

Applications

Note 002 | June 2010

Rapid, Automated Extraction and Purification of Genomic DNA from Saliva using the Akonni TruTip™ Extraction System on the Eppendorf epMotion® 5070

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Abstract

Akonni Biosystems® has developed the simple and effective TruTip automated extraction method designed to isolate genomic DNA from saliva for downstream genetic testing. The tip-based extraction technology is paired with the affordable Eppendorf epMotion 5070 liquid handling system for medium throughput sample processing. Saliva samples preserved with the Oragene® DNA collection kit were pooled and extracted on the epMotion system using TruTips containing a nucleic acid binding matrix. Higher yields of genomic DNA were extracted using the TruTip method when compared directly to industry leaders. Reproducibility studies demonstrated high precision, with low standard deviations and absence of cross-contamination.

Introduction

The purification of genomic DNA is the imperative first step to genetic-based tests including pharmacogenomics, prenatal and newborn screening, parental testing, diagnostic studies and forensics. Though there are numerous clinical sample types from which to isolate genomic DNA, the most common being blood and buccal swabs, saliva is emerging as the sample matrix of choice for many research and clinical laboratories. Saliva offers the high yield and quality of DNA similar to that obtained from blood, but without the invasive collection method and storage stability issues. When collected with the Oragene® DNA kit, saliva is stable at room temperature for years. In response to this growing market, Akonni Biosystems has developed an automated TruTip extraction technology as an affordable and competitive solution for fast and efficient isolation of genomic DNA from clinical samples.

The TruTip technology uses a porous binding matrix embedded in a pipette tip with chaotropic salt chemistry and eliminates the need for vacuum filtration or centrifugation. The purified sample is free of inhibitors and contaminants and ready for downstream detection via PCR. Automation of the extraction process on the epMotion 5070 offers a low cost solution for medium throughput workloads. The simple workflow and user-friendly interface allows program flexibility to suit the user's needs with little training required. 1 to 24 samples can be processed at a time with up to 8 samples extracted in 10 minutes, up to 16 samples extracted in 18 minutes, and up to 24 samples processed in just 30 minutes, or less than 2 minutes per sample. Herein we demonstrate the high precision of the automated extraction system in processing genomic DNA from saliva samples and its advantage over competitors.

Materials and methods

Eppendorf epMotion 5070 equipped as follows:

- Dispensing tool TM1000-8
- Reservoir rack
- Waste tub

Eppendorf consumables:

- Reagent reservoirs: 30 ml
- Sample plate: Deepwell plate 96/2,000µl
- epT.I.P.S. Motion Filtertips, 1,000µl
- EPM TruTips: 1,000µl

Akonni TruTip Extraction System:

- gDNA Saliva Kit Lysis and Binding Buffer
- gDNA Saliva Kit Wash Buffer
- gDNA Saliva Kit Wash Buffer
- gDNA Saliva Kit Elution Buffer

Pooled, de-identified saliva samples collected using Oragene® DNA collection tubes (A, B and C).

Sample, Reagent and Worktable Preparation

Labware was placed onto the epMotion 5070 Worktable, as shown in Figure 1 and Table 1. Pooled saliva samples were dispensed into columns 1, 5 and 9 in the Sample Plate (50 µl each well) as shown in Figure 2. Reagents were prepared and dispensed into 30 ml Reagent Reservoirs as described in Table 2.

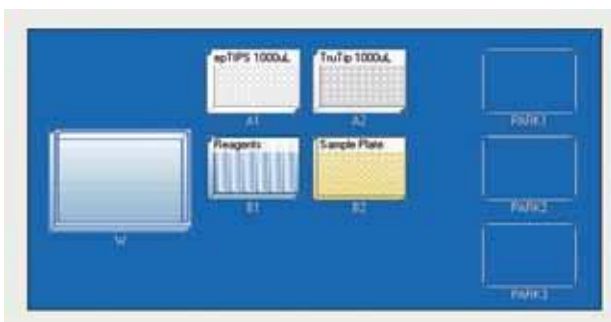


Figure 1: Screenshot from the epMotion Editor showing the epMotion 5070 Worktable setup for the automated method.

Worktable Position	Labware
A1	epT.I.P.S. Motion Filtertips, 1,000 µl
A2	EPM TruTips, 1,000 µl
B1	Reagent Reservoir Rack
B2	Sample Plate, 96/2,000 µl

Table 1: epMotion 5070 Worktable setup, details by position.

Reservoir Rack Position	Reservoir Contents	Reagent Prep Instructions
1	17,005 µl Lysis Buffer	One time only: 95% EtOH added according to kit instructions
2	12,205 µl Wash Buffer 1	One time only: 95% EtOH added according to kit instructions
3	12,205 µl Wash Buffer 2	One time only: 99% Acetone and 95% EtOH added according to kit instructions
4	1,405 µL Elution Buffer	None
5	Empty (input 8,205 µl)*	Reservoir for drying
6 and 7	Empty	Unused

Table 2: Contents of the Reagent Reservoirs in the Reagent Reservoir Rack. Volumes denote minimum volume required for 24 sample program.

