**BIO 510 Exam I –open book (NEB catalog only) NAME (please print)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. (2.5 pts) You want to use PCR to produce a linear double-stranded DNA. Both oligonucleotides primers for PCR must begin (at the 5’ ends) with the same 6 base pair restriction endonuclease site. After gel purification you need to cut the PCR product with the restriction enzyme that recognizes the sequence present in the oligonucleotide-defined DNA ends. You have the option to use the either ApaI, HindIII, NcoI, or SalI restriction sites in the oligonucleotide primers. Assume that each of these primers works efficiently for PCR

 A. Which enzyme would be the best to use for your cloning experiment? Apa1

 B. Why would you select this enzyme? You need to use an enzyme that can cleave a restriction site at the very ends of a DNA molecule. **NEB page 330 chart shows that only Apa1 is efficient at cleaving near the end of DNA molecules. Source: lecture topic & NEB catalog**

2. (2.5 pts) Dr. Rapidpace wants you to prepare 1 microgram of linear pTXB1 plasmid (page 348). You have only one unit of enzyme and one microgram of supercoiled plasmid and must have the linear DNA ready in 1 hour You have the option to use any ONE of the following enzymes: EcoRI, SalI, XhoI or SapI.

 A. Which enzyme would be the best to use? SapI

 B. Why would you select this enzyme? You need an enzyme that is efficient at cutting supercoiled DNA. **NEB page 310 chart shows that only SapI is efficient at cutting supercoiled DNA. Source: lecture topic & NEB catalog**

3. (2.5 pts) You need to ligate a linear plasmid DNA with a DNA fragment. Both DNAs were cut with the same restriction endonuclease. However, you ran out of phenol and have no chemical of enzymatic substitute to remove your restriction endonuclease from the DNA digestion. You have the option of using any one of the following enzymes for cleavage: ApeKI, BcoDI, HinDIII or BglII. Assume that a) each of the four restriction endonucleases produces plasmid & insert DNA with appropriate ends for ligation (that is, the digestion pattern is fine for your experiment and b) any one of the four restriction enzyme digestion buffers will be compatible with T4 ligase activity by the simple addition of 0.5 mM ATP.

 A. Which enzyme would be the best to use? HindIII

 B. Why would you select this enzyme? You need an enzyme that you can temperature inactivate prior to adding DNA ligase. **NEB charts on pp278-281 show that only HindIII can be readily temperature inactivated. Source: lecture topic & NEB catalog**

4. (3 pts) You want to convert plasmid pUC19 (page 350) into a vector that can be used to produce single-stranded DNA and transform both yeast and E. coli. What genes or other DNA elements must be added to pUC19 to accomplish this? Be specific in your answer (e.g., provide the names of any genes you want to add).

For single-stranded DNA production, you need to provide a single stranded DNA replication origin such as the fd origin used in pTZ18u (or an M13 origin). For use in yeast, you need to provide a selectable marker (e.g., URA3) as well as a yeast origin of replication (such as present on the two micron circle). **Source: lab exercise & assigned reading**

5. (2.5) You want to clone human genomic DNA into plasmid pUC19 and transform E. coli. You can use either strain BR111 (F’ ΔproAB, thi-1) BR222 (ΔproAB, thi-1), BR333 (ΔproAB, thi-1, ΔhsdR) or BR444 (F’ΔproAB, thi-1, ΔDam). Note: the strain names are made up (not in the NEB catalog) and the symbol “Δ” means that the indicated gene is fully deleted.

 A. Which E. coli strain would be the best to use? BR333

 B. Why would you select this strain? ΔhsdR mutation will prevent cleavage of DNA unmethylated at the EcoK consensus. **Source: lecture, NEB catalog, assigned reading**

**BIO 510 Exam I –closed book NAME (please print)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. (2 pts) Define “ionizing radiation”. Be specific in your response & be sure to tell what is ionized. Ionizing radiation is a nuclear decay that can stimulate the removal of an electron from a second atom that it hits – thus ionizing the second atom by removing a negative charge. **Source: lecture topic**

2. (1 pt) Provide one example of ionizing radiation. Examples include X-rays, beta particles, other types of nuclear decays. **Source: lecture topic & lab exercise**

3. (2 pts) Very thin high density foils (such as lead) absorb the high energy (beta, particle) emitted from 32P but are not recommended for use as shielding due to the resulting Bremsstrahlung radiation. What is Bremsstrahlung radiation? This refers to (dangerous & penetrating) X-rays released from lead due to beta particle bombardment. **Source: lecture topic, old exam**

4. (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? We added glycerol and DMSO as cryoprotectants. **Source: lab topic & exercise**

