Improving Student Outcomes with an Adaptable Molecular Cloning Course-Based Undergraduate Research Experience

Post-Questionnaire

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For each of the following questions, select the best answer based on your knowledge of molecular biology and molecular cloning. Bubble the letter corresponding to your choice on your Akindi scantron.

1. A PCR reaction mixture does *not* include:

    a. all four deoxynucleoside triphosphates.
    b. DNA containing the sequence to be amplified.
    c. DNA ligase.
    d. heat-stable DNA polymerase.
    e. oligonucleotide primer(s).

2. Which of the following statements about PCR reactions is *not true*?

    a. It is not until the third cycle of PCR that a duplex product of the correct length is produced.
    b. Bases that were not present in the template, but that are present in the primer, can appear in the PCR amplicon.
    c. PCR amplicons are always linear.
    d. PCR amplicons can be circular. In other words, a circular plasmid with no nicks in the backbone can be produced with PCR.
    e. Both circular and linear DNA molecules can be utilized as a template for PCR.

3. What is the purpose of a DpnI restriction digest on the PCR-amplified vector sequence, prior to a Gibson Assembly reaction?

    a. To purify the amplicon from the PCR reaction components such as the DNA polymerase
    b. To fragment the linear amplicon into small pieces which aids in the efficiency of Gibson Assembly
    c. To reduce the number of false positives in antibiotic selection that result from presence of circular vector template
    d. To make the linear vector amplicon have greater homology to the gene insert

4. In a molecular cloning reaction with Gibson Assembly, the three enzymes required are:

    a. RNA polymerase, an endonuclease, and DNA ligase
    b. DNA polymerase, a 5' exonuclease, and DNA ligase
    c. DNA polymerase, a 3' exonuclease, and DNA ligase
    d. DNA polymerase, an endonuclease, and an exonuclease

5. In a molecular cloning reaction with Gibson Assembly, the purpose of having homologous regions on the vector and insert PCR amplicons is:

    a. To allow for recombination between double stranded regions of a vector and insert that are homologous
    b. To allow for annealing between long overhangs/'sticky ends' between vector and insert, which leads to assembly of a recombinant circular plasmid
    c. To allow for annealing between double stranded regions of a vector and insert that are homologous
    d. To allow for an endonuclease to recognize the same sequence in both a vector and insert

6. Gibson Assembly reactions are incubated at 50°C for 15 minutes. At the end of this incubation, which of the following is most likely to be true?

    a. The Gibson reaction mixture will contain only the recombinant plasmid of interest
    b. The Gibson reaction mixture may contain the recombinant plasmid of interest in addition to linear vector and linear insert with long overhangs/'sticky ends'
    c. The Gibson reaction mixture will contain only linear fragments with long overhangs/'sticky ends'
    d. The Gibson reaction mixture will contain only blunt-ended linear vector and blunt-ended linear insert

7. Which of the following features of a cloning vector is utilized to select for cells that take up DNA in a DNA transformation?

    a. Multiple cloning site
    b. Origin of replication.
    c. Antibiotic resistance marker(s)
    d. Ribosome binding site
    e. *lacZ*α gene

8. In blue-white screening utilizing a vector with a *lacZα* gene, which of the following is *not* true?

    a. The competent cell line utilized must contain a deletion of the gene encoding the *lacZα* subunit
    b. The molecule X-Gal is colorless when it is added to the agar media
    c. The molecule IPTG is required in the media to induce transcription of the *lacZα* gene in the vector backbone
    d. Colonies that contain cells transformed with recombinant plasmid containing gene of interest within the *lacZα* gene will appear blue on media with X-Gal and IPTG

9. If a vector and insert do not have necessary homologous regions for Gibson Assembly cloning, how are they typically installed?

    a. With primers and polymerase during the Gibson Assembly reaction
    b. With primers and DNA polymerase during PCR prior to Gibson Assembly
    c. With an exonuclease during the Gibson Assembly reaction
    d. With primers and a restriction enzyme prior to Gibson Assembly

10. Which of the following statements is true about insert and vector sequences that can be utilized in Gibson Assembly?

    a. Both the vector and insert must be circular, double stranded sequences
    b. The vector must be a circular double stranded sequence, and the insert must be a linear double stranded sequence
    c. The vector must be a linear double stranded sequence, and the insert must be a circular double stranded sequence
    d. Both the vector and insert must be linear, double stranded sequences

For questions 11–17, bubble in the letter that corresponds to the column that best matches your understanding of each term.

	A.	**B.**	**C.**	**D.**
11. DNA polymerase	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means.*	*I know what this term means, and could easily explain it to someone else.*
12. Exonuclease	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means.*	*I know what this term means, and could easily explain it to someone else.*
13. Ligase	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means*	*I know what this term means, and could easily explain it to someone else.*
14. Transformation	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means*	*I know what this term means, and could easily explain it to someone else.*
15. Blue-White Screening	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means*	*I know what this term means, and could easily explain it to someone else.*
16. PCR Screening	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means.*	*I know what this term means, and could easily explain it to someone else.*
17. Gibson Assembly	*I have no idea what this term means.*	*I have heard this term before.*	*I have some familiarity with what this term means.*	*I know what this term means, and could easily explain it to someone else.*

For questions 18–28, bubble in the letter that corresponds to the column that best matches your comprehension of each molecular biology technique.

	A.	**B.**	**C.**	**D.**	**E.**
18. I am comfortable performing DNA sequence analysis, including *in silico* cloning of plasmids.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
19. I am comfortable performing molecular cloning reactions with Gibson Assembly.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
20. I am comfortable conducting experiments with polymerase chain reaction (PCR).	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
21. I am comfortable performing transformations with DNA samples.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
22. I am comfortable performing restriction digest reactions.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
23. I am comfortable performing molarity calculations, including using the equation $C_1V_1=C_2V_2$ to develop recipes for molecular cloning reactions.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
24. I am comfortable performing PCR screening to determine identify of cloned plasmids.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
25. I am comfortable performing blue-white screening to determine successfully cloned plasmids.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
26. I am comfortable designing primer sequences for PCR reactions.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
27. I am comfortable performing agarose gel electrophoresis and analyzing gel images.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
28. Based on my current understanding of molecular biology and molecular cloning, I would be comfortable in pursuing a career in which these techniques are involved.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
29. I did my part to learn as much as possible in this course.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*
30. Overall, I would rate this course as a valuable learning experience.	*Strongly Disagree*	*Disagree*	*Neutral*	*Agree*	*Strongly Agree*