*A positive volume must be input into the software for position 6 to compensate for volume of air pipetted during the drying step.

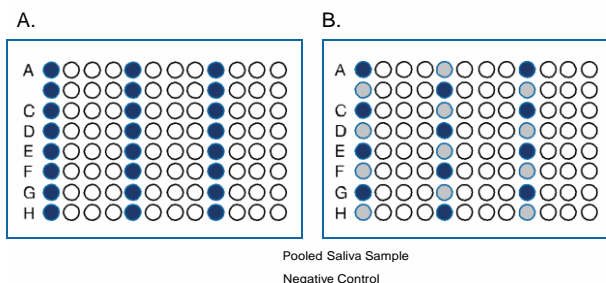


Figure 2: Sample plate layout. A.) 24 sample set-up with all positive samples. B.) 24 sample set-up with staggered positive and negative samples.

Automated Method Overview

The automated method uses the Akonni TruTips to extract genomic DNA from saliva samples in a 24-sample format. The method begins by dispensing all reagents into the Sample Plate: 700 µl Lysis and Binding Buffer is dispensed into wells 1, 5 and 9 and mixed with the saliva sample; 500 µl Wash Buffer 1 is dispensed into wells 2, 6 and 10; 500 µl Wash Buffer 2 is dispensed into wells 3, 7 and 11; 50 µl Elution Buffer is dispensed into wells 4, 8 and 12.

The dispensing tool then picks up 8 TruTips from position A2 on the Worktable and begins the extraction process for the first set of 8 samples in column 1 of the Sample Plate (Figure 2). First, the liquid in column 1 is aspirated and dispensed five times. This step lyses the cells and binds the nucleic acid to the TruTip matrix. Second, the tips move to column 2 of the Sample Plate to aspirate/dispense the Wash Buffer 1 one time. Third, the tips move to column 3 of the Sample Plate to aspirate/dispense the Wash Buffer 2 three times. These two wash steps remove proteins and other contaminants from the bound nucleic acid. Fourth, the tips move to Reagent Reservoir 5 to aspirate/dispense fifteen times with air to dry the TruTip matrix. Finally, the tips move to column 4 in the Sample Plate to aspirate/dispense the Elution Buffer five times. The extracted and purified nucleic acid is now in the elution buffer in column 4. This set of TruTips is discarded into the Waste Bin and a new set is picked up from position A2 on the Worktable.

The extraction process is repeated on the 8 samples in column 5 on the Sample Plate and final extracted and purified sample is eluted into column 8. This set of TruTips is discarded into the Waste Bin and a new set is picked up from position A2 on the Worktable. The extraction process is repeated on the samples in column 9 on the Sample Plate and final extracted and purified sample is eluted into column 12. Eluted samples in columns 4, 8 and 12 are ready for analysis.

Analysis of Extracted DNA

Analysis of genomic DNA in the extracted samples in Sample Plate A and B was performed using a real-time PCR on the Roche LightCycler® 480 system using the Applied Biosystem™ (AB) Quantifiler® Human DNA Quantification Kit (20 µl master mix to 5 µl sample). All extracted samples were analyzed in duplicate. The thermal cycling conditions were as follows: 1 cycle 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds, 60 °C for 1 minute. Fluorescence curves were analyzed using the Absorbance Quantification tool in the LightCycler software to calculate the threshold cycle (Ct) value for each independent curve. The Ct for a specified amplification plot occurs when the fluorescent signal increases beyond the value of the threshold setting. Concentration was calculated based on AB Human DNA Quantifier Standard Curve.

Results

Competitor Comparison

Purification using the automated TruTip extraction method was compared directly to purification of the same pooled saliva samples using Qiagen® Blood or Body Fluid Kit. The TruTip extraction streamlined protocol does not require the 10 minute incubation step with proteinase K at elevated temperature (56 °C) that is in the Qiagen extraction protocol. Using a 50 µl input and elution volume, the TruTip gDNA Saliva Kit yielded double the amount of DNA isolated from the Qiagen Kit (Figure 3).

