Video Article

A Practical and Novel Method to Extract Genomic DNA from Blood Collection Kits for Plasma Protein Preservation

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Abstract

Laboratory tests can be done on the cellular or fluid portions of the blood. The use of different blood collection tubes determines the portion of the blood that can be analyzed (whole blood, plasma or serum). Laboratories involved in studying the genetic basis of human disorders rely on anticoagulated whole blood collected in EDTA-containing vacutainer as the source of DNA for genetic / genomic analysis. Because most clinical laboratories perform biochemical, serologic and viral testing as a first step in phenotypic outcome investigation, anticoagulated blood is also collected in heparin-containing tube (plasma tube). Therefore when DNA and plasma are needed for simultaneous and parallel analyses of both genomic and proteomic data, it is customary to collect blood in both EDTA and heparin tubes. If blood could be collected in a single tube and serve as a source for both plasma and DNA, that method would be considered an advancement to existing methods. The use of the compacted blood after plasma extraction represents an alternative source for genomic DNA, thus minimizing the amount of blood samples processed and reducing the number of samples required from each patient. This would ultimately save time and resources.

The BD P100 blood collection system for plasma protein preservation were created as an improved method over previous plasma or serum collection tubes[1], to stabilize the protein content of blood, enabling better protein biomarker discovery and proteomics experimentation from human blood. The BD P100 tubes contain 15.8 ml of spray-dried K2EDTA and a lyophilized proprietary broad spectrum cocktail of protease inhibitors to prevent coagulation and stabilize the plasma proteins. They also include a mechanical separator, which provides a physical barrier between plasma and cell pellets after centrifugation. Few methods have been devised to extract DNA from clotted blood samples collected in old plasma tubes[2-4]. Challenges from these methods were mainly associated with the type of separator inside the tubes (gel separator) and included difficulty in recovering the clotted blood, the inconvenience of fragmenting or dispersing the clot, and obstruction of the clot extraction by the separation gel.

We present the first method that extracts and purifies genomic DNA from blood drawn in the new BD P100 tubes. We compare the quality of the DNA sample from P100 tubes to that from EDTA tubes. Our approach is simple and efficient. It involves four major steps as follows: 1) the use of a plasma BD P100 (BD Diagnostics, Sparks, MD, USA) tube with mechanical separator for blood collection, 2) the removal of the mechanical separator using a combination of sucrose and a sterile paperclip metallic hook, 3) the separation of the buffy coat layer containing the white cells and 4) the isolation of the genomic DNA from the buffy coat using a regular commercial DNA extraction kit or a similar standard protocol.

Video Link

The video component of this article can be found at https://www.jove.com/video/4241/

Protocol

1. Sample Collection

1. Collect blood samples from individuals with proper informed consent. For each individual, draw 4 to 5 ml of blood into a P100 tube (BD Diagnostics, Franklin Lake, NY, USA). For comparison purposes, simultaneously collect blood in an EDTA tube.
2. The BD P100 tubes contain 15.8 ml spray-dried K2EDTA and a lyophilized proprietary broad spectrum cocktail of protease inhibitors to prevent coagulation and stabilize the plasma proteins. They also contain a mechanical separator. After collecting the whole blood, keep both tubes at room temperature for 60 min to overnight until processing occurs.
3. Centrifuge the BD P100 tubes at 2,500 g for fifteen minutes at room temperature. Transfer the plasma collected above the mechanical separator into micro centrifuge tubes.
4. Store the P100 tubes, containing the blood compacted below the separator, at -80 °C until you are ready to begin the DNA extraction.

