**2016 BIO 510 Final** **NAME** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. (7 pts total) Assume that you want to use reverse-transcriptase PCR (rtPCR) measure the efficiency of intron removal from yeast *RPS17a* pre-mRNA. To do this, you need to know the relative amount of the spliced *RPS17a* mRNA (intron removed) and unspliced *RPS17a* pre-mRNA (intron present) in the RNA sample. Your PI wants the PCR step (that is, after cDNA synthesis) to include only 2 oligonucleotide primers, so these must be able to amply both the mRNA and the pre-mRNA with this pair. **The major intron portion of *RPS17a* is shaded in yellow.**

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’

A) (2 pts) Starting with 1 microgram of total yeast nucleic acid isolated by breaking the cells with glass beads. Describe each enzymatic step needed to measure the mRNA/pre-mRNA ratio in this sample by rtPCR. Describe the following: DNaseI treatment to remove genomic DNA; cDNA synthesis with reverse transcriptase, PCR steps with the oligos outlined above to amplify both mRNA and pre-mRNA

B) (2 pts) Use the DNA sequence above to design two 18 nucleotide primers that will simultaneously monitor both the spliced and unspliced *RPS17a* RNA. **UNDERLINE** in the sequence where each primer is located.

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

 Primer 1) any top strand sequence in white. e.g., 5ATCGACTTAATTCTAAGA…..3’

 Primer 2) any bottom strand sequence in white, e.g., 5CCTCTAACTGGACCCTTT3’

C) (2 pts) Your bench mate did the same experiment but forgot to include 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? For your response **draw a diagram** of the predicted gel image of his and your results – be sure to identify each student and label each lane and indicate the predicted positions of the mRNA and pre-mRNA. Use band thickness to indicate relative RNA abundance. Show the separation of pre-mRNA and mRNA. The benchmate’s sample would appear to have more pre-mRNA due to PCR from genomic DNA. Some genomic DNA might also be present.

D) (1 pt) An alternate 3’ splice site is occasionally used in the splicing of *RP51a* pre-mRNA and is shown above in green. If the major *RP51a* spliced product is 850 nts, what is the size of the mRNA when the alternate 3’ splice site is used? It would be shortened by six nucleotides (844)

2. (12 pts) Using the following hybridization data, determine the location and direction of transcription for the three RNA transcripts. 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 transcript extends into an adjacent (second) restriction fragment. ***Put an arrowhead 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 9.2 kb

BamH1-> Cla1 3.1 kb

Msp2-> Pst1 none

BamH1-> Msp2 3.1 kb

Cla1-> BamH1 9.2, 2.1

Pst1->Gas1 3.1 kb

BamHI->TspI none

Msp2->BamH1 2.1 kb

Pst1->Msp2 3.1 kb

GasI->Tsp1 none

Tsp1-> BamH1 2.1 kb

BamH1—2.1>-Pst1------Tsp1--<3.1------------Msp2--------------Gas1------9.2>----------Cla1

3. (4 pts-total) You isolated a yeast gene called *STR1* that encodes a transcription factor. You want to identify other yeast proteins that interact with the Str1 protein using the yeast two-hybrid assay. You have the option to clone the *STR1* open reading frame (ORF) into either the pACT2 vector or the pAS2 vector and have access to yeast cDNA libraries made in either of the complementary vectors (that is, if you choose to clone *STR1* into pACT2, you can use the pAS2-cDNA library to screen for interacting factors and if you clone *STR1* into pAS2, you can use the pACT2-cDNA library to screen for interacting factors).

Which plasmid would be best to clone the *STR1* ORF into, pACT2 or pAS2? Describe the scientific rationale for choosing this vector rather than the other one.

 Since the STR1 product is a transcription factor, it will stimulate transcription anytime brought into the correct DNA context. Since pAS2 is a fusion to the Gal4 DNA binding domain a pAS2-STR1construct is expected to stimulate reporter gene transcription in the absence of a Y2H binding partner. Consequently, you mist clone into pACT2 and use the pAS2 DNA library

4. (2 pts) **Draw a diagram** which illustrates the alternative splicing products of an intron with alternative 5’ splice sites in a gene with a single intron. Label the image fully to show the unspliced pre-mRNA and the two predicted alternatively spliced products. Diagram – two boxes (exons) separated by a line (intron). Use the left hand start of the line as one 5’ splice site & splice to the end of the line (3’ splice site). The alternative 5’ splice site must be upstream (5’) of the 3’ splice site but can reside either in exon I or in the intron.

