

Purification of Genomic DNA from Saliva Using Oragene® Discover Collection Kits and the Akonni TruTip® Extraction System on the Hamilton Microlab® STAR Liquid Handling Workstation: Rapid, High-Throughput Nucleic Acid Extraction

Application Note

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Abstract

Akonni Biosystems has developed the simple and efficient TruTip automated extraction solution designed to isolate genomic DNA from Oragene DNA saliva collection kits in preparation for downstream genetic testing such as amplification, sequencing and microarray detection, currently for research use only (RUO). The tip-based extraction technology is paired with the advanced, proven and reliable Hamilton Microlab STAR liquid handling workstation for high throughput sample processing. High-quality genomic DNA was extracted using the TruTip method with comparable yields to industry leaders. Reproducibility studies demonstrated high precision with low standard deviations and no cross-contamination.

Introduction

The purification of genomic DNA is the necessary first step to genetic-based tests including pharmacogenomics, prenatal and newborn screening, genotyping, diagnostic studies and forensics. Though genomic DNA can be isolated from numerous clinical sample types, saliva is emerging as the sample matrix of choice for many research and clinical laboratories. Saliva offers the high quality of DNA similar to that obtained from blood, but without the invasive collection method or storage stability issues. When using the Oragene line of saliva collection kits from DNA Genotek, genomic DNA samples are stable at room temperature for years. Akonni Biosystems has developed an automated, high-throughput TruTip extraction technology as a simple, affordable and competitive solution for fast and efficient isolation of human genomic DNA from clinical samples (RUO).

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. Automating the extraction process on the Hamilton Microlab STAR platform offers a dependable, cost-effective solution for high-throughput workloads. The flexible platform can be customized for specific user requirements and workflows. TruTip technology's compact extraction workflow allows ample remaining deck space to integrate upstream or downstream processes in the same run. The 96-channel arm allows for high-throughput processing of 96 samples in less than 30 minutes, for 1,536 samples per eight-hour shift. In the following studies, we have demonstrated the high precision of the automated TruTip extraction technology in processing genomic DNA from low-volume saliva samples and its advantage over competitors.

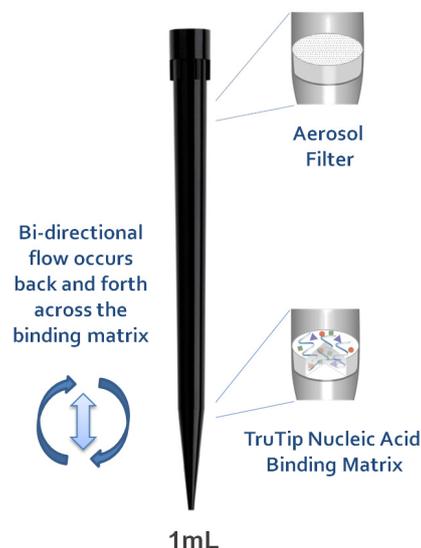
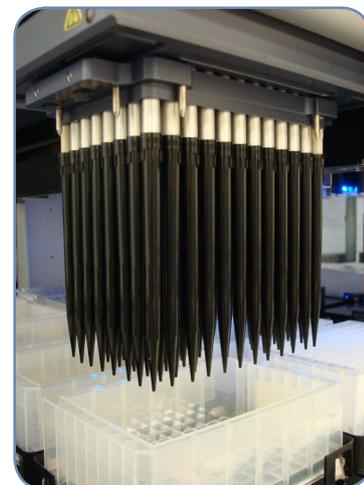


Figure 1: TruTip diagram

System and Materials:

Hamilton STAR equipped as follows (deck layout in Figure 2)

8-channel liquid handling arm

96-channel head

2 x Tip Carriers (TIP_CAR_480BC)

3 x Sample Carriers (SMP_CAR_32_EPIL)

2 x Plate Carriers (PLT_CAR_L5AC)

1 x Multiflex Carrier containing:

- 1 x Rack Carrier (rackformfx_car_L5_rgt5)

Other Consumables

Part Number

5 x 1 mL Hamilton filtered CO-RE 96-tip rack

Hamilton, cat# 235905

1 x 50mL Reagent Trough

Hamilton, cat# 187297

4 x 2 mL Deep 96-Well Plate, 4

USA Scientific, cat# 1896-2800

4 x Reagent Trough

Fisher, cat# 14-222-412

Oragene® Discover Saliva Collection Kit

DNA Genotek, cat# OGR-500

5 x Reagent Trough

Fisher, cat# 14-222-412

Akonn TruTip Extraction gDNA Blood Kit Consumables

1 mL Hamilton LPT TruTips (rack of 96)

