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Quantitative and Qualitative Chemical Extraction of Deoxyribo Nucleic Acid DNA from Human Cell Organelles

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Abstract

The extraction of deoxyribo nucleic acid DNA from biological cell organelles is a complex chemical procedure. The DNA resides in the cell organelles nucleus and mitochondria of biological samples such as blood, bone and tissue. The cell has a cell membrane enclosing the cytoplasm containing cell organelles. The DNA is the self replicating inheriting molecule and encodes all the information of the function of cell organelles. The process of DNA extraction is a chemical cascade of lysing the cell, exposing the DNA to the lysis buffer, separation of DNA from bonded proteins and eluting the DNA in a preservative solution. The cell membrane is lysed in a salt solution of Tris, EDTA and NaCl. The EDTA chelated the inorganic ions of the cell membrane and destabilized it. Tris maintain the pH at 8.0 and also interacted with the lipopolysaccharide of the membrane for further destabilization and hypertonic NaCl solution squeeze out the cell contents. The SDS with polar head and non-polar tail interacted with double layer nuclear and mitochondrial membrane for lysis. The exposed DNA from the cell organelles has boned protein molecules. The serine protease proteinase K digests the protein part of DNA protein complex. The proteins unfolded for interaction of its non-polar groups with non-polar phenol and separated out in the phenol solution. The protein separated DNA solution was mixed with phenol, chloroform and isoamyl alcohol to further denature the residual protein and separate it out with the lipids. The isoamyl alcohol reduces the foaming of phenol and chloroform mix. The DNA was precipitated out from the separated solution by ethanol and sodium acetate. This study investigated the quantity and quality of extracted DNA from cell organelles by spectrophotometric and gel electrophoresis analysis.

Keywords: Phospholipid, cell organelles, Tris, EDTA, NaCl, SDS, serince protease proteinase K, phenol, choloroform, isoamyl alcohol, ethanol, sodium acetate.

Introduction

The DNA is enclosed in a complex environment of protein, lipids and other organic and inorganic molecules inside the cell organelles nucleus and mitochondria. The cell is covered by phospholipid bilayer membrane with the non-polar tails inside and polar head outside the membrane. The DNA extraction is a chemical method of cell lysis, protein cleavage and elution of DNA[1-3]. The phospholipid bilayer of cell membranes is dissolved by the lysis solution on formation of water soluble complexes with the aqueous solution facing on both sides of membrane. The degradation of cell membrane flows out the cell contents of dissolved membranes, cellular proteins, lipids alongwith cell organelles containing DNA. The cell organelles have double phospholipid bilayer membrane having outer and inner membrane with in between perinuclear space that makes it more rigid than cell membrane. The detergent SDS lysed the nuclear and mitochondrial membrane by interaction of its non-polar chain with the non-polar tails of phospholipid bilayer. The DNA comes out in the solution of lysis buffer with other cell components. The differential mass centrifugation settle down the cell debris and supernatant contained the DNA bonded with protein. The protein was cleaved with proteinase K enzymatic digestion. The cleaved protein fragments and lipids were eluted out in phenol, chloroform and isoamyl alcohol solution and DNA is precipitated by sodium acetate in ethanol. The eluted DNA was dissolved in Tris-EDTA buffer, where tris maintain the pH and EDTA chelate the inorganic ions to make DNA stable for long time. The quantity and quality test were performed by spectrophotometer reading at 280, 260 and 230 nm and 0.8% agarose gel electrophoresis. The components of the chemicals for the DNA extraction are assessed for the best quantity and quality yield of DNA. The extraction is dependent on the concentration specific chemical cascade of lysis buffer containing Tris, EDTA and NaCl with Tris pH was maintained by HCl to 8, followed by enzymatic digestion of DNA bonded proteins, elution of proteins and lipids by phenol, chloroform, isoamyl alcohol mix and finally the elution of separated DNA by ethanol, sodium acetate.