5. (5 pts) Describe the reaction mechanism of T4 DNA ligase. Be detailed in your answer and be sure to describe the specific function of ATP. First, DNA ligase binds the ATP and undergoes a covalent modification by transferring AMP to the protein and releasing PPi. Next, the ligase-AMP molecule transfers the AMP to the 5’ phosphate at the end of a DNA molecule, reconstituting a high energy bond. Finally, the 3’hydrozyl of an adjacent DNA strand attacks this diphoshate bond releasing AMP and forming the phosphodiester linkage to seal the DNA backbone. **Source: lecture, assigned reading, lab topic & exercise**

6. (2 pts) What is the role of the buffer (such as Tris-HCl) in a restriction enzyme’s digestion solution? To maintain the pH of the solution. **Source: lab topic & reading**

7. (3) Discuss three different properties of a buffer that you would consider before using it in an enzymatic reaction. Assume that the buffer is pure and not contaminated with any other materials. The pKa (or the buffering range), whether the buffer is stable under the conditions of interaction, whether the buffer adversely chemically interacts with any of the reactants (and other possibilities). **Source: lab topic & reading**

8. (1 pt) You want to minimize (that is, reduce) the difference in mobility between supercoiled DNA and linear DNA of the same length. To do this, you might run an agarose gel in (**0.3X**) TAE buffer. **Source: lab topic & exercise**

9. (3 pts) Three bench mates independently transformed *E. coli* with 10 ng of ligated pTZ18u DNA and obtained the following results on ampicillin plates plated with the same amount of cells & DNA:

Bob: blue colonies (42), white colonies (645)

Cheryl: blue colonies (197), white colonies (490)

Ted: blue colonies (687), white colonies (390)

Assume that for all three students, 100% of the DNA was ligated into circular forms prior to transformation. Which student had the best *transformation efficiency*? What is your rationale for this selection (that is, why did you select this student)?

Transformation efficiency is defined as the number of transformants per microgram of DNA. Ted had the greatest number of transformants & hence the greatest transformation efficiency. NOTE: Bob had the greatest % of white colonies but both white and blue colonies are transformants. **Source: lab topic & exercise**

10. (12 pts) Given the following hybridization data, determine the location and direction of transcription for each RNA 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 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 1.5

BglII - >KpnI no bands

KpnI ->BglIII 0.79, 1.8

Pst-Kpn no bands

PstI->EcoRI 0.79

EcoRI 🡨--1.5------BglII -----0.79-----🡪PstI ---1,8----🡪 KpnI

**Source: lab topic and exercise; old exam**

11. (8 pts) Match the solution below with the letter best corresponding to function in the E. coli miniprep procedure. **Match each solution with only one function by placing the correct number along the function.**.

1) 200 mM NaOH, 1% SDS

2) 1 ml 100% ethanol

3) 100 µl 50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl, pH 8.0)

4) 300 µl phenol:chloroform:isolamy alcohol (50:49:1)

5) 150 µl 3M potassium acetate (made by mixing 300 ml of 5M potassium acetate with 57.5 ml of glacial acetic acid and 142.5 ml of sterile water).

6) 1 ml 80% ethanol

7) 30 µl TE with RNase A

8) 200 µl chloroform

a) dissolves DNA pellet and degrades co-purifying RNA\_7\_\_

b) removes residual phenol from the aqueous layer\_\_8\_

c) lyses the E. coli cells\_1\_\_

d) precipitates the nucleic acids\_\_2\_

e) removes residual salt from the ethanol precipitate\_\_6\_

f) resuspends the cells in an osmotically stable environment before lysis\_3\_\_

g) removes protein in a two-phase partition with the cell lysate\_4\_\_

h) precipitates proteins for removal before to the use of organic solvents\_5\_\_

**Source: lab topic & exercise**

12. (2 pts) For our northern blot experiment, which of the following are expected to increase the stringency of hybridization – that means, which steps are expected to reduce the likelihood that the probe will be retained by short regions of base pairing with non-target mRNAs? **CIRCLE ALL THAT APPLY .**ii., add formamide to the hybridization solution, v increase the temperature of the hybridization**. Source: lab topic & exercise**

13. (1 pt) Treatment of DNA with EcoRI methylase will (decrease) the ability EcoRI restriction endonuclease to cleave DNA? **Source: lecture topic and reading**

14. (4 pts) In what way are bacterial restriction/modification systems similar to the immune system of mammals? The immune system recognizes self from non-self and acts to remove potential pathogens from the bloodstream by using antibodies and specialized white blood cells to bind the pathogen and destroy it (thus saving the host). The restriction modification system of bacterial also code DNA as self (through site-specific methylation). Non-self DNA (e.g., unmethylated or inappropriately methylated viral DNA) is destroyed by the restriction endonucleases thus saving the host (similar to the immune response). **Source: lecture topic & reading**

