GENOME371 FINAL EXAM A and B KEY Autumn 2003

NOTE: Final Exams A and B (white and yellow) are identical.

1A) (*3 points*) An increase in the un-replicated plasmids at 30°C. At this intermediate temperature there is a decrease in functional CDC6 protein so the recruitment of helicase to the origin is impaired.
1B) (*3 points*) d. normal red with fewer white sectors.
1C) (*3 points*) c. there is more CDC6 mRNA in mutant cells than in wild type
1D) (*3 points*) b. dominant gain of function

2A) (*3 points*) e. some combination: a and c
a. this mouse gene and the yeast gene are related by evolution, and
c. this mouse gene and the yeast gene share significant amino acid sequence similarity.
Note: The e-value is the probability that the two would be related *by chance,* not the probability that the match is significant.
2B) (*1 point each*)
Remove ADE3: cells with and without the plasmid would be white
Remove LEU2: cannot select for transformants with the plasmid
Remove ARS1: the plasmid cannot replicate
Remove CEN3: the plasmid will fail to segregate properly
Why ade2/ade2? to allow accumulation of red pigment in cells with the plasmid
2C) (*4 points*) These genotypes may be listed in any order. The genotype listed in 2C must match the phenotype shown in 2D.
1. CDC6; plasmid
2. cdc6-KO; plasmid
3. CDC6; no plasmid
4. cdc6-KO; no plasmid

2D) (*4 points*)
Sectored colony: 1. CDC6; plasmid
No growth: 4. cdc6-KO; no plasmid
Solid white colony: 3. CDC6; no plasmid
Solid red colony: 2. cdc6-KO; plasmid

2E) (*4 points total*) Yes (*1 point*)
(*3 points*) The mouse CDC6 protein is "covering" for the absence of the yeast CDC6 protein. As soon as the plasmid gets lost, however, the cell would fail to divide because it can no longer replicate any of its chromosomes. The plasmid copy of the mCDC6 gene has become <u>essential</u> for the survival of the yeast cell!

3A) (*2 points*) a. neo: to provide for the selection of ES cells that have taken up the DNA
(*2 points*) b. tk: to select against cells that have integrated the DNA into random genomic locations.
3B) (*2 points*) yeast CDC6 near 5'UTR: To ensure that the yeast CDC6 gene gets expressed at the right time and place(s) from the mouse promoter.

3C) (*6 points*)
Blot One, 5'UTR probe:
Lane One, ES cells: 3 kb band
Lane Two, modified ES cells: 9 kb band and 3 kb band

Blot Two, yeast CDC6 probe: We accepted two possible answers.
Possible Answer One, if you assumed that the yeast and mouse genes were too divergent to cross-hybridize:
Lane One, ES cells: no signal
Lane Two, modified ES cells: 9 kb band
Alternative answer, if you assumed the yeast and mouse genes would cross-hybridize:
Lane One, ES cells: faint signals at 10 kb and 3 kb
Lane Two, modified ES cells: strong 9 kb band, faint signals at 10 kb, 4 kb and 3 kb

Blot Three, neo^R probe:
Lane One, ES cells: no signal
Lane Two, modified ES cells: 9 kb band and 4 kb band

3D) (*1 point*) mouse 3 (chimera)
3E) (*1 point*) The ES cells died during embryogenesis or did not become part of the skin (fur).
3F) (*1 point*) mouse 1 (albino)
3G) (*1 point*) mouse 2 (agouti)
(2 points) C/c; mCDC6/ KO-yeast CDC6
3H) (*2 points*) Mate two mice that are heterozygous for the KO allele (identified by Southern blot analysis as described in part C). Note: the *albino* locus segregates independently of the *CDC6* locus.
3I) (*2 points*) The yeast CDC6 gene cannot replace the mouse gene; it fails to complement a mouse knock-out allele.

