ZYMO RESEARCH

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Automating High Throughput Genomic DNA Extractions from Any Sample Type

ABSTRACT

The increased popularity of large-scale genomic testing has prompted the need for a high throughput DNA extraction solution for various samples types. To accommodate for this, the *Quick*-DNA™ Magbead Plus kit was implemented on the Tecan DreamPrep NAP as a collaborative effort between Zymo Research and Tecan. This workflow used human blood, saliva, tissue, and cultured cells to assess the yield, quality, and purity of the DNA and cross contamination of samples were tested between each well of the processing plate. All sample types processed using the *Quick*-DNA™ MagBead Plus workflow had consistent and reproducible DNA recovery and purities that were comparable to manual extractions. The implementation of the *Quick*-DNA™ MagBead Plus Kit on the DreamPrep NAP provides a completely walk away DNA extraction solution from a variety of sample types.

INTRODUCTION

The rise in large-scale genomic testing in institutions such as hospitals, core facilities and molecular diagnostics laboratories highlights the need for updated DNA extraction methods to accommodate the higher throughput demand. The main challenge is to ensurehigh yields of good quality and purity genomic DNA (gDNA) from a wide variety of sample types – such as cultured cells, solid tissues, whole blood, saliva and swabs – without sacrificing performance.

The *Quick*-DNA™ MagBead Plus Kit uses a single, universal extraction protocol based on proteinase K digestion to accommodate all sample input types. Its innovative wash system significantly reduces protein and salt contamination, providing a streamlined wash and elution workflow that is easily customizable, while retaining excellent consistency. The resulting high quality, high molecular weight DNA can be funneled directly into complex, sensitive downstream applications including PCR, microarray and next generation sequencing (NGS).

Tecan and Zymo Research have collaborated to establish an automated workflow for the *Quick*-DNA™ Magbead Plus Kit. This protocol has been tested using blood, saliva, tissue and cultured cells to assess the yield, quality and purity of the gDNA, as well as to check for cross-contamination between wells of the processing plate.

This application note describes the implementation of the *Quick*-DNA™ Magbead Plus Kit on the DreamPrep NAP featuring Zymo Research workstation, providing a complete, walkaway DNA extraction solution for a variety of sample types.

MATERIAL AND METHODS

The workflow has been automated on the DreamPrep NAP featuring Zymo Research workstation, a system based on the

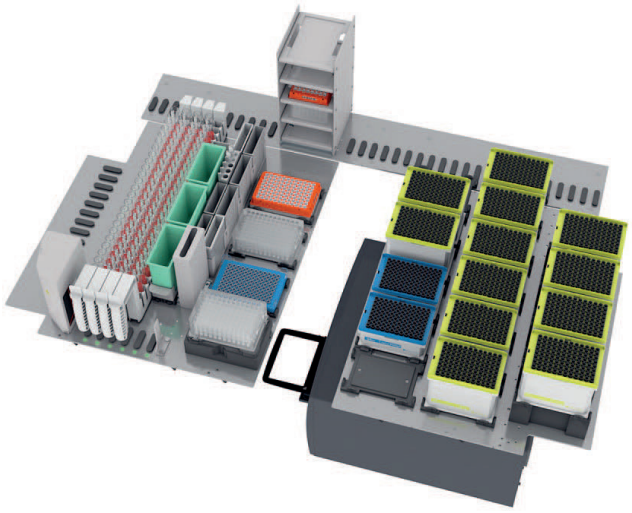

Figure 1: Configuration of the DreamPrep NAP featuring Zymo Research worktable.

