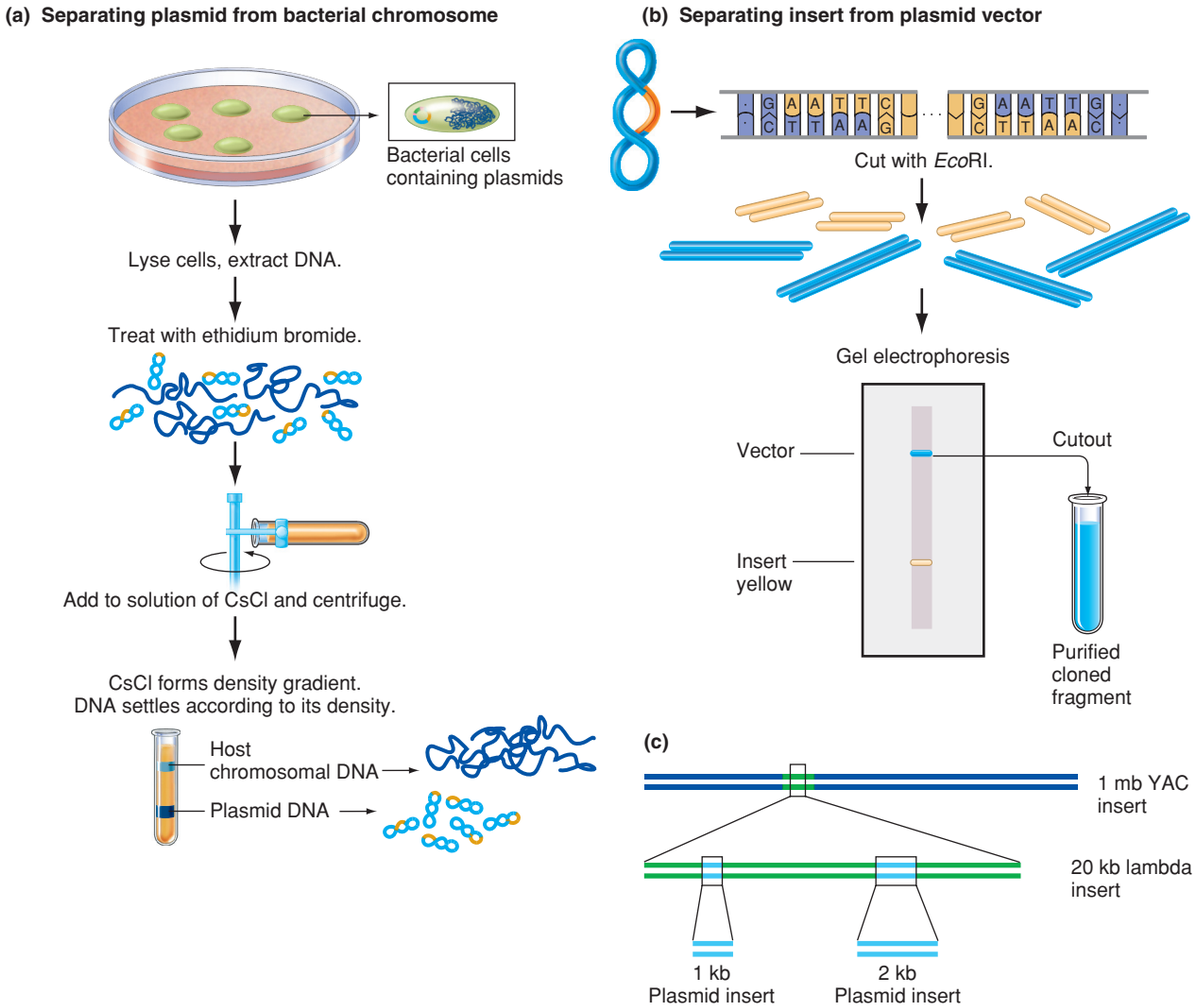

Figure 9.9 Purifying cloned DNA. (a) Separation of plasmid from the bacterial chromosome and other cellular components. In the plasmid-*E. coli* system, the bacterial chromosome is about 4000 kb long, while the recombinant plasmid is from 4–20 kb in length. Cell lysis causes the large bacterial chromosome to fragment into random linear fragments while the small circular plasmid molecules remain intact. As supercoiled plasmid DNA intercalates ethidium bromide, it becomes negatively supercoiled, making it denser than chromosomal DNA. The buoyant density of the plasmids is distinguishable from that of the chromosomes when the two are added to a cesium chloride (CsCl) gradient saturated with ethidium bromide. The band containing plasmid is extracted and purified. **(b)** Separation of the genomic DNA insert from vector DNA. The purified plasmids are digested with the same enzyme that was used to construct the recombinant molecule in the first place, releasing vector and insert fragments that differ in size. With gel electrophoresis, the two types of fragments can be separated from each other, and the band containing the insert can be cut out and purified. **(c)** Subcloning: From YAC clones to lambda clones to plasmids. The large genomes of higher eukaryotes (such as those of mice and humans) can be readily divided into a few thousand YAC clones that each contain 1 Mb of genomic DNA. To study gene-size regions from a particular YAC, however, it is convenient to subclone 20 kb fragments into a lambda vector. To analyze particular regions of a gene, it is useful to perform another round of subcloning, placing fragments that are 2 kb or smaller into a plasmid vector.

same organism. Instead, they can build a **genomic library:** a storable collection of cellular clones that contains copies of every sequence in the whole genome inserted into a suitable vector. Like traditional book libraries, genomic libraries store large amounts of information for retrieval upon request. They make it possible to start a new cloning project at an advanced stage, when the initial cloning step has already been completed and the only difficult task left is to determine which of the many clones in a library contains the DNA sequence of interest. Once the correct cellular clone is identified, it can be amplified to yield a large amount of the desired genomic fragment.