4A) (*3 points*) There is more MSH5 message transcribed in the testis than in the colon. The colon transcripts start with either exon 1A or 1B but the testis transcripts initiate only with 1A.
4B) (*3 points*) An equal amount of message is made in ovary and colon. In the ovary, only exon 1B is used and not exon 1A.
4C) (*3 points*) Different promoters are used to express MSH5 in the gonads of males and females.
4D) (*1 point each*) NOTE: the pedigrees reveal an autosomal ***recessive*** trait.
I-1 from Family One: B I-2 from Family Two: A
II-3 from Family One: C II-2 from Family Two: A
4E) (*3 points*) b. a failure to complement.
4F) (*2 points*) Probability III-2 will be sterile: 1/4.
4G) (*2 points*) Probability III-3 will be sterile: 0 (The *msh5* allele in family two affects only the exon 1A promoter, which is used only in males. Since females use exon 1B, loss of the MSH5 1A promoter will not affect the females).

5A) (*4 points*) c. plasmid with amp^R and no ori.
5B) (*4 points*) The origin is in the 4 kb Bam-Eco fragment and not in the 8 kb fragment.
5C) (*4 points*) The origin is bisected by the HindIII site; both plasmids contain half an origin, which isn't enough for function.

6A) Yeast two-hybrid analysis:

(*6 points*) Clone SPC880 into the GAL4 gene, substituting the mouse gene for the GAL4 activation domain. Make a library of mouse cDNAs in a second clone of GAL4, substituting the cDNAs for the DNA-binding domain. Transform the first clone (the bait) into an **a** mating type yeast strain; transform the library plasmids into an □ mating type yeast strain. Mate these cells and select for HIS3$^+$ and then screen for blue colonies. Such colonies suggest that the SCP880 protein interacts with a protein encoded in the mouse cDNA library thereby restoring activity of the GAL4 transcription factor.

(*6 points*) After finding interacting proteins, recover and sequence the activation-domain fusion gene. Do a BLASTP search with this sequence to find out what mouse gene has been identified. The function of this gene may have already been determined and that would provide a clue to the function of SCP880. One could also look for yeast homologues to these new proteins to see if the function of the yeast proteins has been determined, thus obtaining clues to the process in which these genes participate.

6B) More BLASTP analysis

(*6 points*) Use the sequence of one or both of the yeast proteins (or the mouse SCP880 protein) as a query sequence. Using BLASTP, search for similar sequences in the yeast genome or in other organisms, such as Drosophila, *C. elegans*, *Arabadopsis*, humans, other mammals or vertebrates, or any other taxa.

(*6 points*) If you find a similar gene whose function is known, it could give you insights into the function of the related SCP880 gene. That is, one must know something about the function of these other proteins, either through genetic analysis (mutant studies), biochemical assays, or some other approach that would suggest function.

6C) More mouse knockout experiments

(*6 points*) Knocking out the mouse SCP880 gene causes an early embryonic lethality, yet knocking out the two yeast homologues (individually) produces no phenotype. Perhaps the two yeast proteins are part of a complex where loss of one protein is covered by the other protein, which holds the complex together. Alternatively, the two proteins are redundant and can function for each other. If either of these hypotheses were true, I could knock out part of the mouse protein and perhaps the protein would still function partially. I would knock out the amino terminal and carboxyl terminal portions separately, in two different ES cell experiments. I would transfer the ES cells to blastocysts, implant the embyros, then breed chimeras until eventually I had homozygotes.

(*6 points*) Loss of only one part of the SCP880 protein, either the amino or carboxyl terminus, could give a partially functional protein that would allow the embryo to survive past the early stages. I could then study the phenotype in mice, which would give me a clue to the function of SCP880.

6D) A synthetic lethal screen in yeast

(*6 points*) Knock out one of the two yeast genes. Add back the gene on a plasmid that carries all the markers used for the red-white sectoring assay. Mutagenize the yeast using a transposon-tagging strategy and look for colonies in which the plasmid can no longer be lost. Those strains harbor a second mutation in a gene that likely interacts in the same pathway as YCR039c or YML102w, whichever gene I knocked out originally.

(*6 points*) Use the transposon tag to identify the interacting genes. Usually certain classes of genes interact, most of which function together in a single process. If any of these genes' function is already known, I would have a clue to the function of YCR039c and YML102w and therefore to the function of the mouse SCP880 protein.

Note: Some people made double mutants between YCR039c and YML102w. That is a logical experiment but is not a synthetic lethal screen.