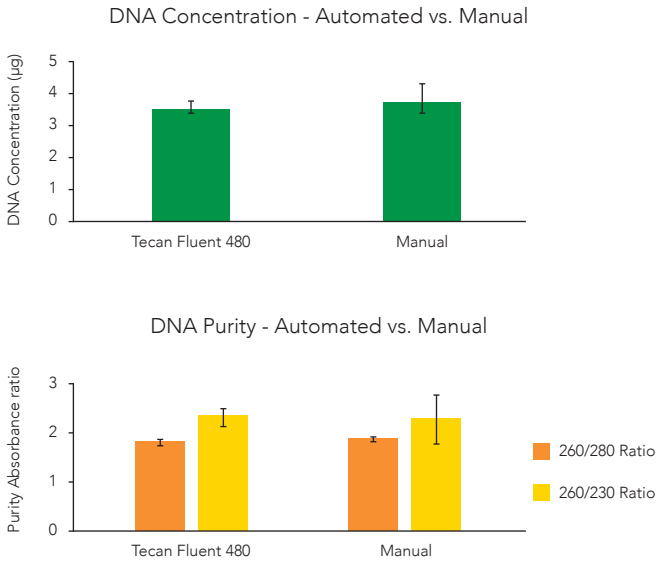

Figure 2: Manual Benchmarking. Average (± standard deviation) DNA extracted from 200 µl human blood using the *Quick*-DNA™ MagBead Plus system scripted on the DreamPrep NAP and manually (n = 8). DNA recovery and purity absorbance ratios from the automated and manual extractions were similar (A260/230; A260/280: >1.8). Absorbance A260/230, and total DNA recovery (µg) were quantified by NanoDrop™ 2000.

Fluent® 480 Automation Workstation. The system is configured for nucleic acid extraction workflows using magnetic bead-based procedures, in combination with Fluent GX Assurance Software. An integrated Infinite® 200 reader in M Nano+ configuration allows quantification and normalization following nucleic acid extraction.

The system is equipped with an Air Flexible Channel Arm™, a Robotic Gripper Arm, FluentID™ and handheld barcode scanners for sample and reagent identification, a BioShake D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX® Enhanced Magnet Plate (Alpaqua).

The automated workflow was tested using the following general protocol. Whole blood samples were added to a 2 ml Nunc™ 96-Well DeepWell™ Plate (Cat. No. 278743)and processed using the automated *Quick*-DNA™ Magbead Plus Kit workflow. DNA was eluted in 50 µl of DNA Elution Buffer and concentration and purity absorbance ratios (A260/230; A260/280) were measured using a NanoDrop™ 2000 UV-Vis Spectrophotometer (ThermoFisher Scientific). The resultant DNA was tested via qPCR, using the Femto™ Bacterial DNA Quantification Kit on the CFX96™ Real-Time PCR Detection System (BioRad Laboratories).

RESULTS

Comparison of automated vs manual workflow

Human blood samples (200 µl) processed with the automated workflow were compared to manually extracted samples to evaluate binding and wash efficiency (n = 8). Averages and standard deviations were calculated to compare consistency between each

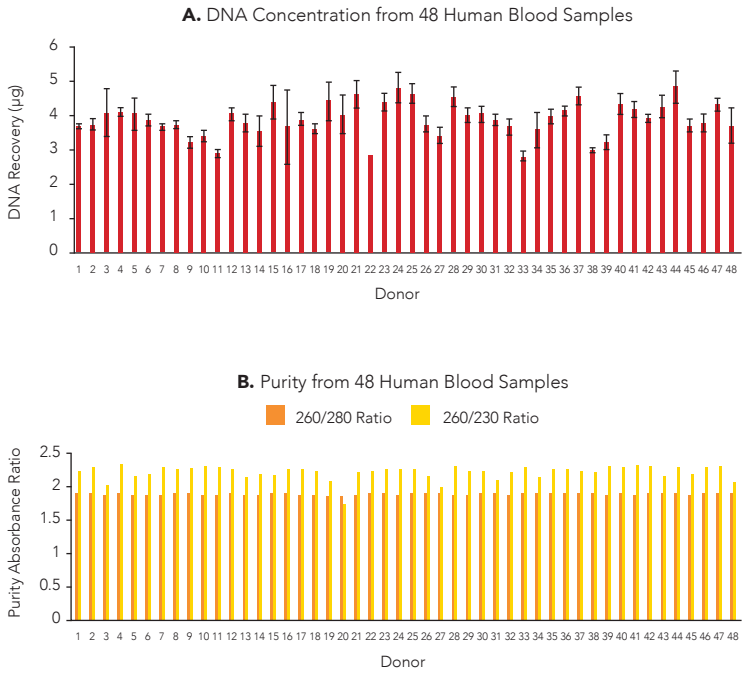

Figure 3: Consistent Yield and Purity. DNA from 200 µl human blood were extracted from 48 individual blood donors (n = 4). Samples were input into the *Quick*-DNA™ MagBead Plus system scripted on the DreamPrep NAP. (A) Total DNA recovery across the 48 donors were at similar amounts. (B) Purity absorbance ratios were consistently high (A260/230; A260/280: >1.8). Absorbance A260/230, and total DNA recovery (µg) were quantified by NanoDrop™ 2000.

