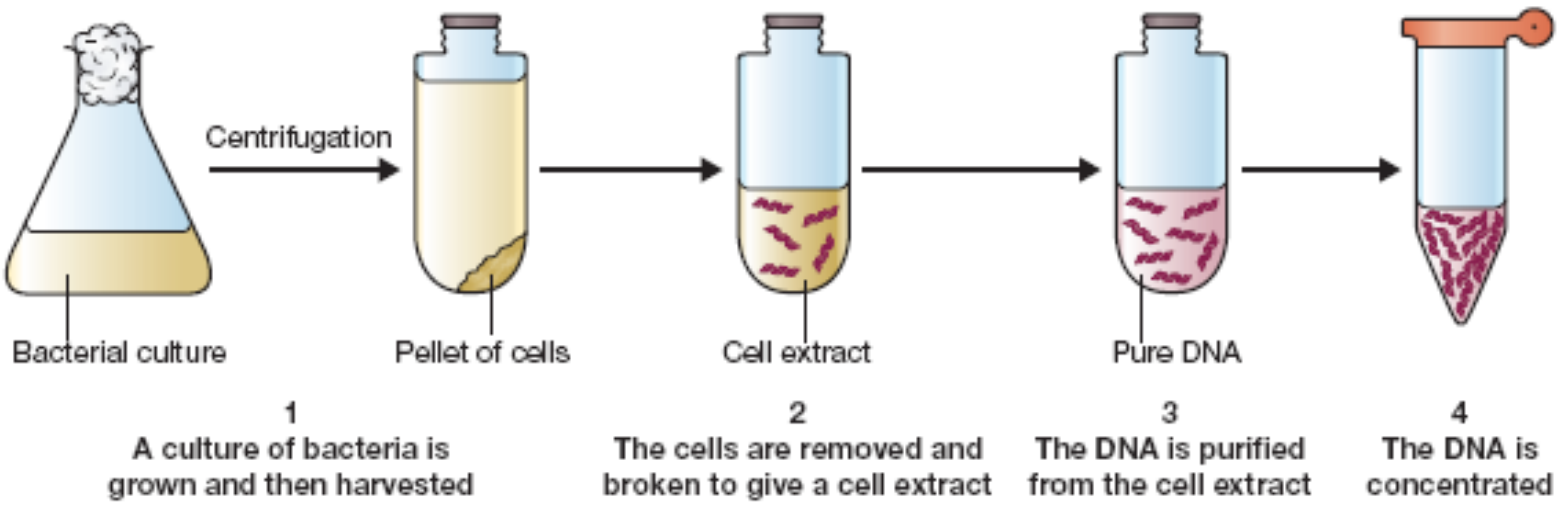
DNA isolation and purification

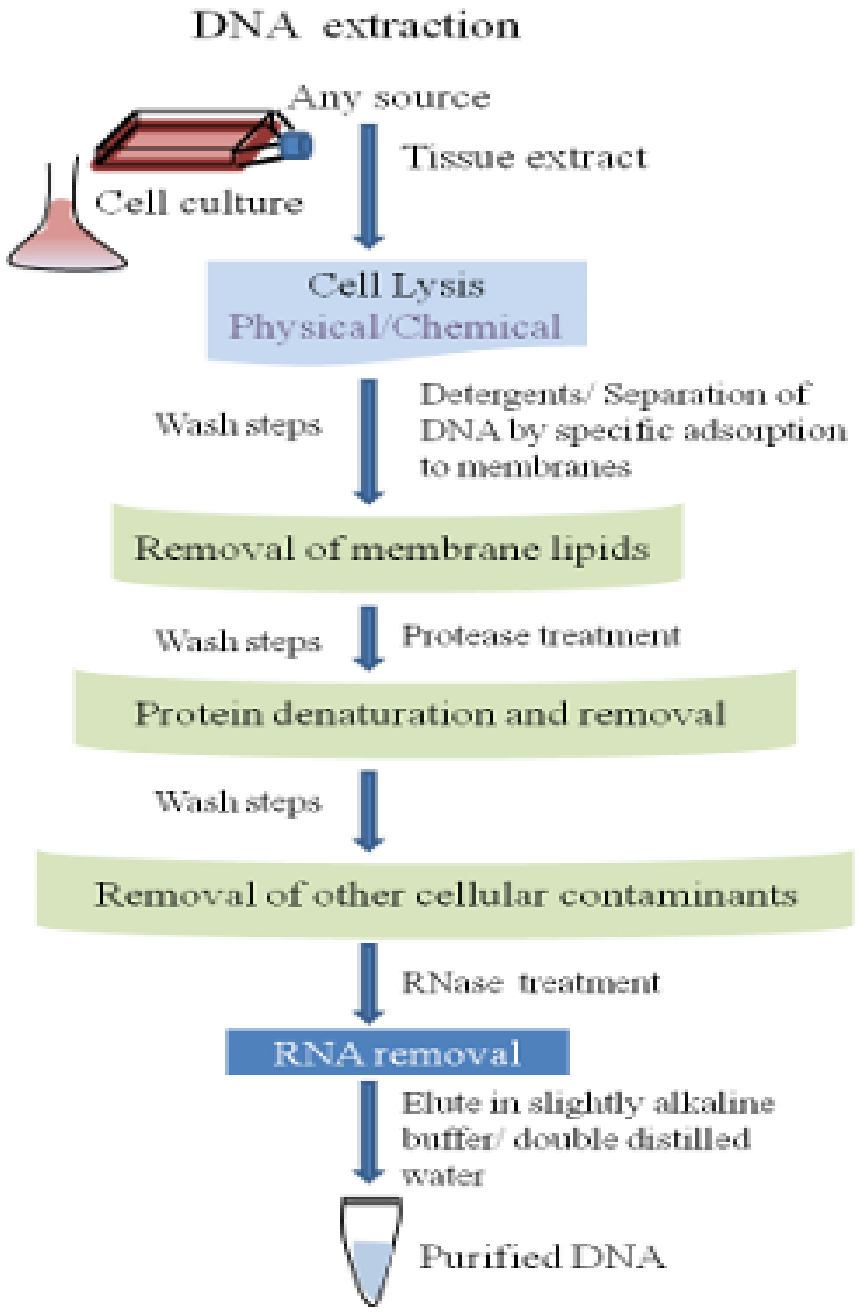
- **3.1 Preparation of total cell DNA**
- **3.2 Preparation of plasmid DNA**
- **3.3 Preparation of bacteriophage DNA**



Source

- Mammalian Cells/Tissues, Blood, Fecal, Hair, forensic
- Bacteria, spores etc
- Fungi
- Algae
- Yeast
- Plants
- Insect

(Fresh, Frozen, FFPE)



Methods

- Organic Extraction
- Silica Based Technology
- Magnetic Separation
- Anion Exchange Technology

For a pure DNA sample, the ratio of absorbance at 260 nm and absorbance at 280 nm (A260/A280) is 1.8. A ratio of < 1.8 indicates the sample is contaminated with protein or an organic solvent such as phenol, often used during extraction processes.

Preparation of Cell Extract

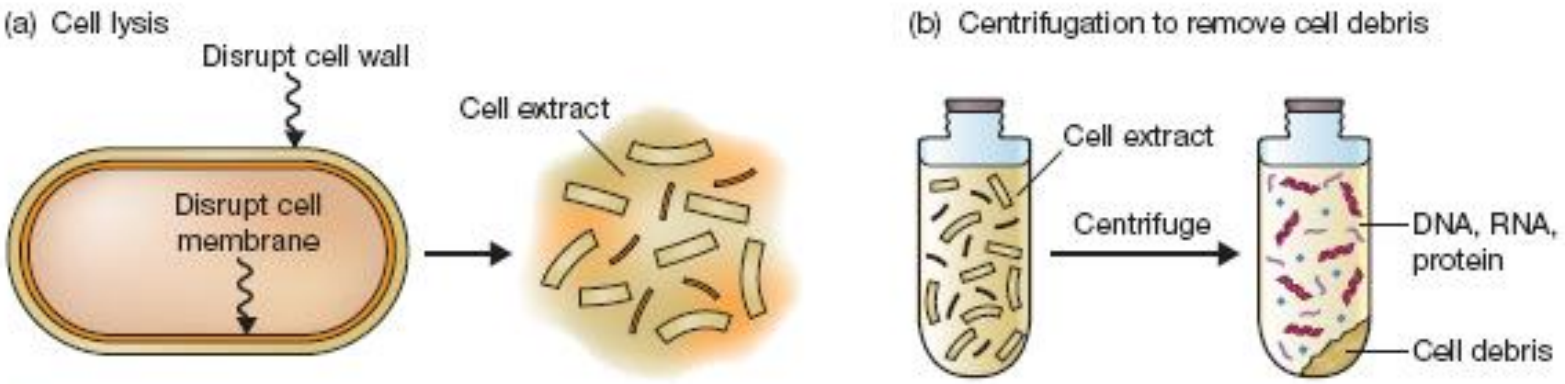


Figure 3.4

Preparation of a cell extract. (a) Cell lysis. (b) Centrifugation of the cell extract to remove insoluble debris.

