

Consistency and Reproducibility of DNA Isolation From Whole Blood Using 96-Well DNA Binding Plate and Liquid-Handling Robot for Samples Under Different Storage Conditions

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OVERVIEW

An optimized protocol for automated DNA isolation using a 96-well DNA binding plate and a liquid-handling robot was developed. Vacuum filtration was used for all liquid evacuation steps.

The protocol was verified for DNA isolation from blood under different storage conditions with multi-well plates from two manufacturers.

Plate performance was monitored by measuring DNA yield and dsDNA purity in each well.

DNA yield ($\mu\text{g}/\text{well}$) from 100 μL of blood was measured by OD₂₆₀ and Pico Green assays.

Percent DNA recovery was calculated with white blood cell (WBC) concentration from each blood sample.

DNA quality was assessed by an A260/280 ratio for each well and gel electrophoresis run for every other well in the plate.

Intra-plate (well-to-well) and inter-plate (plate-to-plate) consistency was evaluated by comparing averages, standard deviation (SD), and CV characteristics of each plate.

Protocol outcomes were found dependable on blood storage conditions.

Fresh blood produced the best gDNA yield at 3.7 μg per well and 86% total predicted recovery from 100 μL of blood.

5-hour lysate blood produced poor gDNA yield at 2.7 μg per well and 63% of total predicted recovery.

Freeze-thaw blood demonstrated a decrease in WBC number and DNA yield compared to fresh samples, 2.3 μg versus 3.7 μg per well, respectively.

The purity of gDNA for all conditions was in the range of industry expectations—A260/280 ratio of 1.7-1.9 from fresh blood and 1.6-1.8 from 5-hour lysate and freeze-thaw blood.

Inter-plate performance was similar for all plates used in the study, varying in the range of one SD for DNA yield and DNA purity characteristics.

INTRODUCTION

There are a number of commercial kits consisting of a DNA binding plate and ready-made buffers for DNA isolation from blood. Pall Life Sciences has recently developed a DNA binding plate for isolation of DNA from a variety of sources. It is necessary to demonstrate that Pall's AcroPrep™ Advance DNA binding plate is capable of interchangeable use with solution kits from other manufacturers that employ glass fiber medium for gDNA isolation and purification. Previous studies proved the viability of processing the Pall filter plate with centrifugation and this study intended that vacuum filtration could be employed when using an automated processing system, such as the Eppendorf epMotion® 5075.

Objectives

- Optimize the automation protocol enabling consistent and reproducible gDNA isolation from human blood using vacuum filtration for all liquid evacuation steps.
- Demonstrate the robustness of the protocol using an automated system and plates from two different manufacturers in conjunction with commercially available buffers.

MATERIALS AND METHODS

I. Materials Required

- Pall AcroPrep Advance DNA Binding Plate (PN 8132)
- Eppendorf DWP (0030 522.109, 2000 $\mu\text{L}/\text{well}$)
- Corning UV/Vis sample collection plate (capacity > 250 $\mu\text{L}/\text{well}$)
- Eppendorf Thermo adapter DWP 96 (960002391)
- Eppendorf EpMotion 5075 vacuum
- E-Gel® Agarose Gel Electrophoresis System (Invitrogen)
- E-Gel 48 1% Agarose gels (Invitrogen G8008-01)
- Quant-iT® Pico Green dsDNA Assay Kit (Invitrogen P7589)

Schematic of epMotion 5075 System Customized for DNA Isolation Protocol

Clamp	1000 μL 50 μL Pipettes	50 μL Tips	1000 μL Tips	Shaker/Heating Thermo Adapter DWP 96 (960002391)
	2 mL Proteinase K	Tub 1-5	Tub 6-7	Vacuum
Waste Bucket	Sample Prep Plate	Corning UV/Vis 96-well plate	2 mL DNA Binding Plate	Vacuum Holder

II. Solutions Required

- AL Lysis Buffer (Qiagen PN 19075)
- Proteinase K (20 mg/mL, Qiagen PN 19131)
- Wash Buffers AW1 and AW2 (Qiagen PN 19081 and PN 19072, both required)
- Elution Buffer (Qiagen PN 19077)
- 100% EtOH

III. DNA Isolation and Purification Protocol

The automation protocol consists of four steps, at each step whole blood or DNA samples are transferred from one plate to another.

