BIO 510 2013 Final Exam NAME\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Please answer all questions except the multiple choice & fill-in-the-blank questions on the attached pages.

1. (3 pts total) What is the “threshold cycle” in a real-time PCR experiment? The cycle where the florescence level is statistically greater than the background due to SYBR green dye binding newly made dsDNA.

Assume you conduct a real-time PCR experiment using A) 0.1 ng, B) 0.5 ng and C) 1.45 ng of the same template DNA. Do you expect the threshold cycle to (decrease) when going from A to B to C?

2. (2 pts) We included 5 mM 3-aminotrizole (3-AT) to the selective plates in our yeast two-hybrid experiment. A) How would expect our results differ if we did not include 5 mM 3-AT? Be specific – tell what would expect to see on the plates and why. If no 3-AT is added, less HIS3 reporter gene expression is required for growth (decreased stringency). We would expect larger colonies or increased numbers of lines giving positive results (for instance, spp382-PXR1 was negative on 3 mM 3-AT but might show colonies in the absence of 3-AT). B) How would expect our results differ if we added 20 mM 3-AT? If more 3-AT is added, greater HIS3 reporter gene expression is required for growth (increased stringency). We would expect smaller colonies and weak activators (the spp382-SQS1 reporter) might show no growth.

3. (2 pts) The Spp382 peptide we purified in class has a molecular weight of 13.3 kDa and a pI of 5.28. What is meant by the term “pI”? In addition to telling what the letters “p” and “I” stand for, explain what the term pI means in terms of a protein’s chemical state. pI is the isoelectric point the pH at which the protein’s net charge is zero.

4. (6 pts total) Assume that you want to use reverse-transcriptase PCR (rtPCR) measure the efficiency of intron removal from the yeast *RPS17* mRNA. To do this, you want to measure the relative abundance of mRNA (intron removed) and pre-mRNA (intron present) in the RNA sample. Your PI wants the PCR step (**after cDNA synthesis**) to include only 2 oligonucleotide primers, so these must be able to amply both the mRNA and the pre-mRNA. **The intron portion of *RPS17a* is shaded.**

5’ATCGACTTAATTCTAAGAAAAGTCAAGATCTCGAGACTAGCAATAACAAAATGGTATGTT3’

3’TAGCTGAATTAAGATTCTTTTCAGTTCTAGAGCTCTGATCGTTATTGTTTTACCATACAA5'

5’AATATGGACTAAAGGAGGCTTTTAAGGACACGTAATATTGAGTCGACATGCGCAATAAAG3’

3’TTATACCTGATTTCCTCCGAAAATTCCTGTGCATTATAACTCAGCTGTACGCGTTATTTC5’

5’TCATACAATAAATAAGTTAAAGAAAACATATGACGATATTTTCAGACGATATGGAACTGC3’

3’AGTATGTTATTTATTCAATTTCTTTTGTATACTGCTATAAAAGTCTGCTATACCTTGACG5’

5’GATACCGAAAAAGTGGTGTGATTGAAATGATACTCATGTTTTGGCTATAGTTGCAGTGGC3’

3’CTATGGCTTTTTCACCACACTAACTTTACTATGAGTACAAAACCGATATCAACGTCACCG5’

5’TTTCCAAAAAATTGGGAGTTAAAATGCCTATAGCAGTATCACCGGAGATTCAGTCTCTTG3’

3’AAAGGTTTTTTAACCCTCAATTTTACGGATATCGTCATAGTGGCCTCTAAGTCAGAGAAC5’

5’TGGATCTTCTTTTTGCATAGTTGAACAAGGGATAATATGGCGCCATGAACTGCCTCTACA3’

3’ACCTAGAAGAAAAACGTATCAACTTGTTCCCTATTATACCGCGGTACTTGACGGAGATGT5’

5’CATTATAATGTGTTTTTGATATCAGTATACTAACAAGTTGAATTGCATTTACAAACTTTT3’

3’GTAATATTACACAAAAACTATAGTCATATGATTGTTCAACTTAACGTAAATGTTTGAAAA5’

5’TATTTTGTATTGCTTTTCGTCATTTTAATAGGGTAGAGTTAGAACCAAGACCGTCAAGCG3’

3’ATAAAACATAACGAAAAGCAGTAAAATTATCCCATCTCAATCTTGGTTCTGGCAGTTCGC5’

5’TGCTTCTAAGGCTTTGATTGAACGTTACTATCCAAAGTTGACTTTGGATTTCCAAACCAA3’

