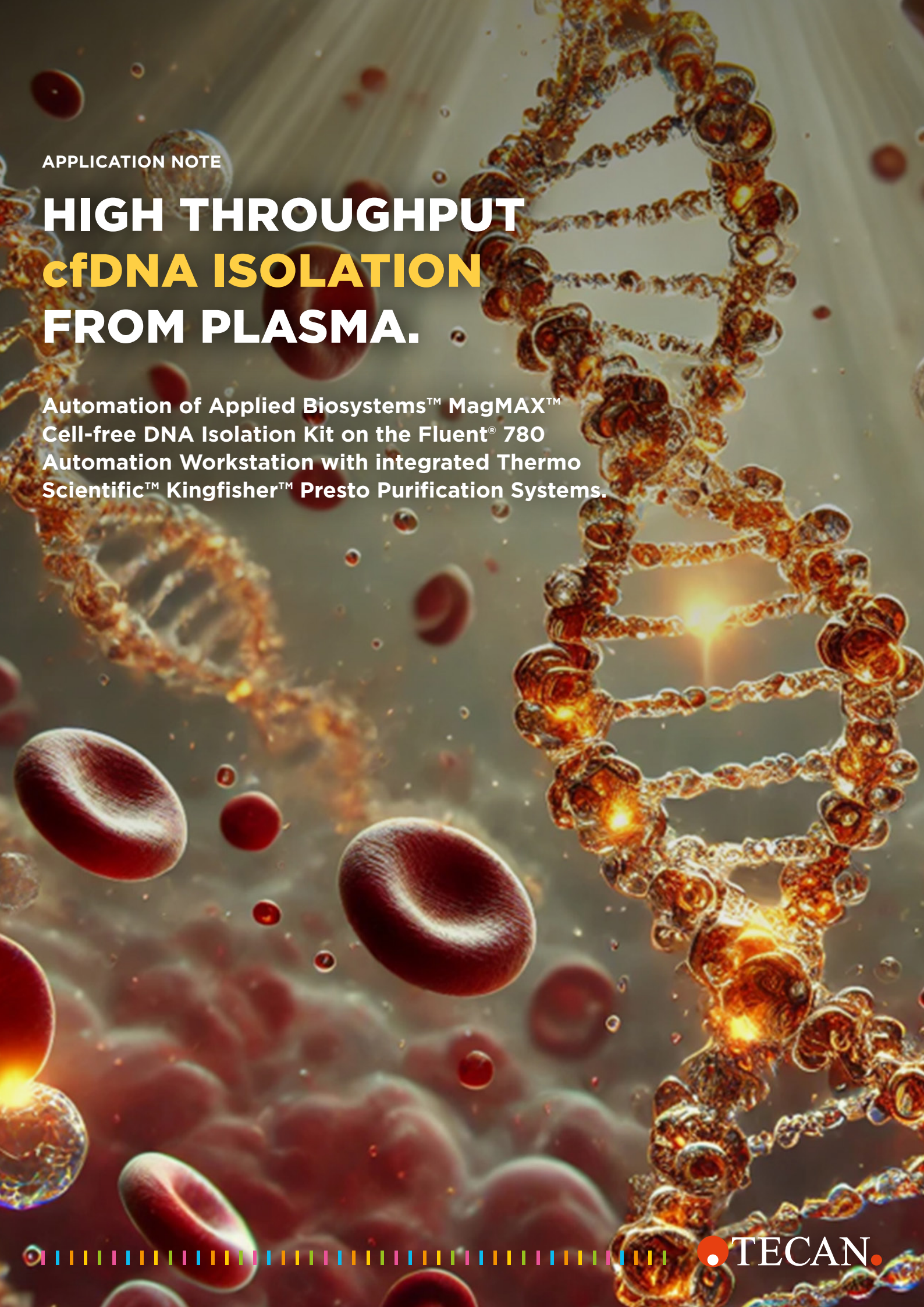


APPLICATION NOTE

# HIGH THROUGHPUT cfDNA ISOLATION FROM PLASMA.

**Automation of Applied Biosystems™ MagMAX™ Cell-free DNA Isolation Kit on the Fluent® 780 Automation Workstation with integrated Thermo Scientific™ Kingfisher™ Presto Purification Systems.**

