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BIO 510 Mid-year Exam 2016 NAME (Please Print)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**1(12 pts)**

The sequence of the *SRF1* gene is presented below. You want to clone the 773 bp shaded region of *SRF1* into the unique SmaI site of the pUC19 vector (**page 357 NEB catalog**). Describe all steps needed to create this recombinant DNA construct.

**(Part A)** Assume that you start with one microgram of the pUC19 vector and 100 nanograms of yeast genomic DNA, a portion of which is shown below. Assume that you have access to any reagents, materials, and machinery that you need. The only exception is that you cannot propose to chemically (that is, non-enzymatically) synthesize the entire *SRF1* gene. **Full credit requires** **that:**

**a) only the desired shaded sequence** is inserted **without any additional sequence**  into the existing SmaI site of pUC19

**b) the desired clone has the insert oriented** such that the insert’s BamHI site (bold & underlined below) is located closest to the pUC19 vector BamHI site and furthest from the pUC19 EcoRI site.

**(Part B)** Tell how you will test your recombinant plasmids to learn which have the correct orientation.

**Discuss in detail** **every enzymatic step** required to accomplish this cloning, you must

**- State which specific enzymes you would use** for each step, name the buffers, tell the temperature and length of incubations, etc.

**- Describe any cleanup steps** needed after one step is completed before the next step is initiated.

**- IMPORTANT: If PCR is to be used**, write out in full the oligonucleotide sequences for both primers to be used for PCR and be sure to mark the 5’-3’ polarity of each.

You need not discuss *E. col*i competent cell preparation, *E. coli* transformation, or any subsequent plasmid “mini-prep” DNA isolation to recover the putative recombinant molecules. However, you must say how you will determine whether or not you have the insert cloned in the correct orientation with respect to the plasmid EcoRI and BamHI sites.

***SRF1***

//…AAAAGTTGGTTTTGGGCAGATCAAAAAACTACGGGCAAAG**ATGTTGGTGGGGCAGCAGTA**

TCATCCATGTCAGGGTGCCCAGTCATGCACGAGTCGTCGTCGTCGTCGCCACCATCCTCT

GAGTGCCCCGTTATGCAGGGAGATAACGATAGAATAAACCCGCTGAACAATATGCCGGAG

TTGGCAGCATCCAAACAGCCTGGCCAAAAGATGGACTTGCCCGTTGATCGGACCATCTCC

GAGCGCAAAGCGGAACAACAACCTCCAACCTTCAAGGAAGTTAGATACGTCTTGGATTTC

TACGGAGGGCCCGACGACGAAAACGGAATGCCTACTTTCCACGTGGATGTCCGTCCTGCC

CTAGATAGTCTAGACAATGCTAAGGACCGGATGACCCGTTTCTTGGACCGGATGATCTCG

GGTCC**GGATCC**CTCTTCGTCCTCCGC….// (**NOTE: BAMHI site is underlined**)

The easiest thing to do is to PCR amplify the shaded region using oligos complementary to the two strands of DNA (upstream 5’**ATGTTGGTGGGGCAGCAGTA3’; downstream 5’AGAGGGATCCGGACC3’).**  The PCR fragment would then typically be purified (on a gel or by a Qiagen column) and phosphorylated with T4 polynucleotide kinase +ATP. The vector should be cut at the SmaI cloning site and then dephosphorylated with shrimp alkaline phosphatase. The insert can be ligated to the vector using T4 DNA ligase then transformed into E. coli. To confirm the orientation of the insert, the recombinant plasmids should be recovered from E. coli then digested with BamHI. The two orientations of the insert will give two different banding patterns, with the wrong orientation essentially excising the insert and the correct orientation essentially linearizing the plasmid.

2. (1 pt) Treatment of DNA with Dam methylase will (**not influence**) the ability EcoRI restriction endonuclease to cleave DNA?