3’ACGAAGATTCCGAAACTAACTTGCAATGATAGGTTTCAACTGAAACCTAAAGGTTTGGTT5’

5’CAAGAGACTTTGTGATGAAATCGCCACTATCCAATCCAAGAGATTGAGAAACAAGATTGC3’

3’GTTCTCTGAAACACTACTTTAGCGGTGATAGGTTAGGTTCTCTAACTCTTTGTTCTAACG5’

5’TGGTTACACCACCCATTTGATGAAGAGAATCCAAAAGGGTCCAGTTAGAGGTATCTCTTT3’

3’ACCAATGTGGTGGGTAAACTACTTCTCTTAGGTTTTCCCAGGTCAATCTCCATAGAGAAA5’

5’CAAATTGCAAGAAGAAGAAAGAGAAAGAAAGGACCAATACGTCCCAGAAGTCTCTGCTTT3’

3’GTTTAACGTTCTTCTTCTTTCTCTTTCTTTCCTGGTTATGCAGGGTCTTCAGAGACGAAA5’

5’GGACTTGTCTCGTTCTAACGGTGTTTTGAACGTTGACAACCAAACTTCTGACTTGGTTAA3’

3’CCTGAACAGAGCAAGATTGCCACAAAACTTGCAACTGTTGGTTTGAAGACTGAACCAATT5’

5’ATCTTTGGGTTTGAAGTTGCCATTATCTGTTATCAACGTTTCTGCCCAAAGAGACAGACG3’

3’TAGAAACCCAAACTTCAACGGTAATAGACAATAGTTGCAAAGACGGGTTTCTCTGTCTGC5’

A) (2 pts) Use the DNA sequence below to design the two 18 nucleotide primers. **UNDERLINE** in the sequence where each primer is located.

Write out here the sequences of each in the 5’-3’ orientation:

 Primer 1) 5’GACTTAATTCTAAGAAAA3’

 Primer 2) 5’TCTCTTTGGGCAGAAACG3’

B) (2 pts) Starting with 1 microgram of total yeast nucleic acid isolated by breaking the cells with glass beads. Describe all the enzymatic steps needed to measure the mRNA/pre-mRNA ratio in this sample by rtPCR. First step is to degrade the chromosomal DNA that co-purifies with mRNA in this preparation. You add RNase-free DNAs in the appropriate magnesium containing buffered solution. After the DNA is digested, the DNase must be removed before moving on – so, PCI extract, then precipitate.

Next, prepare the cDNA. To do this, you anneal either oligo dT or a random primer mixture to the RNA then add reverse transcriptase plus all four dNTPs. Upon completion of the cDNA synthesis, you are ready to perform PCR using the oligos outlined above, Taq DNA polymerase and all four nucleotide triphosphates. Similar to our lab experiments, we will use 25 to 35 PCR cycles for the amplification.

C) (2 pts) Your bench mate did the same experiment but forgot to add the DNase I step. This error will change his results in a predictable way. What difference do you expect to see when you compare his rt-PCR experiment to yours by gel electrophoresis? Increase levels of the full length PCR product (which is from the genomic DNA plus any unspliced pre-mRNA)

Draw a diagram of your predicted gel image of his and hour results – be sure to identify each student and label each lane with the predicted position of the mRNA and pre-mRNA.

 plus DNase minus DNase

genomic - **-**

mRNA **- -**

5. (3 pts) You want to use the Nanostring technology to look for changes in gene expression when the NPT tissue line is exposed to 0.45 mM spitulum. Describe the steps required to do this experiment. In your answer, be sure to describe the basis by which the Nanostring technology works and what information, reagents and resources you need to have available to use this technology and interpret the results. You do not have to go into detail on the RNA isolation steps. Anneal the RNA with the biotin-substituted capture and tagged florescence signal oligonucleotides specific for each mRNA. Bind the annealed mixture to the streptavidin coated slides and remove excess unbound oligos. Align the captured oliog/mRNA complexes in an electric field then identify and quantify the trapped complexes by reading the florescent codes after light stimulation by the Nanostring instrumentation. To perform this experiment, the sequence for every mRNA to be analyzed must be known.

