- 9. Southern blotting is a technique in which DNA fragments are separated by size in a gel and then transferred to a solid support such as a nitrocellulose or nylon membrane. The DNA is fixed to the nylon membrane and hybridized to a labeled DNA or RNA probe. The hybridized probe is then detected by some technique such as autoradiography. Northern blotting is similar to Southern blotting, except that RNA instead of DNA is denatured and then separated on the gel. Southern blotting can be used to identify a DNA fragment that contains a DNA sequence of interest. Northern blotting can be used to determine the steady-state levels of a specific RNA.
- 10. In order to express a foreign gene, a recombinant plasmid would require a promoter for efficient transcription of the foreign gene. A promoter that is inducible would provide even higher expression levels of the foreign gene product. To facilitate purification of the foreign protein, a molecular tag can be added to the recombinant protein. An example of this type of molecular tag is a short sequence of histidine residues (a polyhistidine sequence). The resultant Histagged protein will bind specifically to a bead that has bound nickel atoms. Other proteins can be washed out and the His-tagged protein can be released from the nickel atoms by lowering the pH of the solution. Bacterial cells are limited in their capacity to synthesize complex proteins because of their inability to verform many post-translational modifications, such as glycosylaten that mammalian cells can perform. These posttranslational molifications are essential for the biological activity of the recombinant protein.
- 11. Northern blotting and RTFLCP is useful for analyzing fewer genes because they rely on the creation of specific rad pace v probes or PCR primers. It is less feasible to can elarge quantities (Pehese for whole genome analysis. Studying in the genomes (or chipploring) in this example) is most easily accomplished with a microarray, which uses a chip that can hybridize to hundreds or thousands of genes based on complementary base pairing.
- 12. The expression of mRNA in individual cells can be determined by in situ hybridization in whole cells or tissue sections. Fixed cells are exposed to labeled DNA probes that are complementary to the mRNA of interest. After washing to remove excess probe, the cells can be examined microscopically to detect the locations of labeled mRNA. This process can also be used to identify mRNA locations in embryos.
- 13. Single-nucleotide polymorphisms (SNPs) are changes in a single nucleotide between two individuals. Simple-sequence repeats (SSRs), also known as micro-satellites, consist of a variable number of repeating one-, two-, or three-base sequences. The number of these repeat units at a specific genetic locus varies between individuals. Both types of polymorphisms can be used as molecular markers for mapping studies. The recombination frequency between two polymorphisms can be determined and can serve as the basis for development of a genetic map. In general, the farther two markers are separated on a chromosome, the greater the recombination frequency between those two markers, and vice versa.