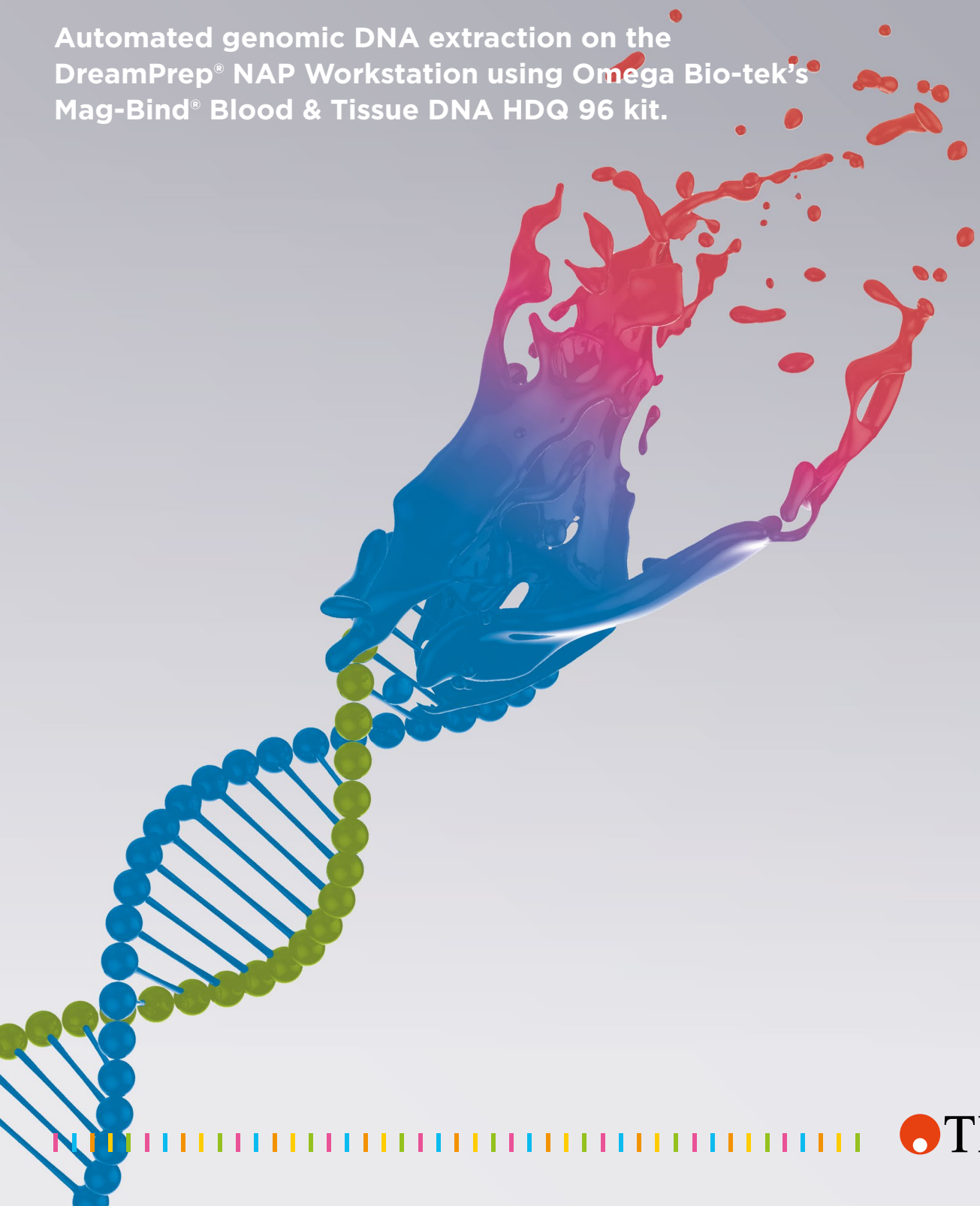
APPLICATION NOTE

STREAMLINED AUTOMATED GENOMIC DNA EXTRACTION FROM WHOLE BLOOD SAMPLES.

