

Cloning Techniques

INSTRUCTIONS:

ANSWER SHEET for Multiple Choice/True or False Questions (Just the LETTER of your choice ONLY!)

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Cloning Techniques

INSTRUCTIONS:

MULTIPLE CHOICE/TRUE or FALSE SECTION:

TRUE or FALSE . Choose "A" for TRUE and "B" for FALSE. DO NOT write "True" or "False" or use "T" or "F" Failure to follow directions will result in loss of points for this section!!!!

1. The genes encoding resistance to ampicillin (amp^r) and tetracycline (tet^r) of pBR322 act have cloning sites for foreign DNA. If one of the antibiotic resistance genes is broken with the DNA of interest, then the bacteria receiving the plasmid will be sensitive to the antibiotic and die if treated with that antibiotic.
2. If the restriction site on one strand is 3'-CTTAGG-5', the complementary strand is 5'-GAATTC-3'.
3. The origin of replication in your vector ensures that you will be unable make large numbers of copies of your cloned DNA because the inserted DNA will contain genes that code for proteins that would eventually be toxic to the host cells.
4. All cells exposed to cloned DNA in the ligation mix of vector and insert DNA will not take it up. The purpose of the antibiotic resistance gene in the vector ensured that only those cells that took up the vector (and with it possibly an inserted piece of donor DNA) would survive when grown up in the antibiotic that corresponded to the antibiotic resistance gene in the vector.
5. When cells take up a pUC plasmid with an insert in the multicloning site, the cells were then able to make functional E-galactosidase because the insert DNA enabled the plasmid to code for this enzyme.
6. *EcoRI* recognizes a specific site and leaves a staggered cut known as a 5' (5 prime) sticky end.

MULTIPLE CHOICE: Choose the BEST answer from the choices given.

7. One of the cloning vectors, pBR322, has a number of important properties. Which of the following properties is NOT characteristic of pBR322?
 - A. pBR322 is derived from the bacteriophage IR322.
 - B. It carries genes encoding resistance to ampicillin (amp^r) and tetracycline (tet^r)
 - C. This vector can optimally accommodate foreign DNA of up to 5 to 10 kb.
 - D. B and C
 - E. All are characteristics of pBR322
8. The cloning vector you used in your cloning experiments was _____.
 - A. pZT18
 - B. pUC18
 - C. pUC57
 - D. pED18
 - E. pUC19
9. The cloning vector you used in your experiment belongs to what class of cloning vectors:
 - A. bacteriophages
 - B. YACs
 - C. BACs
 - D. plasmids
 - E. cosmids
10. The multi-cloning site (MCS) in the cloning vector used in your experiments was located in _____.
 - A. an ampicillin resistance gene
 - B. the *lacZ* gene
 - C. a tetracycline resistance gene
 - D. the origin of replication
 - E. none of these

11. The biochemical activity of restriction enzymes is _____.
 A. repairing the nicks in the sugar/phosphate backbone made by the enzyme **E**-galactosidase
 B. reforming the phosphodiester backbone at specific sites in a DNA sequence
 C. the hydrolysis of the phosphodiester backbone at specific sites in a DNA sequence.
 D. to activate a DNA repair enzymes that would repair the nicked sugar/phosphate backbone.
 E. B and C
12. For running the gels, we will need 2 liters of 1X TAE buffer. You have a concentrated stock solution of TAE that is 20X. How much of this concentrated stock 20X TAE stock solution will you need?
 A. 10 ml B. 20 ml C. 50 ml D. 100 ml E. none of these
13. The percentage of the agarose gel that you ran your ligated DNA and digested vector and insert DNA on was _____.
 A. 1.2% B. 1% C. 0.8% D. 1.5% E. none of these
14. The source of the DNA (donor DNA) that you were trying to clone into your vector was _____.
 A. bacteriophage IX174 B. calf thymus DNA C. bacteriophage lambda DNA D. plasmid DNA E. none of these
16. Cleavage of DNA by restriction enzymes leaves a variety of 3"-OH / 5"-PO4 "ends" on the target DNA. Which of the following is not one of them (if any)?
 A. a 5" sticky end B. a 3" sticky end C. a blunt end D. a cos end E. A, B and C. F. A, B, C, and D
17. The enzyme(s) used to digest your vector and donor DNA for cloning was (were) _____.
 A. *EcoRV* B. *EcoRII* C. *EcoRI* D. *BamHI* E. *BanII* F. C and D
18. After your vector had accepted the insert DNA you treated it with an enzyme to "seal" the pieces together. This enzyme was _____.
 A. DNA polymerase B. RNA polymerase C. DNA repairase D. RNA ligase E. DNA ligase
19. The enzyme that "sealed" the insert DNA into the vector DNA in Question 18 was actually performing what function?
 A. It repaired the nicks in the sugar/phosphate backbone made by the restriction enzymes
 B. It was used to inactivate the restriction enzyme so that it would no longer cut the DNA.
 C. It induced the cell to activate a DNA repair enzymes that would repair the nicked sugar/phosphate backbone.
 D. B and C
 E. A and B
20. The vector you used in your cloning experiment had a (an) _____ origin of replication.
 A. low copy number B. medium copy number C. high copy number D. conjugative E. omnibus
21. The *lacZ* gene in your vector codes for _____.
 A. the β -galactosidase gene
 B. the α (alpha) subunit of β -galactosidase C.
 the ω (omega) subunit of β -galactosidase. D.
 resistance to the antibiotic lacromycinZ E.
 none of the above

