E1. A. Four generations: 7/8 light, 1/8 half-heavy
Five generations: 15/16 light, 1/16 half-heavy

B. All of the DNA double helices would be 1/8 heavy.

C. The CsCl gradient separates molecules according to their densities. ^{14}N-containing compounds have a lighter density compared to ^{15}N-containing compounds. The bases of DNA contain nitrogen. If the bases contain only ^{15}N, the DNA will be heavy; it will sediment at a higher density. If the bases contain only ^{14}N, the DNA will be light; it will sediment at a lower density. If the bases in one DNA strand contain ^{14}N and the bases in the opposite strand contain ^{15}N, the DNA will be half-heavy; it will sediment at an intermediate density.

E2. A. You would probably still see a band of DNA, but you would only see a heavy band.

B. You would probably not see a band because the DNA would not be released from the bacteria. The bacteria would sediment to the bottom of the tube.

C. You would not see a band. Ethidium bromide stains the DNA and you are actually seeing the ethidium bromide when the gradient is exposed to UV light.

E3. You might be able to determine the number of replication forks and their approximate locations. For chromosomes with a single origin, you can determine that replication is bidirectional. However, you do not get any molecular information about the DNA replication process.

E4. You would need to add a primer (or primase), dNTPs, and DNA polymerase. If the DNA were double stranded, you would also need helicase. Adding single-strand binding protein and topoisomerase may also help.

E5. You would need to add a DNA template with a 3′ overhang that was complementary to the telomere sequence. You would also have to add telomerase and dNTPs.

E6. This is a critical step because you need to separate the radioactivity in the free nucleotides from the radioactivity in the newly made DNA strands. If you used an acid that precipitated free nucleotides and DNA strands, all of the radioactivity would be in the pellet. You would get the same amount of radioactivity in the pellet no matter how much DNA was synthesized into newly made strands. For this reason, the perchloric step is very critical. It separates radioactivity in the free nucleotides from radioactivity in the newly made strands.

E7. No, because the hydrolysis of the deoxynucleoside triphosphates provides the energy for the synthesis of new strands.

E8. A. The left end is the 5′ end. If you flip the sequence of the first primer around, you will notice that it is complementary to the right end of the template DNA. The 5′ end of the first primer binds to the 3′ end of the template DNA.

B. The sequence would be 3′–CGGGGCCATG–5′. It could not be used because the 3′ end of the primer is at the end of the template DNA. There wouldn′t be any place for nucleotides to be added to the 3′ end of the primer and bind to the template DNA strand.

E9. For a DNA strand to grow, a phosphoester bond is formed between the 3′ —OH group on one nucleotide and phosphate group on the incoming nucleotide (see Figure 11.10). If the —OH group is missing, a phosphoester bond cannot form.

E10. A. Heat is used to separate the DNA strands, so you do not need helicase.

B. Each primer must be a sequence that is complementary to one of the DNA strands. There are two types of primers, and each type binds to one of the two complementary strands. The arrowheads are at the 3’ end of the primers.

C. A termophilic DNA polymerase is used because DNA polymerases isolated from nonthermophilic species would be permanently inactivated during the heating phase of the PCR cycle. Remember that DNA polymerase is a protein, and most proteins are denaturated by heating. However, proteins from thermophilic organisms have evolved to withstand heat. That is how thermophilic organisms survive at high temperatures.

D. With each cycle, the amount of DNA is doubled. Since there are initially 10 copies of the DNA, there will be 10×2^{27} copies after 27 cycles. $10 \times 2^{27} = 1.34 \times 10^9 = 1.34$ billion copies of DNA. As you can see, PCR can amplify the amount of DNA by a staggering amount!