Automated genomic DNA extraction on the DreamPrep® NAP Workstation using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ 96 kit.



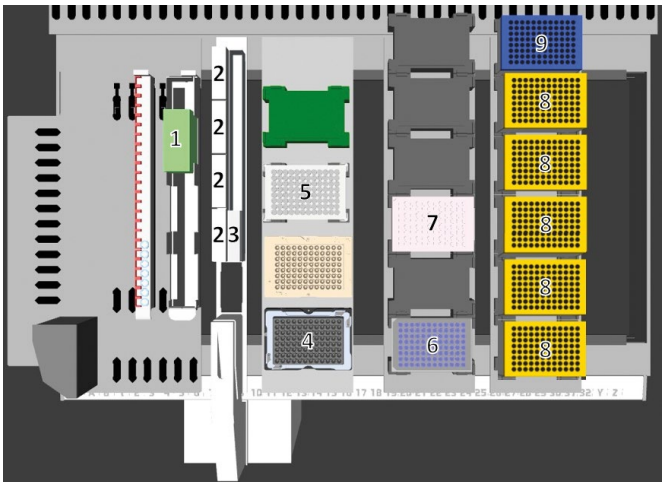
TECAN.

INTRODUCTION.

Blood serves as a valuable biospecimen for acquiring genomic DNA in a wide range of genomic-based downstream applications, spanning both clinical and medical research contexts. To meet the demands of most downstream applications, it is crucial to extract high-quality, high-yield DNA in a high throughput manner using methods that are both reliable and reproducible. Integrating the Mag-Bind Blood & Tissue DNA HDQ 96 Kit (RUO) from Omega-Bio-tek on the Tecan DreamPrep NAP Automation Workstation introduces an efficient solution for extracting DNA from 250 µl blood samples, significantly reducing the need for manual intervention and streamlining the high throughput process. In this application note, we present the automated solution along with validation studies to demonstrate the performance of the system. The automated workflow's results were compared to those obtained with the manual protocol, evaluating the yield, purity, and integrity of the extracted DNA. Ensuring elimination of cross-contamination is paramount for the implementation of any automated workflow. Hence, the automated workflow was further assessed for potential instrument-based cross-contamination across different wells of the plate. Our results indicate that this automated workflow can extract high-quality, high molecular weight DNA 96 samples of 250 µl whole blood each arrayed in a 96-well format in 90 minutes.

MATERIALS AND METHODS.

The Mag-Bind Blood & Tissue DNA HDQ 96 Kit (M6399) is designed for the isolation of high-quality genomic DNA from various sample types utilizing magnetic bead-based extraction techniques. This workflow was automated on the DreamPrep NAP workstation to extract and purify genomic DNA (gDNA) from 1-96 250 µl aliquots of human whole blood. The DreamPrep NAP platform is based on the Fluent® 480 Automation Workstation and has been specifically configured for the automation of magnetic bead-based nucleic acid purification workflows. The system includes tools such as an eight-channel Air Flexible Channel Arm™ (Air FCA), a Robotic Gripper Arm™ (RGA), a BioShake™ D30-T elm for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua), which allows for efficient magnetic separation in DNA and RNA isolation procedures. Additionally, a user-friendly experience is guaranteed by integrating Fluent Control® software and the intuitive touchscreen-guided operation with visual TouchTools™ commands, reducing the need for extensive training. For efficient and reliable sample processing and tracking, it includes FluentControl™ GX Assurance Software. Furthermore, all experiments were conducted using the necessary consumables listed below to process a batch of 96 samples (deck layout shown in Figure 1).



1. 320 ml trough for liquid waste
2. 100 ml reagent troughs
3. 25 ml reagent troughs
4. Qinstruments BioShake D30-T elm
5. Alpaqua Magnum FLX Magnet Plate Magnet
6. Nunc™ 96 Deep-well 2.0 ml Sample Plate
7. Standard 96-well PCR microplate (Elution Plate)
8. 1000 µl filtered conductive disposable tips SLAS format
9. 200 µl filtered conductive disposable tips SLAS format

Figure 1: Tecan DreamPrep NAP deck layout for extraction of DNA from blood samples with an input volume of 250 µl per well.

