DNA plasmid minipreps - How it works:

Solution I: 50 mM glucose, 10 mM EDTA and 25 mM Tris-HCl, pH 8.0
Solution II: 0.2 N NaOH, 1% SDS
Solution III: 3 M potassium acetate, 2 M acetic acid

Solution I converts the bacteria to spheroplasts.

- Glucose prevents immediate osmotic lysis of the bacteria and helps prevent shearing of the DNA.
- EDTA disrupts the outer membrane by chelating calcium (allowing lysozyme to enter, if employed) and magnesium (in order to inhibit cation-dependent nuclease activity).
- Tris acts as a buffer for Solution II to prevent irreversible denaturation of ccc (covalently closed circular) DNA above pH 12.6.
- Lysozyme degrades the rigid mucopeptide layer of the cell wall. RNase A or RNase T1 may be added to reduce the amount of RNA copurified with the plasmid DNA.

Solution II

- NaOH/SDS lyses the spheroplasts and partially denatures nucleases.

  At a pH of 12.0-12.5, linear plasmid DNA and chromosomal DNA are denatured. The ccc DNA is not affected unless the pH is > 12.5.

  Proteins are also denatured at pH 12.0-12.5, eliminating most nucleases.
- SDS lyses the cells and denatures protein.

Solution III

- Potassium acetate is added to pull out cell debris, SDS, SDS-protein complexes and chromosomal DNA as a white curd-like precipitate. If added too quickly, air can be trapped and prevent good sedimentation.
- The lower pH neutralizes the alkali in Solution II and causes the chromosomal DNA to form an insoluble complex that precipitates in high salt.
- The positively charged potassium ions are used in ethanol precipitation to shield the negative charge on the phosphate backbone of the DNA. As a result, non-ionic, hydrophobic interactions take place which provoke aggregation of the DNA.
- Potassium acetate is more effective than sodium acetate, but can be replaced with an equal volume of 7.5 M ammonium acetate.

Precipitation at -70 degrees will precipitate out polysaccharides as well as nucleic acids and should be avoided during plasmid preps.

Quantities of the 3 solutions are calibrated to prevent irreversible denaturation of the ccc plasmid DNA.

Chloroform extraction may be employed to remove cell wall carbohydrates.

Some protocols employ proteinase K to eliminate endonuclease I, but proteinase K preps may be contaminated with nucleases and phosphodiesterases.