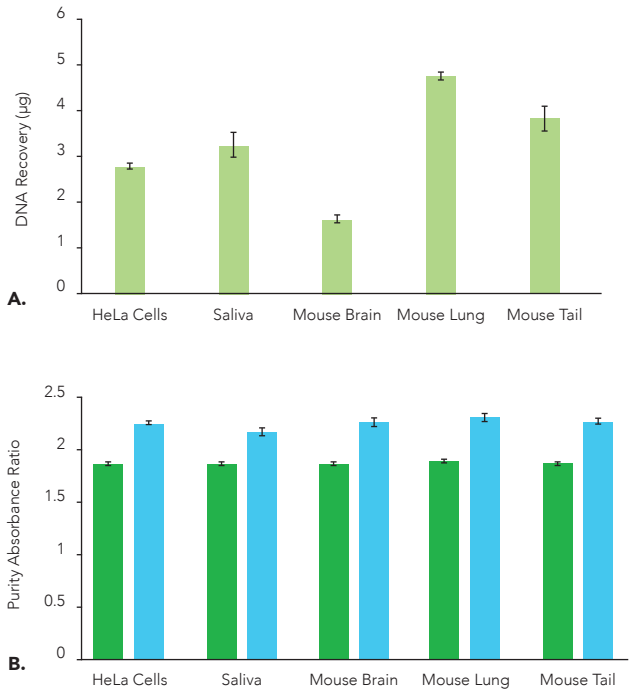

Figure 4: Any Sample Type. DNA from 1 million HeLa cells, 200 µl human saliva, and 15 mg mouse brain, lung, and tail were extracted using the *Quick*-DNA™ MagBead Plus system scripted on the DreamPrep NAP (n=8). (A) Total DNA recovery and (B) purity absorbance ratios were consistently high (A260/230; A260/280: >1.8). Absorbance A260/230, and total DNA recovery (µg) were quantified by NanoDrop™ 2000.

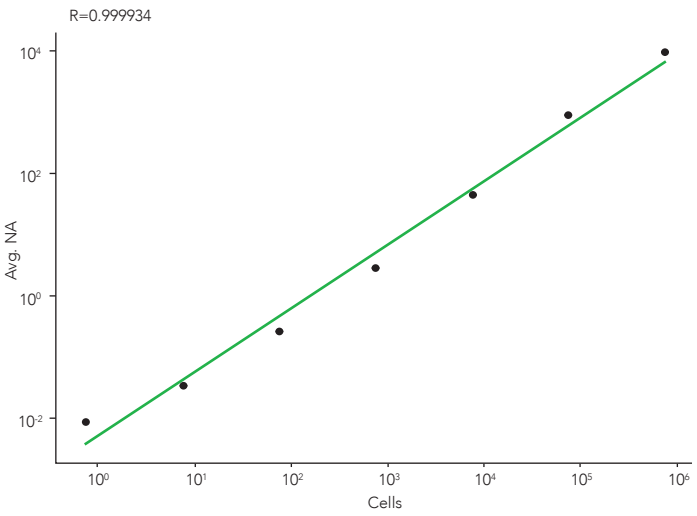

Figure 5: Low Biomass. Plot showing linear recovery of DNA extracted from HeLa cells ranging from 1 million cells to 1 theoretical cell via serial dilution (R^2 = 0.9899). Samples were amplified by qPCR using the Zymo Research Femto™ DNA Quantification kit on the CFX96™ Real-Time System (BioRad Laboratories) along with a standard curve. This indicates that the system can accurately detect samples with as little as 1 cell for the sample input.

method (Figure 2). DNA recovery and purity absorbance ratios from the automated and manual extractions were similar (A260/230/A260/280 = >1.8).