- Automation set-up – All solutions, buffers, and disposables are in place as per schematics. Blood samples are loaded into sample prep 2 mL plate.
- Cell lysis and DNA isolation – Blood samples are lysed, DNA is incubated with chaotropic salt buffer in 2 mL sample prep plate.
- DNA purification – DNA bound to the glass fiber media on DNA binding plate, impurities washed out using two subsequent wash buffers.
- DNA elution – DNA is released from the DNA binding plate into collection plate with TE buffer.

DNA Evaluation

- DNA yield, $\mu\text{g}/\text{well}$, was measured by OD₂₆₀ and Pico Green assays. Percent DNA recovery was calculated from WBC concentration in each blood sample.
- DNA quality was assessed by A260/280 ratio for each well and gel electrophoresis run for every other well in the plate.
- The protocol robustness has been demonstrated using multiple plates from two manufacturers by comparing average yield, SD and CV (%) characteristics of each plate. Six plates from Pall and three plates from Manufacturer Q were used in the study.

RESULTS

DNA Isolation From Blood Under Different Storage Conditions

An optimized protocol for automated DNA isolation using Pall's AcroPrep Advance DNA binding plates and a liquid-handling robot was developed. Vacuum filtration was used for all liquid evacuation steps.

Results presented in Table 1 are representative of Pall DNA binding plate performance with blood from the same donor, but under different storage conditions: fresh EDTA blood, freeze-thaw blood, and lysate that was stored for 5 hours before DNA isolation. 24 wells of the filter plate were used for each condition.

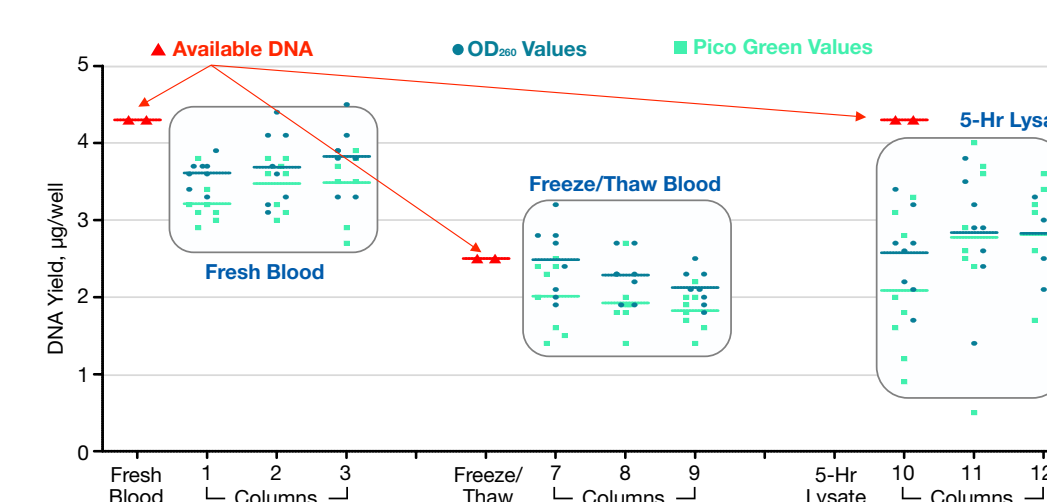
Table 1
Performance of Pall DNA Binding Plate with Blood Under Different Storage Conditions

	Fresh	Freeze/Thaw	5 Hour Lysate
DNA Available (μg)	4.3	2.5	4.3
Purity (A260/A280)	1.8 +/- 0.1	1.7 +/- 0.1	1.7 +/- 0.1
Total Yield (μg)	3.7 +/- 0.5	2.3 +/- 0.5	2.7 +/- 0.7
% Recovery	86	92	63
CV (%) Recovery	14	22	26

The performance of the plate was found dependable on blood sample condition. Fresh blood produced the best gDNA yield at 3.7 μg per well/86% total recovery. Freeze/thaw blood demonstrated significant decrease in WBC count; although the gDNA yield was 92%, the amount of recovered DNA was only at 2.3 μg per well. 5-hr lysate blood produced poor gDNA yield at 2.7 μg per well/63% of total recovery. The purity of gDNA for all conditions was in the range of industry expectations – 1.7-1.9 from fresh blood; 1.6-1.8 from 5-hr, lysate, and freeze thaw blood.

In Figure 1, the distribution of the DNA yield data from blood samples under different storage conditions is presented. DNA yield, $\mu\text{g}/\text{well}$, from 100 μL blood in each well was measured by OD₂₆₀ (blue dots) and Pico Green (green dots) assays. Available DNA (red triangles) was calculated based on WBC amount in 100 μL blood assuming 7 μg DNA per cell.