Material and Methods

The chemical buffers were prepared and autoclaved at 121^0C except proteinase K before use. The molecular biology grade chemicals from Sigma-Aldrich were used. i. 1M Tris- HCl, pH 8.0: 121.1grams Tris base was dissolved in 800 ml of distilled water. The adjustment of pH to 8.0 was performed with concentrated HCl and distilled water added to make final volume of 1 litre. ii. 0.5 M EDTA, pH 8.0: 186.1 grams Ethylene diamine tetra acetate (EDTA) was dissolved in 800 ml

of distilled water and stirred on a magnetic stirrer vigorously. The pH was adjusted 8.0 with NaOH pellets and distilled water was added to make final volume of 1 litre. The EDTA dissolved with the pH adjustment. iii. 5M NaCl: 292.2 grams NaCl was dissolved in 800 ml of distilled water and distilled water added to make final volume of 1 litre. vi. Lysis Buffer I: 30 mM Tris-HCl pH 8.0, 5mM EDTA, 50 mM NaCl 15 ml of 1 M Tris, 5 ml of 0.5 M EDTA and 5 ml of 5M NaCl were mixed and distilled water added to make final volume of 1 litre. v. Lysis Buffer II: 75 mM NaCl, 2mM EDTA, pH8.0 15 ml 5M NaCl and 4 ml 0.5M EDTA was mixed and distilled water added to make final volume of 500ml. vi. 20% SDS: 200g sodium dodecyl sulfate was dissolved in 800 ml of distilled water with heating for dissolution and distilled water added to make final volume of 1 litre. vii. 20 mg/ml Proteinase K: 200 mg Proteinase K was dissolved in 10ml of distilled water and aliquots of 0.5 ml tubes were made. The enzyme was not autoclaved and stored at -20°C. viii. Tris Equilibrated Phenol, pH 8.0: 100g phenol was melted at 65° and mixed thoroughly with 200 mg 8-hydroxyquinoline. The solution was further mixed with half volume of 1M Tris-HCl (pH 8.0) thoroughly on magnetic stirrer. The solution settled down for separation of immiscible phases and aqueous upper phase was discarded. This process was repeated till the aqueous phase pH reached 7.5 and finally 10ml of 0.1M Tris buffer was added. The solution was stored at 4ºC. ix. Chloroform: Iso amyl alcohol (24:1); 2.4 liter of chloroform mixed thoroughly with 100 ml of isoamyl alcohol in dark brown bottle to avoid any reaction of solution with light. x. 3M Sodium acetate, pH 5.2: 40.8 g Sodium acetate tri hydrate was dissolved in 80 ml of distilled water. The adjustment of pH to 5.2 was performed by glacial acetic acid and distilled water added to make final volume of 1 litre. xi. 70% Alcohol: 70 ml absolute alcohol was mixed in 30 ml distilled water. xii. TE Buffer: 10mM Tris-HCl, pH 8.0: 10 ml 1 M Tris-HCl, pH 8.0, 0.2 ml 0.5 M EDTA and 990 ml distilled water were mixed. xiii. 10mg/ml Ethidium Bromide Solution: 100 mg ethidium bromide was dissolved in 10 ml of deionized water and it was wrapped with aluminum foil to avoid any reaction with light. xiv. 50X TAE Buffer, pH 7.2: 242 grams Tris and 100 ml 0.5M EDTA were dissolved in 500 ml of distilled water. 57.1 ml of glacial acetic acid was added and distilled water added to make final volume of 1 litre. xv. 6X Glycerol Gel loading solution: 0.15 % Bromophenol blue, 0.15% Xylene Cyanol FF, 5mM EDTA, 30% Glycerol. 15 mg Bromophenol blue, 15mg Xylene Cyanol FF, 100 µl 0.5 M EDTA (pH 8.0) and 3 ml glycerol were dissolved in final volume of 10 ml with distilled water.

