Forensic DNA Extraction Using an Automated, Magnetic Particle-Based Workflow

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Abstract

DNA of the highest yield and quality provides data integrity beyond reproach to include or exclude persons from a criminal case. During forensic analysis, however, DNA processing is often complicated by a variety of environmental and substrate influences as well as the ever-present risk of human error and variability when manually processing samples. Additionally, time-consuming tasks such as repetitive pipetting slow overall laboratory efficiency and contribute to reduced turnaround times. A novel combination of assay magnetic particle-based chemistry and hardware technology offsets the risks posed by external influences during DNA extraction and purification. When integrating this workflow onto an automated assay ready workstation, risks of human errors and variations present in manual workflows are eliminated, and analysts are free to focus on other tasks while up to 96 samples are automatically processed in microplate format. The resulting high yield, high purity DNA is suitable for sensitive downstream applications such as capillary electrophoresis or massive parallel sequencing. Here, we demonstrate this automated DNA extraction and purification workflow when processing a variety of common human identification sample types and concentrations. We compare the automated workflow to a lower throughput workflow and show that the combination of assay chemistry, specialized technology, and assay ready workstation provide downstream results via capillary electrophoresis that are sensitive, precise, and accurate.

Samples and Sample Dilutions

Samples

Dried blood samples on swabs were prepared in-house from one male donor with a known genotype. Saliva samples on swabs were prepared in-house from one female donor with a known genotype. Standard Reference Material® 2391c Component F (single-source male cells on 903 paper) was procured from NIST. Known, non-probative crime scene samples were prepared in-house mimicking sample types that are commonly encountered in crime laboratories.

Sample Dilution

Whole blood and phosphate buffered saline (PBS) were used to manually create a seven-point sample titration using 1:3 serial dilutions ranging from undiluted whole blood to a 1:729 dilution. A combination of 10 µL of sample at each dilution point and 30 µL of PBS were added to a non-stick RNase-free 1.5 mL microfuge tube. The process was repeated using saliva samples (five-point titration from 1:3 to 1:243 dilution). A total of 40 µL of each sample were added to separate cotton swabs and air dried before extracting. Each dilution point was extracted in triplicate.

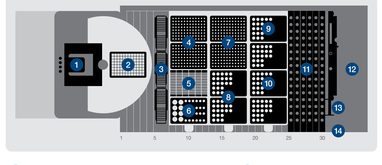
Sample Identifier	Sample Description
NP1	Swab from drink can
NP2	Swab from drink can
NP3	Swab from cell phone
NP4	Swab from cell phone
NP5	Cigarette butt
NP6	Cigarette butt
NP7	Chewing gum
NP8	Chewing gum
NP9	Semen 1:10
NP10	Semen 1:10
NP11	Semen 1:10
NP12	Semen 1:100
NP13	Semen 1:100
NP14	Semen 1:100
NP15	Swab from coffee cup

Sample Identifier	Sample Description
NP16	Swab from coffee cup
NP17	Swab from coffee cup
NP18	Swab from coffee cup
NP19	Swab from coffee cup
NP20	Buccal swab
NP21	Buccal swab
NP22	Buccal swab
NP23	Buccal swab
NP24	Swab from baseball hat
NP25	Swab from baseball hat
NP26	Blood on denim
NP27	Blood on denim
NP28	Blood on denim
NP29	Blood on denim
NP30	Blood on cotton

Sample Identifier	Sample Description
NP31	Blood on cotton
NP32	Blood mixture 1:10
NP33	Blood mixture 1:10
NP34	Blood mixture 1:1
NP35	Blood mixture 1:1
NP36	Hair root x3
NP37	Hair root x2
NP38	Saliva on 3mm FTA punch
NP39	Saliva on 3mm FTA punch
NP40	Saliva on 3mm FTA punch
NP41	Saliva on 3mm FTA punch
NP42	Saliva on 3mm FTA punch
NP43	Saliva on 3mm FTA punch
NP44	Saliva on 3mm FTA punch
NP45	Saliva on 3mm FTA punch

System Design and Workflow

ID NIMBUS Presto Deck Layout for Use with the PrepFiler Automated Forensic DNA Extraction Kit



1. ThermoFisher KingFisher Presto
2. Deep Well Plate Position
3. 200 mL Reagent Troughs
4. 1,000 µL Tips
5. 60 mL Reagent Troughs
6. Reagent Tubes
7. 300 µL Tips
8. Stacked Deep Well Plates
9. KingFisher 96 Tip Comb
10. Deep Well Plates
11. Microtube Carriers
12. Waste
13. CO-RE® Paddles
14. Barcode Reader

Automated Workflow for the PrepFiler Automated Forensic DNA Extraction Kit on the ID NIMBUS Presto

Materials and Methods

Sample Lysis

A volume of 302.4 µL 1.0 M DL-Dithiothreitol was added to 30.2 mL of the Lysis Buffer included in the PrepFiler Automated Forensic DNA Extraction Kit. The samples and prepared lysis buffer were then placed directly into PrepFiler™ LySep™ columns that were inserted into a capless sample tube, and incubated for 40 minutes on a shaker at 70 °C, at 900 RPM. The column/tube assemblies were centrifuged for 2 minutes at 10,000 x g to transfer the lysates to the collection tubes while the substrates were retained in the columns. The lysates were then manually transferred from the collection tubes to a KingFisher™ 96 Deep-well Plate before placing on the ID NIMBUS Presto for automated downstream DNA extraction and purification.