- Lysozyme- digests the polymeric compounds that give the cell wall its rigidity.
- EDTA removes magnesium ions that are essential for preserving the overall structure of the cell envelope, and also inhibits cellular enzymes that could degrade DNA
- SDS-Detergents aid the process of lysis by removing lipid molecules and thereby cause disruption of the cell membranes

Preparation of DNA from Cell Extract-Organic extraction or Enzyme digestion

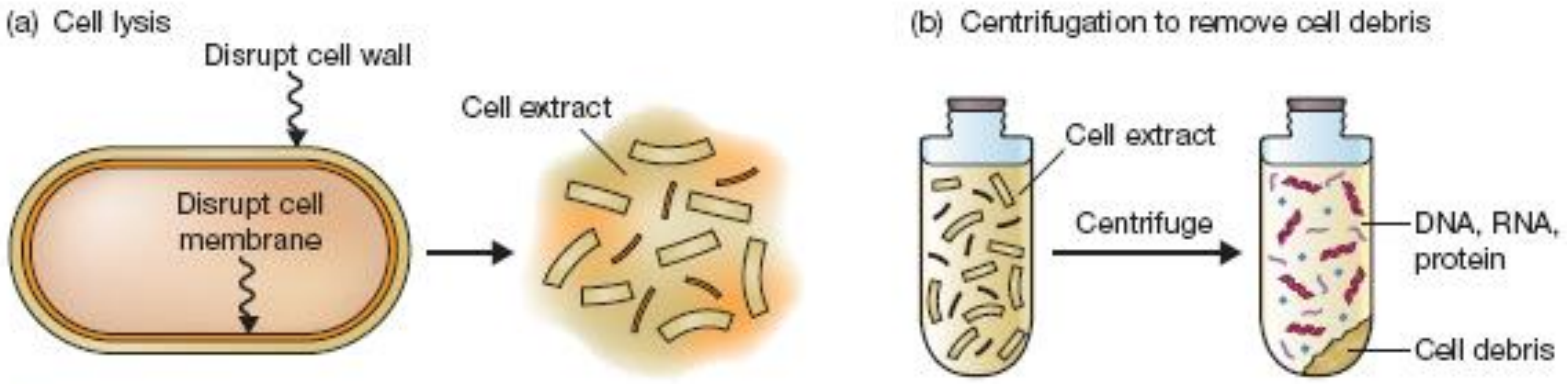
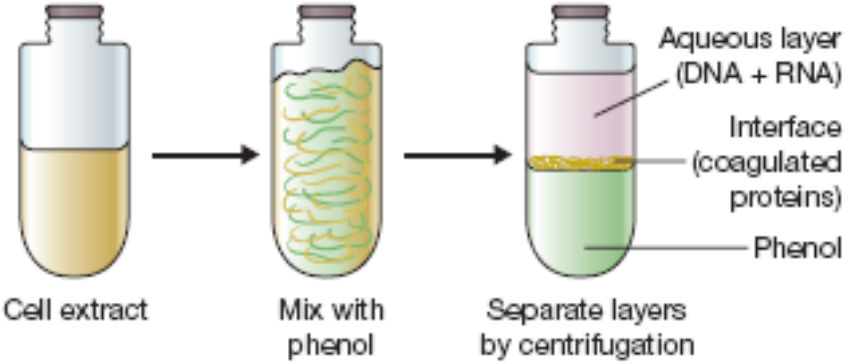


Figure 3.4

Preparation of a cell extract. (a) Cell lysis. (b) Centrifugation of the cell extract to remove insoluble debris.

Figure 3.6

Removal of protein contaminants by phenol extraction.