5. (2 pts) Baker’s yeast protein coding genes are all given a unique open reading frame (ORF) identifier. Where is ORF **YBR0427W** located in the yeast genome? Your answer should provide: the chromosome number, position relative to the centromere and neighboring genes, and whether the gene is transcribed from the top (Watson) strand or bottom (Crick) strand of the yeast chromosome. It is the 427th ORF to the right of the centromere on chromosome II – transcribed from the top strand.

6. (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 & charge

7. (1pt) The alpha (α) phosphate of ATP is:

a) transferred to a lysine residue within T4 DNA ligase protein,

b) transferred to the 5’ phosphate of the DNA strand to be ligated,

e) answers a and b are correct

8. (18 pts) Describe 1) the natural biological substrate (note: **X-gal** is NOT a correct answer for any of these enzymes) and 2) the reaction products for each enzyme:

1. *E. coli* lon gene product – protease that activates other bacterial proteases to degrade denatured or unnatural proteins
2. T7 gene 1 product – viral RNA polymerase that stimulates transcription from T7 promoters
3. Trypsin – low specificity protease that cleaves after lysine and arginine residues
4. RNase H – cleaves RNA strand in DNA/DNA hybrids to release nucleosides or short oligos
5. β- lactamase – cleaves beta lactam ring in penicillin-like compounds (inactivates the drug)
6. β-galactosidase – cleaves lactose to glucose + galactose
7. Terminal deoxynucleotide transferase – non-template directed DNA polymerase acting on free 3’ends of DNA
8. DICER endonuclease – cleaves large dsRNA precursors into 22-27 bp dsRNA
9. ADAR – adenosine deaminase acting on RNA – coverts A to I in the RNA chain

9. The full-length yeast *RP51A* gene was isolated from a yeast genomic DNA library using a DNA hybridization probe.

A) (2 pts) Describe in detail what a genomic DNA library is and how such a library is used to recover a gene as described above. Genomic library is composed of genomic DNA (typically (semi) randomly cut into pieces by shearing or with low specificity enzymes) inserted into a plasmid or viral vector. Describe a nucleic acid hybridization approach to isolate a gene from a collection of E. coli transformants.

B) (3 pts) The yeast *RP51A* gene contains an intron within its open reading frame. Describe in detail every way how the structure of *RP51A* would differ if cloned from a cDNA library rather than a genomic DNA library. The cDNA would lack the RP51A promoter and intron and any regulatory sequence downstream of the poly A site. The cDNA will typically have a poly A tail.

10) (2 pt) Which of the following will likely have a big impact on the electrophoretic mobility of DNA in an agarose gel (circle all that apply)

b) conformational differences in linear and circular forms of the DNA

c) protein bound to the DNA

e) differences in the lengths of the DNA molecules

11. (6 pts - total) Design an experiment to identify genes acting in the process of pre-mRNA splicing using an **dosage suppression** scree. Start with the *prp38-1* mutant strain, ts192. In your answer, you must clearly:

 1 pts) describe all mutations or genetic markers 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.)

Start off by a description of the chromosomal prp38-1 allele. Describe a DNA library to be used that is amplified to high copy number within yeast (or where a gene is driven by the GAL1 promoter – either method works). Note the selectable marker you will use on the plasmid and state that the corresponding chromosomal gene is removed.

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

Easiest to select for the plasmid (on medium lacking uracil, for instance, if a URA3-marked high copy number plasmid is used for the library) at 37C.

 (4 pts) state how you will test whether or not the genes found in your study themselves encode proteins important for pre-mRNA splicing. Here, it will be important to clearly describe the assays that you will use to learn if the identified gene is required for splicing or not. Tell how you would 1) recover the plasmid from yeast, 2) subclone to determine which gene on the library fragment is responsible for dosage suppression, 3) generate conditional lethal mutants of the newly isolated gene & score these in an otherwise WILDTYPE background by northern blot for changes in pre-mRNA accumulation when the product of the newly defined dosage suppressor protein.

12. (4 pts) Provide two biochemical rationales 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? You might 1) stabilize Prp38-1 protein to increase its abundance or promote an active conformation or 2) compensate for a weakened Prp38-1 based protein-protein interaction due to mutation by increasing the abundance of its binding partner.

13 (1 pt). What is the frequency of cleavage expected for each of the following restriction endonucleases? Both are (1/4)6 or 1/4096

**EarI** 5’ CTCTTC(N)13/

 3’ GAGAAG(N)45/

**HinDIII 5’A/AGCTT3’**

 3’TTCGA/A5’

14. (1 pt) What is an intein?

Your answer should describe what biochemical properties are evaluated to define a peptide as being an intein in nature. That is, your response should NOT discuss intein use as applied to our protein purification experiment but describe how a scientist would tell whether or not a newly discovered structure had the characteristic of an intein. An auto-excising peptide segment (cleavage & ligase-like activity) within the body of a larger protein

15 (3 pts total). The intein used in our BIO 510 lab experiment is only partially functional due to a mutation intentionally placed into the corresponding gene.