Automated DNA Purification Using ID NIMBUS Presto

The ID NIMBUS Presto prompted users to load all consumables and PrepFiler reagents included in the PrepFiler Automated Forensic DNA Extraction Kit onto designated deck positions and also prompted user input on the number of samples and volumes. The KingFisher 96 deep-well plate containing the lysate was loaded onto the ID NIMBUS Presto deck in the appropriate position. The robot automatically added 16.5 µL PrepFiler magnetic particles and 180 µL Binding buffer to each well containing sample. Specialized CO-RE paddles automatically transferred the binding plate to the on-deck KingFisher Presto for processing, where magnetic rods sheathed inside a tip comb were independently lowered and raised to agitate and/or immobilize the magnetic particles in solution during binding, and later, during washing and elution. Concurrently during each step of purification, the liquid handler sequentially prepared the two Wash Buffer A plates, one Wash Buffer B plate, the final elution plate, and when applicable, transferred the prepared processing plates from the NIMBUS deck to the Presto purification processing site. Elution was completed in a user-defined volume of 65 µL.

DNA Quantification and Amplification

DNA levels were quantified using the Quantifiler™ Trio DNA Quantification Kit, and a 7500 Real-Time PCR System. DNA was amplified using the GlobalFiler™ PCR Amplification Kit and a ProFlex™ 96-well PCR System. A standard GlobalFiler PCR protocol was used with 29 cycles.

Capillary Electrophoresis

10 µL of a prepared size standard mixture were added to each reaction plate well along with 1 µL of amplified DNA sample or GlobalFiler Allelic Ladder. A 3500 xL Genetic Analyzer was used for electrophoresis using a 1.2 kV, 24 s injection for the contamination study and a 1.2 kV, 18 s injection for all other studies. Data analysis was performed with the GeneMapper™ ID-X v1.6 software using a 50 RFU peak amplitude threshold for the contamination study and a 175 RFU threshold for all other studies.

Cross-Contamination and Accuracy Analysis

Samples containing undiluted blood, undiluted saliva, and NIST reference samples were lysed and manually dispensed into separate wells of a 96-well plate in a checkerboard pattern, alternating between reagent blanks and blood, saliva, or NIST reference samples. The automated extraction and purification workflow proceeded as described. The reagent blanks and NIST sample were quantified and amplified to ascertain any potential for cross-contamination.

Sensitivity, Repeatability, and Reproducibility

Serially diluted and lysed blood and saliva samples were loaded onto the robot and automatically extracted. The samples were quantified to determine detection sensitivity and linearity of concentration across each dilution series. Small Autosomal C_T data derived from the blood and saliva dilution series samples were used to evaluate repeatability of automated extraction and purification, while an additional three replicates from the blood dilution series samples were extracted in a second automated extraction run to ascertain process reproducibility.

Known and Non-Probative Sample Analysis

Several mock crime scene samples representing those typically processed in forensic laboratories, including single-source, mixture, inhibited, low-template, and semen, were automatically processed.

Results and Discussion

Cross-Contamination and Accuracy Analysis

Thirty-three of the reagent blanks (RB#) produced an undetected result with a Threshold Cycle (C_T) greater than 40 for all targets. One reagent blank produced a small autosomal of C_T 39.8 (0.2 pg/µL) that did not generate a downstream STR profile. Therefore, the ID NIMBUS Presto does not introduce cross-contamination during extraction.

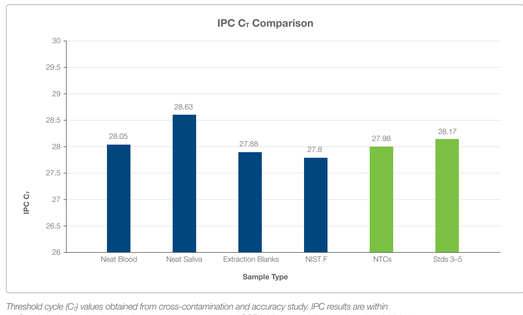
IPC C_T values for blood and saliva samples were within ±1 C_T unit of Quantifiler Trio standards 3 – 5 and the quantification Negative Template Controls (NTCs), thus indicating effective removal of PCR inhibitors during the automated extraction process.

(IPC) C_T values for the extraction blanks were within the same ±1 C_T unit, confirming that PCR inhibitors were not introduced into the samples by the consumables or any other part of the robotic system.