2.1 From blood of P100 tube (P100_DNA)

1. The P100 tubes are stored at -80 °C after plasma extraction. Within 3 to 4 months, retrieve the P100 tube containing the compacted blood from the freezer and thaw at 37 ° in a water bath for 5 min (**Figure 1A**). Remove any residual plasma that sits above the separator. Add 2 ml of 17% sucrose solution (gradient solution tested in house - unpublished data) in the P100 tubes above the separator (**Figure 1B**). Centrifuge the tubes at room temperature for 20 min at 2,500 g; this process pushes the mechanical separator to the top of the tube and yields three layers of solution including the buffy coat (**Figure 1C**). Manually extract the separator using a steriled hooked metal paperclip (**Figure 1D**).
2. After retrieving the mechanical separator from each tube (**Figure 1D**), remove and discard the top sucrose layer. Transfer the middle layer, representing the buffy coat (BC) layer, into a sterile 15 ml conical tube. Add Phosphate Buffer Saline (PBS) to increase the buffy coat volume to 3 ml. Extract the DNA from the buffy coat using reagents from the Qiagen Puregene Blood Kit as per the manufacturer's recommendation; except a centrifugation speed of 2,500 g (instead of 2,000 g).
3. To lyse and remove the red blood cells (RBC), add 9 ml of RBC lysis to 3 ml of buffy coat. Invert each tube several times and incubate at room temperature for 10 min with occasional inverting during incubation. Spin each mixture down at 2,500 g for 5 min and discard the supernatant. Treat the remaining pellet in each tube with 3 ml of cell lysis solution and vortex for 15 sec. Treat the lysate with 1 ml of protein precipitation solution and vortex for 15 sec.
4. Centrifuge at 2,500 g for 5 min and transfer the supernatant into a fresh 15 ml conical tube containing 3 ml of 100% isopropanol. Invert the new tubes 10 times and centrifuge at 2,500 g for 3 min. Discard the supernatant and add 1 ml of 70% ethanol. Invert the conical tubes a few times to dislodge and wash the DNA pellet. Centrifuge at 2,500 g for 1 min. Allow the pellet to dry for 3 min (place the tube upside down) at room temperature and then suspend the DNA with 250 ml of rehydration solution at 37 °C for 10 min and transfer to a pre-labeled 1.7 ml microtube. Store the tubes at -80 °C until use.

2.2 From Blood of EDTA Ttube (EDTA_DNA)

1. The whole blood for routine immunohematology testing is collected in plastic vaccutainer tubes spray-coated with 10.8 mg of K_2EDTA and stored at -80 °C. Because the tubes do not contain a mechanical separator, the DNA extraction does not include the steps involving the mechanical separator (2.1.1 and 2.1.2).
2. Within 3 to 4 months of blood storage, the DNA is extracted using the KingFisher Flex (Thermo Fisher Scientific, Waltham, MA, USA). It is an automation DNA extraction system based on magnetic particle technology and consists of cell lysis / DNA binding, several washing steps and DNA elution.
3. The kit used in the DNA extraction is the KingFisher Flex 24 DNA Blood kit (Qiagen, Germany).

3. DNA Quantity and Quality Assessment

The quantity, quality and integrity of the genomic DNA were assessed by a combination of method that includes picogreen, spectroscopy and electrophoresis.

1. The concentration of the genomic DNA is determined by Nanodrop and picogreen quantification.
2. The purity is determined from the 260/280 ratio measurement on the Nanodrop spectrophotometer.
3. The sample (500 ng) is also loaded on 1% agarose gel to assess the integrity (degradation) of the DNA.

4. Genotyping Assays and Quality Control

1. Five P100_DNA and their corresponding EDTA_DNA samples are randomly selected for further analysis using the custom Immunochip (Immuno DNA Analysis BeadChip). Immunochip is an Infinium genotyping chip containing 196,524 polymorphisms and is designed for immunogenetic studies[5].
2. For each sample, 200 ng of DNA is amplified, fragmented, precipitated and resuspended in the appropriate hybridization buffer.
3. The denatured samples are hybridized on Immunochips at 48 °C for a minimum of 16 hr.
4. After hybridization, the Immunochips are processed for single-base extension reactions and stained.
5. The immunochips are then imaged using Illumina Hiscan Bead array reader and the normalized bead intensity data is loaded into the Illumina GenomeStudio software and converted into genotypes. Genotypes are called using the autocalling algorithm of GenomeStudio. The quality control involves exclusion of single nucleotide polymorphisms (SNPs) with a call rate <95%.
6. The SNP call rate is used as a measure of the immunochip assay efficiency. It is calculated as the percentage of the total number of assays on the chip (196,524 SNPs encoded on each chip) that achieve sufficient signal intensity and quality to receive an automated genotype assignment. High quality DNA (260/280 ratio of 1.8 or higher and absence of degradation) is expected to achieve at least the same call rate as the HapMap control DNA included in the immunochip assay. Quality control involves exclusion of SNPs with a call rate <95% in each data set.

