High Molecular Weight DNA Extraction and Purification from Whole Blood - Fully-automatable and High-Throughput

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Introduction
Extraction of genomic DNA (gDNA) from fresh 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). Scaling down fresh blood volumes while scaling up processing capabilities is desirable to maximize laboratory throughput. At present, most DNA extraction methods are challenging to fully automate because centrifugation or vacuum equipment is still a necessary requirement. Additionally, such conventional column and magnetic-based workflows require relatively large reagent volumes for bind, wash, and elution steps, demanding the use of deep well plates and other specialized consumables, such as plate vortex/incubators. To circumvent sample and process-related challenges, Covaris has adapted the 96 onTUBE AFA Plate for a fully automated, high-throughput DNA extraction and purification protocol starting with 30 µl of EDTA-stabilized human blood and using Adaptive Focused Acoustics (AFA®) technology for the extraction step.

Materials and Methods
- 30 µl of whole blood per sample was added to each well of a 96 onTUBE AFA Plate and 60 µl of either Covaris AFA Conditioning Buffer or Covaris truPOP Buffer containing magnetic beads (GE Healthcare) and protease K (Qiagen) was pipetted on top. Eight technical replicates were used for each donor (nine column in a 96 onTUBE AFA Plate per donor).
- Cell lysis was done by subjecting the samples to AFA for 30 seconds per column. After AFA-extended lysis and homogenization, lysisates were incubated 15 minutes at 56 °C, followed by Covaris Blind Buffer, mixing and short incubation at RT. Magnetic separation of the bead-bound DNA followed. Bead-bound DNA was washed sequentially and finally eluted in 50 µl TE, pH 8.0.
- Fragment size distribution was determined using the Fragment Analyzer (Advanced Analytical Technologies), and DNA yields were determined using Quant-IT fluorometric quantification (ThermoFisher Scientific) as well as qPCR (KAPA hgDNA Quantification and QC Kit, Richco).
- DNA purity was analyzed by evaluating A260/A280 ratio (Nanodrop, ThermoFisher Scientific) as well as by qPCR (KAPA hgDNA Quantification and QC Kit, Richco).

Table 1: Side-by-side comparison of whole blood extraction with truTRAC and other commercial column and magnetic-based kits in kit and 

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>AFA</th>
<th>truTRAC</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA yield (µg)</td>
<td>397.9</td>
<td>387.3</td>
<td>385.4</td>
</tr>
<tr>
<td>2000</td>
<td>AFA</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>20000</td>
<td>BFA</td>
<td>1.0</td>
<td>0.8</td>
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</tbody>
</table>

**Figure 1:** DNA extractions from whole blood in the onTUBE via the LE220R plus Focused-ultrasonicator. The total processing time from sample load to DNA elution is approximately 60 to 90 minutes.

**Results**
- AFA-extended extraction of gDNA from 30 µl of human whole blood was performed in a truly fully-automated workflow in the 96 onTUBE AFA Plate. This new PCR plate-like consumable was specifically designed to be compatible with liquid handling robotics and the Covaris R230 and LE220R plus Focused-ultrasonicators. Since these instruments treat 8 samples (1 column) in parallel, 96 blood samples are lysed in less than 10 minutes.
- More importantly, the entire workflow from “blood in” to “DNA out” is being performed in the same consumable and only clean, eluted DNA is transferred at the end.
- The choice between two lysis buffers allows extraction and purification of either low molecular weight or high molecular weight DNA. (Figure 2A) AFA Conditioning Buffer extracts DNA with an average fragment length of 1 kb, (Figure 2B) Covaris truPOP Buffer yields DNA that averages 40 kb in length.
- 12 and 5 individual blood samples were subjected to extraction using Lysis Buffer (Figure 2A) or (Figure 2B), respectively. Yields (detailed in Table 2 and 3) averaged 508 ng (+/-175) and 504 ng (+/-190), respectively.
- DNA purity and quality as assessed by absorption (260 nm/280 nm ratio) and by amplifiability (KAPA PCR Q-score; Q129/Q41) from all samples and regardless of the lysis buffer chosen, is indistinguishable from purity and quality of commercially phenol/chloroform extracted human gDNA.
- The workflow is robust as demonstrated by the low %CV between replicates of 8 per donor. CVs are ranging from 3 to 9% (AFA Conditioning Buffer) and 5 to 11% (Covaris truPOP Buffer).
- The fragment size distribution (mode) of the purified DNA is very repeatable between replicates. It ranges between 1 to 1.4 kb (AFA Conditioning Buffer) and 36 to 41 kb (Covaris truPOP Buffer). CVs of the fragment size distribution modes are remarkably tight (Table 2 and 3).
- An on-deck or accessible adjacent Focused-ultrasonicator is used at multiple steps within the entire workflow. In addition to lysis, AFA can also be used to mix magnetic beads during binding and elution (data not shown; see Covaris Technical Note “Magnetic Bead-based Clean Up Using AFA energetic”) but also to fragment DNA to size after it is eluted from magnetic beads (Figure 3).

**Conclusions**
- Integration of Focused-ultrasonicators with liquid handling platforms allows a fully automated, high throughput, DNA extraction and purification protocol starting from 30 µl whole blood in one 96 onTUBE AFA plate (no liquid transfer except for final elution).
- The workflow as presented eliminates the requirement for special vacuum manifolds or plate centrifuges, multiple lysis transfer steps and on-deck storage of large volumes of reagents. Integration of AFA into the entire workflow (mixing, elution, and fragmentation) also allows a drastic reduction of pipette tip waste.
- Choice of lysis buffers allows flexibility to extract DNA to size, i.e., pre-fragmented to 1 kb or as high molecular weight DNA (40 kb).
- The “one plate only” workflow can be extended (optional) to fragment eluted DNA to desired fragment size distribution, saving time, consumables, and eliminating deck layout changes.
- This whole blood extraction & purification protocol is compatible with mainstream laboratory automation systems, such as liquid handlers equipped with an on-deck R230 Focused-ultrasonicator (PN 500611) or an off-deck adjacent LE220R Plus Focused-ultrasonicator (PN 500578).