

Guide Questions for Topic: Recombinant DNA Basics

1. The term “recombinant DNA” refers to _____.
2. What are restriction endonucleases?
3. In nature, restriction endonucleases evolved within _____ and function to protect that organisms against _____.
4. BamHI, HindIII, NotI are three examples of restriction endonucleases. What do they all have in common? How do they differ?
5. You are a researcher in need of the restriction endonuclease HpaI to use in generating a recombinant DNA molecule. What would be the simplest way (and most common) to acquire HpaI enzyme?
6. HindIII is an example of a restriction endonuclease that introduces staggered cuts into DNA. Based on this information, what can you tell me about the products of HindIII cleavage?
7. Why are restriction endonucleases that introduce staggered cuts into DNA especially helpful in generating recombinant DNA molecules?
8. The terms “recombinant plasmid” and “plasmid construct” are largely interchangeable. What do those terms refer to?
9. Plasmid constructs consist of two fundamental sources of DNA, which are _____.
10. Plasmid vectors are most often engineered to contain an origin of replication (ori). Why is this the case? In other words, why is inclusion of an ori essential to the usefulness of plasmid vectors in generating recombinant plasmids?
11. If you inserted a DNA insert into a plasmid that does NOT contain an ori and then introduced the recombinant plasmid into bacteria, what would happen in that bacterial culture over time?
12. Plasmid vectors are most often engineered to contain an antibiotic resistance gene (for example, an ampicillin resistance gene, or Amp^r gene). Why is inclusion of an antibiotic resistance gene critical in order for researchers to generate large quantities of a recombinant plasmid?
13. You are a researcher and have generated a recombinant plasmid in which you inserted the gene encoding the transcription factor CREB 1 into a plasmid vector. You introduce the recombinant plasmid into *E. coli*, add antibiotic, and allow the bacteria to proliferate forming a large bacterial culture. Once you have that large bacterial culture, what final step must you perform before you can begin using your recombinant plasmid in experiments?
14. What role do primers play during a polymerase chain reaction (PCR)?
15. What role does Taq DNA polymerase play during PCR?
16. Why is it essential to subject PCR reactions to 95 degrees Celsius with each round of the reaction?

17. If you subjected a sample of DNA containing 5 copies of the sequence you are amplifying to 3 rounds of PCR, how many copies should you end up with?
18. A cDNA clone refers to _____.
19. During the process of cDNA cloning, mRNA is isolated and then incubated with reverse transcriptase and DNA nucleotides, resulting in production of _____.
20. Why is the addition of “oligonucleotide linkers” to the ends of a cDNA essential to the process of cDNA cloning?
21. What does the term “cDNA library” refer to?