High-Throughput, Low Volume gDNA Extraction from Whole Blood Enabled by Covaris Adaptive Focused Acoustics® (AFA®) and AFA-TUBE™ TPX

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Introduction

Extraction of genomic DNA (gDNA) from whole blood is the first step in multiple translational research and molecular diagnostics applications, such as next-generation sequencing (NGS), multiplex PCR, qPCR, and droplet digital PCR (ddPCR). For example, DNA from whole blood is frequently used as a matched-control for solid tumor somatic mutation profiling (1) and for the detection of clinically-relevant variants in hematological malignancies. An increasing number of clinical studies demonstrate the value of detecting disease-specific biomarkers from blood for diagnosis, treatment monitoring, and cohort study recruitment (2, 3).

Scaling down fresh blood volumes while scaling up processing capabilities is desirable to maximize laboratory throughput. At present, most DNA extraction methods require high volumes (>200 µl) of blood (***Table 1***) and are challenging to automate because centrifugation or vacuum equipment are necessary. Additionally, conventional column and bead-based workflows need larger volumes for wash and elution steps, which require deep well plates and other specialized consumables. Larger volumes are also needed to avoid excessive viscosity of the lysate, which can interfere with magnetic bead separation.

To circumvent sample and process-related challenges, Covaris has adapted the AFA-TUBE TPX Plate for streamlining high-throughput nucleic acid extractions from whole blood using AFA technology. Here, we performed DNA extractions from 12 healthy donors to evaluate recoveries, sample quality, and purity. Our results show recoveries in the range between 376 to 809 ng with an average fragment size of >1 kb. We also show that the extracted and purified DNA is devoid of any detectable PCR inhibiting contaminants.

Taken together, this less than 90-minute AFA-enabled workflow significantly improves blood cell lysis and reduces hands-on time. The workflow can be performed on a liquid handler using the integrated Covaris R230 Focused-ultrasonicator, or off-deck using the LE220-plus Focused-ultrasonicator.

Materials and Methods

Whole blood samples from 12 healthy male donors were used to extract sheared DNA in the 96 AFA-TUBE TPX Plate on the LE220-plus Focused-ultrasonicator. 30 µl of whole blood was mixed with 60 µl of AFA Conditioning Buffer containing magnetic beads (GE Healthcare) and proteinase K (Qiagen). Eight technical replicates were used for each donor (one column in a 96 AFA-TUBE TPX Plate) to evaluate the total DNA yields, purity, and fragment size distribution. Cell lysis was achieved by treating samples with AFA for less than 30 seconds per column. After lysis, samples were incubated at 56 °C for 15 minutes to hydrolyze proteins and then Binding Buffer (Covaris) was added followed by magnetic separation of the bead-bound DNA. The bead-bound DNA was washed and then eluted in a final volume of 50 µl. DNA purity was analyzed by evaluating A260/A280 ratios with Nanodrop (ThermoFisher Scientific). qPCR was performed to assess sample amplifiability, which is expressed as the Q-Ratio – a relative measure of gDNA quality (Roche). Fragment size distribution was determined using the Fragment Analyzer (Advanced Analytical Technologies), and DNA yields were determined using Qubit fluorometric quantification (ThermoFisher Scientific).

The whole blood extraction protocol can be performed using laboratory automation systems with deck spaces required as indicated in brackets [#]. The integrated R230 Focused-ultrasonicator version requires 11 deck spaces to perform the entire workflow for 96 samples. If using an off-deck LE220R-Plus Focused-ultrasonicator, the required deck space is reduced to 7.

Required Material

Equipment

- Covaris LE220R-plus [0] attached to a liquid handling system or Covaris R230 [4] Focused-ultrasonicator
- 96 well PCR plate compatible magnet stand [1]
- 96 well incubator block [1]

Consumables and Reagents

- 200 to 300 µl pipette tips [6]
- 96 AFA-TUBE TPX Plate [1]
- 96 well receiver plate [1]
- 12 column reagent reservoir [1]
- AFA Conditioning Buffer or truPOP™
- Proteinase K
- Magnetic SpeedBeads™
- Binding Buffer
- Wash Buffer 1
- Wash Buffer 2
- Ethanol
- Elution Buffer

	96 truXTRAC® Blood DNA	QIAamp 96 DNA Blood (Qiagen)	Blood DNA Isolation 96-Well (Norgen)
Reagent Volumes (96 samples)	6 to 20 ml	2 to 50 ml	2 to 60 ml
Reagent Reservoirs	1	2	2
Lysate Mixing Step	AFA	Vortex Plate	Vortex in Single Tube
Centrifuge	No	Yes	No
Magnet	Yes	No	Yes
Lysate Transfer Step	No	Yes	Yes
Incubator	Yes (56 °C)	Yes (70 °C)	No
Hands off Automation	Yes	No	No

Table 1. Side-by-side comparison of whole blood extraction with truXTRAC and other commercial column and magnetic bead-based 96 well kits.