Representative Results

DNA quality assessment and concentration measurement

The yields of P100_DNAs were significantly lower than those of EDTA_DNAs (**Tables 1** and **2**). The DNA purity represented by its 260/280 ratio value is similar for both P100_DNA and EDTA_DNA sample set (**Tables 1** and **2**). The quality of the DNA is fairly uniform within each set of sample although some degradation is seen in the EDTA_DNA samples, as indicated by light smear (**Figure 2**). The EDTA_DNAs that showed more signs of degradation also showed reduced DNA concentration.

Genotyping

The genotyping call rate for both EDTA_DNAs and P100_DNAs were compared. They yielded similar call rates and were both comparable to the internal HapMap control DNA (**Table 3**).

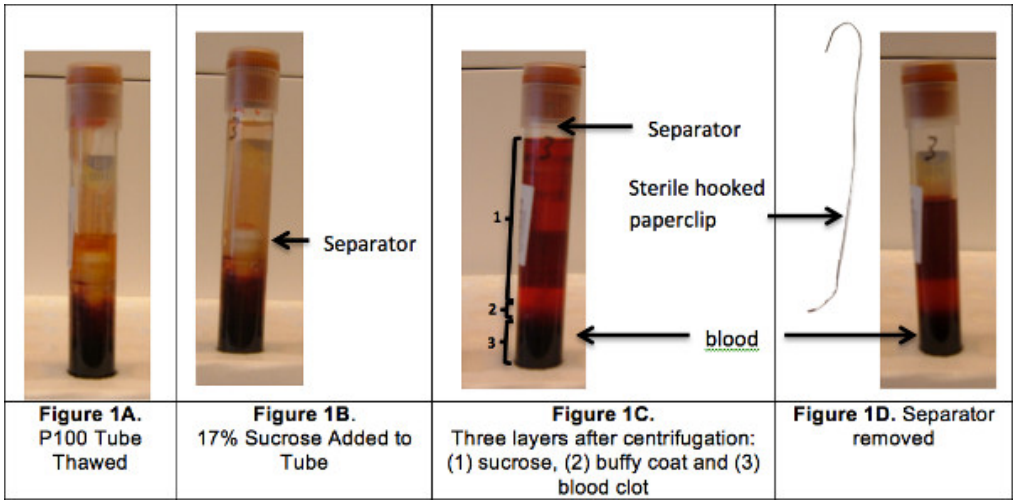

Figure 1A. P100 Tube Thawed

Figure 1B. 17% Sucrose Added to Tube

Figure 1C. Three layers after centrifugation: (1) sucrose, (2) buffy coat and (3) blood clot

Figure 1D. Separator removed

Figure 1. Buffy coat isolation.

Samples	Yield (µg)	Ratio 260/280
P100_01	135	1.79
P100_02	119	1.85
P100_03	57	1.88
P100_04	159	1.86
P100_05	140	1.86
P100_06	150	1.86
P100_07	139	1.74
P100_08	118	1.87
P100_09	51	1.87
EDTA_01	340	1.9
EDTA_02	344	1.89
EDTA_03	129	1.88
EDTA_04	640	1.88
EDTA_05	430	1.88
EDTA_06	673	1.9
EDTA_07	425	1.88
EDTA_08	240	1.86
EDTA_09	86	1.83
Samples	Yield (µg)	Ratio