Consistency of extraction workflow

Frozen human blood samples (200 µl) from 48 individual donors were extracted to evaluate the robustness and reproducibility of the workflow, demonstrating similar DNA recovery rates across all donors (Figure 3). The extraction and quantification process was repeated for quality control (n = 4), with consistently high purity (A260/230/A260/280 = >1.8).

Extracting DNA from different sample types

To evaluate the flexibility of the workflow, a variety of samples types were tested, including 1m HeLa cells, 200 µl of fresh human saliva, and 15 mg of mouse brain, lung and tail (n = 8) (Figure 4). Both, total DNA recovery and purity, were consistently high for all sample types (A260/230/A260/280 = >1.8).

Cross-contamination

Ensuring that cross-contamination does not occur is paramount for any automated workflow. Cross-contamination was assessed by adding *Cryptococcus neoformans* and DNase/RNase-free water to every other well across a 96-well plate in a checkerboard pattern. Eluates from each well were amplified and analyzed to check for C. neoformans DNA in the wells filled with water, demonstrating that no cross-contamination occurred (Figure 6).

Low biomass sample performance

A dilution series was created by 1:10 serial dilution of 1million HeLa cells six times to achieve a range from 1million cells to 1 theoretical single cell. The diluted samples were processed alongside standards of known concentrations, which were used to generate a standard curve. The extracted samples were quantified and plotted to assess the linearity of DNA recovery (Figure 5). This indicated that the system could accurately detect samples with as little as a single cell input with good linearity ($R^2 = 0.9899$).

SUMMARY

The automated workflow for the *Quick*-DNA™ MagBead Plus Kit on the DreamPrep NAP featuring Zymo Research workstation provides a complete, walkaway DNA extraction solution from a variety of sample types, such as blood, saliva, tissue and cultured cells. It offers consistent and reproducible DNA recovery, with purities comparable to manual extraction.

The results presented here demonstrate that the workflow eliminates cross-contamination, generating ultra-pure DNA that can be used for any downstream application, including qPCR and NGS sequencing.

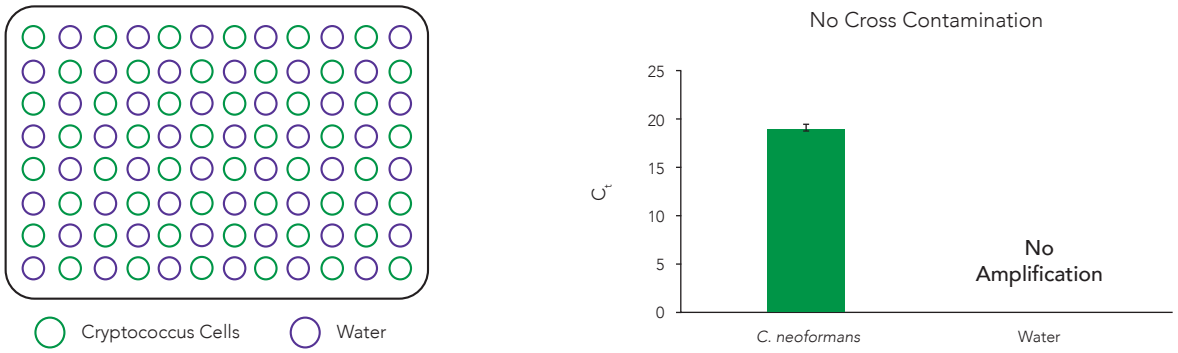

Figure 6: No Cross Contamination. *Cryptococcus neoformans* and DNase/RNase free water was added to every other well across a 96 well plate in a checkerboard pattern. All 96 wells were extracted using the *Quick*-DNA™ MagBead Plus system scripted on the DreamPrep NAP. Eluates from each well were amplified via qPCR (BioRad Laboratories) using the Zymo Research Femto DNA Quantification kit. Wells containing both *C. neoformans* and water were analyzed to check for cross contamination of *C. neoformans* into the wells filled with water.

For Research Use Only. Not for use in diagnostic procedures.

Acknowledgments

This protocol was developed by in collaboration with Tecan (www.tecan.com) and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application. For further information, visit www.zymoresearch.com.