Phenol, chloroform, proteinase K, RNAse

Purification of DNA from Cell Extract- Ion Exchange Chromatography

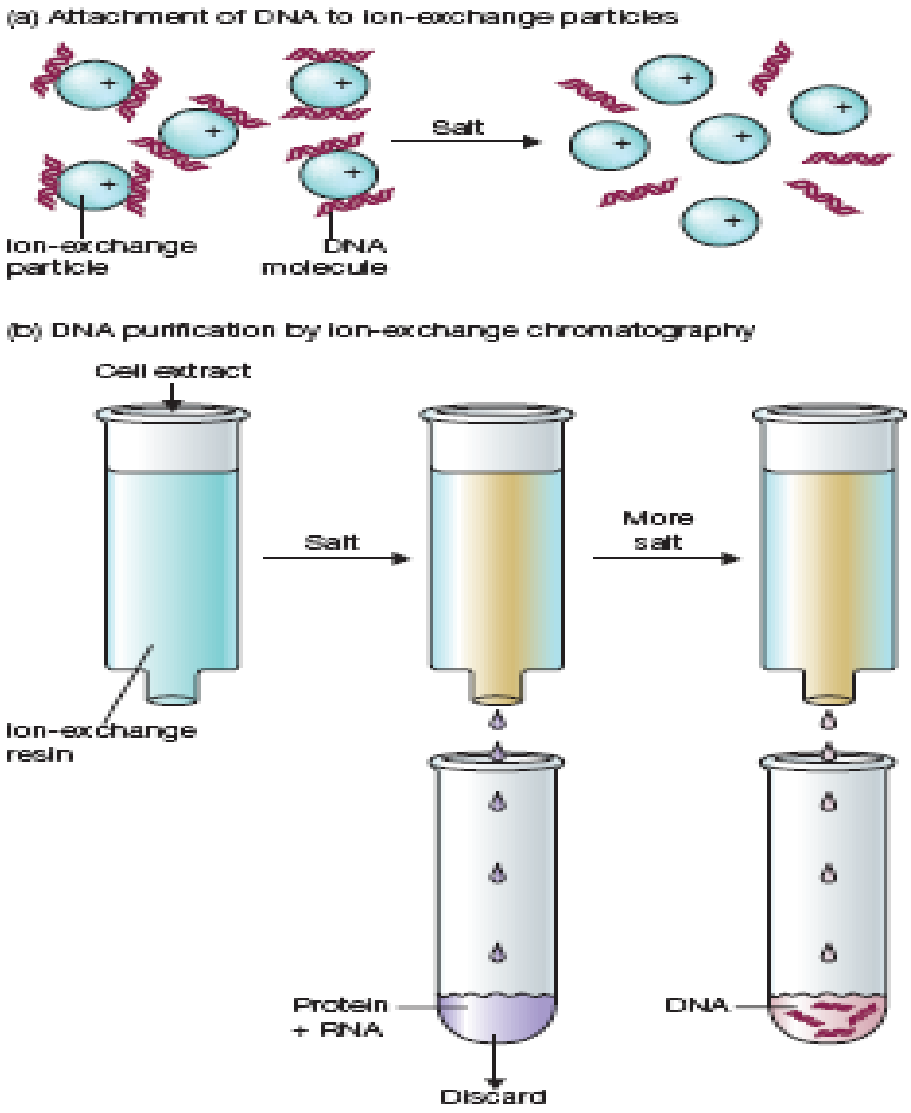


Figure 3.7

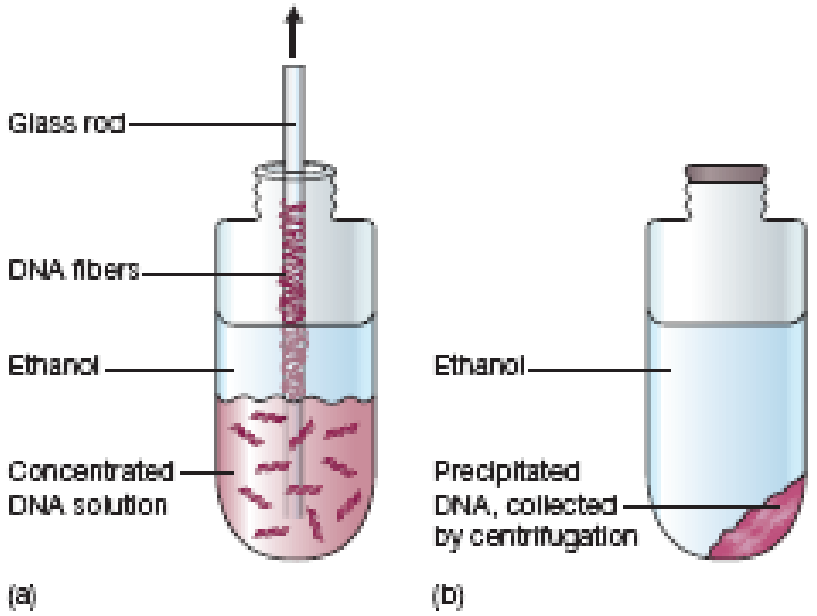
DNA purification by ion-exchange chromatography. (a) Attachment of DNA to ion-exchange particles. (b) DNA is purified by column chromatography. The solutions passing through the column can be collected by gravity flow or by the spin column method, in which the column is placed in a low-speed centrifuge.

DNA binds specifically to the substrate in the presence of low salt, contaminants are removed by wash steps using a low or medium salt buffer, and purified DNA is eluted using a high salt buffer

Concentration of DNA samples

Figure 3.8

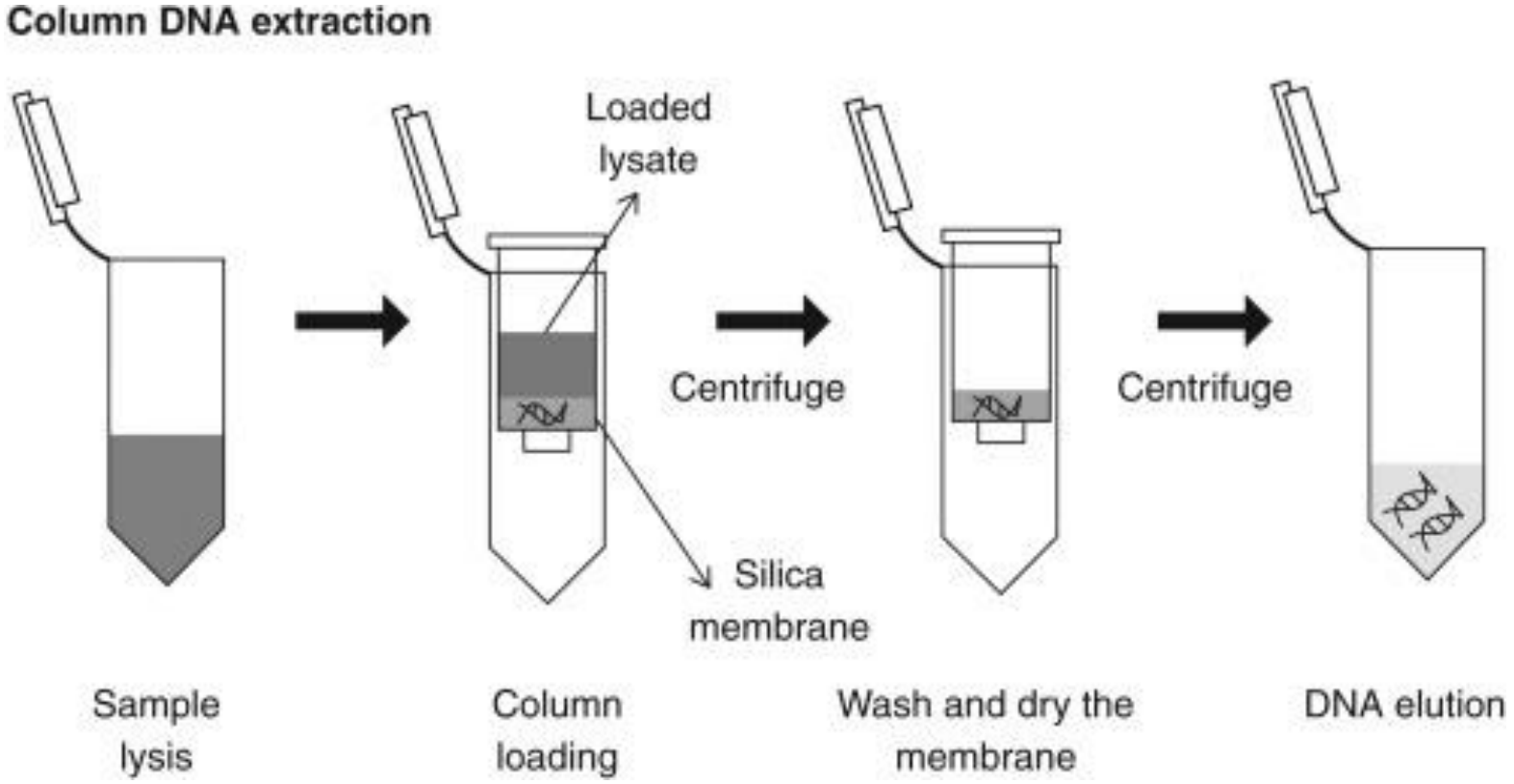
Collecting DNA by ethanol precipitation. (a) Absolute ethanol is layered on top of a concentrated solution of DNA. Fibers of DNA can be withdrawn with a glass rod. (b) For less concentrated solutions ethanol is added (at a ratio of 2.5 volumes of absolute ethanol to 1 volume of DNA solution) and precipitated DNA collected by centrifugation.



absorbance is measured at 260 nm, at which wavelength an absorbance (A260) of 1.0 corresponds to 50 mg of double-stranded DNA per ml.

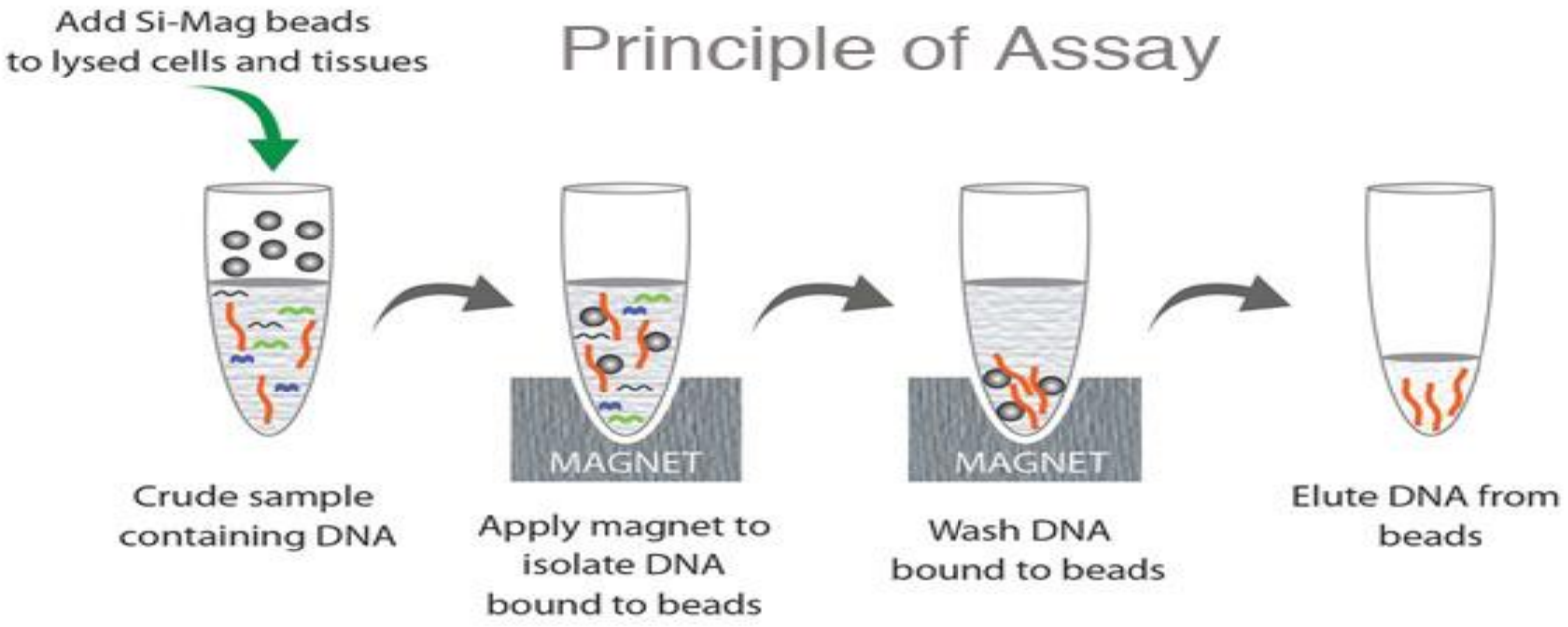
With a pure sample of DNA, the ratio of the absorbances at 260 and 280 nm (A260/A280) is 1.8. Ratios of less than 1.8 indicate that the preparation is contaminated, either with protein or with phenol.