Three 250 µl aliquots were sampled from four different lots of human whole blood and transferred to a 96-well deep well plate on the Tecan DreamPrep NAP workstation for DNA extraction. The lab automation platform was programmed to perform the various liquid handling and magnetic bead-based tasks as required by the Mag-Bind Blood & Tissue DNA HDQ 96 protocol for the extraction of genomic DNA. All consumables and carriers were placed onto the work deck configured as shown in Figure 1 and the gDNA was eluted in 100 µl volume. The extraction workflow was fully automated starting with the sample aliquot in the 96-well plate to the final eluted product. Manual extraction from each sample lot of human blood was performed in parallel and compared to validate the automated purification method.

The purified DNA was quantified using Thermo Scientific's NanoDrop™ 2000c system. Absorbance measurements at the wavelengths of 230, 260, and 280 nm were used to assess the quality of the purified DNA and detect the presence of RNA/protein contamination or salt carryover. The suitability of the

extracted DNA for downstream applications were examined by performing real-time PCR using human-specific primers on 10-fold and 100-fold dilutions of the purified DNA.

The automated workflow was further assessed by carrying out cross-contamination studies. Sample wells containing 250 µl water spiked with ~1 µg of canola plant DNA was alternated with blank wells containing water in a checkerboard pattern. Extraction was carried out using the developed protocol and real-time PCR was carried out on the eluate using canola-specific primers across all the wells of the 96-well plate to check for cross-contamination.

RESULTS AND DISCUSSION.

Comparable DNA Yield – Manual vs Automated.

The concentrations of extracted DNA from manual extraction was found to be comparable to the average DNA yield obtained following the automated protocol across 4 different lots of blood tested. The different average DNA yields ranged from 6-20 µg irrespective of the methodology used. Overall, these results validate the instrument set-up and automated purification protocol (Figure 2).

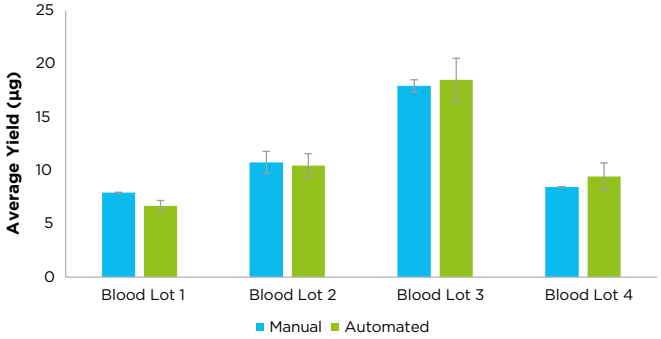


Figure 2: DNA was extracted from 250 µl of blood samples and eluted in 100 µl volume. The DNA yield was determined using Thermo Scientific's NanoDrop 2000c. The average DNA yields from manual and automated extractions were found to be comparable.

Similar DNA Purity Across Manual and Automated Protocols.

DNA purity and quality were analyzed using the A260/A280 and A260/A230 ratios obtained after spectrophotometric analysis (Figure 3). For both manual and automated protocols, the absorbance ratio of A260/A280 was between 1.84-1.91, indicating pure DNA free of contaminating RNA and proteins (Figure 3). The average A260/A230 ratios for the automation protocol were between 1.9-2.3, indicating low salt contamination carryover (Figure 3).

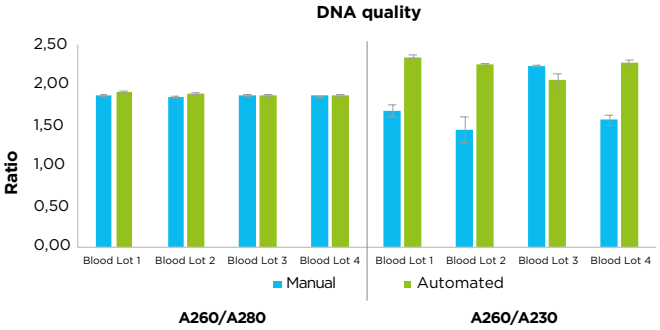


Figure 3: The purity of DNA isolated using manual and automated protocols was analyzed through spectrophotometry focusing on A260/A280 and A260/A230 ratios.