15. (14 pts) Which of the following would be useful for (**put letter alongside process**): 1) purification of DNA \_A\_\_2) creation of unidirectional DNA deletions with Exonuclease III \_\_D\_\_, 3) use of EcoRI methylase \_C\_\_\_, 4) suppressing background in your northern blot hybridization, \_B\_\_\_\_5) use of E. coli DNA ligase\_\_E\_\_\_\_ 6) staining DNA for UV visualization\_\_\_G\_\_\_\_, 7) transcriptional induction of the lacZ gene \_\_F\_\_\_\_

A) B)





 C) D)

E. F. G. 

**Source: lab topic & exercise; lecture topic; old exam**

16. (5 pts) Describe *in detail* the substrate and reaction products of each enzyme:

1. McrB/C nuclease: CpG methylated ds DNA is cleaved at sites of modification.
2. T4 RNA ligase: Single stranded RNA is covalently joined – requires 5’ phosphate and 3’ hydroxyl
3. DNA gyrase: ATP-dependent type II topoisomerase that introduces negative supercoils into DNA
4. DICER nuclease: Double stranded RNA endonuclease generates short (21-24) bp ds`RNA fragments in the RNAi response.
5. Mung bean nuclease: Cleaves single stranded DNA or DNA; removes sticky ends DNA fragments

**Source: lecture & lab topic & reading**

17. (2 pts) Starting out with a single double-stranded genomic DNA template and sufficient primers, enzyme and all other co-factors for successful PCR, predict how may PCR products with ends defined by both primers exist after 4 cycles of PCR? 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). Show your work (that is, how you arrived at this answer). 2n-2N = 8

**Source: lecture topic & reading**

18. (2 pts) You calculated the change in Gibbs free energy value ΔG associated with the folded structure of a natural tRNA and one that you arbitrarily altered by nucleotide substitution. You found that the ΔG value (increased **circle the correct answer**) after nucleotide substitution indicating that the “mutated” version was (less; **circle the correct answer**) stable.

**Source: homework and lab discussions**

19. (2 pts) Define each of the terms (that is, what do the letters stand for) in the following equation for the Gibbs free energy calculation:

ΔG = ΔH- T ΔS

ΔG = change in Gibbs free energy

ΔH = change in enthalpy

T = temperature in degrees Kelvin

ΔS = change in entropy

**Source: homework and lab discussions**

20. (5 pts) When using *C. elegans* in an RNAi experiment, we simply added *E. coli* to the nematode. (5 pts) How did the *E. coli* addition direct degradation of specific target mRNAs (e.g., *CLF1, DPY-1* etc.) within the animal? In your response describe both the role of the *E. coli* and the mechanism of RNAi within the *C. elegans* animal.

The HT115 bacteria have a IPTG inducible T7 RNA polymerase gene and are transformed with the L4440 plasmid which expresses one of the *C. elegans* target gene as dsRNA from flanking T7 promoters. The HT115 expressing the dsRNA are eaten by the C. elegans. Once ingested, the dsRNA is released from the bacteria and enters the cells of the nematode where it is cleaved to 21-24 bp fragments by DICER and then loaded into the RISC complex which binds to and degrades the cellular mRNA corresponding to the target gene.

**Source: lab topic & exercise. Lecture and assigned reading; old exam**

21. (2 pts) Match the RNAi knockdown target with the best description of the observed phenotype compared to the control. Each gene gets a phenotype and each phenotype should be used only once.

Target: a) *CLF1\_\_1\_\_\_\_\_, b) unc-22\_\_3\_\_\_\_, c) dpy-1 \_\_4\_\_\_\_, d) L4440 \_\_2\_\_\_\_*

Phenotype: 1. Great lethality (death) in second generation, 2) no change, 3) twitchy movement and animals held in clusters (look like they were stuck together), 4) short, thick-bodied adults

**Source: lab topic & exercise**

22. (2 pts) In measuring the amount of radioactivity in a sample, we considered both CPM and DPM.

What do the letters in the abbreviations CPM and DPM stand for? Counts per minute (CPM) and decays per minute (DPM)

How are CPM values related to DPM values? DPM are the actual number of nuclear disintegrations that occur each minute. The CPM are the disintegrations detected by a radiation monitoring device. **Source: lab topic & lab exercise**

23. (1 pts) Lecture FALSE (**circle one**). ATP is a required co-factor for *E. coli* DNA ligase (T4 DNA ligase uses ATP, *E. coli* ligase uses NAD). **Source: lecture topic**

24 (3 pts) Discuss three product features that should be considered when comparing competing reagents for use in the BIO 510 lab (as we did when considering the use of ethidium bromide vs Sybr Safe. Toxicity, cost, sensitivity. **Source: lab topic & exercise, old exam**