Silica Membrane



Magnetic Separation

- Magnetic beads are made up of tiny (20 to 30 nm) particles of iron oxides, such as magnetite (Fe3O4), which give them superparamagnetic properties.
- Superparamagnetic beads are different to more common ferromagnets in that they exhibit magnetic behavior only in the presence of an external magnetic field. This property is dependent on the small size of the particles in the beads, and enables the beads to be separated in suspension, along with anything they are bound to. Since they don't attract each other outside of a magnetic field, they can be used without any concern about unwanted clumping.



https://www.cytivalifesciences.com/en/us/news-center/magnetic-beads-a-simple-guide-10001

Plant DNA isolation

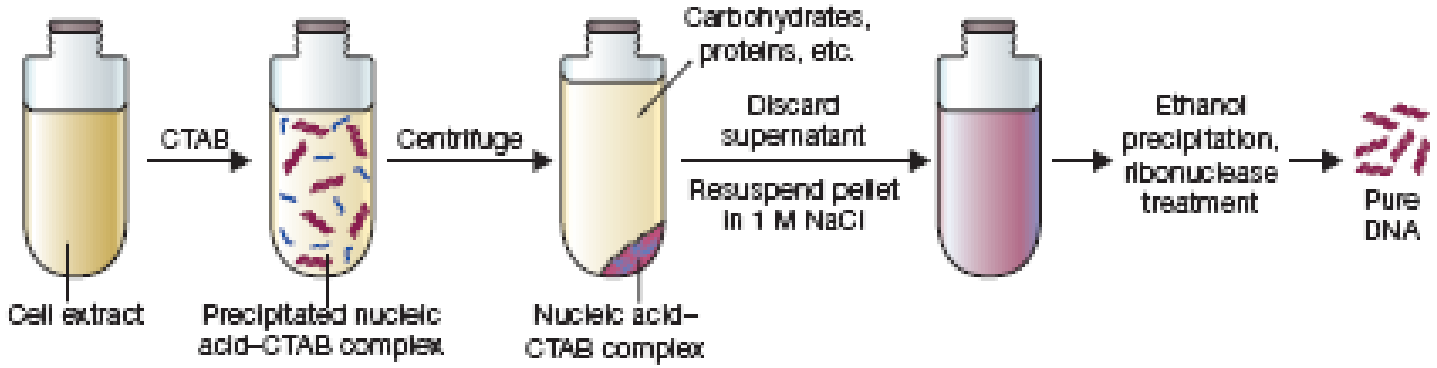


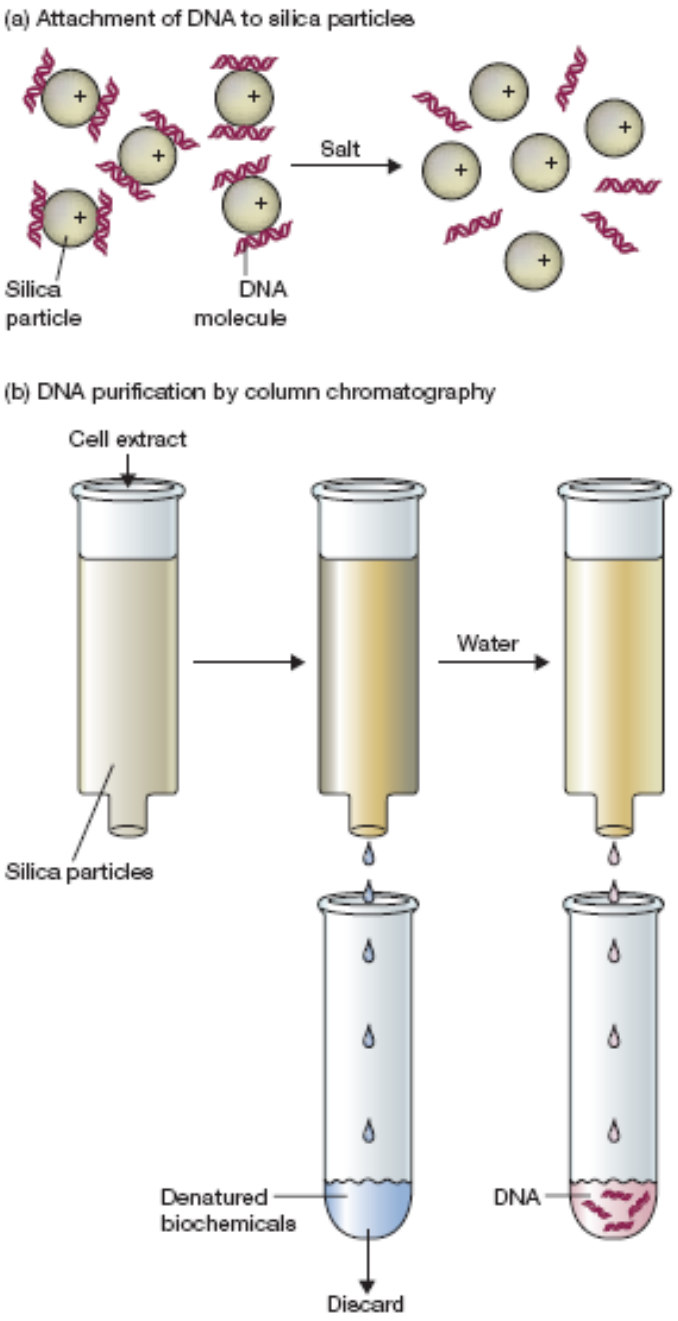
Figure 3.9

The CTAB method for purification of plant DNA.

cetyltrimethylammonium bromide (CTAB)

Guanidium Thiocyanate

Guanidinium thiocyanate, has two properties that make it useful for DNA purification. First, it denatures and dissolves all biochemicals other than nucleic acids and can therefore be used to release DNA from virtually any type of cell or tissue. Second, in the presence of guanidinium thiocyanate, DNA binds tightly to silica particles