Average Ct Values from 10X and 100X Diluted DNA are Comparable Across Automated and Manual Protocols.

Real-time PCR was performed on blood lots 2, 3 and 4 following manual and automated extraction with the DreamPrep NAP. The average Ct values obtained from diluting the purified DNA 10-fold and 100-fold are shown in Figure 4. The Ct values across all dilutions indicate positive amplification and were comparable irrespective of the extraction methodology implemented. ΔCt values obtained from the 10- and 100-fold dilutions were 3.27 and 3.15 for manual and automated protocols, respectively. These values are close to the theoretical 3.3, suggesting inhibition-free DNA extractions using both the automated and manual protocols with Omega Bio-tek's kit and endorsing their suitability for downstream applications.

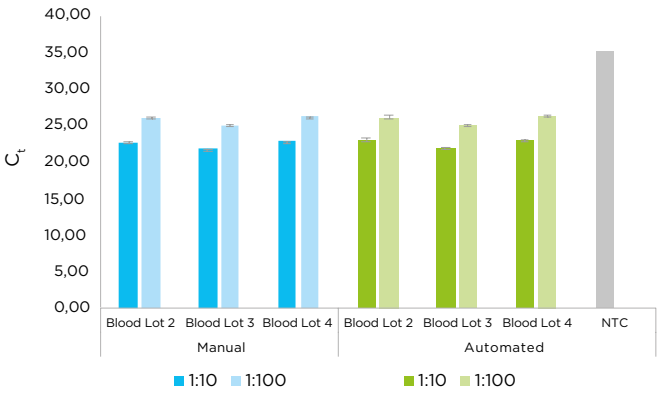


Figure 4: Average Ct values obtained from amplifying the purified DNA following the automated and manual protocols using Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ 96 Kit. A No Template Control (NTC) was added as control to the different blood lot amplifications and to monitor for potential contamination.

TapeStation Analysis of DNA Purified on Tecan DreamPrep NAP.

Additionally, the purified DNA using the automated protocol was analyzed with a TapeStation assay to derive information about the size and integrity of the genomic DNA extracted. DNA Integrity Number (DIN) was determined by the TapeStation 2200 analysis software and typically DNA with a DIN of 10 is considered intact and of the highest integrity. Two samples from four different blood lots were analyzed on TapeStation (Figure 5). Purified DNA following automated protocol is of high molecular weight and migrated as a well-defined band above the largest ladder peak (48,500 bp) with the software analyzing it to be > 60 kb with DIN values >8.4.

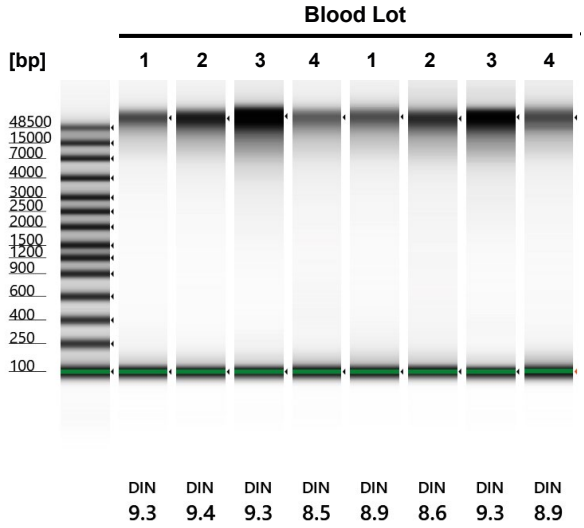


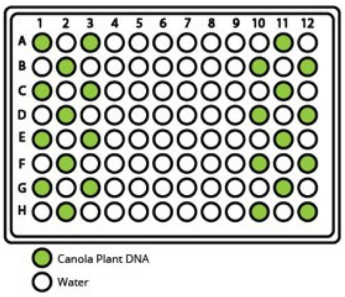
Figure 5: TapeStation Analysis performed on DNA extracted from blood samples using aan automated protocol on the Tecan DreamPrep NAP.

