



An effective method for high quality DNA extraction from frozen blood samples

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ABSTRACT

Today, a number of methods are available for the isolation of genomic DNA from whole blood samples. The ideal goal for all techniques developed for genomic DNA extraction is obtaining high quantities of pure, integral and intact genomic DNA from the sample with minimal traces of impurities. Here we report an effective and standardized protocol for extracting high quality genomic DNA from frozen blood samples. The reason that most of the methods described in literature finds it difficult to extract genomic DNA from frozen blood samples, lead us to evaluate and standardize better methods for the extraction. We have made an attempt to develop a slightly modified protocol that can use in genomic DNA extraction from frozen whole blood samples. The improvised method was developed by standardizing the parameters such as usage of Proteinase K, incubation time and incubation temperature. The described method allows simple, fast and high quality DNA extraction with qualitative parameters maintained from frozen blood samples.

Keywords: DNA extraction, Genomic DNA, Frozen whole blood samples, Incubation temperature, Proteinase K.

INTRODUCTION

Isolation of DNA from blood samples is one of the important preliminary steps done for Molecular diagnosis of a disease. As a reason obtaining a very good quality genomic DNA from the limited blood sample available plays an important role in Molecular Research and diagnosis [1, 2]. DNA extraction from biological samples to obtain pure genomic content is of great importance and acts as a primary step to succeed in various molecular biological techniques such as Polymerase Chain Reaction (PCR), restriction enzyme analysis, mutation detection, genotyping as well as linkage analysis [2]. It's even widely used in determination of genetic abnormalities, epigenetic studies and various diagnostic and preventive tests [3, 4]. For a DNA extraction process to be reliable, it should be a rapid and economical procedure with minimal investments to be put on downstream processes. Even though many procedures have been standardized across the globe, Most of the techniques won't give reliable results when applied for frozen blood samples [5-9]. Unfortunately, there is no particular DNA extraction protocol to meet all these criteria mentioned above [10, 11]. In one hand for instance, some protocols such as Phenol-Chloroform (PC) seems to be more efficient to remove PCR inhibitors comparing to salting out process. On the other hand, the simple and rapid salting out technique (Chelex procedure) could extract considerable amount of DNA which cannot be recovered by Phenol chloroform method [12, 13]. Here we attempted to standardize a reliable method for high quality genomic DNA extraction from frozen blood samples by altering conditions and parameters that could affect the DNA yield and quality.

MATERIALS AND METHODS

Blood samples

Frozen Blood samples were collected from various tertiary care hospitals and diagnostics labs in Perinthalmanna district, Kerala with informed consent. Blood samples stored in -20 °C for more than three months was considered for the study.

Reagents and solutions

- RBC Lysis Solution (Qiagen Autopure™)
- Cell Lysis Buffer (Qiagen Autopure™)
- 100% Isopropanol (Qiagen Autopure™)
- 70 % Ethanol (Qiagen Autopure™)
- DNA Hydration Solution (Qiagen Autopure™)
- Proteinase K (>600 mAU/ml-) Qiagen™

DNA extraction process

DNA extraction process was developed by improvising the techniques described by Atashpaz *et al.* 2010 and Barzegari *et al.* 2010 [14, 15].

1. Dispense 10ml RBC Lysis Buffer to a 15ml Tarson tube containing the frozen blood sample; incubate at 25° C for 5 minutes.
2. Vortex and centrifuge the above sample at 2000 x g for 10 minutes discard the supernatant and vortex the pellet.
3. Dispense 5 ml of RBC lysis buffer to the tube containing the pellet, vortex and centrifuge at 2000 x g for 10 minutes. After centrifugation discard the supernatant and vortex the pellet.
4. Dispense 10 ml Cell Lysis Buffer and vortex for 2 minutes.
5. Incubate at 35° C for less than one hour (45 minutes) and after incubation vortex the sample for 2 minutes.
6. Dispense 3 ml Protein Precipitation Solution vortex for 2 minutes.
7. Centrifuge the tube at 2000 x g for 3 minutes.
8. Dispense 10 ml 100% Isopropanol solution in fresh output tubes and pour the supernatant from step 3 into the output tube.
9. Mix to precipitate DNA, Centrifuge the tube at 2000 x g for 5 minutes.
10. Discard supernatant and drain the tubes for 1 minute.
11. Dispense 5 ml of 70 % Ethanol and centrifuge at 2000 x g for 5 minutes.
12. Discard the supernatant and drain the tubes for 1- 10 minutes.

