Clinical Quality DNA Extraction at Scale: Engineering a Universal Method for Different Sample Types

Authors: Abdimalik Khalif*, Hoai Nguyen*, Jeff Roeder** and Justin Lock*
Affiliations: *Color Genomics, Inc., **Omega Bio-tek, Inc.

Abstract

Next-generation sequencing (NGS) is a powerful and precise tool that is revolutionizing healthcare and driving the development of personalized genomic health products. Most NGS based clinical approaches require stringently purified and high molecular weight DNA as the starting input material for optimal assay performance. Therefore, the implementation of NGS into healthcare systems is challenged by the need to obtain high-quality starting material from a variety of specimen types in a cost-efficient and high-throughput manner. Here, we describe a procedure developed at Color Genomics, a leader in high-quality and affordable genomic healthcare, to extract high-quality genomic DNA (gDNA) from peripheral blood and saliva samples, simultaneously, on the Hamilton Microlab® STAR™ platform using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ magnetic bead-based chemistry. This approach also enables the simultaneous extraction of gDNA from a variety of collection devices, including BD Vacutainer®, PAXgene® Blood DNA Tubes, or Oragene·Dx® saliva collection tubes (various tube sizes, tube shapes and anticoagulants) without compromising gDNA quality, and therefore without increasing lab or labor costs while maintaining throughput.

Introduction

The type of specimen collected from patients differs greatly based on the patient's geographic location (proximity to a healthcare center, state, country, etc.) and the test type requested. For example, large hospital networks often have phlebotomists on staff to collect blood specimens in a tube format most commonly used by that network. In contrast, patients in remote locations are best served by at-home collected samples obtained via the FDA-cleared Oragene·Dx saliva collection device. Hence, Color Genomics is faced with the challenge of extracting high-quality gDNA from collection devices that vary in specimen type (peripheral blood or saliva), tube size (3 mL – 10 mL), anticoagulant (EDTA, Heparin, NaCl, etc.) and tube shape (round or raised bottom). Performing separate automation procedures and extraction chemistries for each specimen type would add significant complexity and cost to the extraction procedure, and also delay patient's care quality. For example, patient samples collected in rarely received specimen types would need to be extracted in respective procedures, thus requiring batching of samples to gather sufficient samples to fill an extraction batch, impacting turn-around time, and leaving patients without vital health information for long periods of time.

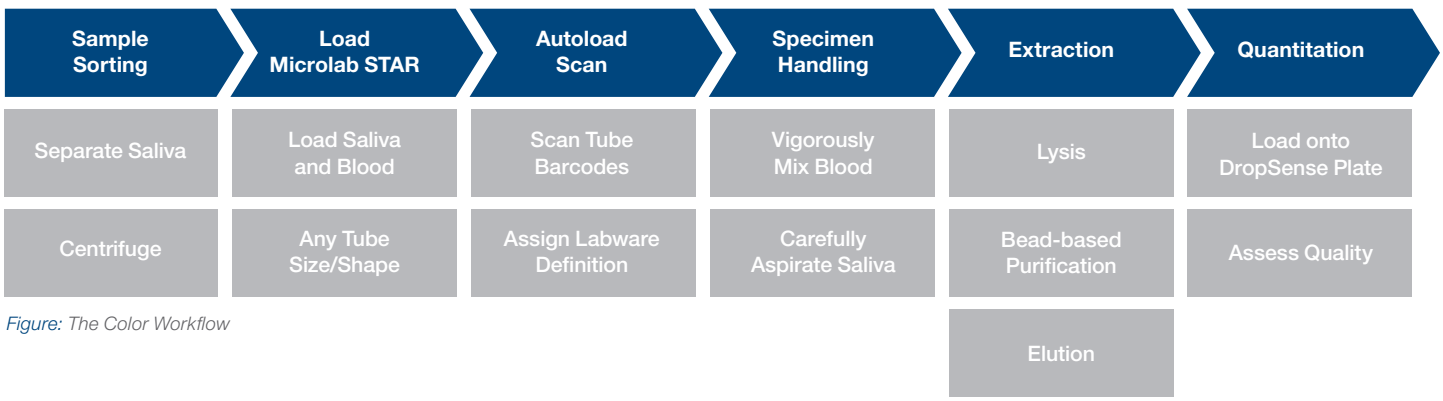


Figure: The Color Workflow

To overcome these challenges, Color Genomics has developed a universal DNA extraction procedure to extract high-quality gDNA from various specimen types, in parallel, using a fully automated and walk-away protocol. The method has two distinct features that allow parallel processing.