500µl of 10 blood samples were taken for DNA extraction and dissolved in 500 µl of lysis buffer I and heat shock was given at 65^0C for 5 min for lysis of the cells. The centrifugation at 10000 rpm for 10 min is given to the cell suspension. The supernatant containing the serum was discarded. The pellet containing the cells were disturbed and dissolved in 500 µl of lysis buffer II. The 1 to 5% SDS, 25 to 125 µg proteinase K and incubation temperature 37 ^{o}C for 24 h and 56 ^{o}C for 2 h were given to the sample mixtures. 500 µl of tris saturated phenol was added to enzyme treated samples and tubes were kept for shaking for 10 min. The samples were centrifuged at 10000rpm for 10 min followed by pellet discard and to supernatant added 250 µl of tris-saturated phenol and 250 µl of 24:1 chloroform: isoamyl alcohol. The samples were kept for shaking for 10 min and centrifugation at 10000 rpm for 10 min and pellet discarded. 500 µl of 24:1 chloroform: isoamyl alcohol was added to the supernatant, and samples were kept for shaking for 10 min and centrifugation for 10 min at 10000rpm. The pellet was discarded and supernatant contained the extracted DNA to be precipitated. The 1 ml chilled absolute alcohol and 17 µl sodium acetate was added to the samples. The mix is slowly swirled to precipitate the DNA. The samples were centrifuged at 20000rpm for 10min to pellet the visible DNA in the form of thread. The DNA pellets were washed thrice with 70% alcohol and once with absolute alcohol. The pellets were dried for 2 hours and then dissolved in TE buffer. The samples were vortexed and incubated at 65^0C for 10 min to dissolve the DNA homogenously. The quantity and quality check of DNA were carried out in spectrophotometer analysis at 230, 260 and 280 nm and 0.8% agarose gel electrophoresis with size separation λ hind III marker.

Results and Discussion

The DNA was extracted from 10 samples. The DNA quantity and quality were checked in spectrophotometer analysis and 0.8% agarose gel electrophoresis. The size standard marker was run along the run in the gel electrophoresis for relative quantification of samples. The spectrophotometric analysis of samples at 230, 260 and 280 nm was carried out for evaluation of quantity and quality of DNA extracted from the samples. The chemical mechanism of cell and nuclear membrane lysis with buffer containing Tris, EDTA, NaCl and SDS, followed by separation of DNA from bonded proteins and lipids by phenol, chloroform and isoamyl alcohol and extraction of DNA in ethanol, sodium acetate precipitation were investigated. The concentration of SDS from 1 to 5%, proteinase K from 25 to 125 µg with the incubation temperature 37 ^{o}C for 24 h and 56 ^{o}C for 2 h in reaction volume were analyzed to estimate the yield of DNA from the blood samples. The yield of DNA in 10 different samples with the above concentration of SDS, proteinase K and enzyme incubation at 37 ^{o}C for 24 h and 56 ^{o}C for 2 h were shown in table 1, 2 and 3 respectively. The 2% SDS, 50 µg proteinase K and incubation at 56^0C for 2 h provide the best average yield of 10.7 µg in all the samples. The concentration of two parameters is kept at optimum to measure the best concentration of one parameter.

The efficiency of optimum concentration of chemicals i.e. 2% SDS, 50 µg proteinase K and incubation at 56^0C for 2 h in cell organelles membrane lysis and cleavage of bonded proteins to DNA is measured by spectrophotometric analysis and gel electrophoresis. The absorption maximum of nitrogenous bases in nucleotides is 260 nm. The UV absorption maximum of proteins is 280 nm, because of tryptophan amino acid. The

absorbance at 280 nm of DNA sample provides the measure of the protein contamination and 230 nm absorbance measure the residual phenol in the sample. The ratio of 260 nm/280 nm absorbance should be 1.6 to 1.8 and 230 nm/260 nm absorbance should be 0.3 to 0.9. Quantitative and qualitative analysis of extracted DNA with 2% SDS, 50 µg proteinase K and 56 ^{o}C incubation for 2 hours is shown in table 4. The concentration of DNA in each sample was calculated by 1 OD equal to 50ng/µl × dilution factor of 100. All the samples passed quality check ratio and free of protein and residual phenol contamination.