Figure 1
DNA Yield (μg per well) Depending on Different Blood Sample Conditions

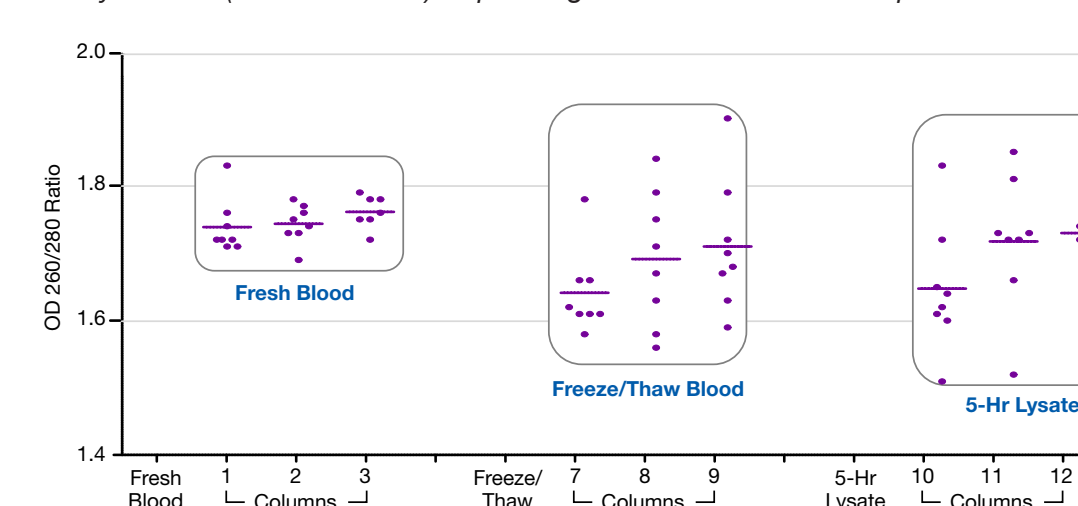


The best DNA yield and well-to-well consistency of 14% CV was achieved with a fresh blood sample; freeze-thaw and 5-hr lysate blood samples resulted in lower gDNA yield along with higher well-to-well variability in the range of 21-26% CV.

RESULTS (continued)

In Figure 2, the distribution of the DNA purity data from blood samples under different storage conditions is presented. DNA quality in each well was assessed by A260/280 ratio for each well (purple dots) and gel electrophoresis run for every other well in the plate (data not shown).

Figure 2
Purity of DNA (A260/280 ratio) Depending on Different Blood Sample Conditions



The quality of gDNA for all blood sample conditions was found in the range of industry expectations, 1.7-1.9 for samples of fresh blood, 1.6-1.8 for freeze-thaw and 5-hr lysate blood. Well-to-well consistency was the best for samples of fresh blood.

Protocol Robustness: Intra-/Inter-Plate Consistency

Protocol robustness has been demonstrated using multiple plates from two manufacturers by comparing averages, SD and CV (%) of DNA yield of each plate. Fresh EDTA blood collected from the same donor on the days of the test was used. In Table 2, the performance of six plates from Pall and three plates from Manufacturer Q are summarized in terms of DNA quality assessed as A260/280 ratio.

Table 2
DNA Purity, Intra- and Inter-Plate Consistency: Plates From Two Manufacturers

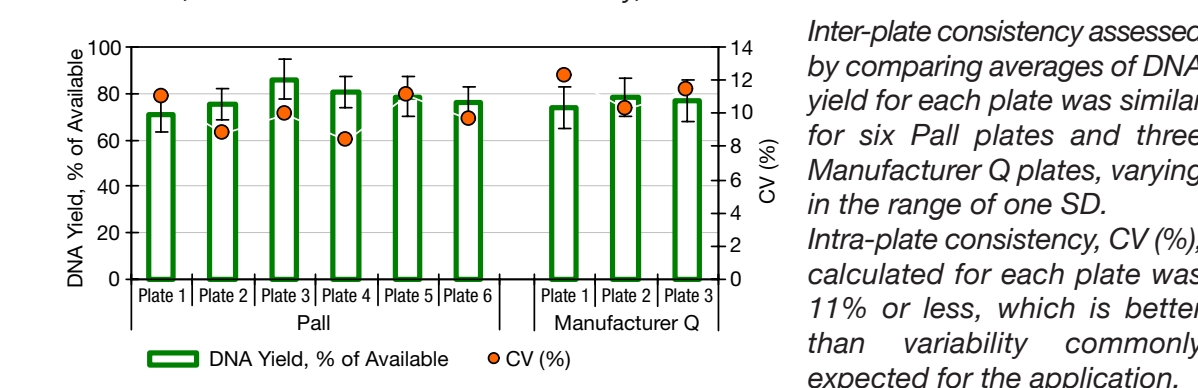
		OD _{260/280} ratio	SD	CV (%)
Pall	Plate 1	1.76	0.05	2.71
	Plate 2	1.83	0.07	3.86
	Plate 3	1.83	0.06	3.07
	Plate 4	1.76	0.11	6.44
	Plate 5	1.76	0.06	3.51
	Plate 6	1.72	0.03	1.99
Average for all Pall		1.78	0.06	3.60
Manufacturer Q	Plate 1	1.78	0.06	3.46
	Plate 2	1.74	0.08	4.79
	Plate 3	1.74	0.04	2.45
Average for all Manufacturer Q		1.75	0.06	3.57