- **The Hamilton Autoload is used to scan the barcodes of specimen tubes, and the scanned barcode is used to query a pre-generated and continuously updated database of known barcodes to identify the collection device type. Based on the collection device type identified, z-axis parameters are assigned to the collection device via on-the-fly generated consumable specific labware sequences.**
- **The DNA extraction chemistry was optimized to accept lysed and unlysed samples (such as Oragene Dx collected saliva or peripheral blood, respectively) and a variety of anti-coagulants. This flexibility was incorporated by optimization of the Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ magnetic bead-based DNA extraction chemistry.**

Combined, these two features allow users to randomly place sample collection devices at any "input" carrier location and have the STAR instrument automatically adjust to accommodate aspiration volumes and mixing parameters required by the specific collection device's processing procedure. Finally, a Trinean DropSense96™ instrument was integrated into the STAR platform environment to automate gDNA quality control after extraction, thus creating a completely walk-away extraction process.

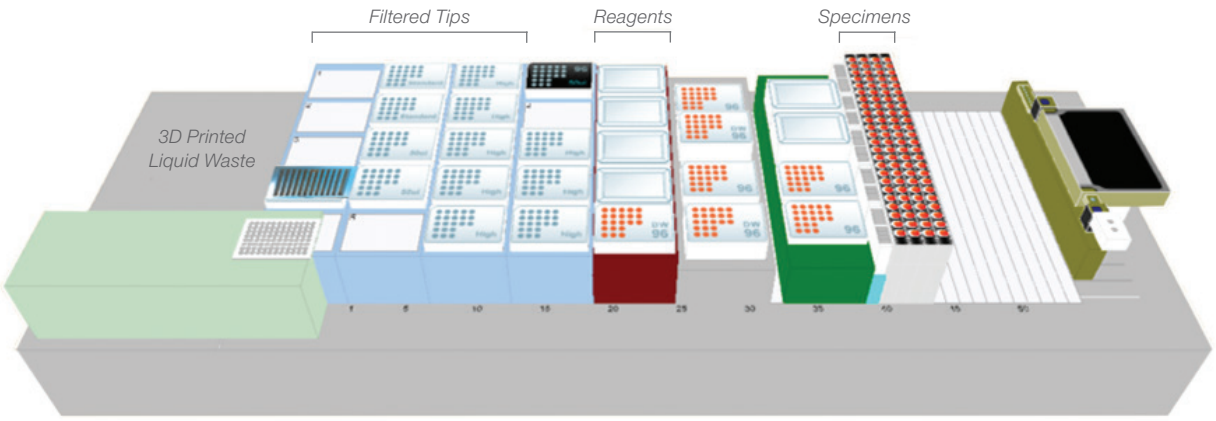
Materials Overview

Equipment	Quantity	Part Number
Microlab STAR Liquid Handling Workstation equipped with Autoload, 8 Independent Channels, CO-RE® 96-Probe Head, and iSWAP® (gripper option for labware transportation)	1	N/A
Deep-Well Plate Carrier (5 SBS Positions)	1	182090
Tip Carrier (5X96 Tips)	4	182085
Multiflex Carrier Base	1	188039
Multiflex Tip Module	1	188040
Multiflex Deep-Well Plate Module	1	188042
Magnum FLX magnet plate	1	OTP-ALPQ-0008
Hamilton Heater Shaker (HHS)	3	199034
3D printed vacutainer adapters	1	N/A
3D printed liquid waste disposal	1	N/A

Reagents: Omega Bio-tek Mag-Bind Blood & Tissue DNA HDQ 96 Kit

Deck Layout

The deck is loaded with tip carriers for 50 µL, 300 µL, and 1000 µL filtered tips. The negative position is extended with an aluminum bracket for placement of a Trinean DropSense96 instrument. The Mag-Bind beads are loaded into a 50 mL reservoir to facilitate vigorous mixing using the 8 channels loaded with 1 mL tips prior to this reagent to sample wells. All other extraction reagents are loaded into open reservoirs with lids to prevent contamination and evaporation. All blood vacutainer tubes and Oragene·Dx saliva tubes are loaded onto the Microlab STAR sample carriers in any order.