TECAN

## INTRODUCTION.

Extraction of cell-free DNA (cfDNA) from biological samples has the potential to provide a non-invasive means of detecting and monitoring various disease states, making the isolation of cfDNA from biological samples – such as plasma, serum, and urine – important for genomics and clinical research. However, the low concentration and fragmented nature of cfDNA present significant challenges in its extraction and analysis, which usually involve the processing of larger sample volumes. Combining the Fluent Automation Workstation with the Thermo Scientific KingFisher Presto Purification System offers a powerful high throughput solution for cfDNA isolation, enhancing workflow efficiency, consistency and reliability for applications in biopharma, biotech, and research.

The Fluent platform provides advanced liquid handling and nucleic acid isolation capabilities within a single workflow. It is designed to handle complex protocols with precision and can process multiple samples simultaneously, offering flexibility in sample volumes and throughput. Equipped with MultiSense™ technology, the Air Flexible Channel Arm™ delivers advanced functionality for precise plasma and buffy coat separation using the proprietary Phase Separator™ technology. Its intuitive software and user-friendly interface simplify setup and operation, reducing the potential for human error and enhancing reproducibility.

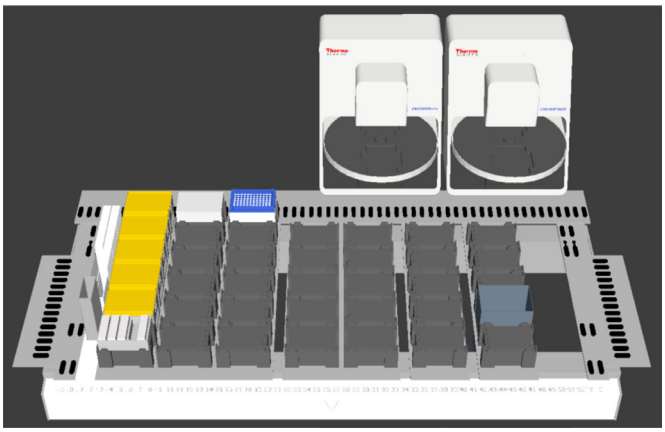
This platform can be seamlessly integrated with the KingFisher Presto purification system, which uses proven magnetic particle-based technology to deliver high-quality nucleic acids and proteins. For example, the Applied Biosystems MagMAX Cell-Free DNA Isolation Kit enables scalable isolation of cfDNA with input volumes ranging from 500 µl to 10 ml and supports a wide range of sample types – including plasma, serum, and urine – making it highly versatile for various laboratory needs. Together with the integrated KingFisher Presto system, the Fluent platform can process batches of 96 or 24 samples at a time, with sample volumes ranging from 50 to 5,000 µl. Additionally, it offers heating capabilities to support applications requiring elevated temperatures.

This scalability allows for the efficient isolation of cfDNA from both small and large sample volumes, ensuring reproducible recovery of high-quality DNA suitable for real-time PCR, digital PCR, and next-generation sequencing. These capabilities unlock valuable insights into oncology research and disease monitoring, paving the way for the future implementation of personalized and precise medical interventions.

## MATERIALS AND METHODS.

The automated cfDNA extraction workflow was carried out on a Fluent 780 workstation equipped with an Air Flexible Channel Arm™, a Robotic Gripper Arm™, a handheld barcode scanner (Honeywell) for reagent and sample barcode tracking, and dual KingFisher Presto systems for heating, shaking, magnetic separation, and isolation of cfDNA (Figure 1). User-friendly FluentControl® Gx Assurance software and intuitive touchscreen guidance via TouchTools™ visual commands ensured straightforward operation, reducing the need for extensive user training.

All experiments were conducted using the consumables listed below to process 96 samples of 4 ml plasma with the MagMAX Cell-Free DNA Isolation Kit.



| Consumable / Labware | Volume or Quantity | Reference |
|---|---|---|
| 1,000 µl filtered conductive disposable tips, SLAS format | ~ 96 tips | (Tecan, #30057817) |
| 200 µl filtered conductive disposable tips, SLAS format | ~ 40 tips | (Tecan, #30057815) |
| KingFisher 24 deepwell plates (for samples) | 8 Plates | (Thermo - 95040470) |
| KingFisher 24 deepwell plates | 20 Plates | (Thermo - 95040470) |
| KingFisher 24 deepwell tip comb and plate | 4 Plates | (Thermo-97002610) |

**Figure 1:** Fluent 780 workstation with dual Kingfisher Presto modules integrated onto the back of the instrument. The worktable accommodates all the consumables and storage positions necessary to process four plates in a walkaway manner.

