Rapid, High Throughput Extraction and Purification of Genomic DNA from Whole Blood Using the Akonni TruTip® Extraction System on the Hamilton Microlab® STAR Liquid Handling System

Application Note

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Abstract

Akonni Biosystems has developed the simple and effective ultra-rapid TruTip® automated extraction method designed to isolate genomic DNA from low volumes of whole blood for downstream genetic testing including any type of PCR, sequencing and microarray detection. The tip-based extraction technology is paired with the advanced, proven and reliable Hamilton Microlab STAR liquid handling system for high throughput sample processing. Comparable yields of high-quality genomic DNA were extracted using the TruTip method when compared directly to industry leaders. Reproducibility studies demonstrated high precision with low standard deviations and high quality.

Introduction

The purification of genomic DNA is the imperative first step to genetic-based tests including pharmacogenomics, prenatal and newborn screening, genotyping, diagnostic studies and forensics. Though there are numerous clinical sample types from which to isolate genomic DNA, whole blood remains a common and more consistent source of genomic material compared to saliva and swab samples and is used by many research and clinical laboratories. Whole blood offers the consistently high yields over wide populations with high quality regardless of the preservative method. Akonni Biosystems has developed an automated, high throughput TruTip extraction technology as a simple and affordable solution for fast and efficient isolation of human genomic DNA from fresh and frozen blood.

The TruTip technology uses a porous binding matrix embedded in a pipette tip (Figure 1) with chaotropic salt chemistry and eliminates the need for costly vacuum filtration, centrifugation, or magnetic rod systems. The purified sample is free of inhibitors and contaminants and ready for any downstream detection method. Automation of the extraction process on the Hamilton STAR platform offers a dependable, cost-effective solution for high throughput workloads. The entire process is performed on-board the instrument including a proteinase K incubation using the Hamilton HHS2 heater/shaker which allows the user to load sample tubes onto the platform and retrieve the final extracted samples at the completion of the run. The 96-channel arm allows for high throughput processing of 96 samples in less than 60 minutes, for a total of 768 samples per 8-hour shift. Herein we demonstrate the high precision of the automated extraction system in processing genomic DNA from low volume whole blood samples and its advantage over competitors.

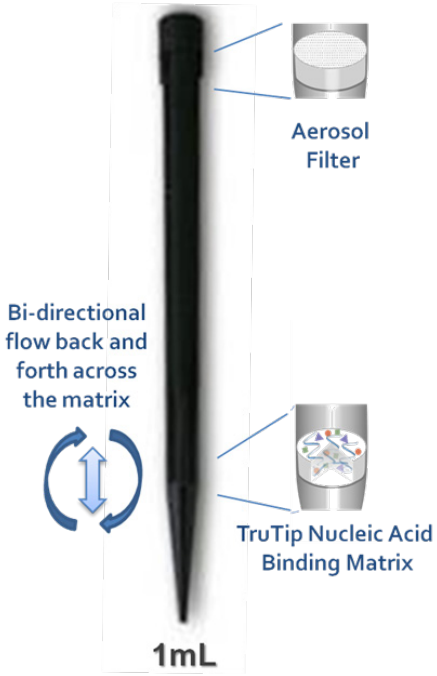


Figure 1: TruTip diagram

HAMILTON

System and Materials:

Hamilton Microlab STAR had been configured with the following components	
Microlab STAR with 8 independent channels	
96 channel CORE Multi Probe Head	

Other Consumables	Part Number
4 x 1 mL Hamilton filtered CO-RE 96 tip rack (Hamilton, cat# 235905)	Hamilton, cat# 235905
1 x 1 mL Hamilton non-filtered CO-RE 96 tip rack (Hamilton, cat# 235904)	Hamilton, cat# 235904
1 x 50 mL Reagent Trough (Hamilton, cat#187297)	Hamilton, cat#187297
4 x Deep Well 2 mL plate, 4 (USA Scientific, cat# 1896-2800)	USA Scientific, cat# 1896-2800
1 x Nunc 96 DWP-2mL (Thermofisher, cat# 278743)	Thermofisher, cat# 278743
5 x Reagent Trough (Fisher, cat# 14-222-412)	Fisher, cat# 14-222-412