Use Fig. 1 to answer the following question: (NOTE: Blue colonies will appear black on this exam)

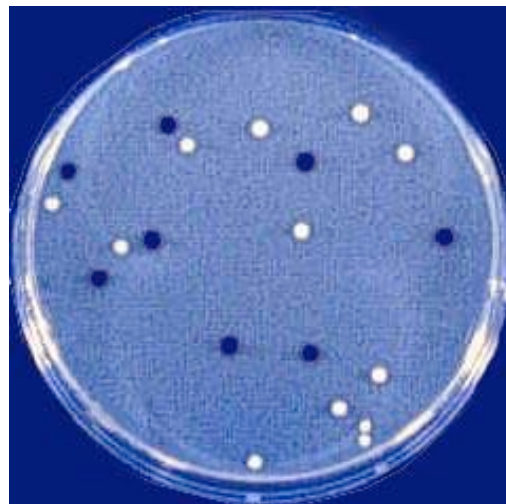
22. The experimenter used your plasmid and cloned some lambda DNA as well. The Fig. 5 is a photo of one of her colony plates. What percentage of the colonies had a DNA insert (a recombinant plasmid)?

A. 40% B. 30% C. 70% D. 60% E. none of these

23. Your host cells were a particular strain of *E. coli* strain named _____.

A. JM109 B. HB101 C. DH5DD. KW251 E. JM103

Fig. 1-Plate with Blue and White Colonies from a Cloning Experiment
(Count ONLY clearly VISIBLE colonies)



MATCHING 1: Match the following definitions on the left to the most appropriate term on the right

24. Multiple, unique restriction enzyme sites for cloning donor DNA („unique” means the enzymes cut the vector only once within the site).

25. The selectable marker that allows cells containing your vector to be isolated

26. Reagent in the agar plates used to indicate that a colony was composed of cells had taken up your vector that also contained a piece of donor DNA (insert DNA)

27. Color of a colony that was composed of cells that contained a vector WITHOUT insert DNA

28. Color of a colony that was composed of cells that contained a vector WITH insert DNA

29. Name of the process of colony selection used in Questions 27 and 28.

- A. Tetracycline
- B. blue
- C. ampicillin
- D. α -complementation
- E. beta-galactosidase
- F. colorless
- G. β -complementation
- H. multi-cloning site (MCS)
- I. X-gal
- J. white

MATCHING 2: Match the vector (left) to the appropriate description (right)

30. plasmids	A. Engineered to only work when recombinant DNA and viral particles are packaged in a test tube and placed on bacterial plates to infect bacteria. Usually hold DNA up to 20 kilobases (kb) in length.
31. cosmids	
32. bacteriophages	B. A plant virus used as a cloning vector.
33. Ti	C. Contains the <i>cos</i> (cohesive termini) sites from phage DNA, a plasmid origin of replication, and genes for antibiotic resistance. It is packaged into a bacteriophage protein coat, but replicates like a plasmid instead of phage DNA. Can hold pieces of DNA between 35 and 45 kilobases.
34. YACs	
35. BACs	D. Covalently closed, circular, extrachromosomal, self-replicating pieces of DNA. This vector can best accommodate DNA of up to 5 to 10 kb.
	E. A centromere allows the chromosome to be transferred to the daughter cells during cell division. A telomere at the end of this vector ensures that the end is correctly replicated and protects against degradation. It has an autonomously replicating sequence (ARS). Useful to clone large DNA fragments, between 200 and 1500 kilobases.
	F. Plasmid from the soil bacterium <i>Agrobacterium tumefaciens</i> used as a cloning vector for plants.
	G. An artificial plant RNA retrovirus used for studying diseases in plants. Can accommodate DNA between 50 to 100 kilobases.
	H. Synthetic vectors that have been used to clone very large fragments of eukaryotic chromosomes, between 100 to 300 kilobases. Used in the analysis of large portions of complex genomes, whole genes, and constructing maps of genomes. Created using a small plasmid, called the F (fertility) factor

Answer 4 out of the 8 following questions with a brief discussion. Circle the number of the ones you want graded. This is NOT a “5 best out of X number”. If you don’t circle the ones you want graded, I will grade the first 5 answers, regardless of their quality (up to you to know your strengths and weaknesses).

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5. Cells that were treated with CaCl_2 in Question 4 still were not quite ready to take up DNA. How were the cells treated to complete the transformation process after the DNA was added?

6. What function did the ammonium acetate serve in isolating plasmid DNA?

7. How would you screen a genomic library for a gene of interest? Give a brief description of the procedure (HINT: Like the one described in the powerpoint)

8. For a gene library, we can easily calculate the number of individual recombinant molecules required to represent an entire genome:

$$N = [\ln(1-P)] / [\ln(1-f)]$$

P – probability of complete coverage (99% or 0.99 confidence)

N – number of individual clones required

f – proportion of genome in average fragment

The genome size is 5,000,000 kb. Your vector is a cosmid that can stably carry up to 30 kb. How many recombinant molecules would be required to represent the entire genome? SHOW YOUR WORK to get credit for this problem (an answer alone will not be considered)