Table-1
Comparative analysis DNA extraction with different concentration of SDS

Sample	1	2	3	4	5	6	7	8	9	10
SDS Conc. (%)	Yield of DNA Extraction (µg)									
1	06	07	05	04	02	03	04	05	06	07
2	10	11	09	12	11	08	13	10	11	12
3	10	10	08	10	10	09	10	11	10	11
4	09	08	07	09	06	08	09	10	09	10
5	07	06	05	04	08	06	08	09	07	08

Table-2
Comparative analysis DNA extraction with different concentration of Proteinse K

Sample	1	2	3	4	5	6	7	8	9	10
Proteinase K Conc. (µg)	Yield of DNA Extraction (µg)									
25	09	08	09	06	05	10	08	07	08	09
50	11	10	12	09	10	12	11	10	12	10
75	10	10	09	10	11	10	10	11	10	09
100	11	10	10	11	09	11	10	10	09	08
125	10	10	09	10	10	10	10	09	08	09

Table-3
Comparative analysis DNA extraction with different incubation temperature and time

Sample	1	2	3	4	5	6	7	8	9	10
Incubation Temp. (^{o}C) for hours	Yield of DNA Extraction (µg)									
37 for 24	10	11	12	09	10	11	12	10	11	11
56 for 2	12	11	10	10	11	10	10	12	09	10

Table-4
Quantitative and qualitative analysis of extraction of DNA on spectrophotometer

Sample	A260	A280	A230	A260/A280	A230/A260	DNA (µg)
1	1.99	1.11	1.19	1.80	0.6	10
2	2.20	1.11	1.32	1.80	0.6	11
3	2.40	1.34	1.20	1.79	0.5	12
4	2.20	1.23	1.32	1.79	0.6	11
5	1.60	0.88	0.80	1.80	0.5	8
6	1.99	1.10	1.19	1.80	0.6	10
7	1.80	1.00	0.90	1.80	0.5	9
8	2.60	1.45	1.82	1.79	0.7	13
9	2.20	1.22	1.32	1.80	0.6	11
10	2.40	1.33	1.44	1.80	0.6	12

The samples were diluted to concentration of 100ng/µl and 2 µl was loaded in the 0.8 agarose check gel in TAE buffer with the loading gel and post run at 80 mV, the samples DNA was stained with 10mg/ml ethidium bromide dye solution. The dye intercalated in DNA was glowed in UV light to visualize the bands of extracted DNA from the samples as shown in figure 1. The quantity and quality of extracted DNA in 10 samples as inferred by gel electrophoresis is shown in figure 2.

The DNA in the biological samples resides in cell organelles nucleus and mitochondria. The cell organelles are enclosed in the phospholipid bilayer membrane. The cell organelles are further enclosed by double layered phospholipid bilayer membranes. The cell organelles nucleus and mitochondria are shown in figure 3 (1) and (2). The structures of Tris, EDTA, phospholipid and SDS are shown in figure 3 (3), (4), (5) and (6).

The chemical mechanism of phospholipid interacting with sodium dodecyl sulphate is shown in figure 4. The interaction lyses the phospholipid layer of cell and organelles membrane.

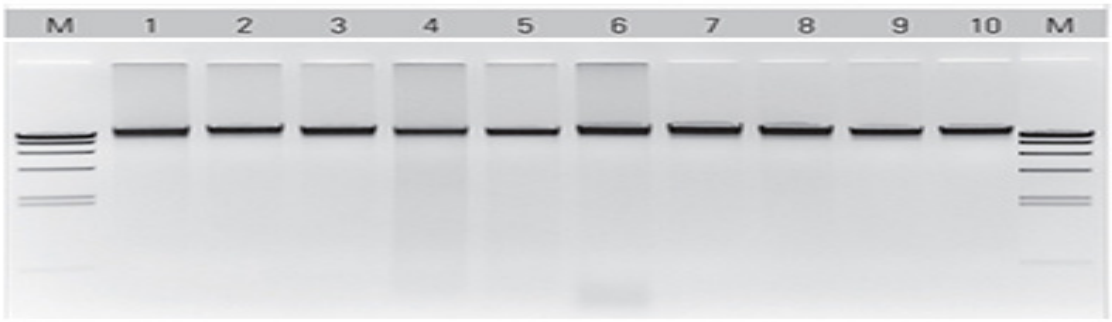


Figure-1
Quantitative and qualitative analysis of extraction of DNA from 10 blood samples with λ hind III markers (M) on 0.8% agarose gel electrophoresis