Method Overview

The linear barcode label attached to input samples is scanned by the Autoload during deck setup. The successfully scanned barcodes are added to an array and, based on the barcode, the sample type is identified and the appropriate labware parameters are established (unsuccessfully scanned barcodes are either manually scanned by the user if possible, or excluded from further processing if the barcode is not readable). Pre-extraction, device specific parameters include vigorous pipette mixing of peripheral blood samples before transfer to the lysis plate. Saliva samples are not mixed at all, preventing the resuspension of pelleted contaminants (food or bacterial particles) common in this sample type.

An aliquot from each sample tube is transferred to a mixture of lysis buffer and proteinase K that has been pre-dispensed into high volume 96-deepwell plates (2 mL). Sample and lysis solution are incubated at 65˚C for 10 minutes on the Hamilton Heater Shaker with vigorous mixing every 60 seconds. Tip mixing is performed at the bottom of sample wells to prevent formation of bubbles and to ensure settled cells are resuspended during the lysis. After lysis is complete, the lysate plates are removed from the heated position and the magnetic particles and binding solution are added to immobilize free nucleic acids. The immobilized DNA is purified with two wash steps: one with a high-salt wash buffer and an additional wash with a low-salt ethanol-based wash buffer. Residual wash buffer and ethanol is removed by dispensing and then immediately aspirating 100 µL of room temperature water into sample wells while the plate is on the Alpaqua® Magnum FLX® Enhanced Universal Magnet Plate. The water wash eliminates the need to dry the Mag-Bind beads before the final elution step, greatly improving the final DNA yield and ensure samples are free of residual reagent contaminants that might negatively impact downstream assay performance. Finally, DNA is eluted from the washed Mag-Bind beads in heated (60 °C) elution buffer (60 µL of 10 mM Tris-HCl). The elution reaction is mixed vigorously by shaking on the Hamilton Heater Shaker (pipette mixing can cause the formation of bubbles and impair elution) before transfer to an Magnum FLX magnet to collect the magnetic particles prior to transfer of the eluted DNA to storage plates.

When extraction is complete and eluted DNA is transferred to storage plates, gDNA quality is assessed by having the STAR aliquot the extracted DNA into a Trinean DropSense96 plate for spectrophotometric based analysis. The DropSense input files are auto-generated by the instrument based on data captured during the deck setup (Sample barcode, operator ID, reagent lot numbers, and instrument ID). The results of the extraction are collated by the method and automatically uploaded to the cloud-based Color Genomics LIMS without the need for any user interaction or manual file handling.

Results

The purified gDNA produced by Color Genomics' extraction procedure performs well in next-generation sequencing, Sanger, MLPA and Array CGH assays. Figures 1 – 4 show comparable performance metrics between the two specimen types. There are, however, three notable, although not unexpected, differences in performance between the two sample types.

- **Extracted gDNA concentration is significantly higher (Unpaired t-Test137 = 4.905, p < 0.001) for saliva compared to peripheral blood samples (Figure 1).**
- **Protein contamination assessed by the A260:A280 ratio was significantly lower for peripheral blood compared to saliva samples (Unpaired t-Test137 = 3.859, p < 0.001) (Figure 2).**
- **The fraction of reads successfully mapped to the reference sequence GRCh37 was significantly lower for saliva compared to peripheral blood samples (Unpaired t-Test137 = 11.01 , p < 0.001). Nonetheless, performance for both samples types was well within the optimal boundaries of next generation sequencing.**

Additionally, adding a low force (2000 rcf for 2 minutes) centrifugation step followed by gentle aspiration prior to cell lysis significantly improved DNA quality (Figure 5) and mitigates the effect of contaminants such as phenolic compounds (common in chocolate, wine, or coffee) in saliva samples affecting downstream applications.