3. ( 1pt) Cleavage by which of the following enzymes produce efficient substrates for exonuclease III? A) KpnI, B) **BamHI, C) SmaI,** D) PstI, or E) ApaI (circle all correct answers)

4) (1pt) Cleavage by which of the following enzymes produces substrates for mung bean nuclease? A**) KpnI, B) EcoRI**, C) SmaI, D) **PstI, or E) ApaI** (circle all correct answers)

**BIO 510 Midterm Exam –Closed book NAME**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. (1pt) The gamma (terminal) 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,

c) released as pyrophosphate,

d) transferred to a lysine residue within *E. coli* DNA ligase protein

e) answers a and b are correct

2. ( 1 pt) The plasmid transformation procedure for *E. coli* and yeast are similar but not identical. Which of the following additions or steps greatly increases yeast transformation efficiency but is expected to inhibit *E. coli* transformation efficiency?

a) heat shock at 42C

**b) addition of 250 micrograms of denatured salmon sperm DNA**

c) use of a selectable marker on the plasmid DNA

d) addition of IPTG and X-Gal to the medium

e) keeping the cells on ice during the initial incubation with DNA

3) (1 pt) The primary feature causing RNA separation during electrophoresis on the denaturing formaldehyde gel we used for our northern blot is:

a) differences in the charge/mass ratios of different RNA molecules

b) differences in the secondary structures of different RNA molecules

c) differences in the number or types of proteins bound to the RNA molecules

**d) differences in the lengths of the different RNA molecules**

e) differences in the amount of formamide bound to the different RNA molecules

4) Yeast strain ts192 contains a temperature sensitive allele, *prp38-1*. At 37C the ts192 strain fails to grow while the wildtype strain grows fine.

A) ( 1 pts) What cellular biological process (for example, transcription, cell wall biosynthesis, translation, etc.) is directly impaired after the ts192 mutant is placed at 37C? Inactivation of Prp38 inhibits pre-mRNA splicing (intron removal).

B) (2 pts) Why does the added heat cause this process to stop in the ts192 strain? Specifically, what molecule is the target (tell me which specific DNA segment or mRNA transcript or protein is sensitive) and in what biochemical way does this target change in response to the increased temperature? This mutation results in an aspartic acid for glycine substitution within the Prp38 protein. This change renders the protein unstable at 37C – with the adopting a normal conformation and active at 23C but denatured and inactive at 37C.

5) The original *PRP38* gene was cloned by screening a yeast genomic DNA library for complementation of the temperature sensitive growth defect.

A) (2 pts) What is a genomic DNA library? A genomic library is a random collection genomic DNA fragments inserted into a vector backbone.

B) (**EXTRA CREDIT** - 2 pts) Assume that you screened the yeast library and found transformants that grow at 37C. Assume such temperature resistant colonies might arise due to two alternative possible mechanisms: 1) the presence of a complementing gene on the transformed plasmid or 2) from a chromosomal event unrelated to the added plasmid. Design an experiment that distinguishes between these two possibilities. That is, design an experiment which will determine whether or not the ability of any individual colony recovered in your selection not only has a plasmid but that growth at 37C is dependent upon the presence of that specific plasmid.

To determine whether the plasmid present in the yeast transformant is conferring the growth at 37C you need to:

 1) IMPORTANT: break open the yeast and recover & amplify the plasmid by transformation into E. coli

 2) transform the naïve (that is, untransformed) ts192 yeast with the recovered transformant

 3) if the plasmid was responsible for the phenotype, then 100% of the transformants should grow at 37. If the plasmid was not responsible, then the yeast will remain temperature sensitive

6) (3 pts) One problem in freezing bacteria is that ice crystals can form during the freezing process and, as water expands upon freezing, the ice causes the cells to lyse (break), killing the bacteria. How did we prevent ice crystal formation during freezing when we prepared TG1 *E. coli* competent cells? The addition of glycerol (and to a lesser extent DMSO) prevents ice crystal formation during freezing.

7. (12 pts) Given the following hybridization data, determine the location and direction of transcription for each cellular mRNA transcript. Assume that no more than one transcript is present in the interval defined by two adjacent restriction sites (that is, EcoRI to PstI, PstI to BglII, BglII to KpnI) and that no transcripts extend into an adjacent fragment. ***Put an arrow head at the 3’ end of the mRNA transcript (5’ -> 3’) and be sure to indicate clearly by a solid line labeled with a transcript length where each transcript resides and its size.***

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

EcoRI –> PstI 4.0

BglII - >KpnI 3.5

KpnI ->BglIII 2.0

Pst-Kpn 3.5

PstI->EcoRI 2.0

EcoRI 🡨4 BglII 🡪2 PstI 🡨3.5 KpnI

8. (2 pts) For our northern blot experiment, which of the following are expected to reduce the stringency of hybridization – that means, which steps are expected to increase the likelihood that the probe will be retained by short regions of base pairing with non-target mRNAs? **CIRCLE ALL THAT APPLY.**

**a) reduce the temperature of the hybridization**, b) add formamide to the hybridization solution, c) remove DTT from the wash solution, **d) increase the sodium chloride concentration in the wash buffer**, e) increase the temperature of the hybridization

9. (1 pt) Treatment of DNA with EcoRI methylase will (increase, **decrease,** or not influence; **circle one**) the ability of the EcoRI restriction endonuclease to cleave genomic DNA?