Plasmid Isolation

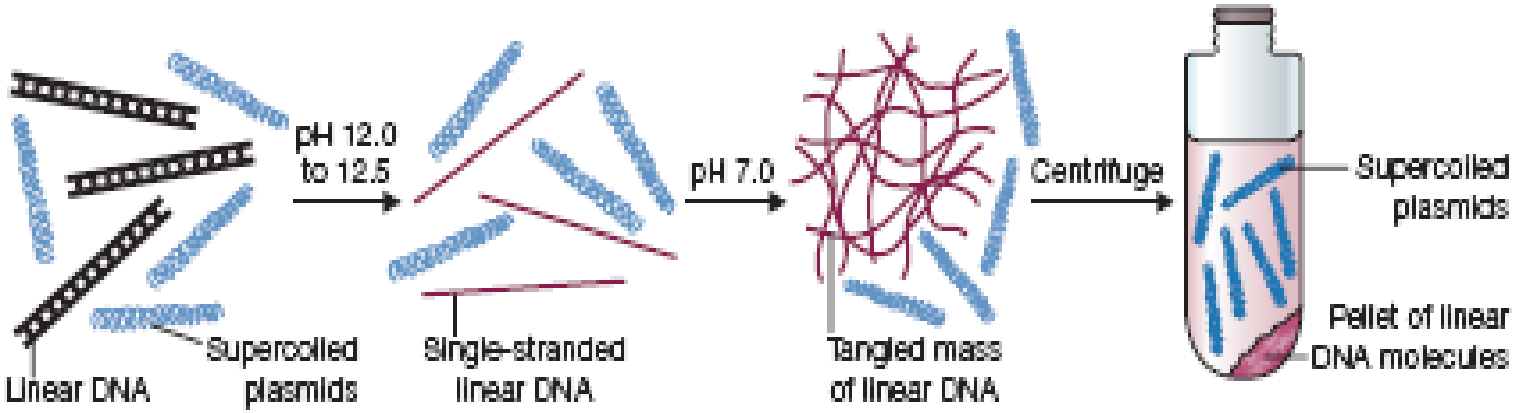
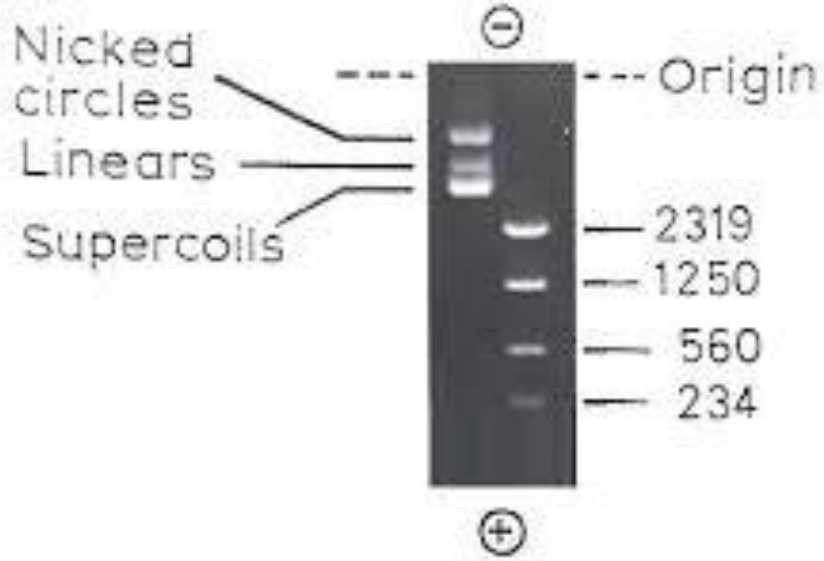


Figure 3.13

Plasmid purification by the alkaline denaturation method.



CsCl Density Gradient Centrifugation

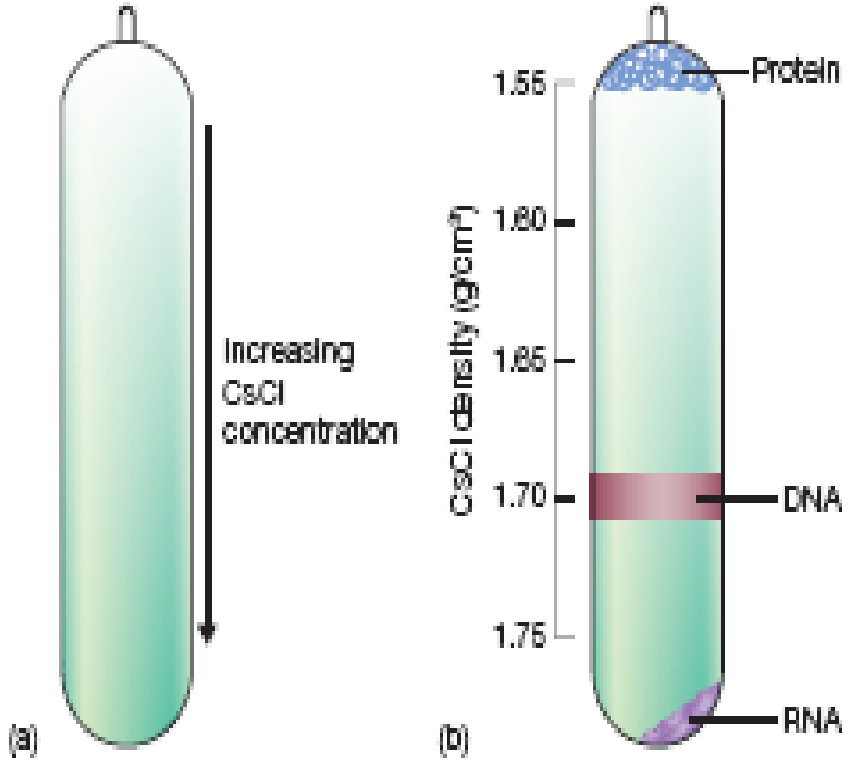


Figure 3.14

Caesium chloride density gradient centrifugation. (a) A CsCl density gradient produced by high speed centrifugation. (b) Separation of protein, DNA, and RNA in a density gradient.

EtBr-CsCl Density Gradient Centrifugation Plasmid DNA Purification

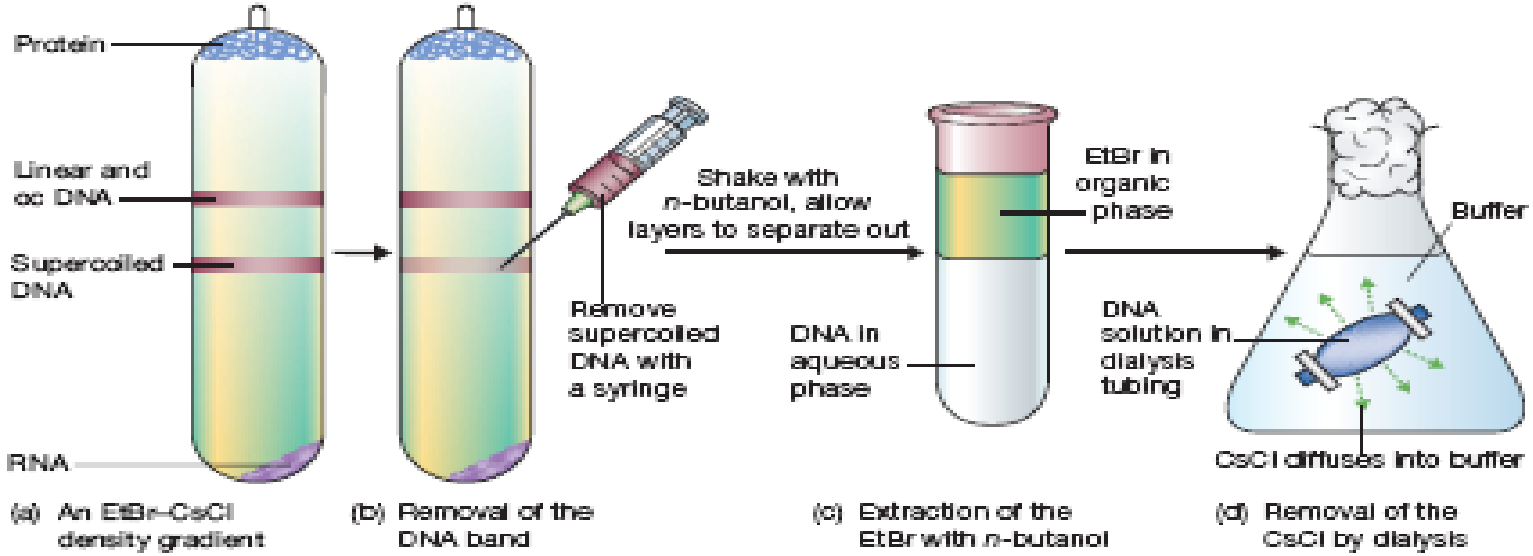
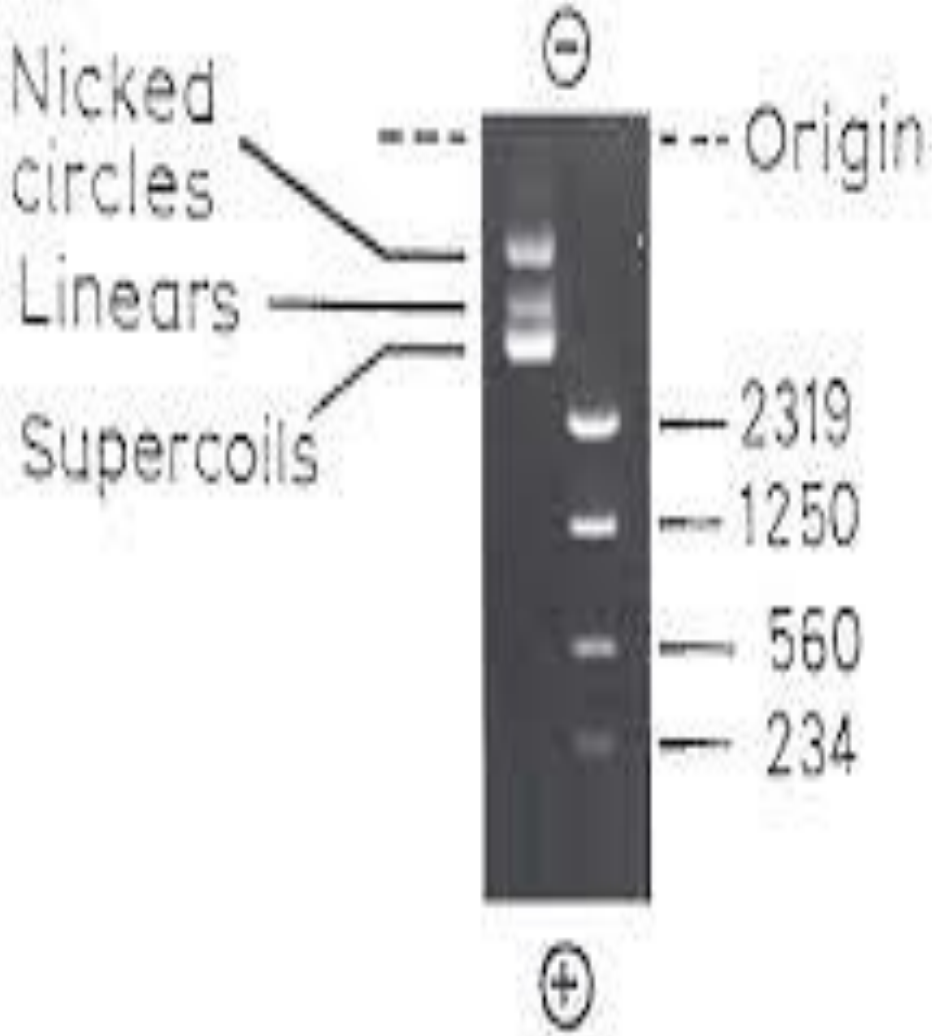