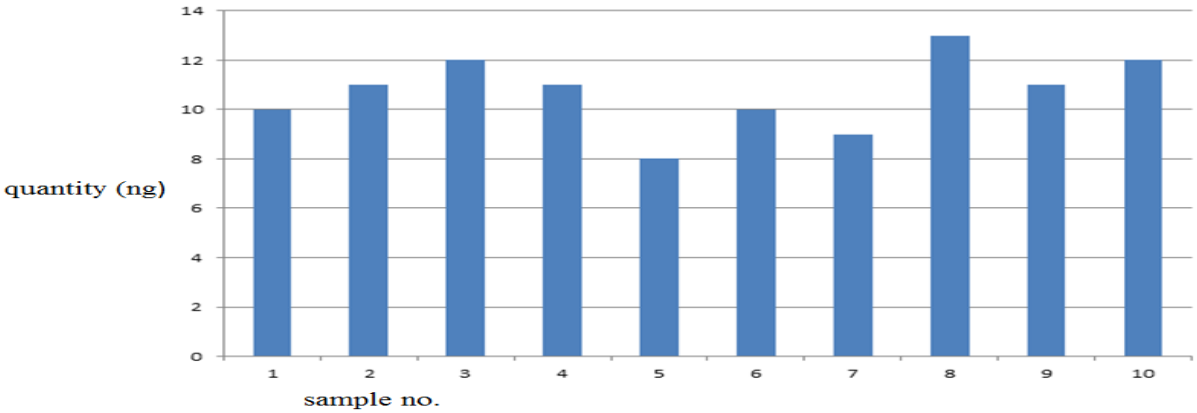
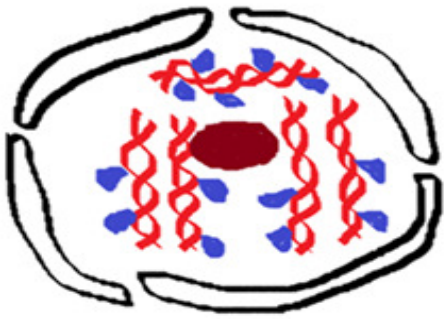
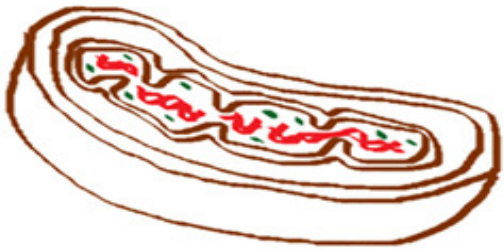


Figure-2
The yield of DNA from 10 blood samples



(1)



(2)