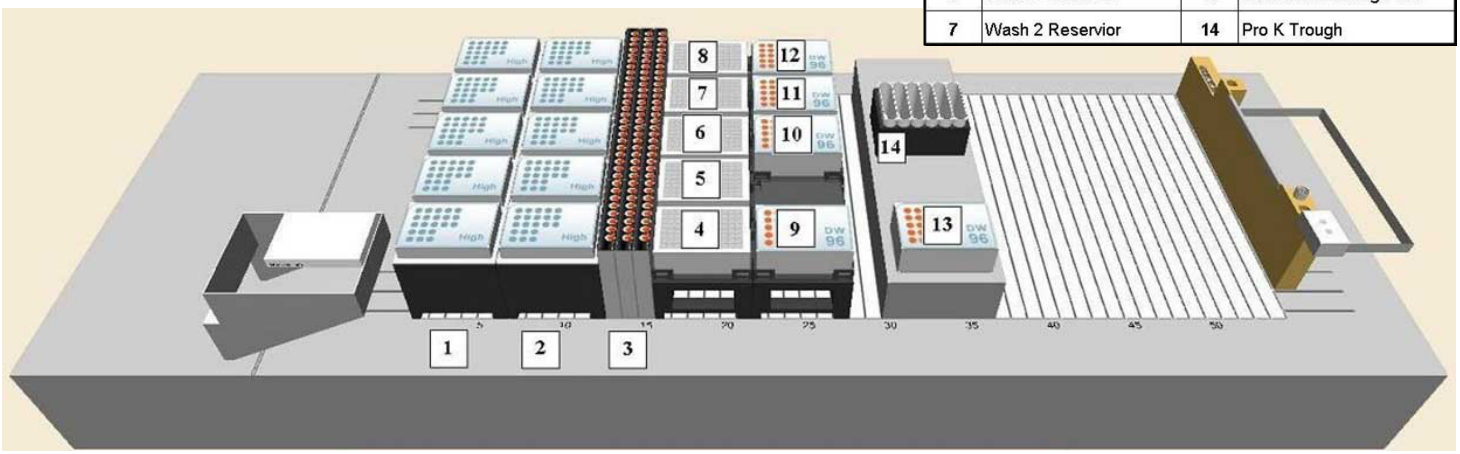
User-Supplied Materials
1 mL Hamilton LPT TruTips (rack of 96)
Akonni Lysis and Binding Buffer F
Akonni Wash Buffer J
Akonni Wash Buffer K
Akonni Elution Buffer A2
95% Ethanol (Provided by user)

Sample, Reagent and Deck Preparation

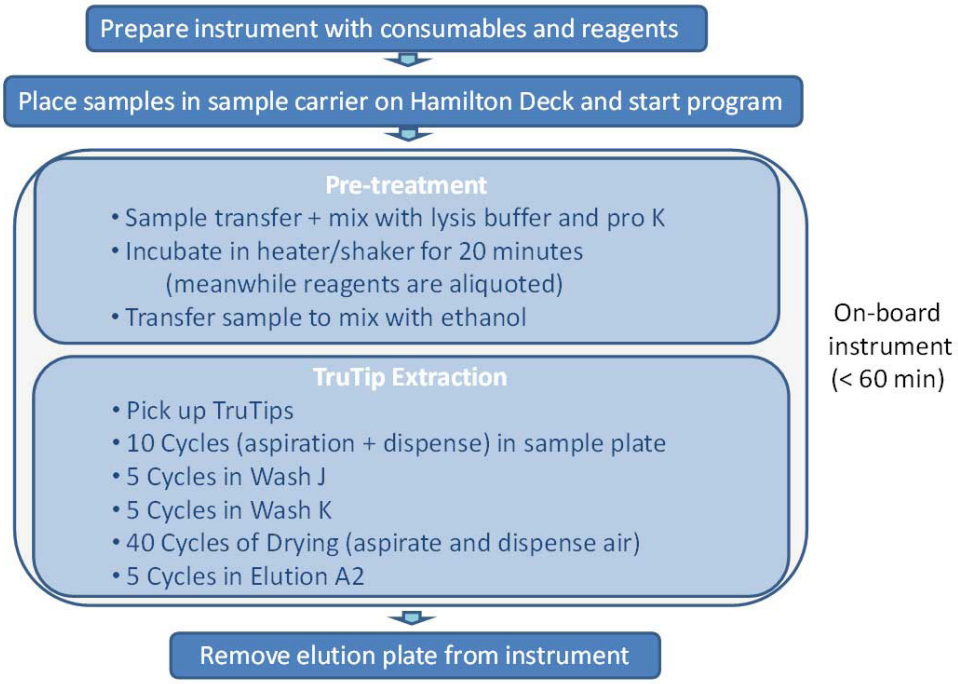
All carriers and consumables were placed onto the Hamilton STAR deck configuration, as shown in Figure 2. Reagents were poured into reagent troughs . Fresh whole blood or thawed frozen whole blood (K2 EDTA preserved) was pooled and aliquoted into 2mL microcentrifuge tubes and placed in sample carriers on the deck (position 3).

