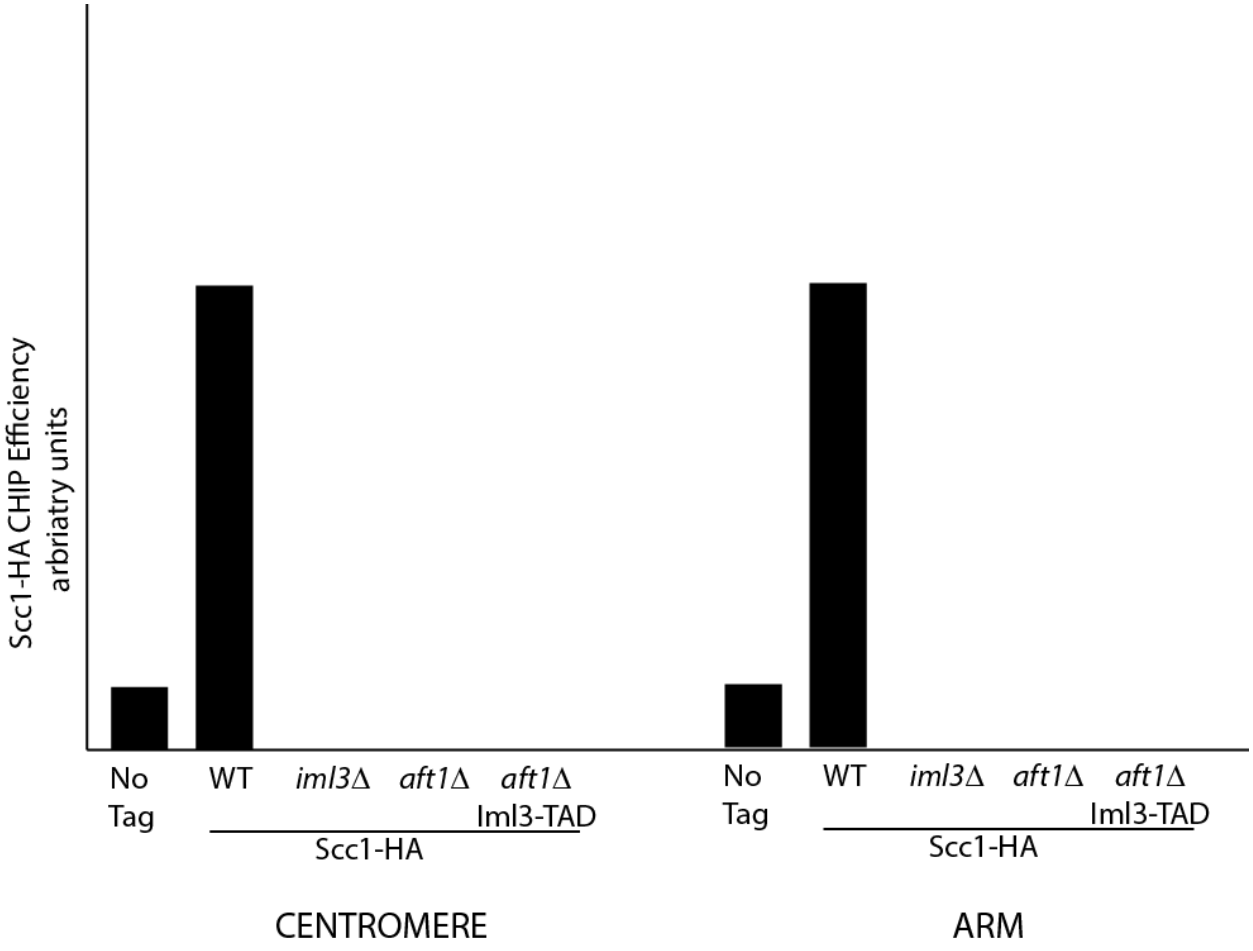
aft1Δchl4Δ

aft1Δsmc3-42

Name:_____

Student #: _____

Question 7. We next decided to analysis Scc1-HA association in cells arrested in metaphase of mitosis in the absence of microtubules. Wild-type (no tag), SCC1-HA, *iml3Δ* SCC1-HA, *aft1Δ* SCC1-HA, and *aft1Δ* IML3-TAD SCC1-HA were arrested in metaphase in the presence of microtubule-depolymerizing drugs benomyl. IML3-TAD is a fusion of Iml3 with the TAD (Transactivation domain) of Aft1. qPCR analysis of Scc1-HA levels were performed at two different regions of chromosome 3, the CENTROMERE and at a region along the ARM. Using the information provided in the text and figures, predict the results of this experiment. Fill in the predicted results in the graph below. (**6 marks**)



Name:_____
Student #: _____

Question 8. Which KDAC is responsible for the deacetylation of Swi4? Explain your answer in 1 or 2 sentences and remember to specifically identify the panel(s)/lane(s) that justify your answer. (**4 marks**)

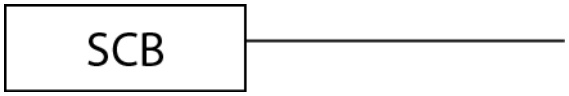
Question 9. The researchers did not indicate in their figure legend how they synchronized their cells for the experiment in **Figure 6D.** Name two possible methods for how they could have synchronized their cells for this experiment (**2 marks)**

Question 10. How does acetylation of Swi4 impact the transcription of *CLN2*? Remember to highlight the correct figure panel(s) in your answer (**4 marks**).

Name:_____
Student #: _____

Question 11. ChIP studies determined that the KDAC responsible for the deacetylation of Swi4 localizes to SCB-promoters. Further it was determined that the KDAC's localization to SCB-promoters is dependent on Whi5 and that Whi5 and the KDAC physically interact. Using this information, along with the information provided in the text and **Figure 6**, propose a model for the **Acetylation-Dependent Regulation of Swi4 and Transcriptional Induction of *CLN2* during G1 Phase of the Cell Cycle**. On the *SCB* promoters below draw what happens in Early G1 versus LATE G1/S and clearly indicate how the text and experiments (**Figure 6**) support your model. (**10 marks**)

EARLY G1



LATE G1/S (high Cln3-CDK1 activity)