6. (12 pts) Using the following hybridization data, determine the location and direction of transcription for the four RNA transcripts. **GIVEN**: Assume that no more than one transcript is present in any interval defined by two adjacent restriction sites (that is, BamH1 to Pst1, Pst1 to Tsp1, Tsp1 to Msp2 etc.) and that no transcripts extend into an adjacent restriction fragment. ***Put an arrow head at the 3’ end of the transcript (5’ -> 3’) and be sure to indicate clearly by a solid line labeled with a transcript length where each transcript resides and label its size.***

**Single stranded Probe (5’->3**’) **Hybridizing Bands on Blot (all in kb)**

ClaI->Msp2 1.0, 1.7 kb

BamH1-> Cla1 9.6 kb

Msp2-> Pst1 0

BamH1-> Msp2 9.6 kb

Cla1-> BamH1 1.0, 1.7, 3.4kb

Pst1->Gas1 9.6 kb

Msp2->BamH1 3.4 kb

Pst1->Msp2 9.6 kb

GasI->Tsp1 1.0 kb

Tsp1-> BamH1 3.4 kb

BamH1--------Pst1------Tsp1--------------------Msp2--------------Gas1-----------------------------Cla1

 3.4> <9.6 1.0> 1.7>

  **<9.6 (also acceptable)**

7. (2 pts) Match the RNAi dsRNA construct used in our C. elegans experiment with the observed phenotype. *CLF1\_A\_\_\_ Dpy-1 \_D\_\_\_Unc-22 \_\_C\_\_\_ sup-12 \_\_B\_\_*

A) Strong larval lethality, few animals survive

B) Changes in the level of alternative splicing of the *egl-15* muscle transcripts

C) Impaired mobility

D) Animals appear bloated due to lipid accumulation

8. (2 pts) A temperature sensitive defect due to mutation might be relieved by genetic complementation or genetic suppression. What is the difference between these two terms? The ts mutant is complemented by the wildtype allele of the mutant gene and suppressed by the impact (due to overexpression or mutation) of gene other than the one that was originally mutated.

9. (4 pts-total) You have isolated a yeast gene called *STR1,* that encodes a potent transcription factor. You want to identify genes that encode proteins that interact with the Str1 protein using the yeast two-hybrid assay. You have the option of cloning the STR1 open reading frame (ORF) into either the pACT2 vector or the pAS2 vector and have access to DNA libraries made in either of the complementary vectors (that is, if you choose to clone into pACT2, you can use the pAS2-library to screen for interacting factors. (1 pt) Which plasmid would you choose to clone the *STR1* ORF into, pACT2 or pAS2? (3 pts) Describe the rationale for choosing this vector over the other. If *STR1* encodes a transcription factor, you must clone it in the Gal4 activation domain plasmid, pACT2. If you clone it into the DNA-domain vector, pAS2, then the Str1 protein will stimulate transcription even in the absence of a pACT-based binding partner (since it is a full transcription factor already)

10. (2 pts) Which of the following is **not** true of 5 floroorotic acid (5-FOA)? Circle all that apply.

a) it increases the frequency of plasmid loss

d) it increases the stringency of the yeast two-hybrid (Y2H) assay

11. (2 pts) What is an intein and what role does it serve in nature? That is, what purpose does the intein serve in cellular biochemistry in nature outside of the scientific lab? An intein is a auto-excising peptide sequence (the peptide equivalent of an intron). Intein excision changes protein structure and function .

12. (2 pts) Which of the following compounds will likely to activate an intein? Circle the letters of all that apply.





A B

13. (3 pts) Outline the biochemical steps required to complete a “Sanger sequencing” reaction In your response, 1) name the specific reagents used in the sequencing reaction, 2) tell how the data are gathered (that is, briefly describe what the machinery does to make the chemistry in part 1 readable) and 3) how the data are interpreted. Prepare single stranded template - either by ssDNA synthesis or by denaturation of dsDNA. Anneal a synthetic ssDNA oligonucleotide downstream of the sequence to be analyzed. Complete 4 sequencing reactions each containing all 4 dNTPs and one of each of 4 chain terminating (dideoxy) ddNTPs. Either domplete the reaction with a radiolabeled oligo or dNTP or choose 4 different fluorescently tagged ddNTPs for the chemistry. Resolve the 4 reactions by gel electrophoresis - 4 reactions separately on 4 adjacent lanes of a gel (with radioactivity) or all together by capillary electrophoreses. Read the signals (radioactivity or florescence) from fastest migrating (or eluting) on as complementary nucleotides of the DNA target.

14. (2 pts) Starting with the following DNA cut at the position of the slashed lines, which of the following inserts will most likely result in an “in frame” protein fusion before and after the insertion site? The methionine used for translational initiation is shaded and underlined. NOTE: there may be more than one correct answer; all presented as the coding strand in a 5’ to 3’ orientation. Circle the correct answer(s).