**Sample preparation.**
4 ml aliquots of 3 individual plasma samples (A, B and C) were pipetted into a 24-well deep-well plate (DWP) in a checkerboard pattern (Table 1). Each Sample was processed in four replicates according to the layout in Table 1 on the Fluent using the Kingfisher Presto instruments, allowing four plates to be processed in parallel. A similar set-up using a standalone, benchtop KingFisher Flex sample purification system was used to compare full and semi-automated extraction workflows.

**Table 1:** Plate layout of Sample sample replicates. One plate was processed on the Kingfisher Flex (N=4), and four plates were processed in parallel using the integrated KingFisher Presto systems (N=16).

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Sample A (Rep 1/4) | | Sample B (Rep 1/4) | | Sample C (Rep 1/4) | |
| 2 | | Sample A (Rep 2/4) | | Sample B (Rep 2/4) | | Sample C (Rep 2/4) |
| 3 | Sample A (Rep 3/4) | | Sample B (Rep 3/4) | | Sample C (Rep 3/4) | |
| 4 | | Sample A (Rep 4/4) | | Sample B (Rep 4/4) | | Sample C (Rep 4/4) |

# RESULTS.

An Invitrogen™ Qubit™ fluorometer (Thermo Fisher Scientific) and Agilent Cell-Free DNA Tapes (ref. 5067-5630) on the TapeStation® System (Agilent) were used to measure average DNA concentrations in pg/µl after extraction. All samples processed with the fully automated workflow exhibited DNA concentrations exceeding 80 % of those obtained with semi-automated processing, representing an equivalent performance between the methodologies (Figure 2).

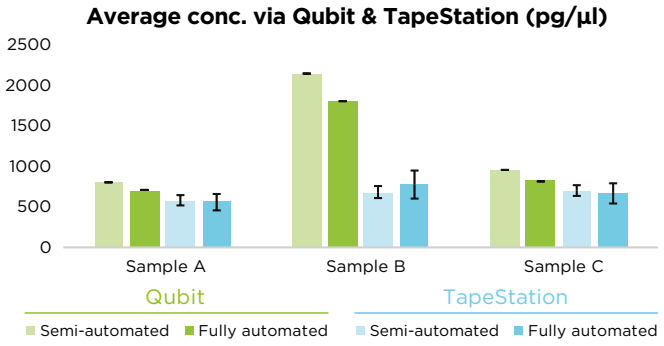



**Figure 2:** Average yields using fully automated (integrated KingFisher Presto purification instrument on Tecan Fluent liquid handler) and semi-automated (KingFisher Flex benchtop purification instrument).

Additionally, the analysis via TapeStation provided cfDNA fragment sizes between 50 and 700bp for both semi- and fully automated methodologies, corresponding to a normal cfDNA range of fragments (Figure 3).

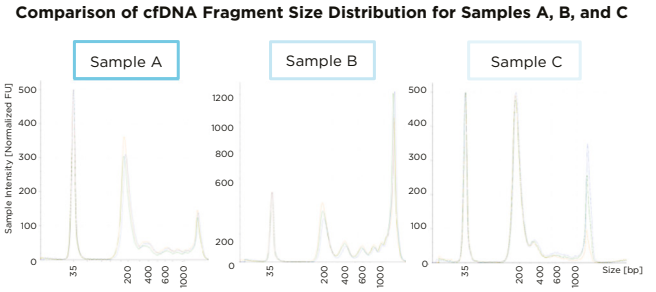



**Figure 3:** TapeStation Electropherogram showing one extraction replicate of sample A, B and C via KingFisher Flex instrument and on the Tecan Presto-Fluent.