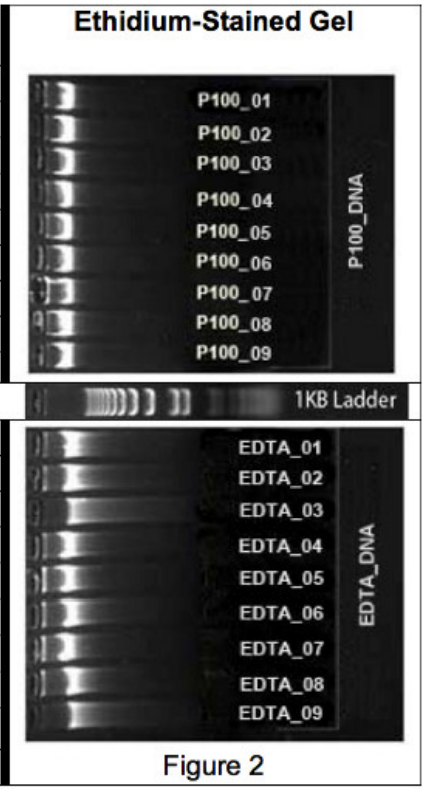

Figure 2

Table 1. DNA sample yield (PicoGreen) and ratio (nanodrop).

	DNA Yield		DNA Purity	
Samples	**P100**	**EDTA**	**P100**	**EDTA**
1	135	340	1.79	1.9
2	119	344	1.85	1.89
3	57	129	1.88	1.88
4	159	640	1.86	1.88
5	140	430	1.86	1.88

6	150	673	1.86	1.9
7	139	425	1.74	1.88
8	118	240	1.87	1.86
9	51	86	1.87	1.83
p value	p = 0.00221		p = 0.09836	

Table 2. Comparison of yield and purity (two-tailed, Paired Sample t-test).

	Genotyping Cell Rate	
Samples	**P100**	**EDTA**
1	97.9	97.5
2	97.9	97.8
4	97.4	97.5
7	97.5	98.2
8	97.9	98
P value	p = 0.67970	

Table 3. Genotyping call rate (two-tailed, Paired Sample t-test).

Samples	Layers	Yield (µg)	Ratio (260/280)
P100_06	Buffy coat	150	1.86
	RBC	4.13	1.73
P100_07	Buffy coat	139	1.74
	RBC	1.00	1.70
P100_08	Buffy coat	118	1.87
	RBC	3.72	1.78
P100_09	Buffy coat	51	1.87
	RBC	0.23	0.98

Appendix. Evaluation of the amount of DNA found in red blood cell (RBC) layer.

Discussion

Many human diseases have genetic causes and exploring the genetic basis of human disease demands an increasing high quality DNA. The most common source of DNA used in genetic studies is anticoagulated whole blood. Because most clinical laboratories perform biochemical, serologic, and viral testing as a first step in phenotypic outcome investigation, the blood is often collected in a plasma tube. After collection of the plasma, the compacted blood is often discarded. Therefore, when DNA is needed to conduct genetic studies or testing, an additional blood draw is required from the same patient. The discarded compacted blood represents a potential source of DNA as it contains white blood cells. Therefore, using compacted blood from plasma tubes offers an opportunity to use the collected blood sample more efficiently, without the need to draw surplus blood or recall the patient for additional blood.

Various methods to extract DNA from the white cells of compacted or clotted blood have been reported[6-10]. Most of the challenges of these methods were associated with the design of the plasma collection tube. Earlier plasma collection tubes contained a separator made of a gel (gel separator) which, when centrifuged, formed a barrier to separate the blood cells (trapped below the separator) from the plasma (located above the separator). To prevent contamination of the blood cells with the gel material, the separator must first be carefully removed from the tube[11]. This is followed by a fragmentation of the blood clot to guarantee efficient DNA extraction. The fragmentation process often results in a significant loss of blood sample and a reduction in DNA yield. To minimize sample loss and improve the process, a small-pore mesh was used to mechanically dispersed the blood clot into small pieces[12], but fragmentation still affected DNA yield and quality and even constituted a health hazard to the technician[13].

