GENOME 371; Autumn 03 Name:_____
Berg/Brewer
Practice Exam IV; 10 December 2003 TAs:_____

PRACTICE EXAM 4 GENOME 371 Autumn 2003

These questions were part of the third and fourth exams from Autumn 2002. Some of the questions are a bit redundant with each other, while others do not represent the material covered in class as well as one might desire, as this year we have discussed some different topics compared to previous years. The final exam will use the same format as our midterm exams this year.

Take the practice exam in a quiet place and only when you are sure you will have time to complete the exam uninterrupted. Time yourself. This exam should require about 75 minutes to complete. STUDY FIRST!

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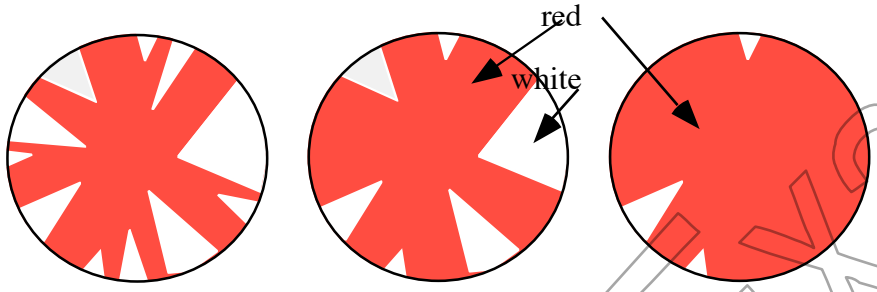
1. (14 pts) The URA3 transposon mutagenesis performed for quiz section resulted in a few mutants that showed variation in the pattern of white sectors in colonies with the normal red color. Choose either mutant #14A or mutant #3C and answer the following questions:



URA3 Transposon Mutant #14A

Wild type cells (ura3, leu2, ade2, ade3) Wild type plasmid (LEU2, ADE3)

URA3 Transposon Mutant #3C

a. Which mutant are you describing? _____

b. What is your hypothesis to explain the change in sectoring frequency in your mutant. As part of your hypothesis you need to include . . .

whether the transposon hopped onto the plasmid or onto a yeast chromosome,

what gene or element the transposon hopped into, and

whether the transposon is interfering with or improving the performance of that gene or element.

c. How would you test experimentally if the transposon hopped onto a chromosome or the plasmid?

2. (12 pts) In drought-stricken areas, crop plants cannot grow because of a buildup of salts in the soil. To determine what genes are needed to tolerate elevated salt in the environment, you decide to study salt tolerance in yeast.

A. You have identified a gene that you think is important for salt tolerance in yeast. You put a wild-type copy of this gene on two plasmids: one is a TRP1-ARS plasmid that lacks a centromere and the other is a TRP1-ARS plasmid that also contains a centromere. You transform trp1 yeast with the two plasmids to create two strains. Both strains grow in the absence of tryptophan on 0.08 M NaCl, but only cells with the cen-less plasmid grow on 0.4 M NaCl. What conclusions does this experiment suggest?

B. How would you determine if the same gene is present in corn or rice? (Both of these genomes are being sequenced.)

C. How would you use the knowledge obtained from yeast to begin thinking about increasing salt tolerance in corn or rice?

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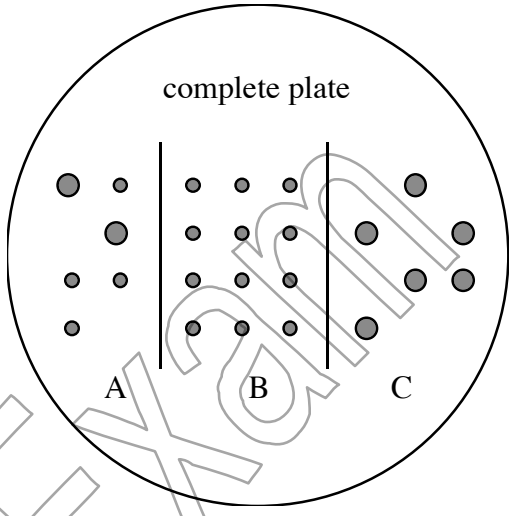
Question 3A, B and C below appeared on the practice exam for midterm two. You will need to know the information from this question to answer question 3D and question 4. The correct responses are shown in bold red below.



3) (14 pts) The yeast genome contains 8 different loci that encode the tyrosine-tRNAs that recognize UAC codons in mRNA. These 8 wild type genes are called sup2, 3, 4, 5, 6, 7, 8 and 11. A mutation in any of these loci that changes the anticodon loop so that it now recognizes a UAG codon is called a "suppressor". SUP3 is one such dominant mutation; SUP11 is another. Haploid cells with either of these mutations are sick and make small colonies on complete plates.