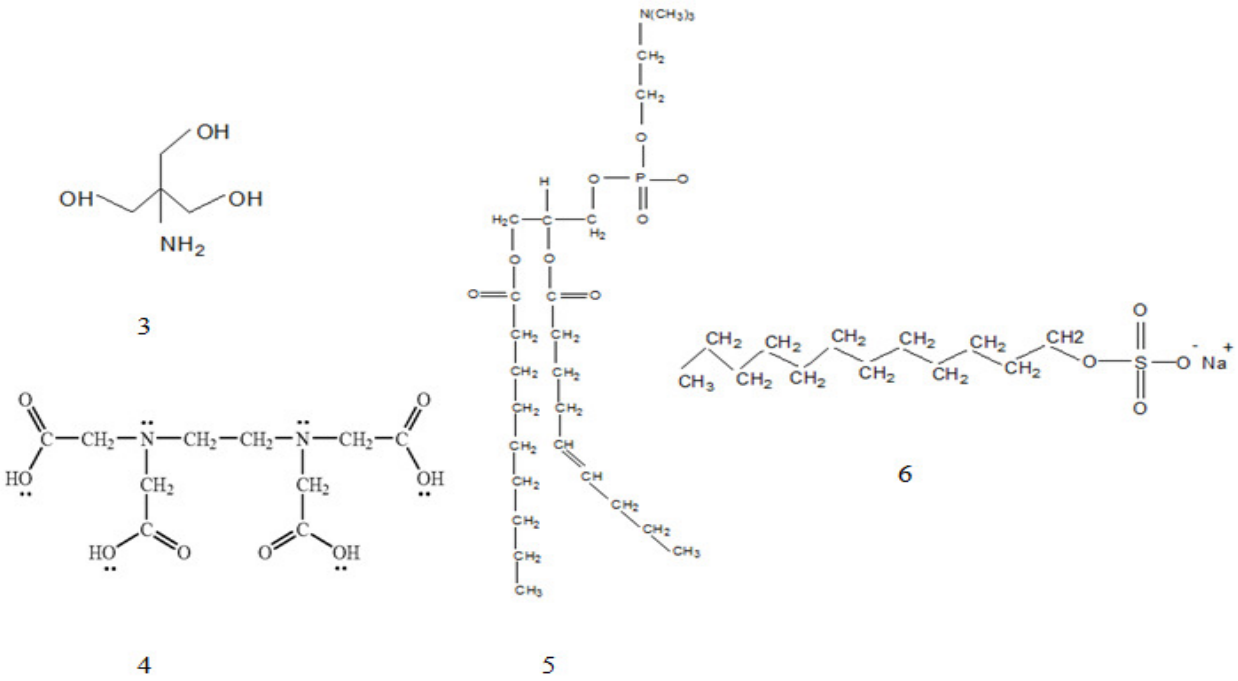


Figure-3
DNA, (2) Mitochondria. The DNA in red, protein in blue, nucleolus in brown and ribosome in green colors are shown. (3) Tris (2-Amino-2-hydroxymethy-propane-1, 3-diol), (4) EDTA (Ethylenediaminetetraacetic acid) (5) Phospholipid, (6) SDS (Sodium dodecyl sulphate)

