**Final Exam Fall 2012 with brief answer guide**

1. (8) total – be sure to answer each of the questions)

a. What sorts of chromosomal sequences do you expect to be found exclusively in the genomic DNA library? *Genomic – non-transcribed DNA (intergenic regions, centromeres, telomeres), promoter regions of genes, sequences downstream of the polyA site, intron sequences.*

c. What specific sequence commonly associated with mRNA will only be found in the cDNA library? *cDNA – none of the above but the cDNA will have the non-encoded polyA tail.*

d-e. What factors do you consider when estimating the **number of clones** that need to be screened to find a gene of interest in 1) a cDNA library and 2) a genomic DNA library?

*) a cDNA library (mRNA abundance or enrichment) and 2) a genomic DNA library (genome complexity and length of DNA insert)?*

2. (10 pts) Which of the following would be useful for (put letter alongside protein or process: 1) \_\_\_B\_\_\_suppressing background in your northern blot hybridization, 2) \_\_C\_\_use of EcoRI methylase, 3)\_\_D\_\_\_ creation of unidirectional DNA deletions with Exonuclease III, 4) \_\_\_E\_\_use of E. coli DNA ligase, : 5) \_\_A\_\_\_\_purification of DNA

A) B)





 C) D)

E.

3. (3 pts) Why do we add the “extra RNA sequence” onto the end of the antisense U1 snRNA probe used in the RNase mapping? The extra RNA will be trimmed off in the hybrid between the U1 snRNA and the probe. This causes a shift in the probe length and provides assurance that the RNase A is actually working.

4. (5 pts) You suspect that the RNA transcribed from a nonessential yeast gene, *BXL1,* is efficiently spliced when yeast are grown on glucose-based medium but poorly spliced when yeast are grown on galactose-based medium. Design an experiment to test this hypothesis. In your answer, be clear to describe the experimental approaches that you will use including controls and tell how the data will be evaluated (that is, what will you see and how will you interpret it) to definitively show whether or not the hypothesis is correct. You have only $1,000 to complete this experiment, so deep sequencing approaches are not available. You might use either a rtPCR or a northern blot approach. If rtPCR, you would make oligonucleotides against the exon sequences flanking the intron and expect to see an increase in the abundance of the longer (intron-bearing) cDNA & likely a decrease in the abundance of the shorter spliced cDNA (from the spliced RNA) from the galactose grown culture. Controls might include conducting the same experiment but leaving out the reverse transcriptase (to show that he longer band was not due to genomic DNA contamination) and a rtPCR against an intronless gene insensitive to the difference in sugar source. The northern blot wound be done similarly except that you will use a probe prepared, for instance, by random prime labeling against the exon (or intron + exon) sequences. Here again, expect an increase in the upper (unspliced) RNA in the galactose sample. A loading control can be either the Ethidium bromide stained rRNA after transfer or hybridization with a probe for an intronless gene insensitive to the sugar source shift.

5. (6 pts) You complete an Illumina-based transcriptome study (that is RNA sequencing) and find evidence for anti-sense transcription across the yeast *CYC1* gene. This means that you find examples of RNA that has the opposite polarity of the natural *CYC1* mRNA. Design an experiment that:

a. provides an alternate approach to demonstrate that both anti-sense RNA really exists in the cellular RNA population. Here you need a RNA “strand sensitive” detection methodology. You could do this by northern blot using + and – strand riboprobes (like we did for the U1 snRNA detection) or by RNase A mapping (like we used for U1 snRNA abundance measurement)

b. allows you to determine the length in nucleotides of the antisense RNA present in yeast, and

Can use the northern blot approach since this shows full-length RNA as long as you include makers for RNA length. An RNase A (or 5’ and 3’ RACE rtPCR) approach will also work but you have to say how you know that your probe(s) detect the full-length molecule (in the case of RNAse A, without the poly A tail).

c. allows you to determine whether or not the antisense RNA is polyadenylated. Compare the signals obtained with RNA before and after oligo dT selection; if polyadenylated, the antisense RNA should be enriched in the oligo dT selected pool and depleted in the RNA not bound to the oligo dT. Hybridization of the RNA in solution with oligo dT followed by RNase H will trim off the polyA tail and this will be seen as a band shift(which can be used to calculate the size difference) on the northern blot.

