# Ammonium acetate induced isolation of plasmid DNA

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## Abstract

The widespread use of phenol chloroform isoamyl alcohol, GuTc(Guanidine thiocyanate) as a protein elimination reagent was found to interfere with subsequent investigation of the isolated plasmid DNA. Thus the current method was designed to avoid the use of hazardous substances such as resin beads, Guanidine thiocyanate, phenol chloroform isomyl alcohol and instead involved the use of readily available ammonium acetate as the protein precipitation solution. This method produced high quality plasmids of supercoiled and open nicked forms without the use of plasmid spin columns .This was found economically efficient and could be used for day-to-day laboratory regimen and even for crucial experiments.

**KEYWORDS:** Plasmid DNA, Ammonium acetate, alkalinelysis, Agarose gel electrophoresis.

## Introduction:

Plasmids hold a significant position in recombinant DNA technology experimentations that utilizes bacterial plasmid DNA for different cloning and expression strategies. The plasmid is an autonomous, extra-chromosomal genetic material and it configures itself as covalently closed circular DNA inside the bacterial cell (Fiers and Sinsheimer.,1962)[1]. Plasmid DNA uptakes a linear double stranded configuration and forms a circular structure, by nicking one strand and joining the other or by the attachment of both the strands, this structure is referred as covalently closed structure, although extracellular plasmid DNA exists in supercoiled form (Sinsheimer.,*et al*,1964; Vinograd.,*et al*,1965)[2 & 3]. Plasmid DNA isolation is one of the most pivotal step for major molecular biology experimentations involving cloning, DNA sequencing, gene therapy , transfection strategies and many more and hence a high degree of purity of plasmid DNA is a prerequisite for such cases.One of the most common techniques for bacterial plasmid DNA isolation is selective alkaline lysis of high molecular weight chromosomal DNA while the plasmid DNA remains double stranded. In this process sodium hydroxide and sodium dodecyl sulphate treated bacterial host cells lyse at an alkaline pH and high molecular weight chromosomal DNA is denatured, on treatment of the lysate with acetate salt.The pH is neutralized, and chromosomal DNA forms an insoluble aggregration (Birnboim and Doly ,1979)[4].High concentration of acetate salt leads to SDS-protein complex precipitation (Kay.,*et al*.1952; marko.,*et al*,1951)[5 & 6] , and also RNA precipitation (Crestfield.,*et al*,1955)[7].

Purification of plasmid DNA by alkaline lysis method , followed by ammonium acetate precipitation method was also reported (MicardD.,*et al* ,1985)[8].In some recent studies plasmid DNA extractions were done using ammonium acetate precipitation for mammalian cell transfections(Saporito Irwin SM.,*et al*, 1997)[9] and reactor scale alkaline lysis that included a selective precipitation step with ammonium acetate (Wright JL., *et al*,2001)[10].Efficient plasmid DNA isolation protocol by chromatography purification methods (StadlerJ.,*et al*,2004)[11 & 12],(Sun B.,*etal*,2013)has been reported but many of them are cumbersome and elaborate methods.

In this study, we are reporting an improved procedure of plasmid extraction by alkaline lysis method, without the use of harmful organic solvents. Ammonium acetate method significantly reduced the loss of plasmid DNA as in resin beads and silica gel columns due to lack of adsorption and also excluded the chances of phenol contamination in the product.

**Materials and methods:**

The present study was conducted in DSR Genome Technologies Pvt. Ltd.

**Bacterial strain:***Escherichia coli* DH5α ( MTCC No.:1652) containing pUC based plasmid was purchased from MTCC, India.

**Media:**Molecular Biology grade Nutrient Agar,Yeast extract type I and Tryptone type I were purchased from HiMedia.

***Reagents/chemicals:*** TGE(Tris Glucose EDTA) buffer, cell lysis solution, neutralization solution, molecular biology grade isopropanol was procured from Merck, molecular biology grade RNase A,TE(Tris EDTA) buffer, TAE(Tris Acetate EDTA) buffer ,Ethidiumbromide, Bromophenol blue and Nalidixic acid antibiotic were purchased from HiMedia,1 Kb gene ruler was obtained from Takara,Clontech ,molecular biology grade Agarose was procured from Lonza Ltd.