Akonn Lysis and Binding Buffer E

Akonn Wash Buffer J

Akonn Wash Buffer K

Akonn Elution Buffer A2

95% ethanol (provided by user)

Sample, Reagent and Workstation Set-up

Oragene-preserved saliva samples were incubated for a minimum of one hour in the water bath at 60°C, as directed by the manufacturer. All carriers and consumables were placed onto the Hamilton STAR worktable, as shown in Figure 2. Reagents were poured into reagent troughs. Oragene Saliva Samples were pooled and aliquoted into 2 mL microcentrifuge tubes and placed in sample carriers on the deck (position 3). Alternatively, the entire Oragene tube can be placed directly on the system in the appropriate rack.

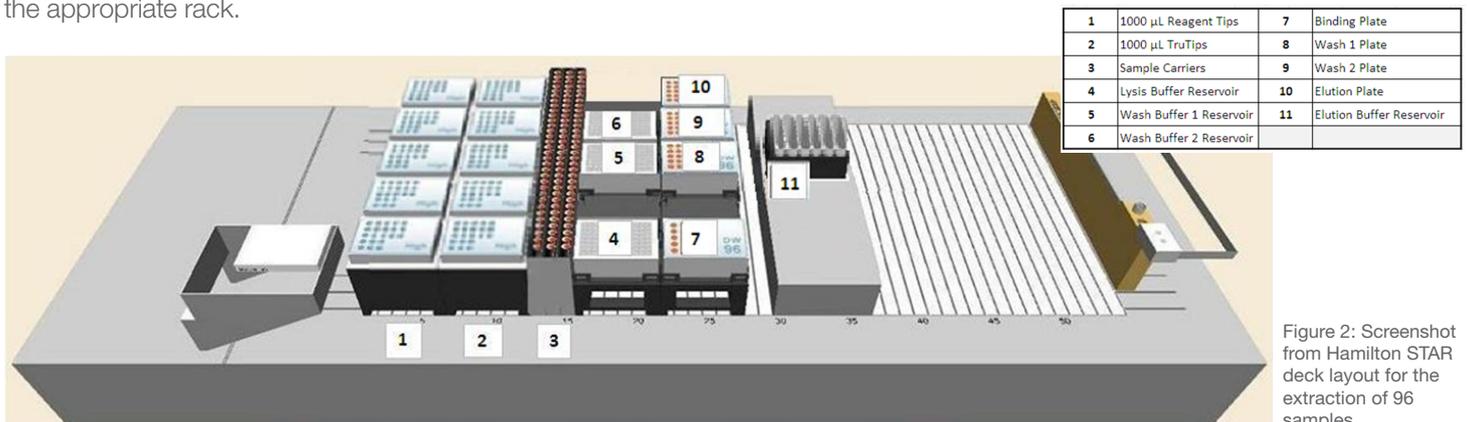
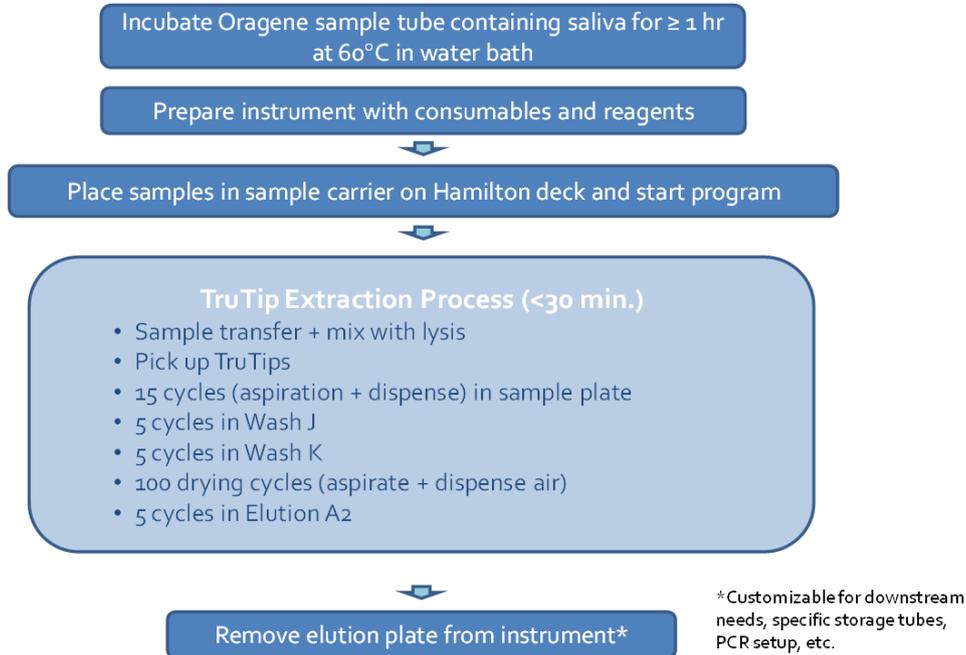