Cross Contamination Study Across 96-well Plate following Automated Protocol.

The layout for the cross-contamination study and subsequent results qPCR results in a 96-well plate format are as shown in Figure 6. Eluates in each well were amplified using qPCR specific for canola DNA to identify any well-to-well contamination across the 96-well plate. The Ct values exhibited in Figure 6 show that there is no cross contamination of purified DNA into wells containing water when using the automated Mag-Bind Blood & Tissue DNA HDQ 96 Kit.

CONCLUSIONS.

By leveraging the automated capabilities of the Tecan DreamPrep NAP workstation and the efficiency of the Mag-Bind Blood & Tissue DNA HDQ 96 Kit, researchers can significantly enhance their DNA extraction workflows. Integration of these two technologies offers a highly reliable and automated high-throughput purification method for genomic DNA (gDNA) extraction from whole blood samples. The automated protocol consistently produces high-quality DNA, comparable to the results obtained using the manual protocol. This workflow enables the processing of a full 96-well plate containing 250 µl blood samples in just 90 minutes in a full walk away manner. Moreover, automation of the Mag-Bind Blood & Tissue DNA HDQ 96 Kit has been proven to eliminate any concerns of well-to-well cross contamination. The high yield and high quality of the purified DNA substantiate its use in various downstream applications, including real-time PCR and next generation sequencing.



	1	2	3	4	5	6	7	8	9	10	11	12
A	20.26	U	20.35	U	U	U	U	U	U	U	20.21	U
B	U	20.28	U	U	U	U	U	U	U	20.20	U	20.29
C	20.23	U	20.24	U	U	U	U	U	U	U	20.27	U
D	U	20.29	U	U	U	U	U	U	U	20.14	U	20.18
E	20.59	U	20.17	U	U	U	U	U	U	U	20.22	U
F	U	20.20	U	U	U	U	U	U	U	20.08	U	20.18
G	20.19	U	20.20	U	U	U	U	U	U	U	20.27	U
H	U	20.44	U	U	U	U	U	U	U	20.25	U	20.00

Figure 6: 96-well plate map showing the locations of samples and subsequent PCR amplification. The Ct values are only detectable in wells containing samples, illustrating no cross-contamination from Omega Bio-tek's automated protocol. (U: Undetermined)

ORDERING INFORMATION.

Product No.	Description
M6399-00	Mag-Bind® Blood & Tissue DNA HDQ 96 kit (1 × 96 preps)
M6399-01	Mag-Bind® Blood & Tissue DNA HDQ 96 kit (4 × 96 preps)
M6399-02	Mag-Bind® Blood & Tissue DNA HDQ 96 kit (24 × 96 preps)

About the authors.

Kiranmai Durvasula (PhD) is a Product Manager at Omega Bio-tek, Inc., leading the efforts to develop and deliver novel nucleic acid extraction products supporting the fields of basic and translation research, diagnostics, pharmacogenomics, etc. Kiranmai obtained her PhD in Chemical Engineering from University of Florida, Gainesville, and received postdoctoral training in cell-based therapies for treatment of insulin-dependent diabetes in Dr Athanassios Sambanis's laboratory at Georgia Institute of Technology. She has authored several peer-reviewed journal papers and is passionate about translating fundamental research platforms into practical technologies.

Sara Amirahmadi is a geneticist by training, she holds a BS in Genetics and an MS in Immunology & Bacteriology. Her skills in genetic analysis and laboratory methodologies were honed during her five-year tenure at Omega Bio-tek. Over the past three years, she has been working as a field application scientist, where her focus has been on automation and streamlining processes to achieve innovative outcomes. Beyond the lab, she finds balance in yoga and the world of books, nurturing both her body and mind.

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omega BIO-TEK

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