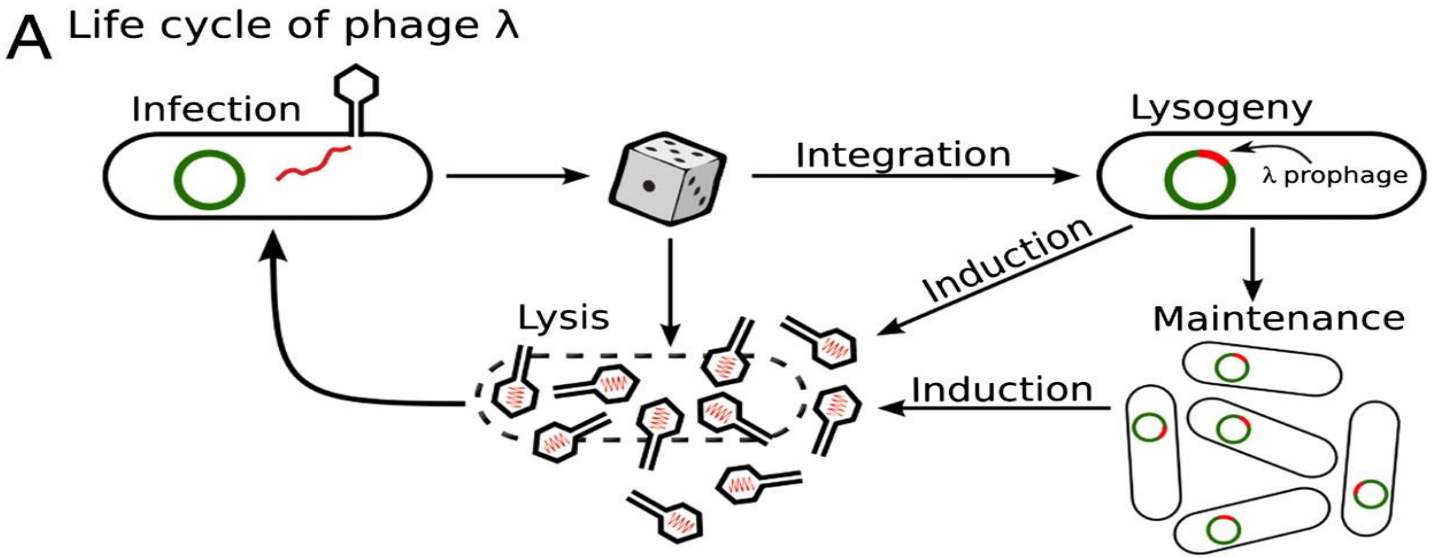
Figure 3.16

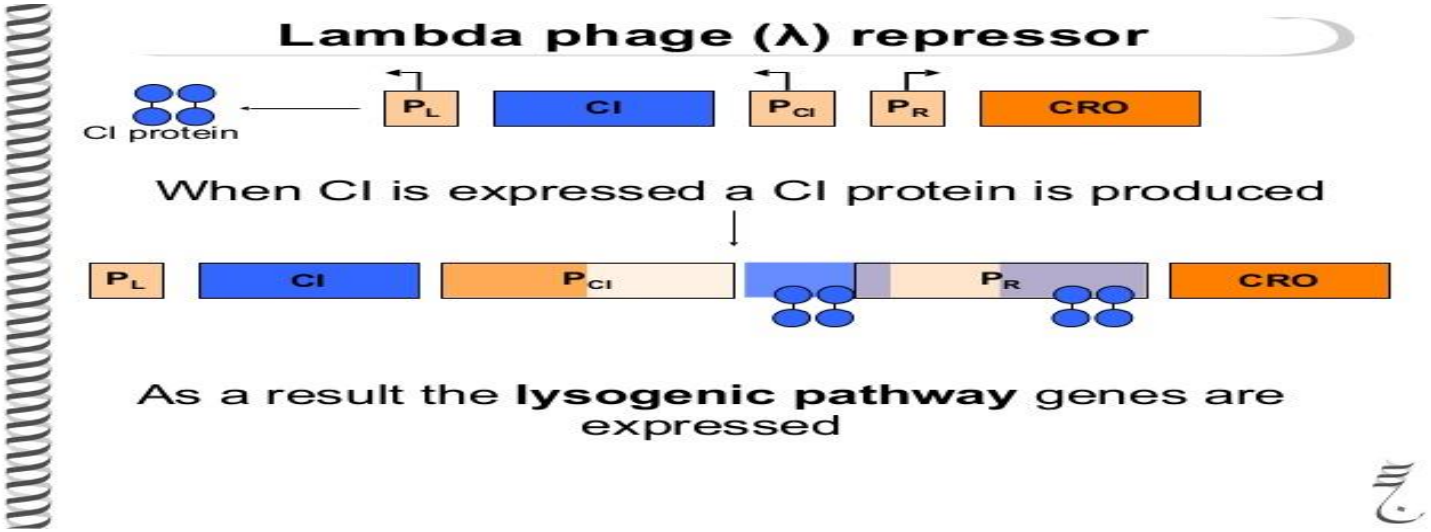
Purification of plasmid DNA by EtBr–CsCl density gradient centrifugation.

