19.1
1. What is recombinant DNA?
   - Combination of DNA molecules that are not found together in nature.
2. What are the general steps to making a recombinant DNA?
   1. DNA to be cloned is purified from cells or tissues
   2. Proteins called restriction enzymes are used to generate specific DNA fragments. They cut at a specific nucleotide.
   3. These fragments are joined to other DNA molecules that serve as vectors or carrier molecules. A vector joined to a DNA fragment is a recombinant DNA molecule.
   4. The recombinant DNA is put back into host’s cell and it replicates making many copies.
   5. As the cell replicates the recombinant DNA introduced is passed on to the progeny cells creating a population of host cells which carry the copies of the cloned DNA.
   6. Now the cloned DNA is recovered from the host’s cells, purified, and analyzed.
   7. Now the cloned DNA can then be transcribed, its mRNA translated, and the encoded gene product isolated and used for research or sold commercially.

19.2 and 19.3
1. How does a restriction enzyme know where to cut the DNA?
   - There are many restriction enzymes and they all have their own specific place where they cut the DNA
   - First they recognize a specific nucleotide sequence on the DNA called recognition sequence and they cut there.
2. How do you know how often a restriction enzymes cleaves a DNA?
   - Every \(4^{\text{# of base recognition sequence}}\) bases
   - For example if a restriction enzyme recognizes a recognition sequences with four bases, it would cut every \(4^4\) bases on the DNA to give many fragments.
3. What are the recognition sequences like?
   - They give the same sequence when read from 5prime to 3prime and in the 3prime to 5prime direction(palindrome)
4. Describe the process of cutting done by EcoRI.
   1) It recognizes the recognition sequences and it digests the DNA there
   2) After it cuts we have single stranded tails (sticky ends) that can form hydrogen bonding with the complementary single stranded tails from the other DNA that is to make the recombinant DNA.
   3) They two complementary single stranded tails hydrogen bond, not covalently, to make the recombinant DNA.
   4) Now we have gaps between the sticky end of one DNA and a base of another. Same thing happens to the top and bottom strands of the DNA.
   5) DNA ligase seals the gaps, covalently bonding the two strands.

19.4
1) Can DNA restriction fragments enter a host’s cell to be cloned?
   - No
2) What do we need for the fragment to be allowed to enter the host’s cell?
   • The fragment needs to be joined to a DNA molecule called a vector.
3) What exactly are vectors?
   • Carrier DNA molecules that transfer and replicate (clone) inserted DNA fragments.
4) What does a vector have to have to qualify as a vector?
   • Must be able to independently replicate itself and any DNA fragment it carries
   • Must contain several recognition sequences to allow insertion of DNA fragments to be cloned.
5) How are you able to put in a vector into a fragmented DNA?
   I. The vector is cut with a restriction enzyme
   II. It is then mixed with DNA fragments that are produced with the same restriction enzymes
   III. Now you have the recombinant DNA.
6) How are you able to tell the DNA with a vector from other DNA after you insert it in the host’s cell?
   • The vector contains a selectable marker gene (usually antibiotic resistance genes or genes for enzymes absent from the host’s cell)

1. What are plasmids?
   • The first vectors developed which have been extensively modified by genetic engineering to serve as cloning vectors.
2. Describe plasmid pUC18.
   • It is small so it can carry large DNA inserts
   • It has an origin of replication and can produce up to 500 copies of inserted DNA fragments per cell.
   • A large number of recognition sequences have been engineered into it in a region called polylinker.
   • This polylinker is contained within a LacZ gene.
   • It allows recombinant plasmids to be easily identified. If the plasmid carries a fragment the lacZ gene is inactivated and thus the cell that this plasmid is in starts to does not make blue colonies and makes blue if the fragment is not there.
3. What are Lambda phage vectors?
   • When genetically modified strains of lambda phage are used as vectors
   • These are good if you want to insert DNA fragments that have very big
4. How is a Lambda phage used as a vector?
   • It has three parts to its chromosome
   • What happens is that using a restriction enzyme, EcoRI, the three parts are cut into three parts, left and right hand and dispensable central region.
   • Now a foreign DNA which was cut by EcoRI is ligated using DNA ligase replacing the central region and connecting the left and right arms.
• Now this recombinant lambda vector are packaged into phage protein heads in vitro and introduced into the bacterial host cell.
• Inside the bacteria the vectors replicate and form many copies of infective phages which all carry the DNA inserts.
5. What are cosmid vectors?
• Hybrid vectors created by combining parts of the lambda chromosome with parts of plasmids.
• They have a cos sequence of the lambda necessary for packing phage DNA into phage protein coats
• Also have plasmid sequences for necessary for replication.
6. How can you tell them apart form something else?
• They have an antibiotic resistance gene to help identify host cells carrying recombinant cosmids.
7. What is the difference between the lambda phage and cosmid vectors?
• Cosmid vectors can 50kb of inserted DNA while the other can carry only about 10-15 Kb.
8. What are shuttle vectors?
• Hybrid vectors that are like cosmid vectors except that they have animal viruses instead of Lambda
• They can replicate in more than one type of host cell

9. What are bacterial artificial chromosomes (BAC)?
• Vectors that can carry huge DNA fragments
• These are used for eukaryotes that have very big DNA
• They carry F factors which are independently replicating plasmids that transfer genetic information during bacterial conjugation.
• They also contain polylinker, at least one antibiotic resistance marker and flanked promoter sequences used to generate RNA molecules for the expression of the cloned genes.
10. What are expression vectors?
• Vectors engineered to produce many copies of a selected protein in a host cell.

11. How does the expression vector, pET, in E coli work? (page 463)

19.5
1. What are the steps required to make recombinant DNA molecules and transfer them to an E coli host cell.
• DNA to be cloned is isolated and cut into fragments by restriction enzymes.
• The fragments are ligated into plasmid molecules that have been cut with the same restriction enzyme, creating a recombinant vector.
• The recombinant vector is transferred into an E coli host cell, where the recombinant plasmid replicates to form dozens of copies.
• The bacteria are plated on nutrient medium, where they form colonies and are screened to identify those that have taken up the recombinant plasmids.

2. T/F After the recombinant vector is introduced into the host cell and the cell replicates giving many cells with a cloned copy of plasmid, the colony that is made and they plasmids that they contain are identical clones.
   • True

3. Which prokaryotic host is commonly used?
   • A strain of bacterium E coli called K12.

19.6
1. Which host cell is used for the cloning and expression of eukaryotic genes?
   • Yeast Saccharomyces cervisiae

2. Why this yeast?
   • Can be grown and manipulated just as bacterial cells
   • Its genetics has been highly studied
   • Its entire genome has been sequenced and its genes have been identified
   • It posttranslationally modifies the proteins it makes so that they fold into functional form. Bacteria host cells cannot.

3. What is a yeast artificial chromosome (YAC)?
   • Yeast cloning vector that have telomeres at each end and a centromere in the center joined by maker genes (TRP 1 and URA 3)
   • Can clone DNA inserts from 100 to 1000 kb

19.7
1.