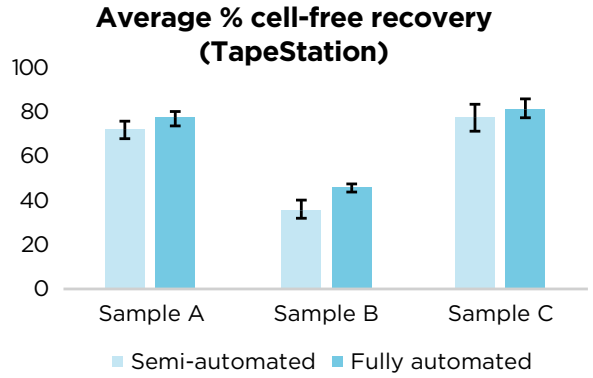



**Figure 4:** TapeStation results showing average % cell-free recovery for both semi- and fully automated approaches.

Further evaluation of DNA quality was performed using TaqMan™ chemistries targeting GAPDH-g1((GAPDH_g1 Assay [HS02758991_g1], Thermo Scientific), Thermo Scientific (TaqMan Universal Master Mixx II, No UNG [4440041], Thermo Scientific) to determine the Ct values (Figure 4). The average Ct values were slightly higher for the fully automated approach, with ΔCt values ranging from 0.220 to 0.437, indicating comparable DNA quality for both methodologies.

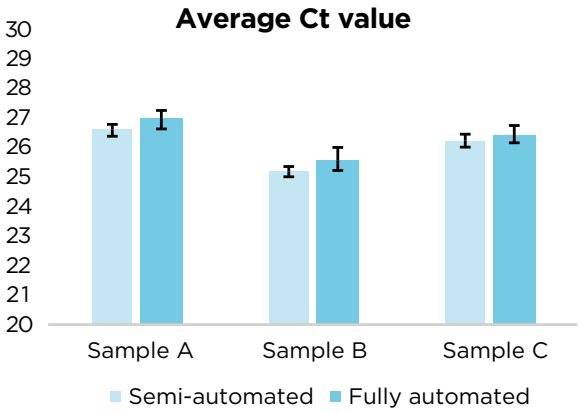



**Figure 5:** Average Ct values generated by GAPDH-g1 PCR analysis for both semi- and fully automated approaches.

## CONCLUSIONS.

The fully automated workflow integrating the Fluent workstation and KingFisher Presto instruments with the MagMAX cfDNA chemistry offers a robust solution for high throughput cfDNA isolation from plasma. This walkaway set-up provides excellent reproducibility and high cell-free DNA recovery for a wide range of sample volumes, yielding similarly high-quality DNA to semi-automated benchtop processing with the KingFisher Flex instrument. Full automation of the extraction process improves efficiency and ensures reliable and reproducible results, making it an ideal choice for laboratories seeking to enhance their cfDNA extraction workflows in support of a wide range of genomics applications.

## ABOUT THE AUTHORS.

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*Luis graduated with a B.S. Degree in Molecular Genetic Technology from The University of Texas M.D. Anderson Cancer Center. He started his career as a Medical Technologist specializing in nucleic acid extraction utilized for downstream genetic testing. In 2016, he transitioned into an engineering role by refining his skills in process design, script development and automation validation. Luis joined Thermo Fisher Scientific in 2021 as an Automation Scientist, working on the system design and development of ultra-high throughput COVID-19 automation. Since 2022, Luis has been working as a Staff Engineer focusing on script development of various nucleic acid extraction kits on automated liquid handlers.*

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*Tiffany May is a R&D Scientist III with Thermo Fisher Scientific. She has over a decade in experience in DNA extraction technology, with extensive experience in high-throughput automation and agribusiness. Her current focuses within Thermo Fisher Scientific are automation and oncology, developing innovative, hands-free solutions to cell-free extraction technologies and workflows.*

***Lokesh Kothandaramaswamy Raguraj***
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*Thermo Fisher Scientific*
*Austin, Texas USA*

*In 2021, Lokesh joined the Sample Prep Workflow and Automation group at Thermo Fisher Scientific. His focus is on developing innovative genomics and oncology sample prep solutions, including MagMAX Nucleic Acid purification kits and automated workflows for KingFisher instruments and liquid handlers. Lokesh brings over 13 years of experience in Clinical Molecular Testing, assay development and holds a master's degree in biotechnology from VIT University in India.*

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403191 V1.0, 2025-01

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