BIOL 352 Lab Practicum II – Study Guide

REVIEW DAY/OPEN LAB – Friday, December 1, 2006

Please utilize this time to review all the demos.

There are 3 additional study guides on the class website (http://www.unlv.edu/staff/wmojica/). I strongly recommend that you study these thoroughly. It might be helpful to refer to these study guides while looking at the demos.

- Practical II General Review Guide
- Practical II Tests
- Study Photos for Practical II

Review the lab manual, lab handouts, quizzes and the lab reports, especially the review questions!!

The following questions cover only the following exercises:
- Polymerase Chain Reaction (study the PCR Handout)
- Bacterial Transformation
- Purification and Identification of Plasmid DNA

******************************************************************************

1. Polymerase chain reaction is a method used to amplify a specific DNA sequence in vitro by repeated cycles of synthesis. Each PCR “cycle” involves 3 steps. Complete the following table with information pertinent to each step.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>What Happens</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. What are the three items that should be added in excess at the start of the PCR process?
   i. 
   ii. 
   iii. 

3. Why is a primer needed at each “end” of the DNA to be amplified?

4. Suppose that you want to amplify the following DNA sequence using PCR. First, you have to prepare two oligonucleotide primers – reverse and forward. If you have all the necessary raw material, including all four dNTPs, what would be your two primers?

   5’ AAGTCCACCGTAAAGCGCCCTAAATGCTTAAG 3’

   3’ TTCAGGTGGCAATTCCGCGGATTTAGGCGGGATTACGAATTC 5’
5. Why is PCR usually performed with Taq (*Thermus aquaticus*) DNA polymerase?

6. What other reagents are necessary for PCR and why?

7. What are some applications of PCR?

8. What is the most common way of seeing the results of a PCR?

9. Gel electrophoresis is a powerful tool for the separation of macromolecules. Once electric current is applied, what characteristics determine the rate of migration of a DNA molecule?

10. The following figure shows an agarose gel. Three fragments of DNA (A, B, and C) have been loaded into wells toward the top of the gel, and the positive pole of the electric field is at the bottom. The sizes of the three DNA fragments are indicated below.

   (a) After a defined period of migration time, where will you see the three DNA fragments?

<table>
<thead>
<tr>
<th>DNA Fragment</th>
<th>Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4000</td>
</tr>
<tr>
<td>B</td>
<td>500</td>
</tr>
<tr>
<td>C</td>
<td>1800</td>
</tr>
</tbody>
</table>

   (b) What dye is usually used to stain the above gel and what would you do to get an image of the DNA bands?

11. Define genetic transformation.

12. What is a plasmid?

13. A cell that is able to take up a molecule of DNA and be transformed is said to be _________________. (Fill in the blank)

14. Relatively few species of bacteria can be naturally transformed. In the lab, what methods did you use to artificially induce competence in *Escherichia coli* cells?

15. In the pAMP plasmid, why is the beta-lactamase gene called a selectable marker?

16. Calculate the transformation efficiency of the following pAMP transformation experiment.

   - Total number of colonies growing on the [+] plasmid, LB] plate = 240
   - Total number of colonies growing on the [+] plasmid, LB/Ampicillin] plate = 75
   - The total volume of plasmid used = 10 μl
   - Concentration of the plasmid used = 0.005 μg/μl
   - The volume of CaCl₂ solution used = 200 μl
   - Volume of LB broth used = 200 μl
   - Volume spread on each plate = 50μl
17. You are given the following plasmid and culture of competent bacteria and asked to perform a transformation experiment. The bacteria are auxotrophic (a mutant bacterium that lacks the ability to synthesize an essential nutrient and must obtain it from the surrounding) that requires the amino acids Throneine (Thr) and Leucine (Leu) to grow. These bacteria, however, produces Histidine (His) and Methionine (Met), two other amino acids required for its growth. Plasmid A contains the genes that codes for Throneine (Thr) and Leucine (Leu).

Plasmid A – Thr+, Leu+
Competent bacteria – His+, Met+

(a) Upon performing the transformation experiment, you plate the cultures (+ plasmid A and – plasmid A) on glucose-minimal salt agar plates lacking/containing the above-mentioned amino acids. Predict the outcome and explain your answer.

Note: +Thr means that the amino acid threonine was added to the glucose-minimal salt medium. –Thr means that the amino acid threonine was NOT added to the glucose-minimal salt medium.

<table>
<thead>
<tr>
<th></th>
<th>- Plasmid A</th>
<th>+ Plasmid A</th>
<th>- Plasmid A</th>
<th>+ Plasmid A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+Thr, +Leu</td>
<td>+Thr, +Leu</td>
<td>-Thr, -Leu</td>
<td>-Thr, -Leu</td>
</tr>
<tr>
<td></td>
<td>-His, -Met</td>
<td>-His, -Met</td>
<td>-His, -Met</td>
<td>-His, -Met</td>
</tr>
</tbody>
</table>

(b) Use the following data to calculate the transformation efficiency.

The number of colonies observed on the [+ Plasmid A, -Thr, -Leu, -His, -Met] plate = 120
The total mass of plasmid used = 0.05 μg
The total mass of plasmid in the cell suspension spread = 0.01 μg
The fraction of plasmid spread = 0.2

18. What is achieved at the end of the “miniprep” (minipreparation) procedure?

19. Explain the molecular and biochemical effects of each of the following reagents used in the miniprep protocol.
   (a) Glucose-Tris-EDTA
   (b) SDS-Sodium Hydroxide
   (c) Potassium acetate/acetic acid
   (d) Isopropanol
   (e) Ethanol
   (f) Tris-EDTA
20. What are restriction endonucleases?

21. (a) Which of the following, that when combined with its complement, could be clipped by a restriction endonuclease?
   i. ATCGATCGTAGCTAGC  iii. GAATTC
   ii. AAGCTTTTCGAA   iv. ACCATTGGTA

   (b) What is such a sequence called?

22. If you digest the following plasmid (4 kilo bases) using the endonucleases EcoRI and HindIII, how many fragments will you get? Label the fragments and state the size of each fragment. Note: The arrows define sites cleaved by various restriction endonucleases. The number between two arrows identifies the size of that section.

   - EcoRI
   - HindIII
   - HaeIII

   1.6 kb
   0.8 kb
   4 kb
   1.2 kb

23. Following is a DNA fingerprint used in a “crime” trial. Lane 1 is the control lane with known markers. Lane 2 contains an evidence sample taken from the victim. Lanes 3, 4, and 5 contain samples taken from 3 different suspects.

   Which individual committed the “crime?”
   A. Suspect I
   B. Suspect 2
   C. Suspect 3
   D. Neither of them