BIOL330 | Final Exam | Name:
Fall 2010 | | Lab Section: W Th

* Numerical answers without evidence of how you calculated them will earn half credit (I must see how you found the answer).
* Volumes meant for pipetting in the lab should reflect the correct number of significant figures.
* Be neat to earn maximum partial credit. Be sure the answer is obvious to ME.
* Final answers should NOT be in scientific notation. Write out the numbers.

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1. From stock solutions of 15X SSC, 13% SDS, 18 mg/ml RNase, and 1.7M Tris make 650 ml of a solution containing 40ug/ml RNase, 1.5% SDS, 5X SSC, and 3.5 mM Tris.

2. How much RNase (12mg/ml) do I add to 450µl to reach 35µg/ml?

3. How would you make 500ml of a 125mM NaOH (mw = 40g)?

4. How would you make up 75ml of a 0.65% agarose solution?

5. Show how to setup a digest of 7.4µg of DNA (350µg/ml) with Sfu I (8U/µl) in 35µl.

6. If a UV-Visible spectrophotometer gives the following absorbencies for 15-fold dilution of a DNA solution, tell about the original DNA: A234 = 0.169, A280 = 0.147, A260 = 0.273. Keep 3 decimals in the answers.

7. Each of 7 PCR reaction has a volume of 30µl and will require 10µl of a master mix that will produce final concentrations of 200µM dNTPs (from 2.5mM), 2.5mM MgCl2 (from 20mM), 1.25 Units of Taq Polymerase (from 5U/µl), and 1X buffer (from 10X). Make up the MM table to deliver these reagents properly.

8. Set up a pipetting table to test the effect of different amounts of Taq DNA polymerase on PCR. Test 0.5, 1, 2, 4 Units of *Taq* per reaction (from a 5U/µl stock). Each reaction will have 15µl master mix, 2ng of *V.fischeri* DNA (from 75ng/µl), and 0.24µM (from 5µM) each primer (lux 7 and lux 8 primers) in a total PCR volume of 40µl. This problem has nothing to do with the previous master mix problem.

9. Write down the lane information for the PCR experiment above (Q8) as you would on a blue sheet:

Lane 1

Lane 2

Lane 3

Lane 4

Lane 5

Lane 6

10. What is the stringency (high or low) of the wash condition using a hybridization of an 80b prolactin gene probe that is 52% G+C. The wash is at 64°C with 2X SSC (20X = 2M NaCl + 0.6M $Na_3$Citrate) and 24% formamide.

**Tm = 81.5°C + 16.6(log M) + 0.41(%GC) – 0.62 (%formamide) - 500/L**

11. Conversions: 2.4 ng/μl = ______________mg/ml

0.5 U/ng = _______________ U/μg

12. A 40μl PCR reaction with 0.24μM primer "A" has how many pmoles of that primer?

13. Draw the following reactions as you would expect to see them on a gel. pGEM is 3200 bases.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Lane 1 λ DNA, Hind III | (-) | | | | | | | | |
| Lane 2 Human genomic DNA in TE | | | | | | | | | |
| Lane 3 Human genomic DNA, Hind III | | | | | | | | | |
| Lane 4 pGEM/Sal I, 1900b PCR amplicon | | | | | | | | | |
| Lane 5 Same as Lane 4, after ligation | | | | | | | | | |
| Lane 6 Amplicons, 6500b and 3900b | | | | | | | | | |
| Lane 7 pGEM | (+) | | | | | | | | |
| Lane 8 λ DNA | | | | | | | | | |

14. How much of an 8X loading dye would you add to 15μl of solution to be loaded in a gel?

15. What is the exact formula for TE? <u>How exactly does this solution protect DNA?</u>

Match the enzymes to the statements. Answers may be used more than once or not at all.
A. Luciferase B. DNase C. RNase D. β lactamase E. Proteinase K F. *Sal* I
G. *Taq* Polymerase H. Ligase I. β Galactosidase J. Lysozyme K. Peroxidase

__________ 16. Breaks peptide bonds. Put all that apply.

__________ 17. EDTA would stop this (these) from working. Think. Put all that apply.

__________ 18. Coded for on pGEM. Put all that apply.

__________ 19. Breaks phosphodiester bonds. Put all that apply.

__________ 19. Makes phosphodiester bonds. Put all that apply.

20. Number the following as they would occur for Southern blotting and probing. Think through the entire process because many steps have been omitted.

_____ DNA bound to Nylon _____ Hydrogen Peroxide destroyed _____ Probe binds

_____ Biotin-Streptavidin bind _____ DNA moves to + pole

_____ TMB reduced _____ NaOH makes ssDNA

21. Tell me what the following settings indicate on each Pipetman Pipet.

| P10 | P1000 | P100 |
|---|---|---|
| 1 | 0 | 0 |
| 1 | 3 | 7 |
| 2….1 | 1….0 | 9….8 |
| ^ | ^ | ^ |

Meaning:

Match the following as they relate to alpha-complementation and cloning:
A. LacZα B. LacZΔM15 C. X-gal D. BCIP E. Ampicillin

_____ 22. pGEM with an insert disrupts this.

_____ 23. The substrate for β Galactosidase.

_____ 24. Bacteria with β lactamase are selected for by this.

25. Name each of the three steps in a PCR cycles, give the approximate temperature, and tell what is happening during that step.

| Name of Step | Temp | What is happening |
|---|---|---|
| | | |

1.

2.

3.

26. A) What does it mean to make bacteria “competent” and B) why did we need to do it to a special laboratory strain of bacteria rather than ordinary bacteria.

27. If our DNA quantification by UV spectroscopy indicated very low purity of DNA and significant protein contamination, here’s what I would do to clean up the DNA (currently in TE). Tell me why each step is necessary and why each reagent is there.

A. Proteinase K at 50°C

B. Phenol:

C. Chloroform:Isa

D. 1/10 vol Sodium Acetate

E. 2 vol cold ETOH

F. TE