Finally, the STR profile obtained from the NIST F standard was determined to be 100% concordant to the expected genotype, confirming accuracy of the automated process.

	1	2	3	4	5	6	7	8	9	10
A	NB1	RB5	NB9	RB13	NS1	RB21	NS9	RB29	NB19	
B	RB1	NB5	RB9	NB13	RB17	NS5	RB25	NS13	RB33	
C	NB2	RB6	NB10	RB14	NS2	RB22	NS10	RB30	NIST F	
D	RB2	NB6	RB10	NB14	RB18	NS6	RB26	NS14	RB34	
E	NB3	RB7	NB11	RB15	NS3	RB23	NS11	RB31		
F	RB3	NB7	RB11	NB15	RB19	NS7	RB27	NB17		
G	NB4	RB8	NB12	RB16	NS4	RB24	NS12	RB32		
H	RB4	NB8	RB12	NB16	RB20	NS8	RB28	NB18		

ID NIMBUS Presto sample layout for cross-contamination and accuracy study. Automated dispensing in a checkerboard pattern of reagent blanks (RB#, grey), lysed neat blood samples (NB#, red), lysed neat saliva samples (NS#, blue), and NIST Standard Reference Material 2391c Component F (NIST F, green) prior to extraction and purification.



Threshold cycle (C_T) values obtained from cross-contamination and accuracy study. IPC results are within ±1 C_T unit, indicating that the robotic system removes unwanted PCR inhibitors and does not introduce inhibitors.

Sensitivity, Repeatability, and Reproducibility

Sensitivity levels were detected down to approximately 1 pg/µL in saliva swabs, approximately 2 pg/µL in blood swabs, and yields were proportional to sample inputs.

C_T values corresponding to DNA concentrations were consistent across each sample type and dilution range and within expected variation ranges of typical extraction and quantitation procedures.

The average heterozygous peak heights from each dilution point demonstrated that full balanced profiles were obtained from saliva DNA extracts out to a 1:81 dilution and from blood DNA extracts out to a 1:243 dilution. Representative STR profiles of single sample replicates from the saliva dilution series and blood dilution series confirm the sensitivity of detection at each serial dilution point (data not shown).

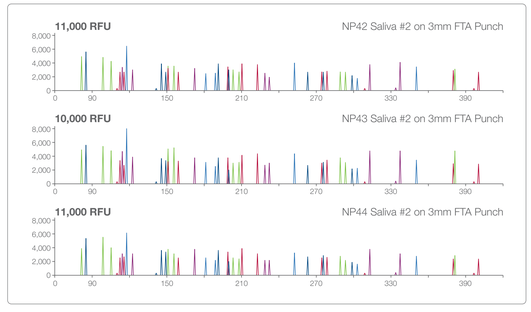
Known and Non-Probative Sample Analysis

Sample NP16 was invalidated as it produced no detectable DNA due to sample collection failure.

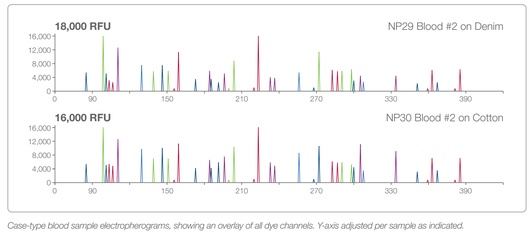
DNA concentrations of the various sample types ranged from 33 ng/µL to 0.005 ng/µL.

IPC C_T values for the mock crime scene samples were within ±2 C_T units of standards 3 – 5 and the quantification NTCs, thus indicating that any PCR inhibitors were effectively removed during the automated extraction workflow.

Partial or full profiles were obtained from all valid sample types as demonstrated in the representative samples below. The average heterozygous peak heights for all samples ranged from approximately 267 RFU (sample NP17) to approximately 11,000 RFU (sample NP27), relative to the corresponding DNA input for each sample.



Case-type reference sample electropherograms, showing an overlay of all dye channels. Y-axis adjusted per sample as indicated.



Case-type blood sample electropherograms, showing an overlay of all dye channels. Y-axis adjusted per sample as indicated.

Conclusion

Extracting and purifying high yield, high quality DNA from a variety of forensic-type samples in a high-throughput, hands-free format is enabled through use of the magnetic particle-based PrepFiler Forensic DNA Extraction Kit from Thermo Fisher Scientific and the ID NIMBUS Presto assay ready workstation from Hamilton. The automated workflow may be used to process samples with a wide range of DNA input amounts and is effective in maximizing DNA yields without PCR inhibitors or cross-contamination from samples containing scant to ample quantities of biological material. Results are robust, repeatable, and comparable to or better than other methods, while walkaway processing reduces active labor time and the risks of human variability and errors. The combination of magnetic particle-based assay chemistry and robotic workstation provides reliable results when obtaining genomic DNA from forensic biological samples to support sensitive downstream analysis using real-time quantitative PCR and PCR for STR profiling.