1) GCG GGG CCC GCG

3) GCG GGG

4) GGG GGG CGC

5) CGC GGG CCC CGC

 5’ATAAGAAATCATGGCT/GGTCAAGTGTTGGACGGCAAAGCATGCGCTCAGCAGTTTAGAAG3’

3’TATTCTTTAGTACCGA/CCAGTTCACAACCTGCCGTTTCGTACGCGAGTCGTCAAATCTTC5’ M A G Q V L D G K A C A Q Q F R S amino acid seq.

All of these contain multiple of 3 nucleotides and hence retain the open reading frame.

15. (6 pts total) You have performed a yeast two hybrid analysis to map the location of Pxr1 interaction with the actin protein You get the following results: Positive interactions: A, C, H) Negative interactions (B, D, E, F, G, I). **NOTE**: In the diagram below, the **boxes represent the fragments that *ARE INCLUDED*** in the yeast two hybrid construct tested. For instance, construct “A” tested the whole protein while construct “B” contained ONLY amino acids 25-70 fused to the Gal4. **This is opposite of how the diagram was used for our lab** experiment (i.e., in lab, but not in this exam, we considered the boxes to be the deleted region).



Based on these observations,

a. (3 pts) Which peptide segment of Pxr1 appears necessary for interaction with actin? The position 1-25

b. (3 pts) Design an experiment to test whether this peptide segment is sufficient for this interaction. Clone the Actin gene into pAS2 as an in-frame fusion with the Gal4 DNA binding domain. Clone Pxr1 1-25 codons as an in-frame fusion with the Gal4 activation domain. Score for *GAL1-HIS 3* transactivation by growth of yeast pJ69-4A transformants on medium lacking histidine and containing 3 aminotriazole.

16. (5 pts) Affinity selection of TAP-tagged proteins from protein lysates can be inhibited by other proteins that bind your protein of interest and mask the TAP tag. You find that 65% of the Prp43 in yeast extract does not bind to your affinity column. Your colleague suggests that this may be due to a sub-population of the Prp43-TAP in your extract that is resistant to affinity purification. Describe an experiment to test this hypothesis and tell how you will interpret the data. If the hypothesis is proved false, suggest an alternative reason for why the 65% of Prp43 was not recovered in your original experiment. Perform the binding experiment as before to remove the 35% of the Prp43-TAP that binds the resin. Take the unbound extract containing the remaining 65% of the Prp43-TAP and rebind it to fresh IgG (or calmodulin) resin. If much of the reapplied extract now “sticks” to the resin, it appears that your recovery conditions contain too little resin or you are not providing sufficient time for the resin to bind. If the re-applied material still does not bind, it is likely to be unavailable – perhaps because the protein A or calmodulin binding domain is masked by another protein.

17. (2 pts) Draw a diagram which illustrates alternative splicing events involving regulated alternative 3’ splice site selection in a gene with two exons and one intron (label each). See lab slide showing the alternative 3’ splice site for *IWR1*.

18. (2 pts) When the downstream 3 splice site is used in the image presented in question 17, the mRNA product is (shorter) and the released intron is (longer).

19. (2 pts) Chitin is best described as a (modified polysaccharide) (circle one)

20. (2 pts) In what basic ways do monoclonal antibodies different from polyclonal antibodies? Monoclonal antibodies are produced from a single immunoglobulin – molecule, typically an activated B-cell fused to a cancer cell (hybridoma). Monoclonal antibodies bind on site on the target protein. Polyclonal antibodies are collections of antibodies produced by multiple different activated B cells. Polyclonal antibodies may bind multiple different sites on the target protein (each antibody at a specific site, but since many different antibodies present, may cover multiple sites).

21. (5 pts) A standard western blot protocol involves the use of a primary and a secondary antibody. Describe the features of each antibody type and explain the roles of each in the western blot experiment. The primary antibody binds to the target protein. The secondary antibody binds to the immunoglobulin of the primary antibody. The secondary antibody is covalently linked to an enzyme or florescent molecule to provide a tool for detection.

22. (3 pts) Baker’s yeast protein coding genes are all given unique open reading frame (ORF) identifiers. Where is ORF **YCL042C** located in the yeast genome? In your answer, provide: the chromosome number, position relative to the centromere & neighboring genes, and whether the gene is transcribed from the top (Watson) strand or bottom (Crick) strand of the yeast chromosome. This is the 42nd open reading frame from the centromere on the left arm of yeast chromosome 3. It is expressed from the Crick strand.

