**Final Exam Fall 2012 NAME\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

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?

c. What specific sequence commonly associated with mRNA will only be found in the cDNA library?

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?

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

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.

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,

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

c. allows you to determine whether or not the antisense RNA is polyadenylated.

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?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b. the isolation of hydroxylamine mutagenized DNA? Which of the listed genes are relevant to the recovery of the mutagenized plasmid DNA? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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,gal, sulA11 Δ(mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10)(TetS) endA1 [dcm];

**TG1** McrA+, McrBC-, EcoKR‑, EcoKM- (hsd\_5), supE, thi,\_(lac‑proAB), F'(traD36, proAB+, lacIq, lacZ\_M15);

CJ236 Genotype: FΔ*(HindIII)::cat* (Tra+ Pil+ CamR)/ *ung-1 relA1 dut-1 thi-1 spoT1 mcrA*

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.

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*

**MGD353-96D** *MATa ura3-52, snR19::KAN, prp43 H219A*

**BY4743** *MATα ura3-52,* McrBC-, lacZ::T7gene1, gal1*Δ*

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.

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
2. Taq polymerase
3. 3-aminotrizole
4. BCIP/NBT
5. Reverse transcriptase

11. (2 pts). The following compound was used in the BIO 510 lab a) to denature double stranded DNA, b) to remove secondary structure in RNA, c) to stain nucleic acids for imaging under a UV light, d) to increase the efficiency of yeast transformation, e) 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

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.*

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 b) phosphoribosylanthranilate isomerase, c) galactokinase, d) imidazoleglycerol-phosphate dehydratase or e) 3-aminotriazole

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?

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.

17. (2 pts) Hydroxylamine is **most likely** to cause (circle only one answer): a) GC->AT mutations, b) large deletions, c) large insertions, d) AT->CG mutations, e) chromosomal translocations

18. (2 pts) Some naturally occurring genes show the low-level use of alternate pre-mRNA splice sites**\***. If one assays the use of alternate splice sites selection by rtPCR, one finds that:

a) the alternately spliced mRNA is always shorter than the primary spliced mRNA

b) the alternately spliced mRNA is always longer than the primary spliced mRNA

c) the alternately spliced mRNA is the same length as the primary spliced mRNA

c) the alternatively spliced mRNA may be either longer or shorter than the primary spliced mRNA

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

**\*** this question refers to any gene in any organism and is not restricted to the experiment we did in the BIO 510 class

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.

20. (2 pts) Which of the following statements is most correct? (circle one) FOA a) causes *URA3*-bearing plasmids to be lost from a cell, b) increases the rate of spontaneously plasmid loss no matter what the plasmid marker is, c) selects for cells that have spontaneously lost a *URA3* bearing plasmid, d) acts as a competitive inhibitor of the enzyme encoded by *HIS3* e) blocks cellular uptake of 3-aminotriazole

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 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.

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?

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 Nanstring technology (but no other technologies). **Which** methodology would you select and **why** do you choose this?

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. a) Ion Torrent, b) 454 Sequencing, c) Illumina Sequencing, d) Solid Technology e) Sanger Sequencing and capillary electrophoresis

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.

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\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

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

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

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

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