10 (10 pts) Which of the following would be useful for (put letter alongside protein or process): 1) purification of DNA **B**\_2) creation of unidirectional DNA deletions with Exonuclease III \_D\_, 3) use of EcoRI methylase **C**\_, 4) inhibiting DNase activity during plasmid isolation hybridization, \_**A\_**5) use of *E. coli* DNA ligase\_**E**\_

A)  B)

C)



D)

 E)

11. (5 pts) Starting out with a single double-stranded genomic DNA template and sufficient primers, enzyme and all other co-factors for successful PCR, **draw all products of three (3) full PCR cycles**. Use the diagram below as a starting point for your image. **IMPORTANT:** In your diagram, be sure to clearly show which molecules have PCR primers attached & which are extended beyond the primer binding site. **See lab manual**

12. (2 pts) How may PCR products with ends defined by both primers exist after 4 cycles of PCR using the DNA described in question 11? By this I mean that you count only double stranded molecules that **begin and end** at the primer binding sites but lack any other genomic DNA. For the purposes of this question ignore the terminal transferase activity of Taq DNA polymerase (that is, assume that this activity is not relevant). **2n-2n = 8**

13) In measuring the amount of radioactivity in a sample, we considered both CPM and DPM.

(1 pt) What do the letters in the abbreviations CPM and DPM stand for?

(1 pt) How are CPM values converted into DPM values for any isotope? For this answer, I am not looking for a specific numerical multiplier (i.e., number) – rather, I want an explanation for what principles determine the conversion factor to be used. Counts per minute and Decays (for Disintegrations) per minute. The conversion of CPM->DPM is a function of the efficiency of the scintillation counter to detect the decay; this depends on the isotope use and the sensitivity of the scintillation counter being used,

14. (1 pt) What is the purpose of the scintillation fluid used in our quantification of 32-P incorporation? The scintillation fluid absorbs energy from the radiocucleotide decay and subsequently releases as secondary emission of light that is captured by the photomultiplier tube of the scintillation counter (or by a secondary scintillant first).

15. ( 3 pts) Describe the basis for biochemical separation in the P6 column we used for probe preparation. That is, how does the column “work”? The P6 acrylamide is composed of small gel beads with holes that allow molecules <6,000 Daltons to enter. In our in vitro transcription reaction, unreactive nucleotides enter the bears and are retarded in migration through the beads when spun in the microfuge (the nucleotides remain in the beads). The full length RNA probe exceeds the 6,000 Da limit and flows rapidly through column and is collected in the tube.

16. (18 pts) Describe *in detail* 1) the **substrate** and 2) the **reaction products** for each enzyme:

1. McrA nuclease – ds endonuclease that cleaves CpG methylated DNA
2. T4 DNA ligase – joins 5’ phosphate and 3’ hydroxyl in nicked DNA (compatible sticky ends or blunt ends of dsDNA)
3. Reverse transcriptase – RNA dependent DNA polymerase – produces an RNA/DNA hybrid
4. RNase H – ribonuclease that cleaves RNA in an RNA/DNA hybrid
5. β- lactamase – enzyme that degrades the beta lactam ring of ampicillin (and similar compounds)
6. β-galactosidase – enzyme that cleaves lactose into glucose plus galactose (or cuts X-gal)
7. Terminal deoxynucleotide transferase – adds ssDNA extensions to the free 3’ end of DNA in a template independent manner
8. DICER endonuclease – cleaves dsRNA precursors of siRNA or miRNA to 22-26 nt ds DNA products
9. Mung bean nuclease – cleaves ss regions of DNA or RNA

17. a (1 pt) We did an in-class comparison of linear and supercoiled DNA resolved under different salt conditions. We found that ds linear DNA rather (**faster** in 3 X TAE compared with 1/3 X TAE (that is, high salt compared to low salt).

b. (2 pts) Supercoiled DNA behaved differently than the linear DNA in this assay. Describe how the ***relative mobility*** of supercoiled DNA vs linear DNA changed when the samples were resolved in 3 X TAE vs 1/3X TAE. The supercoiled DNA ran proportionately much faster in high salt than the corresponding linear DNA indicating that the increased salt helped compress the supercoils further (by neutralizing charge repulsion in the phosphodiester backbone)