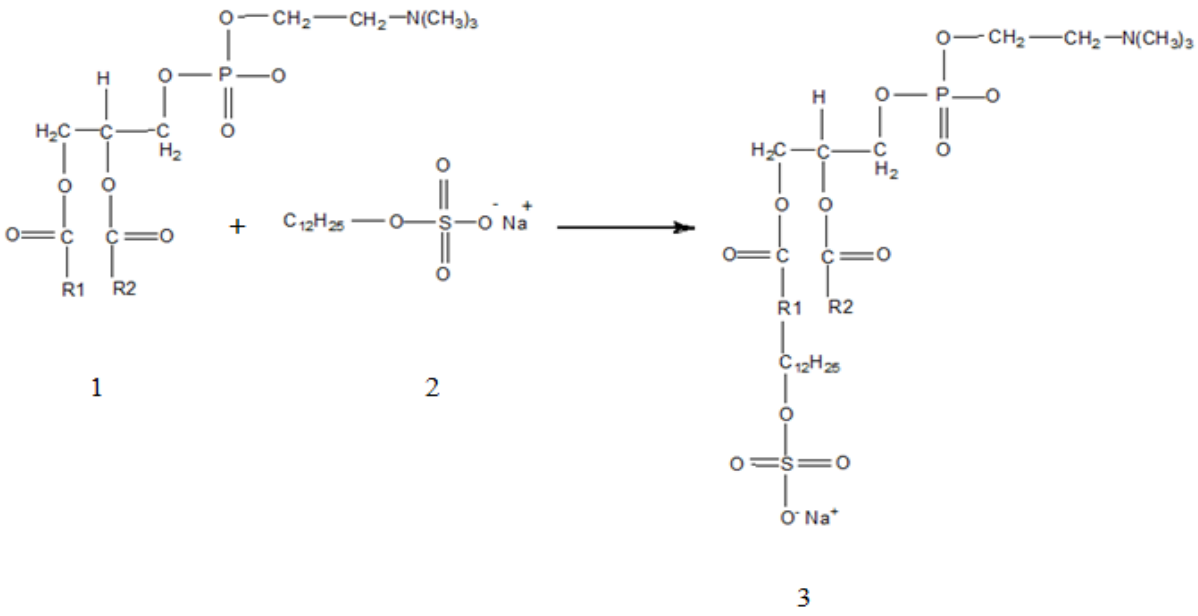


Figure-4
Phospholipid (1) interact with SDS (2) to form Phospholipid SDS complex

The DNA extraction from cell organelles is a complex procedure involving a cascade of chemical reactions[4-8]. The chemical reaction are performed in a stable pH of 8.0 maintained by Tris buffer. The cell organelles are enclosed in a cell membrane composed of phospholipid bilayer. The phospholipid has polar head and non polar tail with polar region is folded inside the membrane. The lysis buffer contain Tris, EDTA and NaCl, all three starts lysing the phospholipid membrane. The Tris maintain the pH and interact with the lipopolysaccharide of the membrane to destabilize, EDTA chelate the inorganic ions of membrane for further destabilization, NaCl in the lysis buffer make it hypertonic to squeeze out the inside material of the cell membrane and polar head of cell membrane to dissolve in polar solvent of lysis buffer. The lysed cell material contains the cell organelles nucleus and mitochondria. The cell organelles have double layered membranes of phospholipid bilayer making them more rigid to lyse with the lysis buffer alone. The detergent SDS is pumped with lysis buffer to lyse the double layered membrane. The non polar tail of SDS interact with non polar ends of the phospholipid to lyse the double layered membrane of cell organelles. The lysis make the enclosed DNA of cell organelles to come out in the buffer solution. The released DNA is bonded with proteins and therefore enzymatic treatment is given with serine protease proteinase K. The peptide bond of bonded proteins is cleaved with catalytic triad of three amino acids serine, histidine and asparatic acid in proteinase K. The proteinase K is acive in the pH range of 4-12 and showed optimum activity at pH 8.0 and also act efficienty in temperature of 37-60^{0}C[9]. The cleaved protein is precipiated in phenol solution by non polar interactions. The choloroform further denature the proteins and also remove the residual lipids and isoamyl alcohol added to phenol, cholorofrom mix for reducing the foaming. The protein and lipid part settled down and DNA is eluted out in aqueous phase by centrifugation. The eluted DNA is precipitated out from the aqeous phase by ethanoal salt precipitation. The water forming the aqueous phase has a high dielectric constant of 80, suggest that force exerted between H^+ and HO^- ion is much less in compared to vaccum, making water a polar solvent and dissolve the DNA containing PO^{3-} ions in the sugar phosphate backbone. The ethanol has low dielectric constant of 1.69 and salt sodium acetate with Na^+ and $CH3COO^-$ ions helps in precipitation of DNA from water. The low dielectric constant of ethanol allow the PO^{3-} ions of DNA to leave out from H^+ ions of water and come close to Na^+ ions of sodium acetate and precipitate as a thread. The precipiation is further faciliatated at low temp, at low dielectric constant by adding ethanol chilled at 4^{o}C. The DNA comes out as solid thread like material and residual salt is washed out by 70 % ehanol and absolute ethanol. The DNA thread is pelleted down by centrifugation and dissolved in 10mM Tris-EDTA buffer. The tris maintain the pH and EDTA chelates the inorganic ions of solution forming a degradation free stable environment for long term storage of DNA. The quanity and quality of DNA is essential for genomic analysis to infer the human genetic identification and diveristy[10-15]. The published studies on the extraction, synthesis, characterization and screening on other chemical systems is referred for the analysis of experimental data in this study[16-20].

Conclusion

The chemical method of quantitative and qualitative extraction of DNA from cell organelles of human blood samples was investigated in this study. The chemical cascade of Tris, EDTA, NaCl, SDS, serince protease proteinase K, phenol, choloroform, isoamyl alcohol, ethanol and sodium acetate yielded pure average 10.7 µg of DNA from human cell organelles nucleus and mitochondria of 10 blood samples. We further propose to investigate extraction of DNA with innovative battery of reagents and enzyme. The extracted DNA is analyzed for human genetic identification and diversity studies.

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