E1. The location of the intron within the cDNA is shown below:

cDNA:

5′-ATTGCATCCAGCGTATACTATCTCGGGCCCAATTAATGCCAGC
GGCCAGACTATCACCCAACTCG…**INTRON**…GTTACCTACTAGTATATCCCATATACTAGCATATATTTT
ACCCATAATTTGTGTGTGGGTATACAGTATAATCATATA–3′

You can figure this out by finding where the sequence of the genomic DNA begins to differ from the sequence of the cDNA. The genomic DNA has the normal splice sites that are described in Figure 12.21.

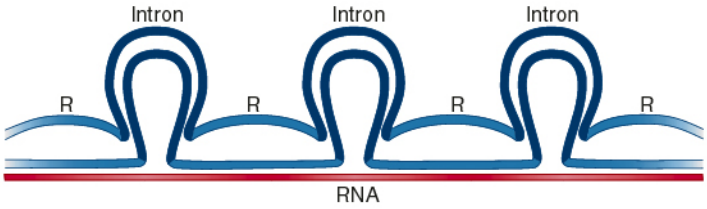
Genomic DNA:

5′–
ATTGCATCCAGCGTATACTATCTCGGGCCCAATTAATGCCAGCGGCCAGACTATCACCCAACTCGGCC
CACCCCCCAGGTTTACACAGTCATACCATACATACAAAAATCGCAGTTACTT**A**TCCCAAAAAAACCTAG
ATACCCCACATACTATTAACTCTTTCTTTCTAGTTACCTACTAGTATATCCCATATACTAGCATATATTTT
ACCCATAATTTGTGTGTGGGTATACAGTATAATCATATA–3′

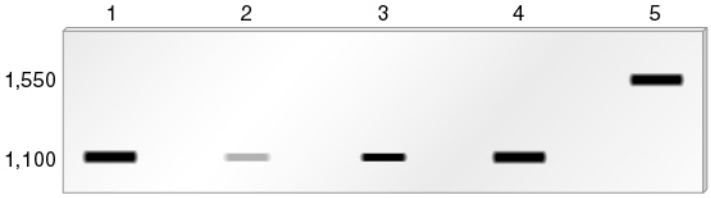
The splice donor and acceptor sites are underlined. The space indicates where the strands in the corresponding RNA would be cut. The branch site is also underlined. The large A is the adenine that participates in the transesterification reaction.

E2. An R loop is a loop of DNA that occurs when RNA is hybridized to double-stranded DNA. While the RNA is hydrogen bonding to one of the DNA strands, the other strand does not have a partner to hydrogen bond with so it bubbles out as a loop. RNA is complementary to the template strand, so that is the strand it binds to.

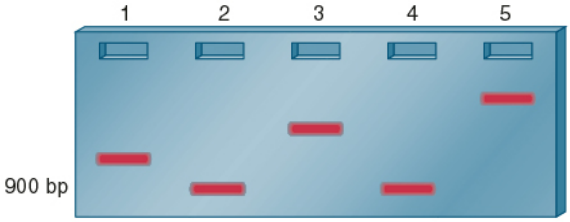
E3.



E4. The 1,100-nucleotide band would be observed from a normal individual (lane 1). A deletion that removed the –50 to –100 region would greatly diminish transcription, so the homozygote would produce hardly any of the transcript (just a faint amount as shown in lane 2) and the heterozygote would produce roughly half as much of the 1,100-nucleotide transcript (lane 3) compared to a normal individual. A nonsense codon would not have an effect on transcription; it affects only translation. So the individual with this mutation would produce a normal amount of the 1,100-nucleotide transcript (lane 4). A mutation that removed the splice acceptor site would prevent splicing. Therefore, this individual would produce a 1,550-nucleotide transcript (actually, 1,547 to be precise, 1,550 minus 3). The Northern blot is shown here:



E5. When the 900 bp fragment is mixed with TFIID (lane 1), it would be retarded because TFIID would bind. When mixed with TFIIB (lane 2), it would not be retarded because TFIIB cannot bind without TFIID. Compared to lane 1, the 900 bp fragment would be retarded even more when mixed with TFIID and TFIIB (lane 3), because both transcription factors could bind. It would not be retarded when mixed with TFIIB and RNA polymerase (lane 4) because you do not have TFIID, which is needed for the binding of TFIIB and RNA polymerase. Finally, when mixed with TFIID, TFIIB, and RNA polymerase/TFIIF, the 900 bp fragment would be retarded a great deal because all four could bind (lane 5).



E6. A. It would not be retarded because ρ protein would not bind to the mRNA that is encoded by a gene that is terminated in a ρ -independent manner. The mRNA from such genes does not contain the sequence near the 3′ end that acts as a recognition site for the binding of ρ protein.

B. It would be retarded because ρ -protein would bind to the mRNA.

C. It would be retarded because U1 would bind to the pre-mRNA.

D. It would not be retarded because U1 would not bind to mRNA that has already had its introns removed. U1 binds only to pre-mRNA.

E7. A. The region of the gel from about 250 bp to 75 bp does not contain any bands. This is the region being covered up; it is about 175 base pairs long.

B. In a nucleosome, the DNA is wrapped twice around the core histones; a nucleosome contains 146 bp of DNA. The region bound by RNA polymerase II plus TFIID and TFIIB would be slightly greater than this length. Therefore, if the DNA was in a nucleosome structure, these proteins would have to be surrounding a nucleosome. It is a little hard to imagine how large proteins such as TFIID, TFIIB, and RNA polymerase II could all be wrapped around a single nucleosome (although it is possible). Therefore, the type of results shown here makes it more likely that the DNA is released from the core histones during the binding of transcription factors and RNA polymerase II.

E8. A.mRNA molecules would bind to this column because they have a polyA tail. A polyA tail is complementary to poly-dT, so the two would hydrogen bond to each other. To purify mRNAs, one begins with a sample of cells; the cells need to be broken open by some technique such as homogenization or sonication. This would release the RNAs and other cellular macromolecules. The large cellular structures (e.g., organelles, membranes, etc.) could be removed from the cell extract by a centrifugation step. The large cellular structures would be found in the pellet, while soluble molecules such as RNA and proteins would stay in the supernatant. At this point, you would want the supernatant to contain a high salt concentration and neutral pH. The supernatant would then be poured over the poly-dT column. The mRNAs would bind to the poly-dT column and other molecules (i.e., other types of RNAs and proteins) would flow through the column. The mRNAs would bind to the poly-dT column via hydrogen bonds. To break the hydrogen bonds between the mRNAs and poly-dT, you could add a solution that contains a low salt concentration and/or a high pH. This would release the mRNAs, which would then be collected in a low salt/high pH solution as it dripped from the column.

B. The basic strategy is to attach an oligonucleotide (i.e., a short sequence of DNA) to the column matrix that is complementary to the type of RNA that you want to purify. For example, if an rRNA contained a sequence 5′–AUUCCUCCA–3′, a researcher could chemically synthesize oligonucleotides with the sequence 3′–TAAGGAGGT–5′ and attach these oligonucleotides to the column matrix. To purify rRNA, one would use this 3′–TAAGGAGGT–5′ column and follow the general strategy described in part A.