The sup3 gene and the sup11 gene are not linked.

A. When a strain that contains a SUP3 mutation is crossed to a strain that contains a SUP11 mutation, the diploid survives, but it is also sick. The tetrads produced by sporulating the diploid are of three basic types illustrated on the right.

What type of tetrads are class A? **tetratype (1:1:1:1, one of each, see below)**

What type of tetrads are class B? **parental type (2 SUP3; sup11 and 2 sup3; SUP11)**

What type of tetrads are class C? **non-parental type (2 SUP3; SUP11 [dead]; 2 sup3; sup11 [large]**

B. What are the genotypes of the four spores that make up the class A tetrad?

large colony: **sup3; sup11**

small colonies: **SUP3; sup11 and 2 sup3; SUP11**

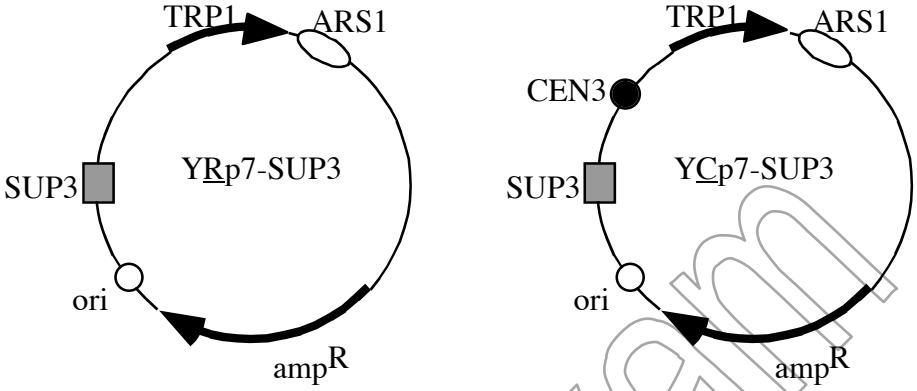
dead colony: **SUP3; SUP11**

C. What is your hypothesis for why some of the spores in type A and C tetrads are dead? Explain.

Two copies of a suppressing tRNA kills the cells. An increase in the ratio of suppressing tRNAs to normal tRNAs causes too many ORFs to have their proteins extended past the stop codon. The extra material at the carboxyl ends of these proteins interferes with the function of these proteins. Essential cellular processes are interrupted and the cells die.

D. What is the term that describes this kind of genetic interaction?

4) (14 pts) The SUP3 mutant allele was cloned into plasmids that can replicate in both E. coli and in yeast. One plasmid is called YRp7-SUP3. If the plasmid also contains CEN3 it is called YCp7-SUP3. See the maps at right.



a. Cells with a trp1 mutation and wild type for all of the tyrosine tRNA genes are separately transformed with each of the plasmids above. Transformants are selected on plates lacking tryptophan.

Results:

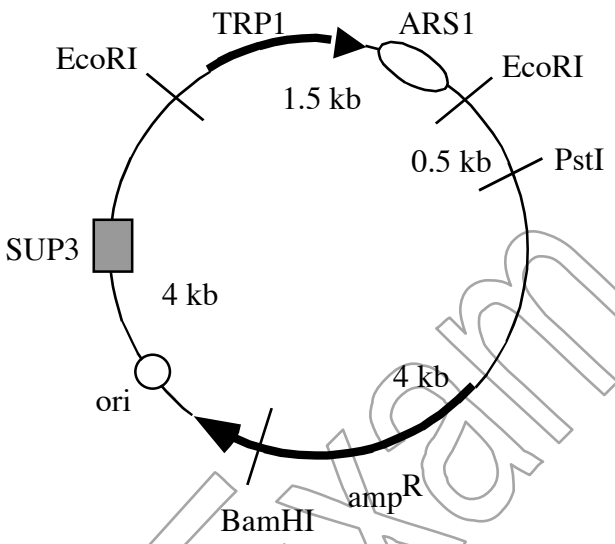
Lots of transformants are recovered that contain YCp7-SUP3 as a plasmid (not integrated).

No transformants that contain YRp7-SUP3 as a plasmid are recovered.