The standardized process

The same procedure mentioned above was carried out with changes made in the incubation temperature and time period of incubation after addition of cell lysis buffer (as mentioned in step 4 in the extraction protocol). In the first step of standardization, samples 2A and 4A was added with 5 µl of Proteinase K. Samples 1A and 4 A was devoid of Proteinase K solution. Incubation time for samples 1 A and 2 A was set as 35 °C for 1 hour and samples 3 A and 4 A were Kept at 55 °C for overnight incubation. The concentration and purity of genomic DNA obtained was measured using NanoDrop 2000™ UV-Vis Spectrophotometer. In the second step of standardization, after the cell lysis buffer addition step, the samples 5A, 7A, 9 A, 11A were added with 5 µl of Proteinase K solution and samples 6A, 8A, 10 A, 12A were devoid of Proteinase K. These samples were incubated at 45° C for different time periods. The samples 5A and 6A was incubated for 1 hour, the samples 7A and 8A for 2 hours, the samples 9A and 10A for 4 hours and samples 11A and 12A for overnight. The DNA concentration and purity was checked by NanoDrop 2000™ UV-Vis Spectrophotometer. In the third step, the samples 13A, 15A, 17 A, 19A were added with 5 µl of Proteinase K and samples 14A, 16A, 18 A, 20A were devoid of Proteinase K. These samples were incubated at room temperature for different time periods. The samples 13A and 14A was incubated for 1 hour, the samples 15A and 16A for, the samples 17A and 18A for 4 hours and samples 19A and 20A for overnight. The DNA concentration and purity was checked by NanoDrop 2000™ UV-Vis Spectrophotometer.

RESULTS AND DISCUSSION

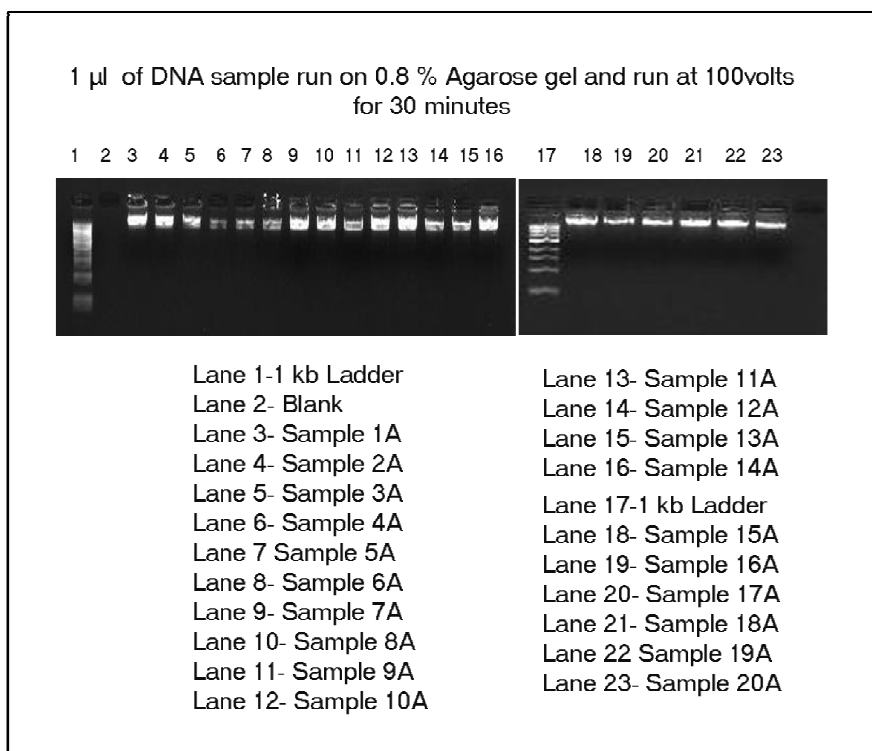


Figure 1- Represents the agarose gel picture of the DNA samples run on 0.8% agarose gel

Table 1-Represents the DNA concentration and purity of different samples measured using NanoDrop 2000™ UV-Vis Spectrophotometer

Sample No:	DNA concentration (ng/ µl)	OD 260/OD280 ratio
Sample 1A	62 ng/µl	1.88
Sample 2A	52 ng/µl	1.89
Sample 3A	48 ng/µl	1.81
Sample 4A	21 ng/µl	1.82
Sample 5A	20 ng/µl	1.8
Sample 6A	20.5 ng/µl	1.87
Sample 7A	52 ng/µl	1.83
Sample 8A	48 ng/µl	1.81
Sample 9A	54 ng/µl	1.84
Sample 10A	48 ng/µl	1.79
Sample 11A	50 ng/µl	1.81
Sample 12A	36 ng/µl	1.81
Sample 13A	36 ng/µl	1.81
Sample 14A	42 ng/µl	1.82
Sample 15A	95 ng/µl	1.8
Sample 16A	50 ng/µl	1.81
Sample 17A	39 ng/µl	1.88
Sample 18A	48 ng/µl	1.82
Sample 19A	32 ng/µl	1.81
Sample 20A	48 ng/µl	1.82

Among different protocols carried out for standardization of the technique, the sample 15A showed maximum DNA concentration and purity. The standardization process was repeated twice to confirm the conditions optimum for isolation. The results obtained suggests that best yield of genomic DNA from the frozen blood samples was obtained when the samples were added with 5 µl of Proteinase K solution after the cell lysis step and when undergoing an incubation for two hours at room temperature (35 °C) prior to the protein precipitation step.

CONCLUSION

This study represents a reliable approach for DNA extraction from frozen blood samples. As good quality of DNA was obtained by this process similar to those obtained from fresh blood samples this could be suggested as

an effective DNA extraction method from blood samples stored in -20 °C for a period of three months and more. Furthermore modifications can be done on this technique applying the conventional Phenol Chloroform principle; where by the same technique can be performed at lesser investment rates.

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