(1 pt) What feature or features of a natural intein are LOST in the intein used in BIO 510?

One of the two cleavage sites was mutationally inactivated. Under reducing conditions, the remaining site could still be cleaved by the natural pathway to release our recombinant protein.

(2pts) Why was it important to make this change (i.e., mutation) for the success of our experiment? In your answer, fully describe how our results would be expected to differ if a fully functional intein was used. The intein would be removed & most of t the CBD-Spp382(1-121) would remain bound to the column.

16. (2 pts) The RISC complex contains an endonuclease (, Ago2, **circle the correct answer**) that

b) cleaves mRNA bound a siRNA

17. (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: B, C, D, G, H) Negative interactions (E, F, I). **NOTE**: In the diagram below, the top line shows the structure of the entire protein. In constructs B-I (there is no construct A). the **boxes represent the fragments that *ARE EXCLUDED by deletion*** in the yeast two hybrid construct tested. For instance, construct “B” lacks amino acids 25-70 but has the reset of the protein sequences (1-24 and 71-271 all present). This is the same as we interpreted B-I in the BIO 510 lab.

Based on these observations,

A. (3 pts) Which peptide segment of Pxr1 is necessary for interaction with actin? Most likely 101-225

B. (3 pts) Design an experiment to test whether this peptide segment is sufficient for this interaction. Simply clone PXR1 codons 101-225 in one of the Y2H vectors (e.g., pACT2) and clone the full-length actin gene into the second Y2H vector (pAS2). Next, describe how the Y2H assay is performed (e.g., using minus histidine 3AT medium in a background bearing a GAL1-HIS3 reporter).

18. (2 pt) The following statement is (FALSE circle one): The addition of 3 aminotriazole (3 AT) is expected to inhibit the growth of the following yeast strain on YPD (rich) medium. **NOTE**: this is the full genotype of the strain I want you to consider.

Genotype: PJ75-5B ***MATa*** *trpl-901 leu2-3,112 ura3-52 ade2 his3-200 ga14 Δ ga18OΔ GALl-CYC1**GAL2-ADE2 GAL7-lacZ*

Why or why not 3-AT inhibit growth under these conditions? HIS3 gene expression is only needed when cells must make their own histidine for survival. The YPD medium is nutrient rich and supplies all the needed amino acids. Under these conditions, HIS3 gene expression is not relevant.

19. (2 pts) Nitrous acid converts cytosine into (uracil,; circle correct answer) and converts adenine into (hypoxanthine,; circle correct answer).

20. (1 pt) In a standard western blot of yeast proteins (no protein A-tagged proteins present), the secondary antibody is conjugated to an enzyme or a florescent molecular and directly binds:

b) the primary antibody,

21 (4 pts) We used the TAP tag to identify the Tda1-TAP protein band on a western blot and we used the chitin-binding protein/intein fusion to affinity purify Spp382(1-121) protein from E. coli cells. Outline a step-by-step experimental approach to affinity purify the Tda1 protein from a whole cell lysate (that is broken cells) using a yeast strain that expresses Tda1-TAP. Describe the TAP epitope (protein A-TEV-calmodulin binding domain). Break the cells, select recombinant protein on IgG-agarose, elute with TEV protease, select again on calmodulin agarose (calcium present), release purified protein with EGTA.

22. (4 pts). You performed a nitrous acid mutagenesis on pTZ18u and identified one mutant that lost lacZ gene function. You isolated this plasmid and sequenced the lacZ gene and found two different mutations in the same gene. Each mutation results in one amino acid substitution in the β-galactosidase protein, specifically, alanine 76->glycine and aspartic acid 112->proline. Design an experiment that will unambiguously determine if A76G and D112P are individually sufficient to inactivate β-galactosidase or if both A76G and D112P must be present simultaneously in the same protein to inactivate β-galactosidase. Be detailed and specific in how this experiment will be completed. Complete two inverse PCR reactions - 1) introduce the A76G codon change in plasmid one and 2) introduce the D112P codon change in plasmid two. Next transform these two mutants, the appropriate controls (unmutated pTZ18u – expect a signal; A76G and D112P double mutant, expect no signal) into TG1 E. coli. Select on ampicillin plates with IPTG/X-gal to learn if lacZ activity is present or not.

23 (3pts) Provide the name of each structure and the use of each in our Bio510 experiment

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**A** **C**

**B**

DTT, nitrous acid, 5-FOA

24. (EXTRA CREDIT – 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. Lots of good ideas were presented.