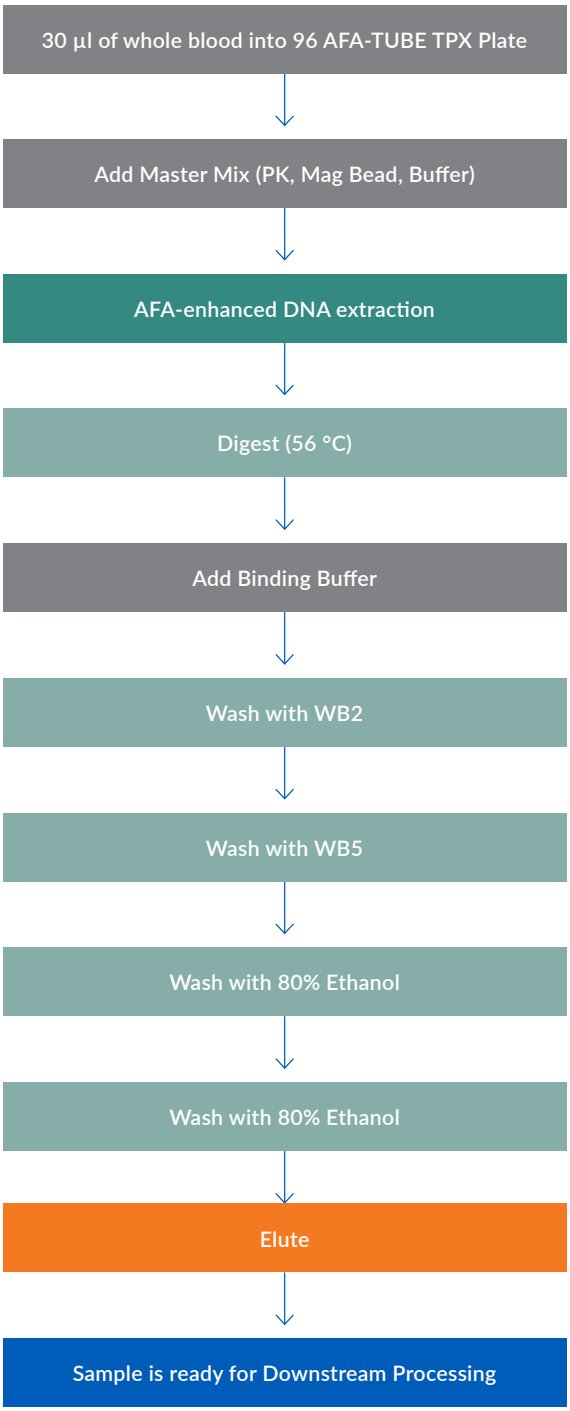

Figure 1. DNA extraction from whole blood in the AFA-TUBE TPX on the LE220-plus Focused-ultrasonicator. The total processing time from sample load to DNA elution is approximately 60 to 90 minutes.

Results

This study demonstrates the advantages of AFA-enhanced cell lysis and lysate homogenization for the extraction and purification of DNA from whole blood. Using the 96 AFA-TUBE TPX Plate, the entire workflow can be performed in the same consumable. By assessing 12 individual blood samples (N=8 per donor), pure, PCR inhibitor-free DNA in the range of 376 to 809 ng was extracted and purified. Coefficient of variance (CV) values of DNA yields within replicates from the same donor blood were <10% demonstrating that the process is highly repeatable. In order to assess DNA purity, 260 nm/280 nm ratios were determined, which were all within the range of DNA devoid of residual protein (1.7 to 1.9). The Q-Score for DNA derived from whole blood samples proved that the presence of PCR inhibitors was minimal and comparable to scores generated from highly pure gDNA sheared to the same size (***Table 2***). The mode of fragment size distributions for the extracted DNA ranged between 1 to 1.3 Kb when processed in the AFA Conditioning Buffer, with remarkable repeatability between replicates (***Figure 2A, Table 3***). Alternatively, we were able to demonstrate high molecular weight DNA extraction (above 20 Kb) using our truPOP lysis buffer with a single donor sample (***Figure 2B***). Importantly, it should be noted that due to AFA-enhanced lysis, extracted DNA can be sheared to insert sizes required for sequencing (150 to 500 bp) by adjusting the AFA settings during lysis.

Donor	Average Yield (ng)	%CV on Yield	Average 260/280 nm	Q129/Q41 Ratio
A	416	7.9	1.9	0.91
B	776	7.7	1.9	0.85
C	387	7.4	1.8	0.86
D	506	7.3	1.8	0.80
E	809	8.6	1.8	0.86
F	376	4.0	1.8	0.95
G	481	8.1	1.9	0.81
H	395	6.1	1.9	0.82
I	453	8.8	1.9	0.83
J	391	3.8	1.8	0.84
K	330	5.9	1.7	0.87
L	778	7.8	1.8	0.90
Control DNA	N/A	N/A	1.8	0.85

Table 2. Total yield, 260/280 values, and Q-scores were determined for DNA (30 µL of whole blood) extracted with AFA Conditioning Buffer from 12 healthy male donors and commercially available hgDNA (Promega) sheared to 1 kb.