6. (6 pts) Below are the genotypes of three bacterial strains. Which strain is best for:

a. blue-white (beta-galactosidase-based) colony screening of in *E. coli*? Which of the listed genes are relevant to the blue-white colony screen? **TG1** \_(lac‑proAB), F'(proAB+, lacIq, lacZ\_M15);

b. the isolation of hydroxylamine mutagenized DNA? Which of the listed genes are relevant to the recovery of the mutagenized plasmid DNA? CJ236 Genotype: *ung-1 dut-1*

c. High level protein expression from the pTXB1-Spp382 (1-128) construct? Which of the listed genes are relevant to the protein production and recovery? **ER2566** F- λ- fhuA2 [lon] ompT lacZ::T7gene1,sulA11

7. (3.5 pts) You have two cultures of *E. coli*, A and B. Assume that the major replicative DNA polymerase of *E. coli* is composed of 4 interacting protein subunits, called I, II, III, and IV. You hypothesize that the subunit stoichiometry of the major replicative DNA polymerase differs in A and B, with strain A having one copy each of subunits I, II, III and IV and strain B having one copy each of I, III and IV and three copies of subunit II in the active enzyme. Discuss how mass spectroscopy can be used to test this hypothesis – specifically to learn if the DNA polymerase subunit stoichiometry’s differ in strains A and B. While you do not have to describe the mechanics of mass spectroscopy machine operation, outline your experiment, state whether you prefer a MALDI-TOF or ESI MS/MS approach and, most importantly, how you will determine the ***relative levels*** of the DNA polymerase subunits in strain A and B. Mass spectroscopy can be made quantitative using heavy atom labeling or by post-labeling of the isolated proteins(e.g., ICAT). In principle, either MALD-TOF or ESI MS/MS can be used. As an example, you could grow strain A bacteria with 15N- and strain B with 14N-containing methionine (or any amino acid). Harvest equal amounts of the two cultures, break the cells and mix the two samples. Then, by ESI MS/MS (for instance) use the 15N- to 14N ratios for subunits I, III, and IV for normalization (since these are known to be in equal abundance). If subunit II is really is really 3 times more abundant in strain B, then the 15N/ 14N ratio will decrease by the expected 3-fold (due to the increase 14N value).

8. (2 pts) You want to determine where the Sqs1 protein binds Spp382 (that is, what site on Spp382 is in contact with Sqs1) by the yeast two-hybrid (Y2H) approach.

Which one of the following yeast host strains would you select to use for this study and why do you select this one over the others?

**PJ69-4A** *MATa trpl-901, leu2-3,112, ura3-52, his3-200, ga14 Δ ,ga18Δ, LYS2::GALl-HIS3, GAL2-ADE2, met2::GAL7-lacZ* Because this is the only strain with GAL4-sensitive reporters for the Y2H assay (also has the needed genetic markers for the pACT and pAS2 plasmids, plus is a gal4 mutant – all good)

9. (8 pts) Assume that a single site for Sqs1 binding exists on Spp382 and that you have no knowledge of where the binding occurs or information on the Spp382 protein structure other than its length of 708 amino acids. Ideally, you want to map the site of interaction within a 50 amino acid region. How would you design your experiment to make it most likely to find the unknown site of interaction using the ***fewest number*** of constructs? Describe the specific yeast two hybrid (Y2H) constructs that you willd make and discuss the rationale for this choice. To simplify your experiment, you can assume that any construct that you make that retains the full binding site will give you a positive Y2H result and that any construct that removes even one amino acid from the binding site will give you a negative Y2H result.

 Easiest done by a “cut the universe in half” approach. So, the first construct might contain the amino half of Spp382 (codons 1-354) cloned into pACT and full length Sqs1 in pAS2. If the N-terminal half of Spp382 binds Sqs1, you need not check the C-terminal half (codons 355-708). If the N-terminus does not interact, try the C –terminus of Spp382. The C-terminal half of Spp382 will either bind Sqs1 (in which case you know that the binding site is somewhere between Spp382 codons 355 and708) or not, which means that you have bisected the Sqs1 binding site and that this must exist somewhere within the 100 codons flanking position 354. You continue this approach of cutting the interacting domain in half each time to narrow down the site of association.

10. (5 pts) Describe the properties of each of the following and tell what purpose each served in our BIO 510 lab experiments?

1. RNase A – cleaves single stranded RNA after pyrimidines, used in RNase protection study to measure U1 snRNA abundance, and also in the *E.coli* miniprep to degrade the RNA that co-purifies with our plasmid
2. Taq polymerase – thermostable DNA polymerase used for PCR (e.g., with our genomic yeast DNA to determine with strain had the deletion)
3. 3-aminotrizole – an anti-metabolite that is a competitive inhibitor of the *HIS3* gene product; we used this to reduce background in our Y2H on the medium lacking histidine
4. BCIP/NBT – The substrate & enhancer of the alkaline phosphatase used in our western blot.
5. Reverse transcriptase – RNA-dependent DNA polymerase used in our rtPCR experiment to prepare cDNA for PCR.