1	1000 µl Reagent Tips	8	Elution Buffer Reservoir
2	1000 µl TruTips	9	Binding Plate
3	Blood Sample Carriers	10	Wash 1 Plate
4	Lysis Buffer Reservoir	11	Wash 2 Plate
5	95% EtOH Reservior	12	Elution Plate
6	Wash 1 Reservoir	13	Incubation/Binding Plate
7	Wash 2 Reservior	14	Pro K Trough

Figure 2: Screenshot of Hamilton STAR deck layout for extraction of 96 samples.



Automated Method Overview



Results and Discussion

Yield and Quality

Purification of whole blood samples using the automated TruTIp extraction method was compared directly to an industry leader's manual spin column extraction kit for blood and body fluids. Figure 3A illustrates the comparative yields of the two extraction methods. Yields observed for TruTip were 30% higher compared to the manual spin column kit. These results correspond well with typical yields from this extraction method, between 4-6 µg. The TruTip extraction process generates higher molecular weight genomic DNA with less shearing compared to the spin column method as shown in Figure 3B. Though the TruTip protocol does not include a separate RNase step, no RNA is visible in gel shown in Figure 3B. Absorbance spectra from isolated products result in 260/280 and 230/230 ratios that further indicate the high purity of the extracted samples (Figure 4). A variety of anticoagulants (EDTA, heparin, citrate) have been tested with equal quality products.

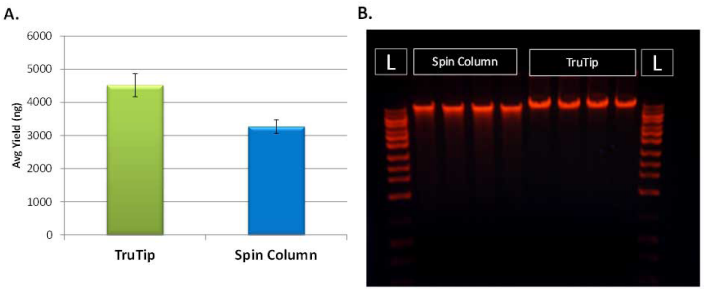


Figure 3: A) Replicate extractions of 200 µL pooled frozen blood using the TruTip gDNA Blood Kit (n=16) and the spin column kit (n=16). Yields were determined from the absorbance at 260 nm using the NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific Inc.). B) Representative product gels comparing the two extraction methods. 1% TBE Gels (Lonza) were run at 96 V for 60–90 minutes. The ExACTGene 24kb Max DNA Ladder (Fisher Scientific) was run as a standard.