Figure 2: Screenshot from Hamilton STAR deck layout for the extraction of 96 samples.



Workflow Overview



Results and Discussion

Yield and Quality

Purification 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 3 illustrates the comparative yields of the two extraction methods with seven individual saliva donors. Yields observed for TruTip were 20% higher on average compared to the manual spin column kit. Absorbance spectra from isolated products resulted in 260/280 ratios that further indicate the high purity of the extracted samples (table in Figure 3). The TruTip extraction process generated higher molecular weight genomic DNA, as shown in Figure 4.

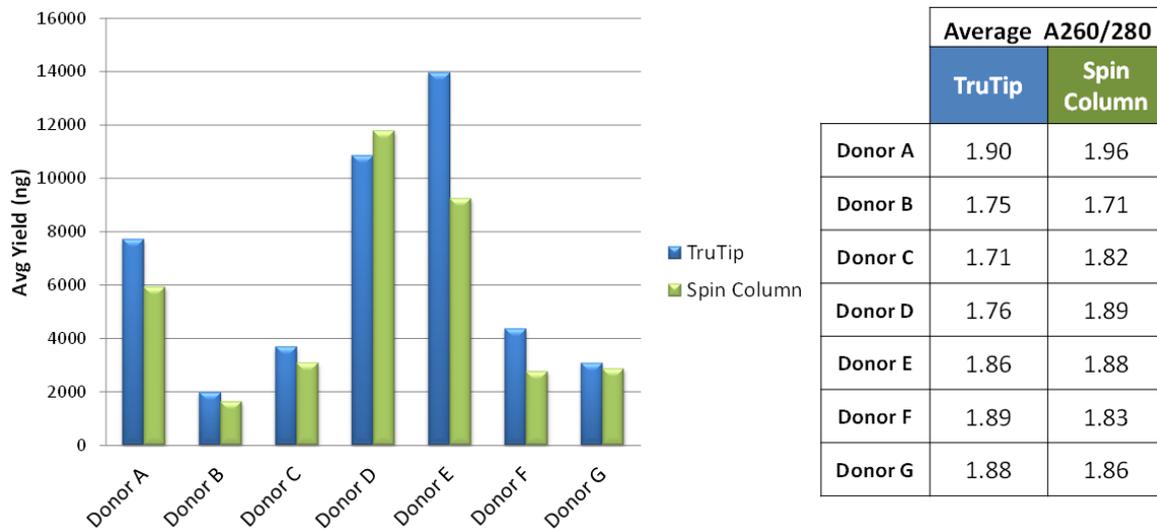


Figure 3: Comparison of extraction yields from the two isolation methods for seven individual donors. Replicate extractions of 400 μ L individual donor saliva samples were extracted using the TruTip gDNA Blood Kit (n=3) and the spin column kit (n=3). The table (right) shows 260/280 ratios for each sample. Yields and purity ratios were determined from the absorbance at 260 nm and 280 nm using the NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific).

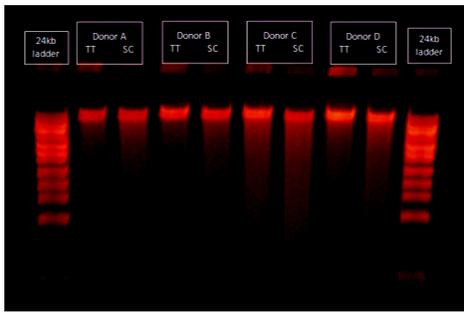


Figure 4: Representative product gel comparing TruTip (TT) to spin column (SC) extraction methods using four donor samples from Figure 3. On percent TBE Gels (Lonza) were run at 96 V for 60 minutes. The ExACTGene 24kb Max DNA Ladder (Fisher Scientific) was used as a standard.