Inter-plate consistency was similar for six Pall plates and three Manufacturer Q plates, plate averages varied in the range of one SD. Intra-plate consistency, CV (%), was ~6% or less for all plates, better than variability commonly expected for the application.

RESULTS (continued)

In Figure 3 below, the performance of six plates from Pall and three plates from Manufacturer Q is summarized. Each green column represents a plate performance as the average of DNA yield as percent of available DNA from 96 wells (with the exception of plate #5 represented by 24 wells). Orange dots show CV% for a plate, error bars show one SD range.

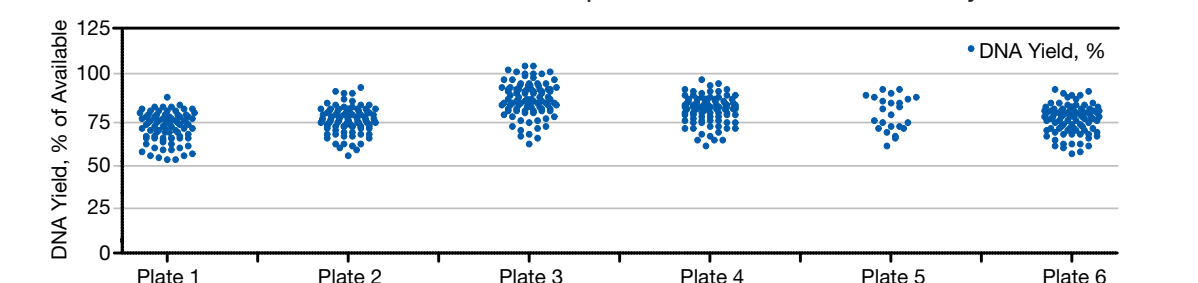
Figure 3
DNA Yield, Intra- and Inter-Plate Consistency, Plates From Two Manufacturers



Inter-plate consistency assessed by comparing averages of DNA yield for each plate was similar for six Pall plates and three Manufacturer Q plates, varying in the range of one SD. Intra-plate consistency, CV (%), calculated for each plate was 11% or less, which is better than variability commonly expected for the application.

In Figure 4, the distribution of individual well performance as DNA yield presented as percent of available DNA, is shown for six Pall DNA binding plates; dsDNA was measured by Pico Green assay while available DNA was calculated based on the amount of WBC per well assuming 7 μg DNA per cell.

Figure 4
96 Data Points for All Plates with an Exception of Plate #5 Presented by 24 Data Points



The plate averages varied in the range of 71-86% of DNA yield; 3-SD distribution of individual well data points was between 50-100%.

CONCLUSIONS

- An optimized protocol for automated DNA isolation using Pall's AcroPrep Advance DNA binding plates and a liquid-handling robot was developed using vacuum for all liquid evacuation steps.
- Robustness of the protocol was demonstrated using plates from two manufacturers:
 - Inter-plate consistency, DNA yield, and purity was similar for six Pall plates and three Manufacturer Q plates varying in the range of one SD.
 - Intra-plate consistency, CV (%), was 11% or less for DNA yield and ~6% for DNA purity as measured A260/280 ratio, better than commonly expected for the application.
- Protocol was verified for blood samples under different blood storage conditions:
 - Fresh blood produced the best gDNA yield at 3.7 μg per well, 86% total recovery; freeze-thaw and 5-hr lysate blood resulted in decreased amount of recovered DNA.
 - The purity of gDNA for all blood storage conditions was in the range of industry expectations—1.7-1.9 from fresh blood, 1.6-1.8 from stored blood.