More importantly, density gradient centrifugation in the presence of ethidium bromide (EtBr) can be used to separate supercoiled DNA from non-supercoiled molecules. Ethidium bromide binds to DNA molecules by intercalating between adjacent base pairs, causing partial unwinding of the double helix (Figure 3.15). This unwinding results in a decrease in the buoyant density, by as much as 0.125 g/cm^3 for linear DNA. However, supercoiled DNA, with no free ends, has very little freedom to unwind, and can only bind a limited amount of EtBr. The decrease in buoyant density of a supercoiled molecule is therefore much less, only about 0.085 g/cm^3. As a consequence, supercoiled molecules form a band in an EtBr–CsCl gradient at a different position to linear and open-circular DNA (Figure 3.16a).


Nicked
circles
Linears
Supercoils
Origin
2319
1250
560
234

Lambda Phage





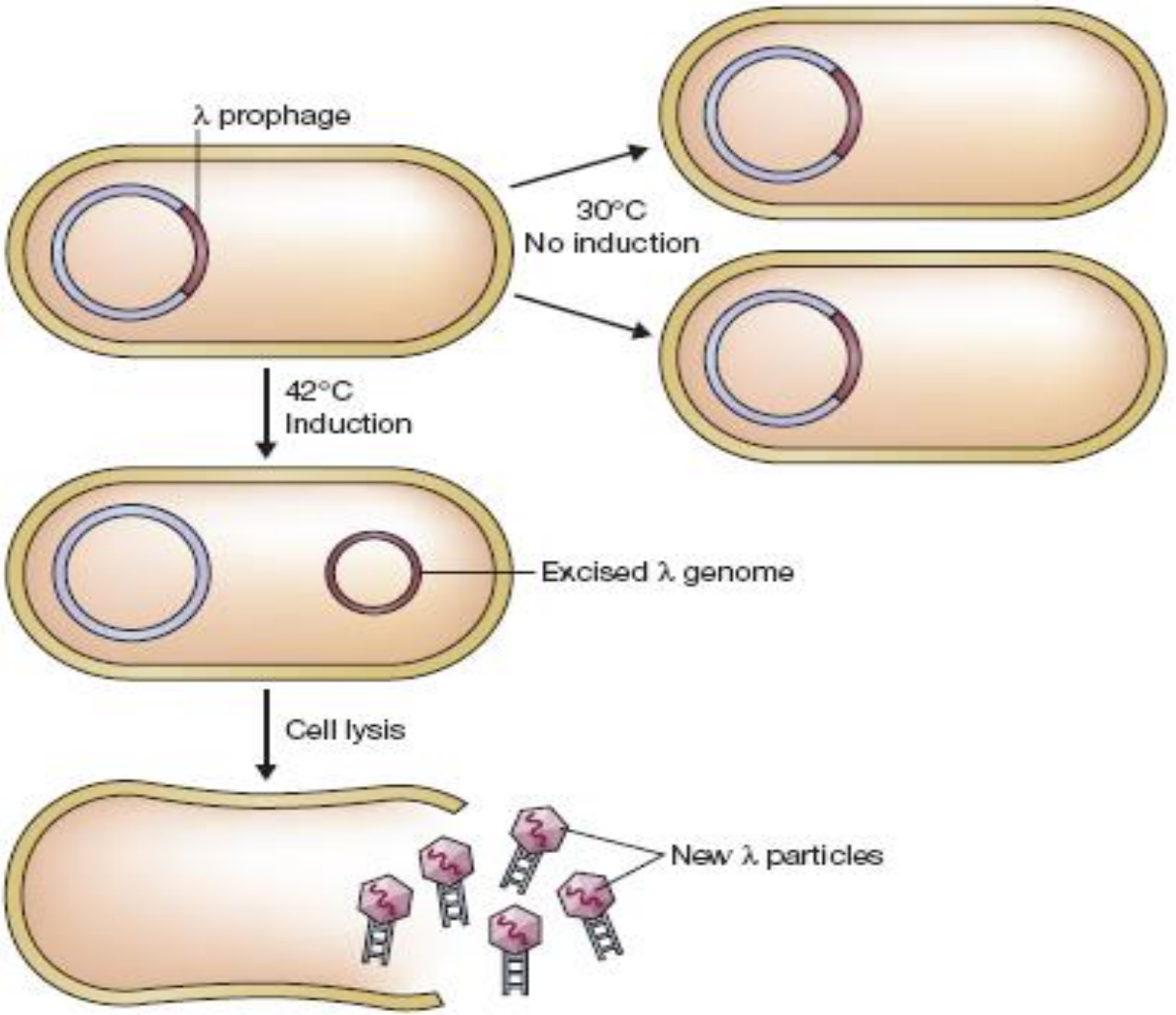


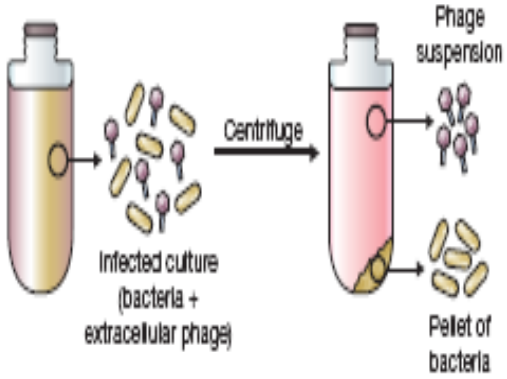
Figure 3.19

Induction of a λ *cIts* lysogen by transferring from 30°C to 42°C.

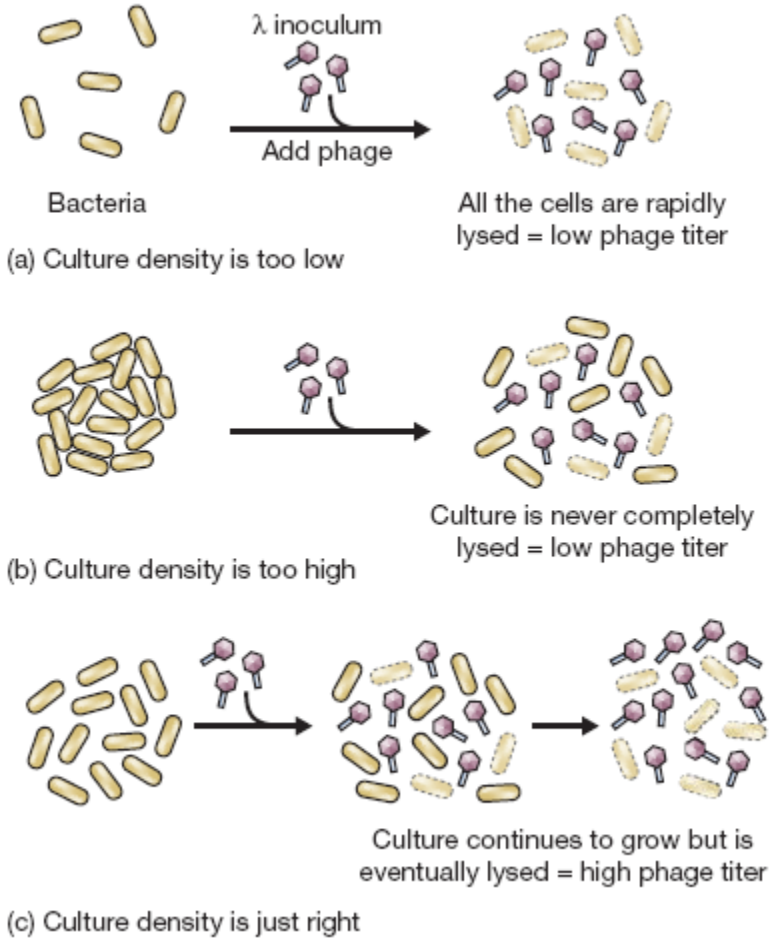
Lambda Phage Isolation

Figure 3.18

Preparation of a phage suspension from an infected culture of bacteria.