The BD P100 tubes from BD bioscience are the latest generation of plasma collection tubes evaluated in multicenter immunologic research study[14]. Like the previous plasma tubes, they contain anticoagulants to prevent coagulation and protease inhibitors to stabilize the plasma proteins. The major innovation of the BD P100 tube resides in the mechanical separator (not a gel separator) inside each tube. The mechanical separator also provides a physical barrier between the plasma and cell pellet after centrifugation, but unlike the gel separator, offers no risk of sample contamination by a gel. The protocol applied in this study does not include a fragmentation; therefore there is no health hazard risk.

The DNA was extracted from the buffy coat, using a commercial kit protocol (see DNA extraction). The compacted blood in the BD P100 tubes were cryopreserved for a period of 3 to 4 months prior to DNA extraction. DNA extracted from EDTA tubes (EDTA_DNA) were used as a control in the assessment of the yield and quality of their corresponding P100_DNA. From previous studies [12,13,15] , the yields of EDTA_DNA samples were significantly higher than that of DNA from blood of previous plasma tubes. In this study, for the same amount whole blood collected from

each patient, the buffy coat of the compacted blood yielded less DNA (**Table 2**, p = 0.00221). During the DNA extraction from the blood in the new P100 plasma tubes, some white blood cells from the buffy coast are lost to the red blood cell layer (below it) but represents insignificant amount of DNA compared to those from the buffy coat (see appendix table). The variation in yield seen with EDTA_DNAs is also mimicked with the P100_DNA suggesting that it is sample dependent. Samples with higher volume would normally yield more DNA and samples with lower volume and/or slightly degraded DNA would yield less DNA.

Analyses on agarose gel (**Figure 2**) revealed that EDTA_DNAs show signs of degradation (smear) not seen in their corresponding P100_DNAs. In particular, two EDTA_DNA samples (EDTA_01053 and EDTA_06030) with the lowest yield show more smear than others. In their study, Wong *et al.*[11] reported decreased DNA yield with increased storage time. Since all the blood samples in our repository were subjected to the same storage condition and the sample subset used in this study was randomly selected, storage length and condition are unlikely to account for the differences seen on the agarose gel between P100_DNAs and EDTA_DNAs. The fact that only EDTA_DNAs show degradation could be associated to the lack of protease inhibitors in the EDTA tubes.

The purity of the P100_DNAs and EDTA_DNAs were not significantly different (**Table 2**, p = 0.09836). This shows that good quality DNA can be efficiently extracted from P100 plasma tubes using a routine commercial kit. The fact that slightly degraded EDTA_DNA samples also show good purity, suggesting that purity is not a good measure of DNA sample integrity.

The samples used were recruited for the genetic studies of inflammatory bowel disease. Therefore, to compare the performance of the extracted DNA samples, the immunochip[5] was selected as the genotyping testing platform. The immunochip has been used as a fine mapping genotyping platform for immunogenetics [16,17]. The genome-wide nature of the SNP genotyping assays was used as a stringent measure of DNA performance. The average genotyping call rates for all DNA test and HapMap control samples, across all chips, were 97.76% and 97.4% respectively, showing comparable excellent performance of the DNA from both blood collection tubes (**Table 3**, p=0.67970).

Conclusion

DNA can be isolated from the compacted blood of BD P100 tubes, facilitating genomic testing from the same blood sample drawn for plasma proteomics studies. Our results extend the research utility of the P100 tube. The fact that DNA in the cellular content of blood collected in P100 is usable for genomic analyses can help simplify sample acquisition for studies in which both proteomic and genomic analyses will be performed. Therefore, less blood is needed from each human subject and an associated cost and effort savings for the study sponsors and staff is improved. There are several advantages demonstrated:

- Less blood is required per human subject when both genomic and proteomics studies need be performed. This is of special value for patients with disease conditions (such as leukemia) that require careful limitation of the volume of blood that can be drawn.
- Only one blood tube is required during sample acquisition and processing steps, simplifying the overall protocol.
- A routine commercial kit has proven efficient in extracting DNA from blood collected in BD P100 tubes.
- The cost savings associated with the use, acquisition and processing of one tube is increased.

Disclosures

No conflicts of interest declared.

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