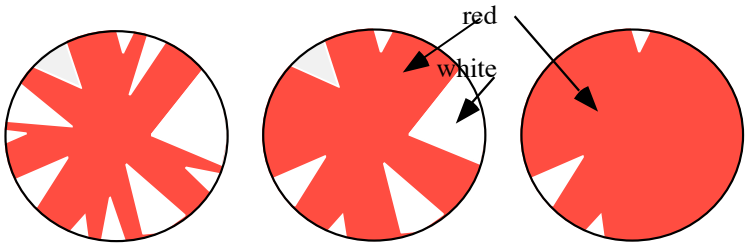
GENOME 371; Autumn 03 Name: KEY
Berg/Brewer
Practice Exam IV; 10 December 2003

PRACTICE EXAM 4 KEY GENOME 371 Autumn 2003

1.

1A. ANSWER if you chose #14A:

1B. HYPOTHESIS: The transposon hopped into the plasmid near ARS1, interfering with its efficient use as a replication origin. If the inefficient origin results in a failure to replicate the plasmid before mitosis, the one copy of the plasmid will go to one pole and the other pole will get none – hence, a white sector will result.



URA3 Transposon Mutant #14A | Wild type cells (ura3, leu2, ade2, ade3) Wild type plasmid (LEU2, ADE3) | URA3 Transposon Mutant #3C

1C. TEST: Pick white cells from a colony of mutant 14A. Streak them onto –uracil plates. If they grow, then my hypothesis is wrong (the URA3-bearing transposon hopped into the chromosome and is present even when the plasmid is not). If the cells do not grow, then I'm right; the URA3-transposon was on the plasmid and when the plasmid gets lost, so does URA3.

1A. ANSWER if you chose #3C:
1B. HYPOTHESIS: The transposon hopped into a chromosome near the promoter for an origin recognition protein. The mutation causes an increase in the amount of protein so that the chance that the plasmid fails to replicate is lower than wild-type cells.
1C. TEST: Pick the white cells from a colony of mutant #3C. Streak them onto –uracil plates. If they grow then my hypothesis is correct (they have lost the plasmid but not URA3). If they fail to grow then my hypothesis is wrong (URA3 was lost when the plasmid was lost).

2A. The CEN-less plasmid can become high copy in some cells, allowing vast overproduction of the salt tolerance protein. Cells with the CEN plasmid have only a single extra copy and don't make enough tolerance protein to overcome the high salt.

2B. To determine if the same gene is present in rice or corn, I would perform one of two experiments. I would use the yeast protein in a BLASTP search of the rice and corn genomes. Alternatively, I would use the yeast sequence as a probe on a Southern blot with corn and rice DNA (a "zoo" blot).

2C. If corn and rice had the salt tolerance gene, I would clone it out and use it to create a construct that somehow overproduces the salt tolerance protein. If corn and rice did not have the gene, I would put the yeast gene into these species and see if it conferred resistance to high salt.

3D. synthetic lethality

4A. Yrp7-SUP3 has no centromere and once it gets into a trp1 cell it will fail to segregate properly. The copy number of the plasmid will increase. The more copies of the plasmid per cell, the more copies of suppressing tRNA the cells will have. Two copies of SUP3 per cell is lethal (see previous question). Ycp7-SUP3 has a centromere so it segregates properly. This plasmid will maintain one copy of SUP3 per cell and these cells will live (but make small colonies).

4B. Use Yrp7-SUP3 as a cloning vector to isolate bits of the yeast genome. Any plasmid that gets a centromere will produce a small colony. All of the other bits of DNA cloned into this plasmid will not allow correct segregation of the plasmid and the cells will die on plates lacking tryptophan due to excessive SUP3 tRNAs.

5A. Do a partial digest with PstI.

5B. Cut the plasmid with PstI.

5C. ligase

5D. ampicillin

5E. ...yeast strain with genotype **trp1 sup3 (and wt for all other tRNA genes),** and selected for yeast coplonies on **– tryptophan** plates.

6A. What two questions was Phil asking?

1) Are the colonies due to reversion of the trp1 gene back to TRP1, or, do they have a recombinant DNA molecule?

2) Do the colonies contain a free plasmid, or, did the plasmid integrate into a chromosome?

6B. Colonies #1 and #2 have a free plasmid. Both plasmids are larger than the starting plasmid so they must contain additional DNA (a centromere). The insert in colony #1's plasmid is larger than the insert in colony #2's plasmid. The plasmid in colony #3 integrated into a yeast chromosome and doesn't have an inserted centromere.

7A. If the mutation was due to an insertion of the transgene, the *E. coli lacZ* gene should segregate with the mutant phenotype. That is, all subsequent animals that had the limb deformity defect should have the *E. coli lacZ* gene, and vice versa. The presence of the *lac*Z gene could be recognized by Southern blot analysis, PCR analysis, or an XGAL assay for β-galactosidase activity on fixed mouse tissue.

7B. Isolate DNA from mutant mouse cells, do a partial digest with a restriction enzyme (e.g. EcoRI), and clone the DNA into a bacterial plasmid that does not have the *lacZ* gene in it, only a selectable marker such as ampicillin and an *E. coli* origin of replication. Transform the library into *E. coli* and plate on amp plates with XGAL. Choose blue colonies. They would have the *lacZ* gene and DNA flanking the insertion. Alternatively, clones containing the *lacZ* gene and the flanking DNA could be identified by assaying hybridization of *lacZ* sequences to the library of genomic clones produced from cells of the mutant mouse (colony hybridization).

7C. Cross mice heterozygous for the *lacZ* insertion to heterozygotes from the *ld* strain. Look for the 1/4 homozygotes; do they have limb defects (failure to complement)? Or, sequence the DNA of the *ld* strain looking for mutations in the gene that you identified by cloning the *lacZ* insertion and its flanking DNA. Finally, test if a wild-type copy of the gene mutated by the *lacZ* insertion can rescue the *ld* mutant phenotype.

7D. You would expect transcripts in the embryo and adult because the mutant mice show defects in limbs and kidney. The existence of transcripts in other tissues is OK. It could be that the gene functions in other tissues but the loss of the gene results in a subtle phenotype that you haven't detected. Alternatively, some other gene could be expressed in the other tissues and be redundant. Finally, the transgene could disrupt some of the transcripts but not all, leading to phenotypes in only some tissues.