***Plasmid isolation:*** Lyophilized *E. coli* DH5α host cells were revived using LB media having 50 µg/ml Nalidixic acid concentration. *E. coli* were grown for 16-18 hours at 105 rpm and 37$^{o}$C.1.5 ml of the overnight grown culture was centrifuged at 6000 rpm for 5 minutes at 4$^{o}$C to harvest the bacteria . The cell pellet was resuspended in 100 µl of ice cold TGE buffer(25mM Tris, 50mM Glucose,10mM EDTA pH=8), lysed with 200 µl of cell lysis solution(0.2 N Sodium hydroxide,1% SDS),kept in ice for 3-5minutes and then to the lysate 300 µl of neutralizing solution(7.5 M Ammonium acetate) was added and inverted gently few times.The white precipitate was removed after centrifugation at 8000 rpm for 15 minutes and the supernatant was transferred to fresh vials to which equal volume of pre-chilled isopropanol was added, this was incubated for 10 minutes on ice and then centrifuged at 10000 rpm for 20 minutes at 4$^{o}$C.After the supernatant was decanted the pellets were air dried and resuspended in 20 µl of 1XTEbuffer along with 5 µl of RNase A and incubated at 37$^{o}$C for 20 minutes.

***Agarose gel analysis***: Agarose gel analysis was done to check the purity and integrity of the extracted plasmid DNA. A 1% agarose gel was made,0.001% Ethidium bromide was

added, 1 Kb gene ruler and the extracted plasmid DNA were loaded into the wells along with bromophenol blue, and was visualized in UV transilluminator.

***DNA quantification***: Estimation of plasmid DNA quantity was done by dilution of the extracted DNA samples in Nuclease free water, the absorbance was measured at 260 nm and 280 nm using a spectrophotometer (BioPhotometerPlus,Eppendorf).

**Results and discussion:**

On subjection of bacterial cell suspension to a highly alkaline environment, the high molecular weight bacterial chromosomal DNA and proteins are denatured. When the lysis is carried out at a pH environment of 12-12.5(Birnhoim and Doly,1979)[4], it leads to an irreversible denaturation of the high molecular weight chromosomal DNA, the cell lysate is neutralized by the addition of a high molecular weight acidic salt which acidifies the alkaline environment. This slight change in pH of the environment leads to rapid selective renaturation of the small molecular weight plasmid DNA but is unable to renature the bacterial chromosomal DNA.

The use of Ammonium acetate for protein and high molecular weight DNA precipitation has been already reported (Aidar M. *et al.*,2007)[13] for the purification of buccal DNA. The use of Ammonium acetate for selective precipitation of bulk RNA and high molecular weight DNA from plasmid DNA has been reported to be of high effectiveness. Thus it was observed that when equal volume of 7.5 M Ammonium acetate is added to cell lysate, instead of half volume of 3M Potassium acetate, the recovered plasmid DNA were quantified to be of about a concentration that is in close proximity to the concentration of plasmid DNA extracted by DNA extraction kit as shown in Table 1.

In the plasmid DNA extraction method,the three main contaminating macromolecules are protein,RNA and chromosomal DNA, so with the use of Ammonium acetate , the co-precipitation of these contaminants is highly ensured .The bands visualized confirmed high yield of plasmid DNA extracted by ammonium acetate method(Figure 1.a) and simultaneously confirmed the presence of plasmid DNA in similar concentrations in both the modified method and method using potassium acetate as neutralization solution(Figure 1.b).The two bands of plasmid DNA observed in a single lane corresponds to open nicked forms and supercoiled forms of plasmid DNA respectively

**Conclusion:**

The data revealed that the present protocol utilizing ammonium acetate neutralization solution can be adapted for obtaining a good yield of plasmid DNA, much the same as protocols utilizing potassium acetate.The optical density ratios at 260/280 nm indicated the mere presence of contamination and impurities. This described protocol is easy, simple, cost effective and rapid method for isolation of plasmid DNA from bacterial cells which has reasonable yield of high grade plasmid and can be used at par with commercially available plasmid isolation kits using potassium acetate as neutralization solution.

**Table and Figures:**

**Table 1: Quantification of DNA samples by Ammonium acetate method (modified method) and potassium acetate method of plasmid isolation.**

| Sl.Nos | Sample ID | Plasmid isolated using modified protocol | Plasmid isolated using potassium acetate |
|---|---|---|---|

| | | *ng/µL* | *260/280* | *ng/µL* | **260/280** |
|---|---|---|---|---|---|
| **1** | PL001 | 203 | 1.69 | 200 | 1.72 |
| **2** | PL002 | 154 | 1.76 | 180 | 1.75 |
| **3** | PL003 | 264 | 1.81 | 278 | 1.79 |
| **4** | PL004 | 166 | 1.74 | 236 | 1.73 |