11. (2 pts). The following compound was used in the BIO 510 lab as a mutagen



12. (2.5 pts) Three students perform a northern blot experiment in which the very same amount of a two RNA preparations from yeast strains A and B. The hypothesis is that stain A has 10% of the amount of *PSP1* gene’s mRNA compare with strain B. The samples were loaded on a single gel and transferred to an single membrane which was cut into thirds for two RNA hybridizations. A single RNA probe preparation was made and shared between the students for this experiment. Students #1 and #2 each used 49 µl of the available probe leaving student #3 with only 2 µl. The results obtained from phosphoimaging are presented below. Assume that the RNA transfer efficiency & crosslinking was 100% efficient in each case and, other than the amount of probe used, that there were no procedural mistakes were made. Assume also that the results radioactive signals are all within the linear sensitivity range of the phosphoimager and are fully reproducible. Provide an explanation for how the difference in probe abundance can result in these two very different hybridization results.

Student 1 Student 2 Student 3

A B A B A B

 <PSP1 mRNA

 <Loading control RNA

Student 3 shows roughly equal signals in A and B rather than the much greater signals in B seen in student’s 1 and 2 samples.

One way this result can occur is if the amount of probe becomes limiting in student #3’s hybridization– much less probe than the corresponding mRNA target bound to the paper. In such a case, the signals are no longer proportional to the mRNA abundances, since the few probe strands available find lots of open (single stranded mRNA) targets over A and B. When the probe is in “excess” (such as with student’s 1 &2) the mRNA hybridization saturates and the signals are proportionate to the mRNA actually bound on the membrane. Think about this in the following (more extreme) way. Assume that there are two wells dug in a football field, one 50 foot deep, the other 500 ft deep. Two students are allowed to wander over the field blindfolded. While most of their time is spend safely walking over the field (which is very large compared to the well surface; similar to the limited sites of hybridization on the much larger membrane surface), occasionally a student will fall into one or the other well – in both cases he/she gets stuck. If you performed this experiment each night for 30 days (removing the fallen students each day), at the end of a month’s time there would be roughly similar numbers of students recovered from the 50 foot well and the 500 foot well (this is similar to student 3’s result in your question). If, on the other hand, you put 100,000 blindfolded students on the field each night, many students will fall into both wells and saturate each – but the 50 foot well only holds 10 people while the 500 foot well holds 100 (like the results for students 1 and 2 in your question).

13. (5 pts) Describe the steps involved for **one** of the following DNA sequencing methodologies. Sanger di-deoxy sequencing **or** pyrophosphate sequencing. State the enzymes and reagents involved and tell how the sequence information is obtained. *You will get credit for only* ***one*** *description so do either Sanger or pyrophosphate sequencing, but not both.* See the class notes for detailed descriptions

14. ( 2 pts) 5-fluoro-orotic acid (5-FOA) is an antimetabolite that inhibits the growth of yeast that express the gene encoding (circle one) a) orotidine-5'-phosphate (OMP) decarboxylase

15. (2) Define the features of plagiarism as this relates to any scientific writing. That is, what specific things would be present in a document that would demonstrate that the document is plagiarized? The use of any text or image from another source in your composition without direct attribution of the original source.

16. (6 pts) Describe how SYBR green was used in our realtime PCR experiment to determine the amount of a target DNA in an unknown sample. In your answer, be sure to tell a) what SYBR green does and b) how this is observed in the data obtained by realtime PCR results. In your answer tell how the realtime PCR results are analyzed to determine the actual amount of target DNA present. SYBR green is a dye that shows increased florescence when it binds double stranded DNA. The florescence signal is monitored as a function of PCR cycle by the realtime PCR machine. When sufficient PCR amplification occurs that the newly made ds DNA adds substantially to the SYBR green florescence, the “threshold” is reached. To determine the DNA amount in an unknown sample, the threshold cycle of the unknown sample is compared to a standard curve prepared by PCR of equivalent DNAs of known concentration and scoring the threshold of each as a function of the input DNA.