Based on the observations in Question 3 and your understanding of the differences between these two plasmids, explain the different transformation results obtained with these two plasmids. Why were there lots of transformants with YCp-SUP and none with YRp7-SUP3?

b. Using the information in Question 3 and Question 4a, how could the plasmid on the left (YRp7-SUP3) be used to clone new centromeres by SELECTION (instead of a SCREEN)? Just explain the **logic** here; the details of the cloning are in the next question.

5) (12 pts) Phil, a graduate student in John Carbon's lab, decided to clone the rest of the yeast centromeres (John cloned CEN3 by chromosome walking). Phil started with YRp7-SUP3 (right) as his vector and made a library of the yeast genome in E. coli. What did he do at each of the steps below?



a. Cut genomic DNA with _____.

b. Cut the plasmid DNA with _____.

c. Combined the two DNA samples and treated with _____ to repair the phosphodiester backbone.

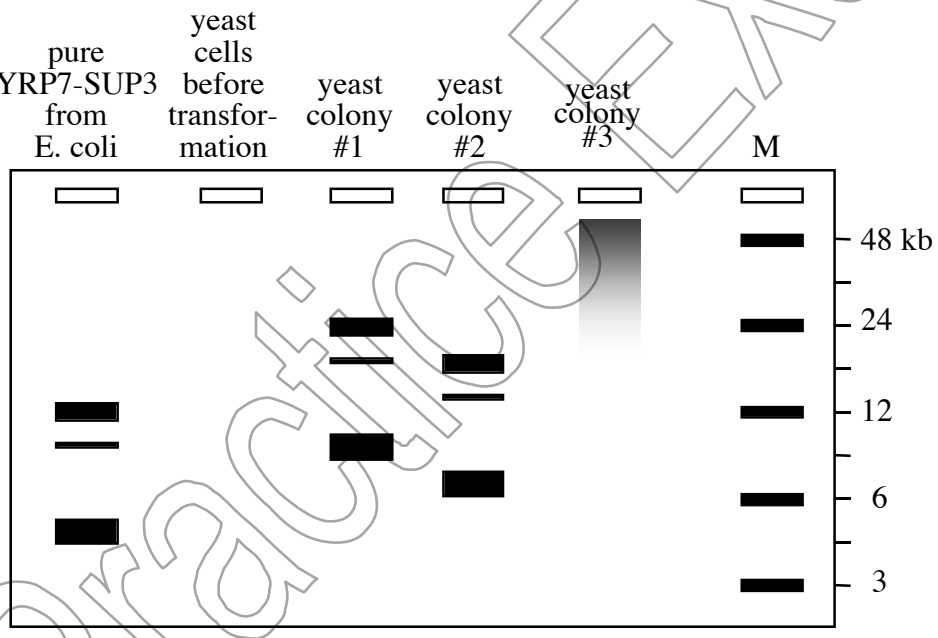
d. Transformed wild type E. coli and selected for colonies on _____ plates.

e. Isolated all of the plasmids from the library (all 50,000 of them as one pool), transformed the plasmids into a yeast strain with the genotype _____, and selected for yeast colonies on _____ plates.

6) (12 pts) Phil got 17 colonies. To begin characterizing them he isolated DNA from each yeast colony, ran the DNA on a gel without treating it with restriction enzymes, and probed the Southern blot with the "ampR-ori" part of the plasmid.

a. What **two** questions was Phil asking? (Why did he do this particular experiment?)

b. Below are the results of the Southern blot from three of his colonies and two controls.



What did the results of the Southern blot tell Phil about colonies 1, 2, and 3? Be specific.

7. (22 points) When DNA is injected directly into a fertilized mouse embryo, the DNA can insert at random into any of the chromosomes. Subsequent matings produce animals homozygous for the transgene. Sometimes an interesting phenotype is generated by the insertion event. In one case, after injection of a plasmid carrying the *E. coli lacZ* gene driven by a mouse promoter, investigators identified a recessive mutation that causes limb defects and kidney malfunction.

A. The mutant phenotype could be due to insertion of the transgene in a particular region of the genome or a chance mutation that arose in the mouse. How would you distinguish these two possibilities?

B. The mutation in this example was in fact caused by insertion of the transgene. How could you use this transgene insertion as a tag for cloning the mutated gene?

C. The mutation was mapped to chromosome 2 of mice in a region where a mutation called *limb deformity* (*ld*) had previously been identified. Mice carrying this *ld* mutation are available from a major research laboratory. How could you determine if the *ld* mutation is in the same gene as the transgenic insertion mutation?

D. Analysis of transcripts from the *ld* gene showed that different mRNAs (formed by alternative splicing) are found in embryonic limbs, adult kidneys and several other tissues. Is this finding consistent with the observed mutant phenotype?