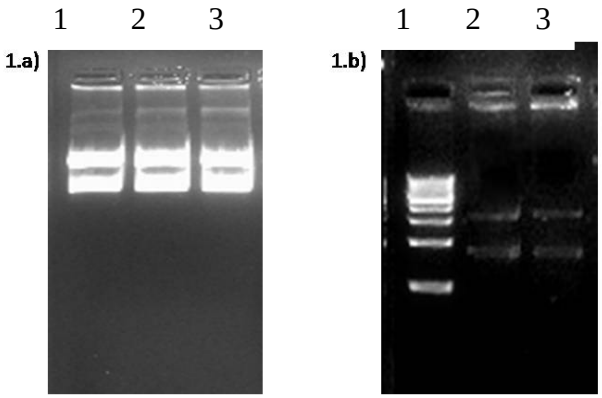


**Figure 1.a and 1.b** : 1 % Agarose gel electrophoresis of isolated plasmid DNA samples.
**Figure 1.a : Lane 1,2,3:** Plasmid DNA samples isolated by ammonium acetate method.
**Figure 1.b : Lane 1**-1Kb DNA ladder,
**Lane 2**-Plasmid DNA isolated by ammonium acetate method,
**Lane 3**-Plasmid DNA isolated by potassium acetate method .

**References:**


1. Fiers, W., and R. L. Sinsheimer. (1962). *J. Mol. Biol*. 1962;5:424.
2. Young ET, 2nd, Sinsheimer RL.(1964), Novel intra-cellular forms of lambda dna. *J Mol Biol.* 1964 Dec;**10**:562–564.
3. Vinograd J, Lebowitz J, Radloff R, Watson R, Laipis P.(1965) The twisted circular form of polyoma viral DNA. *ProcNatlAcadSci U S A.* 1965 May;**53**(5):1104–1111
4. Birnboim HC &Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl Acids Res* 7: 1513–1523.
5. Kay, E.R.M., Simmons, N.S. and Dounce, A.L. (1952), An improved preparation of sodium deoxyribonucleate *J. Am. Chem. Soc.*, 74:1724-1726
6. Marko, A.M. and Butler, G.C. (1951) The Isolation Of Sodium Desoxyribonucleate With Sodium Dodecyl Sulfate *J. Biol. Chem.*, 190: 165-176.
7. Crestfield, A.M., Smith, K.C. and Allen, F.W. Y(1955), The Preparation And Characterization of Ribonucleic Acids From Yeast, *J. Biol. Chem.*, 21: 6185-193.

**8.** Micard D, Sobrier ML, Couderc JL, et al.(1985) Purification of RNA-free plasmid DNA using alkaline extraction followed by Ultrogel A2 column chromatography,*AnalBiochem* 1985 Jul; 148(1) :121-6.

**9.** Saporito-Irwin SM, Geist RT, Gutmann DH.(1997) Ammonium acetate protocol for the preparation of plasmid DNA suitable for mammalian cell transfections.*Biotechniques*. 1997 Sep;23(3):424-7

**10.** Jason L. Wright, Martin Jordan & Florian M. Wurm (2001) Extraction of plasmid DNA using reactor scale alkaline lysis and selective precipitation for scalable transient transfection.*Cytotechnology* 2001 35: 165–173

**11.** Stadler J, Lemmens R, Nyhammar T.(2004) Plasmid DNA purification.*J Gene Med.* 2004 Feb;6 Suppl 1:S54-66

**12.** Sun B, Yu X, Yin Y, Liu X, Wu Y, Chen Y, Zhang X, Jiang C, Kong W (2013) Large-scale purification of pharmaceutical-grade plasmid DNA using tangential flow filtration and multi-step chromatography.*J Biosci Bioeng.* 2013 Sep;116(3):281-6.doi: 10.1016/j.jbiosc.2013.03.015.

**13.** Aidar M, Line SR.(2007) A simple and cost-effective protocol for DNA isolation from buccal epithelial cells,*Braz Dent J*. 2007;18(2):148-52.

**14.** Atul Kumar, Sachidanand Singh, AkashTomar(2010) Development of Midi-Plasmid Isolation Kit and Cost Optimization of DNA Molecular Weight Ladder: Economy of Laboratory vs.Commercial Products, *Advances in Applied Science Research*, 2010, 1 (3): 129-146

**15.** Sambrook, J. and Russell, D.W. (2001)*Molecular Cloning: A Laboratory Manual*, *Cold Spring Harbor Laboratory Pres*s, Cold Spring Harbor,NY.3rd Ed, pp. 1.32, 1.35, 1.38.

**16.** T. F. Roth and D. R. Helinski(1967), Evidence for circular dna forms of a bacterial plasmid.*ProcNatlAcadSci* U S A. Aug 1967; 58(2): 650–657.

**17.** Tarczynski, M.C., Meyer, W.J., Min, J.J., Wood, K.A. and Hellwig, R.J. (1994) Two minute miniprep method for plasmid DNA isolation.*Biotechniques*., 16(3): 514–519