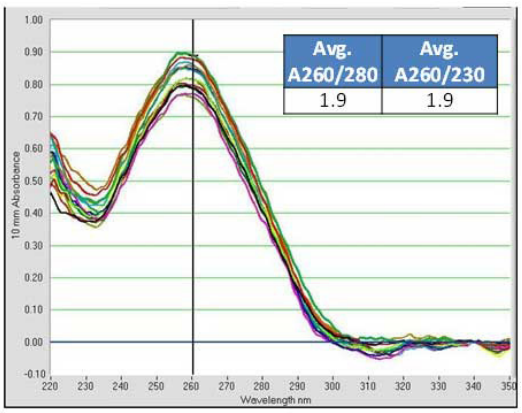


Figure 4: UV-Vis Absorbance spectrua of replicate TruTip extractions (n=16) from the NanoDrop 1000 instrument.

Reproducibility and Repeatability Study

Extraction uniformity for TruTip quality and heating efficiency was tested in a reproducibility study of 96 simultaneous extractions. Pooled fresh whole blood distributed into the 96 sample wells on a single plate. The duration of the protocol was < 60 minutes from start to finish with minimal hands-on time. Results demonstrated high precision of this extraction method with low standard deviation using the automated system (Figure 5). A repeatability study was performed by two operators processing 16 samples separately on three different days using frozen whole blood. Results demonstrated very uniform recovery independent of operator and were reproducible over the course of the three day study (Figure 6).

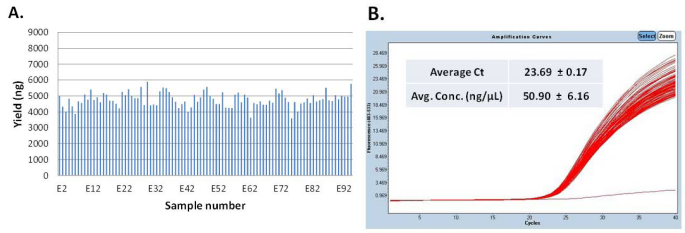


Figure 5. TruTip reproducibility study results (n=96, pooled fresh blood) as determined by A) 260 nm absorbance readings on the NanoDrop™ 1000 Spectrophotometer and B) Real-time qPCR on the Roche LightCycler® 480 system with the Applied Biosystem™ (AB) Quantifiler® Human DNA Quantification.

Repeatability Study

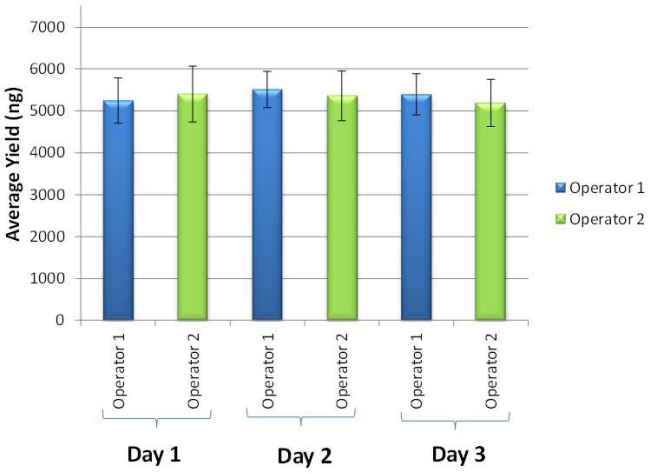


Figure 6. Repeatability study results (n=16 pooled frozen blood per run for each operator). Yields were determined by real-time qPCR using the Roche LightCycler® 480 system with the Applied Biosystem™ (AB) Quantifiler® Human DNA Quantification. Concentration was calculated based on AB Human DNA Quantifier Standard Curve.

Conclusion

Processing of whole blood in a high throughput format is greatly simplified using the TruTip technology on the Hamilton STAR system. The TruTip protocol demonstrated successful extraction and purification of high molecular weight genomic DNA from whole blood with competitive yields, quality and reproducibility.

Features and Benefits

- **Fast extractions – 96 in less than 60 minutes, for a total of 768 samples per 8-hour shift**
- **High quality extraction products with high reproducibility**
- **Simple deck layout with throughput flexibility on the STARlet, STAR and STARplus platforms**
- **Eliminates the need for costly vacuum filtration, centrifugation, or magnetic rod systems**
- **Fully automated protocol performed on-board the instrument allows user to set-up and walk away**

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