18. (2 pts) Which of the following steps is expected to increase the number of yeast transformants of YCplac33? (circle all that apply)

a) heating the YCplac33 plasmid DNA at 100C for 10 minutes prior to the transformation, b) addition of ampicillin to the selective agar medium, c) addition of 5-fluoro-orotic acid (5FOA) to the selection medium, **d) addition of PEG to the DNA + cells transformation mix, e) heat shock the transformed cells at 42C for 15 minutes prior to platin**g, f) none of the above

19. (3 pts) Mark each of the following genes/reagents as useful in a i) selection procedure or ii) screening procedure

A. addition of IPTG & X-gal to bacterial plates\_\_screen\_\_\_\_

B use of yeast medium lacking leucine in our YCplac33 plasmid transformation selection

C addition of ampicillin to our bacterial plates selection

20. (2 pts) What was the purpose of adding salmon sperm DNA to your northern blot hybridization experiment (be specific). The salmon sperm DNA covalently binds the membrane surface not already bound by the transferred yeast RNA. This “blocks” our radiolabeled RNA probe from binding directly to the membrane (so the probe is retained only where it can base pair with RNA present on the membrane).

21. (3 pts) The EcoK restriction modification system is composed of 3 protein-coding genes, *hsdM, hsdR, hsdS*. What is the function of each of the encoded proteins? Be specific & define terms as needed.

*hsdM, encodes the DNA modification (methylation) subunit*

*hsdR, encodes the DNA restriction endonuclease subunit*

*hsdS*, encodes the specificity factor needed to direct the methylation and endonuclease subunits to the correct DNA sequence

22. (3 pts) Dr. Nubitz suspects that the rat Htep cell line expresses 15-fold more Blt1 mRNA than found in rat liver, kidney, heart or brain. Describe in detail an experiment to test this hypothesis using an **RNase protection** assay. In your response, describe how the experiment is performed, discuss any reagents or enzymes to be used, show the results will be obtained, and how you will interpret your results. Draw a diagram to illustrate what the results of a successful experiment might look like.

**IMPORTANT:** The transcribed region of Blt1 is shown in yellow. For simplicity, make a probe 20 nts in length ***Show the RNA transcript you will make by underlining the appropriate 20 nt region of the Blt1 locus below***.

5’AGCATCCCCAAGAGTCC**AGACAGTAACGAGTTCTGGGAGTATCCT**TCTCCACAACAGATG

TACAATGCTATGGTTAGAAAGGGCAAGATTGGCGGTAGCGGCGAAGTCGCCGAAGATGCA

GTGGAGTCCATGGTGCAGGTCCACAACTTTCTAAATGAAGGGTGCTGGCAGGAAGTGCTC

GAATGGGAAAAACCGCACACAGATGAAAGCCACGTGCAGCCTAAGTTGCTGAAATTCATG

GGGAAACCGGGCGTATTGAGCCCTCGTGCTCGCTGGATGCACCTGTGCGGCCTACTGTTT

CCGTCCCATTTTAGCCAAGAACTACCATTCGACAGGCACGACTGGATTGTACTCCGAGGC 3’

Prepare a radioactive single stranded antisense probe. The probe must be longer than the target mRNA. Here I underline a region that extends outside of the transcribed region to indicate this. The probe can be made in a number of ways (chemically synthesized or prepared as a T7 transcript as we did by cloning into pTZ18u).

Isolate total RNA from the appropriate tissues. Hybridize the radiolabeled ssRNA probe with the tissue RNA to form RNA/RNA hybrids. Add RNase A or S1 to the hybridization to degrade all unhybridized probe and to trim the probe down to the corresponding mRNA coverage area (needs to be done to know that the RNase actually worked in the experiment). Resolve the RNA products on a polyacrylamide gel and determine the intensity of the bands using a phosphoimager or X-ray film. See Friday PowerPoint slides for representative image.

23. (2 pts) How did you make use of the oxidation/reduction of Eu and of BaFBr to acquire data from your northern blot experiment? The radiation emission causes oxidation of the Eu and reduction of the BaFBr on the phosphoimager screen (the “plastic” film we put in contact with the membrane) – this amount of this oxidation/reduction depends on the amount of radiation in the sample. The phosphoimager red laser (635 nm) scans the screen and the laser reverses the oxidation/reduction resulting in the release of blue light at 390 nm which is read and quantified by the phosphoimager. How much blue light is released is proportional to the amount of radioactivity in your sample.