Reproducibility and Repeatability Study

TruTip quality was tested through extraction uniformity in a reproducibility study of 24 simultaneous extractions using pooled saliva. A repeatability study was also performed by running the method with the same sample over three consecutive days. Results from both experiments demonstrated highly uniform recovery, both tip to tip and over the course of multiple days as shown in Figure 5.

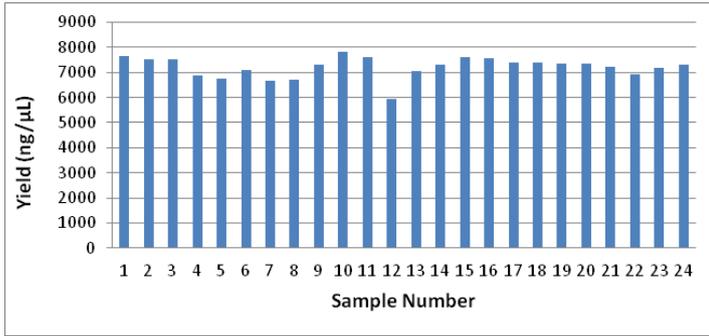


Figure 5. Average concentration of extracted products from 24 pooled saliva samples. Yields were determined by 260 nm absorbance readings on the NanoDrop™ 1000 Spectrophotometer.

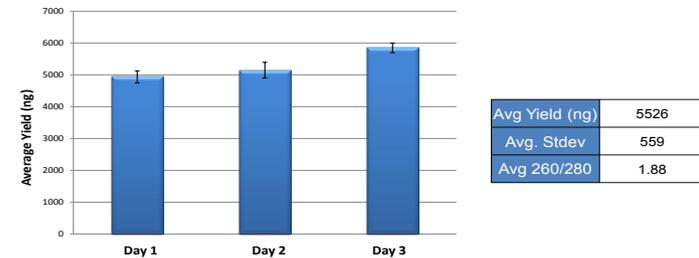


Figure 6. Average yield of extractions over three days (n=8 pooled Oragene saliva samples per day). Yields were determined by 260 nm absorbance readings on the NanoDrop™ 1000 Spectrophotometer.

Cross-contamination Study

A cross-contamination study was performed by alternating positive and negative samples across the sample plate for a total of 24 pooled saliva samples and 24 negative samples (1X PBS). Results demonstrated no carry-over between samples when processed on the Hamilton STAR automated system (Figure 7).

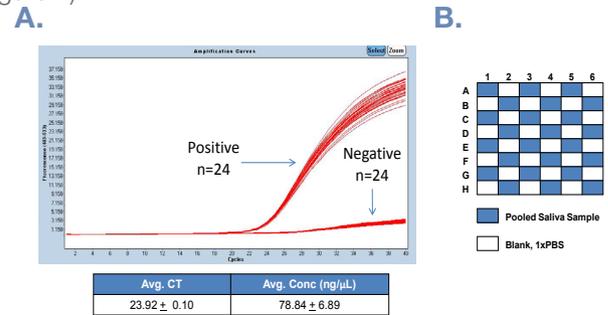


Figure 7. A) Real-time PCR results from the cross-contamination study. Twenty-four pooled saliva samples and 24 negative samples (1X PBS) were positioned in a checkerboard pattern in the sample plate according to plate map (B). Extracted products were amplified using the Quantifier® Human DNA Quantification Kit by Life Technologies.

Conclusion

The workflow for high-throughput extraction of human genomic DNA from saliva is greatly simplified using Oragene Collection Kits with TruTip technology on the Hamilton Microlab STAR system with extraction of up to 96 samples in less than 30 minutes. The DNA is extracted reproducibly and is of high quality and purity compared to industry-standard spin column extractions. This kit is currently released for RUO.

Features and Benefits

- ▶ **Fast extractions:** 96 samples in less than 30 minutes, for a maximum throughput of 1,536 samples per eight-hour shift
- ▶ **High-quality, pure nucleic acid isolated with excellent reproducibility**
- ▶ **Simple deck layout with throughput flexibility on the NIMBUS, STARlet, STAR and STARplus platforms**
- ▶ **Easily integrated with upstream or downstream processes**
- ▶ **Eliminates the need for costly vacuum filtration, centrifugation, or magnetic rod systems**
- ▶ **Fully automated protocol allows user to set up and walk away**

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