17. (2 pts) Hydroxylamine is **most likely** to cause (circle only one answer): a) GC->AT mutations

18. (2 pts) Some naturally occurring genes show the low-level use of alternate pre-mRNA splice sites**\***.

d) the alternately spliced mRNA may be either longer, shorter, or the same size as the primary spliced mRNA

19. (4 pts) Professor Mixup included 50 mM DTT in his *E. coli* breakage buffer and in the chitin agarose binding buffer during an experiment when he wanted to isolate Spp382 (1-121) expressed from the pTXB1-Spp382(1-121). Based on his error, describe what protein bands he would expect to find in 1) the soluble fraction obtained in the chitin elution step 2) the boiled chitin agarose beads remaining after the chitin elution step. The early addition of DTT is expected to cause intein cleavage and the release (and subsequent loss) of the Spp382(1-121) peptide prior to chitin-agarose chromatography. When the protein sample is bound to chitin, only the CBD-intein segment will bind. The soluble eluted sample is expected to have no bands (since no additional cleavage occurs) and the boiled chitin beads release the CBD-intein segment. Spp382(1-121) is lost from the preparation since it was never bound to the column.

20. (2 pts) Which of the following statements is most correct? (circle one) FOA c) selects for cells that have spontaneously lost a *URA3* bearing plasmid

21. (5 pts) Dosage (or high copy) suppression, extragenic suppression and synthetic lethality are three genetic approaches that can be used to identify genes involved in a given cellular pathway. Describe what each screen involves – that is, provide an example of how each screen is designed. For instance, in an extragenic suppression screen, “I would start out with (state the starting strain characteristics)…. and then identify candidate interacting factors by (state what steps are needed to identify interacting genes). In your response, be sure to describe any features of the strain genotype relevant to your screen. Look at the handout provided in class.

22. (3 pts) Define what is meant by the terms “primary antibody” and “secondary antibody”. In addition to “order of use” issues, what characteristics distinguish a primary from a secondary antibody? The primary antibody is raised in a particular animal against the protein of interest (e.g., a rabbit anti-yeast Prp43 antibody). The secondary antibody is raised in a different organism, binds the primary antibody, and is typically conjugated to an enzyme (alkaline phosphatase, horseradish peroxidase) or a florescent dye (e.g., goat anti-rabbit IgG-alkaline phosphatase conjugate).

23. (2 pts) You have identified a rare and aggressive form of human cancer and suspect that it results from the activation of a previously uncharacterized cellular pathway. Unfortunately, you have no idea what genes are involved. You have the option to use either Affymetrix whole genome array hybridization or Nanostring technology (but no other technologies). **Which** methodology would you select and **why** do you choose this? Nanostring technology is limited to scoring a few hundred genes and is best used when you know the genes of interest and want to score the levels of corresponding RNA. The Affymetrix arrays will allow you to score all genes in parallel independent of whether you suspect involvement or not – so, it is much better for this purpose.

24. (2pts) You have completed an in vitro mutagenesis experiment to create an A to T mutation in the first codon of the actin gene. This gene is present on a plasmid and you want to confirm that the mutation was correctly introduced. **Which** sequencing technology be most appropriate to use and **why**? ***Circle*** the correct technology ***then state the reason*** why you believe that it is the best technology to use for this application. Sanger Sequencing and capillary electrophoresis. This is a simple single run sequencing experiment on a purified gene – the high throughput sequencing approaches are of no value here (and are much more expensive).

25 (2 pts). Captain Retrievit recovered a previously unknown lizard from the forests of Borneo. This lizard changes color with light and is white in sunlight and black in the dark. The basis for this color change is unknown but presumably mediated by changes in gene expression. You can either use Illumina based RNA sequencing or DNA microarrays to identify the genes whose expression differs in the light and dark. Which technology is most appropriate to answer this question? In considering your response, ignore the issue of cost. Be sure to say **why** the technology you choose is better for this study. DNA microarrays require you to know the sequence of the genes – you do not, since this is a “previously unknown lizard”. The Illumina sequencing works great for this purpose since you do not need a completed genomic sequence to measure relative mRNA levels.

26. (3.5 pts) Mark each of the following genes/reagents used in lab as best described as most useful in a A) selection procedure or B) screening procedure

i. addition of IPTG & X-gal to bacterial plates\_\_\_\_B\_\_\_\_\_\_\_\_\_\_\_\_

ii addition of kanamycin sulfate to the M13KO7 infected cultures \_\_\_\_\_\_A\_\_\_\_\_\_\_\_\_

iii use of the GFP/RFP dual reporter in the Asd-1 RNAi knockdown \_\_\_\_\_B\_\_\_\_\_\_\_

iv use of yeast medium lacking uracil in our transformation \_\_\_\_\_\_A\_\_\_\_\_\_\_\_

v addition of 5FOA to our yeast medium \_\_\_\_\_\_A\_\_\_\_\_\_\_\_

vi addition of ampicillin to our bacterial plates \_\_\_\_\_A\_\_\_\_\_\_