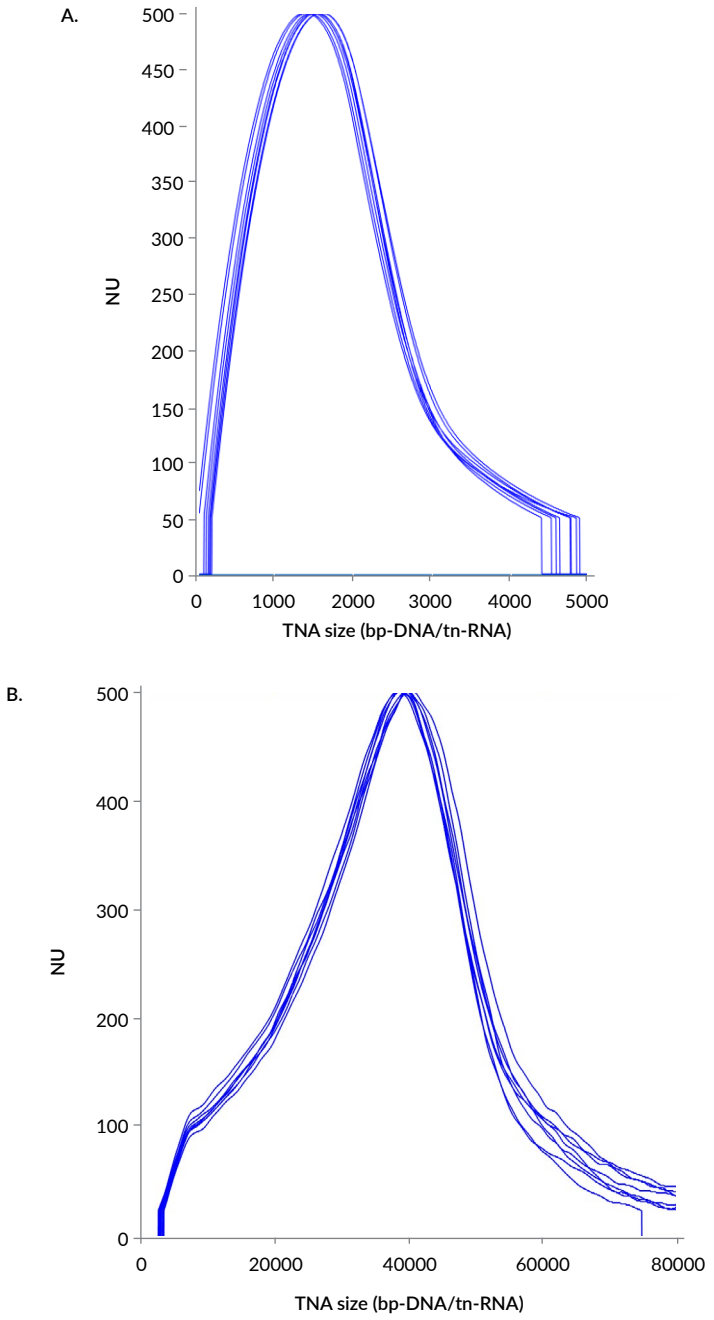

Figure 2. DNA fragment size distribution: Extracted and purified DNA was analyzed on the Fragment Analyzer. ***A.*** An overlay of the technical replicates for the fragment size distribution from Donor K (N=8). DNA fragment size modes are between 1 to 1.3 Kb when processed using the AFA Conditioning Buffer. ***B.*** Fragment size distribution from Donor L using Covaris truPOP Buffer (N=8) with a mode of 39 Kb. DNA yields using truPOP Buffer were comparable to yields achieved with AFA Conditioning Buffer (815 ng, %CV 7.8).

Donor	Average Fragment Mode (bp)	%CV on Fragment Mode
A	1310	2.7
B	1025	3.3
C	1066	3.6
D	1021	5.2
E	945	4.0
F	1049	4.9
G	1216	7.3
H	1122	10.5
I	979	8.1
J	1179	10.5
K	1431	2.6
L	1156	4.4

Table 3. Average fragment mode size and %CV for the fragmentation profiles obtained from DNA extracted with AFA Conditioning Buffer from 30 µL of whole blood from 12 healthy donors.

Conclusions

Covaris has established a fully automated, high-throughput, low volume gDNA extraction and purification protocol from whole blood using AFA-enhanced lysis in the 96 AFA-TUBE TPX Plate. Importantly, the protocol eliminates the requirement for special vacuum manifolds or plate centrifuges and multiple lysate transfer steps. This extraction protocol allows for an optional fragmentation step during lysis to generate smaller DNA fragments. Taken together, this will enable direct coupling of the extraction and purification steps required for downstream applications, such as next generation sequencing.

In previous development work, high molecular weight (>30 Kb) DNA extraction conditions were successfully established for bacteria and yeast samples. Our preliminary results show that using AFA with truPOP enables high molecular weight DNA extraction from whole blood, and this protocol is still being validated using multiple donor samples. Finally, although the bead mixing and elution was performed manually in this pilot study, Covaris intends to optimize AFA-mediated micro-mixing and DNA elution to further enhance high throughput nucleic acid extractions.

References

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