23. (4 pts) What does the term “eipgenetics” mean? Stable gene expression resulting from covalent modification of the DNA (e.g., DNA methylation) or associated chromatin (histone methylation, acetylation, phosphorylation).

24. (2 pts) MALDI-TOF and ESI-MS/MS are two alternative mass spectroscopy approaches applied to proteomic studies. (MALDI-TOF) uses laser excitation to ionize peptides. (ESI-MS/MS circle one) generally provides more detailed peptide information resulting from greater numbers of peptide ionization states.

25. (2 pts) Mass spectra are generally graphed as relative intensity (Y axis) against the m/z ratio (X axis). What values do the “m” and “z” terms define? Mass and charge

26. (3 pts total) Which of the following three strains would be a best choice to express the yeast Pxr1 protein in *E. coli* using the pTXB1 plasmid (1pt)? Explain why this is the best choice (2 pts)

**Strain HG125**: F- λ- fhuA2 [lon] ompT lacZ::T7 gene 1 gal sulA11 Δ(mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10)(TetS) endA1 [dcm]

Only this strain expresses the T7 RNA polymerase gene needed for pTXB1 expression.

27. (2 pts) What are “inclusion bodies” and why are inclusion bodies generally not desirably when trying to purify enzymes? When recombinant proteins expressed in E. coli produce insoluble aggregates. These are typically misfolded and denatured proteins likely to lack enzymatic activity.

28. (5 pts) What is the specific molecular target of each of the following affinity matrixes used for protein purification.

Streptavidin-agarose biotin

Chitin-agarose chitin binding peptide

IgG-agarose protein A

Calmodulin-agarose calmodulin binding domain peptide

Nickel-NTA-agarose His6

39. (6 pts - total) Design an experiment to identify genes acting in the process of pre-mRNA splicing using an **extragenic suppression** approach starting with the *prp38-1* mutant strain, ts192. In your answer, you must clearly:

 1) 1 pts) describe all mutations in any chromosomal or plasmid-based genes used in this study. The description should include the characteristic of the mutant allele (e.g., temperature sensitive, lethal, fully functional, partial loss of activity, etc.)

 2) (1 pt) clearly state what culture conditions (e.g., media type & temperatures) will be used

 3) (2 pts) state how the wildtype alleles of any mutants found in your study will be cloned

 4) (2 pts) state how you will test whether or not the genes found in your study actually encode proteins important for pre-mRNA splicing. Here, it will be important to clearly describe the assay that you will use to learn if the identified gene is required for splicing or not.

See lab PowerPoint slides and assigned reading – in essence, select for spontaneous (or induced) suppressor mutants of ts192 by colony growth at 37C. Identify the subset of recessive mutations by complementation analysis. Clone the responsible wildtype allele by transformation of the suppressor strain with a yeast DNA library of WT DNA, then using replica plating, find transformants that regain the ts phenotype. Recover the plasmids from yeast, amplify in E. coli then sequence the DNA to identify the gene (characterize subclones if more than one gene present). If the newly identified gene is needed for splicing, then loss of function alleles will result in the accumulation of unspliced pre-mRNA. This could be tested by northern blot, for instance, using a geneX::KAN background where a complementing GAL1-GeneX allele is transcriptionally repressed on glucose medium.

30. (2 pts) Provide one biochemical rationale for how “dosage suppression” might work. That is, how might the enhanced expression of one gene suppress the impaired function of a second (i.e. different) gene? If the two factors (A, B) interact to form a critical complex (AB), then decreased complex abundance due to weakened affinity resulting from mutation of one factor (aB) might be offset by increased frequency of interaction due abundance of the other factor (excess B).

31. (3 pts) Offer two specific suggestions on how we can improve the BIO 510 course in future offerings. The suggestions can be for changes in course content or organization but should retain the current class hours and credit. Credit will be given only for positive, thoughtful responses.

4. (3 pts) Col EI is a naturally occurring plasmid of E. coli. Discuss how Col EI replication is regulated to maintain copy number in *E. coli*. Be specific about the roles of trans-acting RNAs and proteins that modulate Col EI replication activity.

8. (5 pts) Design an experiment to purify Prp43-TAP through the tandem affinity purification protocol. Be specific of what resins you will use and any steps needed to release the protein from these resins.

****16 (2pts) Provide the name of each structure and the use of each in our Bio510 experiment

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