Plasmid DNA Isolation from *E. coli* cells (Followed as per Quiagen guideline and Quiagen kit)

DNA yield depends on the quality of the cell lysate used. Preparation of a cleared cell lysate is therefore a critical step in the purification procedure, which should be carefully designed to provide optimal lysis conditions.

After harvesting and resuspension in P1 buffer (containing RNase A), the bacterial cells are lysed in NaOH-SDS (Buffer P2). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA from the cell without release of cell wall-bound chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. The lysate is neutralized by the addition of acidic potassium acetate (Buffer P3). The high salt concentration causes KDS* to precipitate, and the denatured proteins, chromosomal DNA, and cellular debris become trapped in salt–detergent complexes. Plasmid DNA, being smaller and covalently closed, renatures correctly and remains in solution. Since any SDS remaining in the lysate will inhibit binding of DNA to QIAGEN resin, the solution must be thoroughly but gently mixed to ensure complete precipitation of the detergent. (* Potassium dodecyl sulfate.)

Separation of plasmid from chromosomal DNA is based on coprecipitation of the cell wall-bound chromosomal DNA with the insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAGEN resin and will elute under the same salt conditions. RNase A, which is added at the beginning of the procedure, digests the liberated RNA efficiently during the alkaline lysis. The resulting RNA fragments do not bind to QIAGEN-tip

(purification matrix present on a small tube) under the salt and pH conditions present in the lysate. The precipitated debris is removed by centrifugation, producing a cleared lysate for loading onto the QIAGEN resin. It is important that the lysate is clear at this stage to ensure good flow rates and, ultimately, to obtain protein-free plasmid DNA preparations.

The cleared lysate is loaded onto a pre-equilibrated QIAGEN-tip by gravity flow. The salt and pH conditions of the lysate and the superior selectivity of the QIAGEN resin ensure that only plasmid DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained and appear in the flow-through fraction. The QIAGEN-tip is then washed with buffer PE containing 70% ethanol followed by recovering of plasmid DNA by EB buffer containing 10 mM Tris-HCl (pH 8.0).