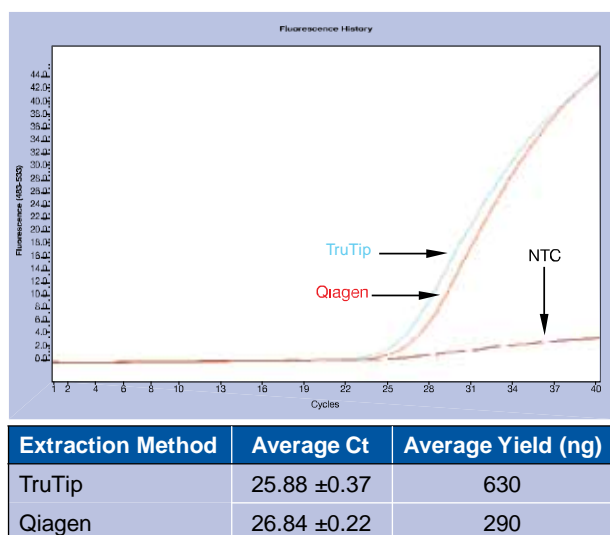


Figure 3. Replicate extractions of 50 µl pooled saliva A using the TruTip gDNA Saliva Kit (single epMotion plate, n=24) and the Qiagen Blood or Body Fluid Kit (n=8). The same input and elution volume was used for both kits for direct comparison; however, the Qiagen kit recommends 200 µl elution.

Reproducibility and Cross-Contamination Study

A reproducibility study was performed by extracting the same sample from each of the 24 sample wells on a single plate. Results demonstrated high precision of this extraction method with low standard deviation using the automated system (Table 3). A cross-contamination study was performed by alternating positive and negative samples across the sample plate for a total of 12 pooled saliva samples and 12 negative samples (H₂O). Results demonstrated no cross-contamination between samples when processed on the epMotion 5070 automated system (Figure 4).

Column 1		Column 4		Column 8	
Sample	Ct	Sample	Ct	Sample	Ct
EP1	25.57	EP9	26.76	EP17	25.83
EP2	26.17	EP10	26.01	EP18	26.08
EP3	26.48	EP11	26.32	EP19	25.78
EP4	25.96	EP12	26.12	EP20	25.62
EP5	26.54	EP13	26.29	EP21	26.08
EP6	26.09	EP14	25.76	EP22	25.86
EP7	26.08	EP15	25.85	EP23	26.07
EP8	26.22	EP16	26.43	EP24	25.65

Avg. Cp	Avg. Conc. (ng/µl)	Avg. Yield (ng)
26.07 ±0.30	10.8 ±2.3	540

Table 3. epMotion reproducibility studies. 24 replicate extractions of 50 µl pooled saliva B were run on a single plate according to the plate map A (Figure 2).

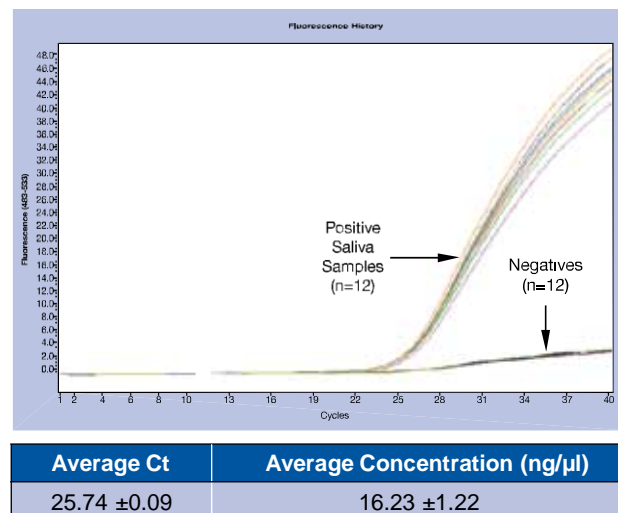


Figure 4. epMotion cross-contamination studies. 12 pooled saliva samples (C) alternated with 12 negative samples (H₂O) were run on a single plate according to plate map B (Figure 2).

Conclusion

The integration of the Akonni TruTip with the epMotion 5070 resulted in a complete system for automated extraction and purification of genomic DNA from saliva. The flexible method is designed for medium to high throughput by processing 1 to 24 samples on a single plate using an inexpensive liquid handling instrument. A full plate of 24 samples is processed in 30 minutes. All reagent transfer steps are included in the method for an easy workflow with little set-up time required.

The user-friendly procedure effectively removes inhibitors and yields amplification-ready DNA without the need for centrifugation or vacuum filtration steps. The TruTip protocol demonstrated successful extraction and purification of DNA from saliva samples at yields greater than Qiagen's analogous spin column purification protocol but with a streamlined procedure. Reproducibility studies yielded highly repeatable results, with no detectable cross-contamination.

Ordering information

Description	Catalog No.
Eppendorf	
30 ml Reagent Reservoirs	960050100
Reservoir Rack	960002148
Sample Plate: Deepwell plate 96/2,000 µl	0030502302
Dispensing tool TM 1,000-8	960001061
epT.I.P.S. Motion Filtertips, 1,000 µl	960050100
Akonni Biosystems	
EPM TruTip gDNA Saliva Kit (EPM Tips, 1,000 µL, 96 SPT) For use with the epMotion Automated Pipetting System. Includes TruTip SPT tips and buffers for 96 extractions.	300-20120

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