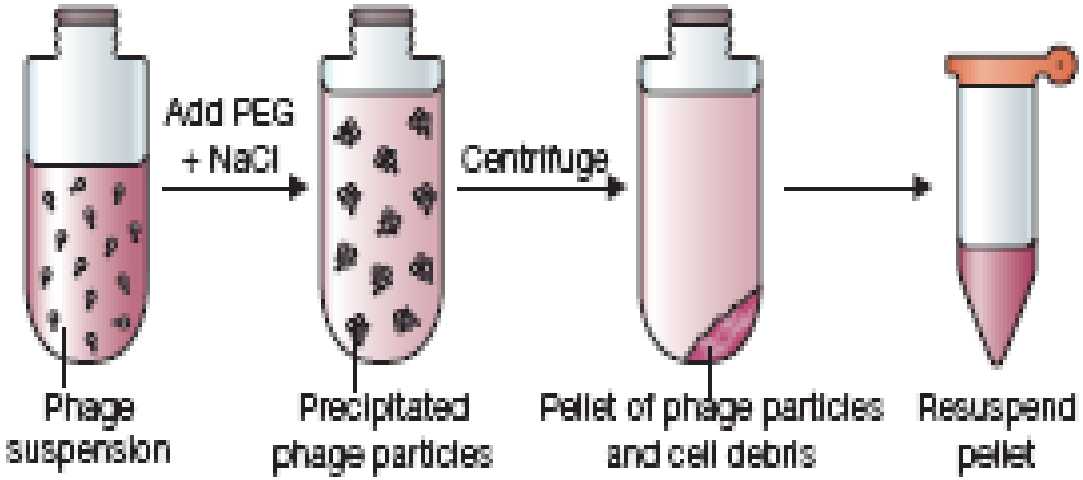
phage DNA is subject to several pitfalls. The main difficulty, especially with λ, is growing an infected culture in such a way that the extracellular phage titer (the number of phage particles per ml of culture) is sufficiently high. In practical terms, the maximum titer that can reasonably be expected for λ is 10^{10} per ml; yet 10^{10} λ particles will yield only 500 ng of DNA. Large culture volumes, in the range of 500–1000 ml, are therefore needed if substantial quantities of λ DNA are to be obtained.



Lambda Phage Isolation

Figure 3.21

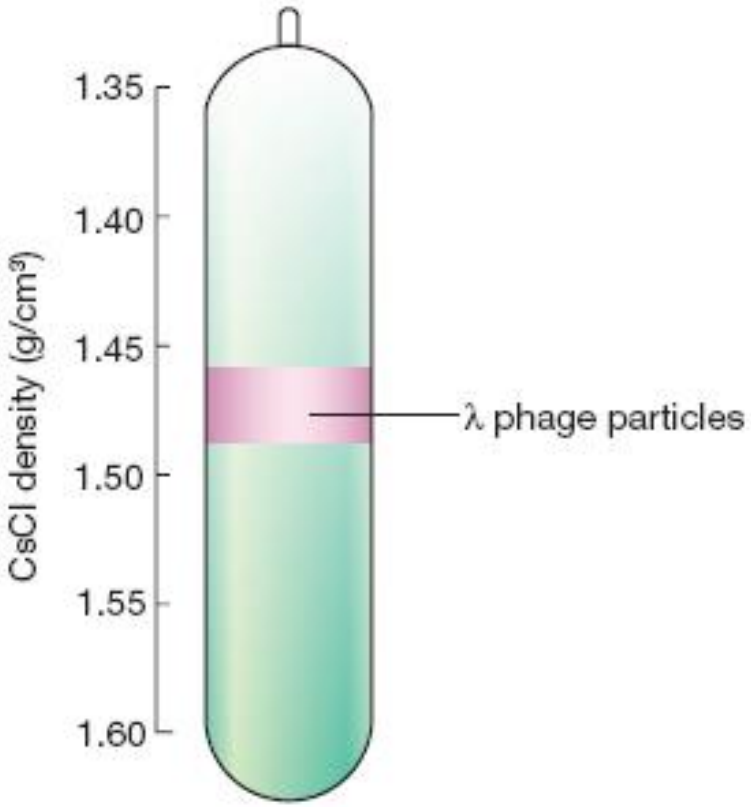
Collection of phage particles by polyethylene glycol (PEG) precipitation.



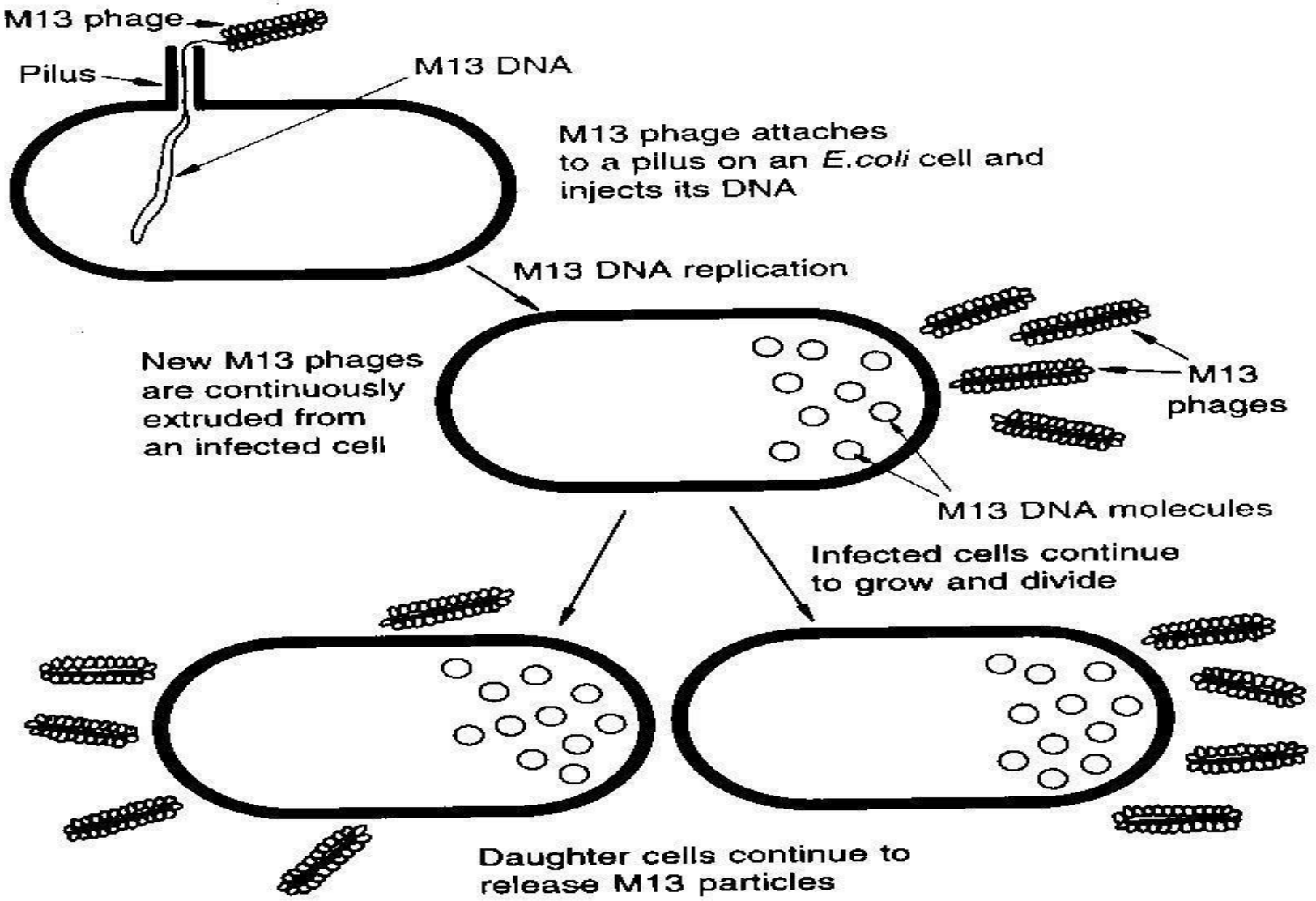
Phage particles are so small that they are pelleted only by very high speed centrifugation.

Collection of phages is therefore usually achieved by precipitation with **polyethylene glycol (PEG). This is a long-chain polymeric compound which, in the presence of** salt, absorbs water, thereby causing macromolecular assemblies such as phage particles to precipitate. The precipitate can then be collected by centrifugation, and redissolved in a suitably small volume (Figure 3.21).

CsCL purification of Lambda Phage



M13 phage DNA



M13 phage DNA preparation