DNA Concentration from Two Sample Types

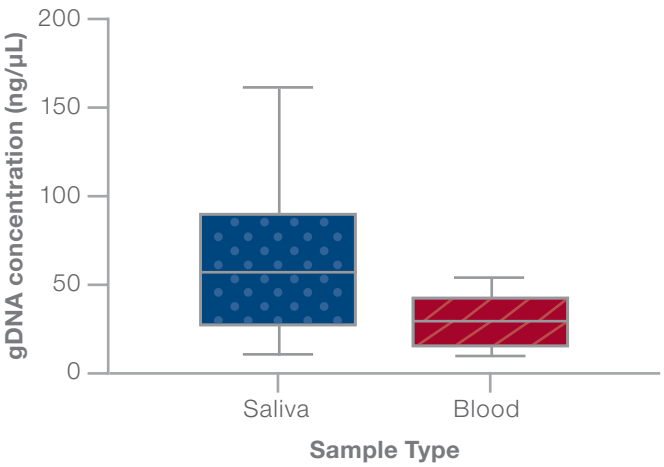


Figure 1: The concentration of DNA extracted from two specimen types using Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ chemistry and automated in parallel the Microlab STAR platform. The method extracted DNA from 300 µL of starting material (saliva, n = 500 or peripheral blood, n = 95) and eluted purified nucleic acids in 60 µL of 10 mM Tris-HCl before quantification using the Trinean DropSense96 instrument. Whiskers show the 10th and 90th percentiles, there is low variability in respective sample types.

DNA Purity from Two Specimen Types

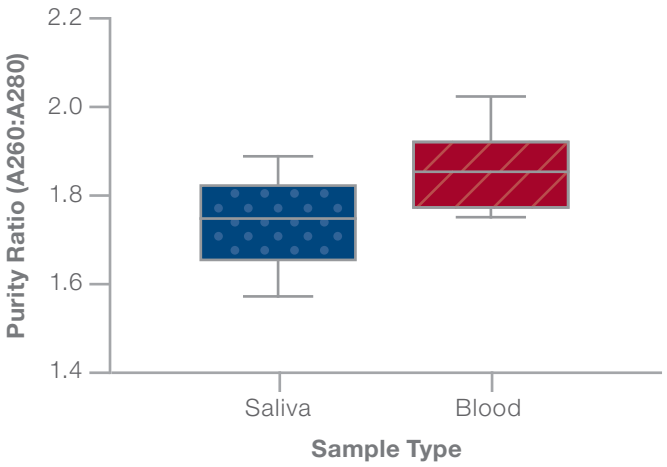


Figure 2: The purity of DNA extracted from two specimen types using Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ chemistry and automated in parallel using the Microlab STAR platform. The method extracted DNA from 300 µL of starting material (saliva, n = 500 or peripheral blood, n = 95) and eluted purified nucleic acids in 60 µL of 10 mM Tris-HCl before measurement of DNA purity via spectrophotometry (A260:A280) using the Trinean DropSense96 instrument. Whiskers show the 10th and 90th percentiles.

Total Fraction of Reads Mapped to Human Genome

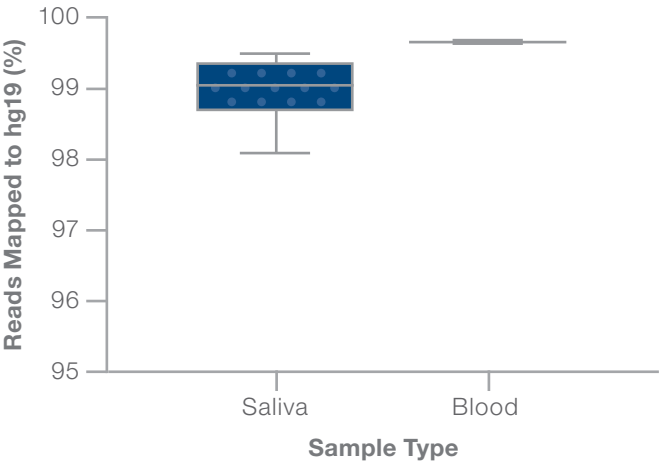


Figure 3: The fraction of NGS reads successfully mapped to the human reference after library preparation and capture using input gDNA from two specimen types (saliva, n = 500, peripheral blood, n = 95). Specimens were extracted with Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ chemistry automated in parallel on the Microlab STAR platform. Generated NGS data was aligned with BWA to the GRCh37 (hg19). Whiskers show the 10th and 90th percentiles, there is very little variability in mapping rates for peripheral blood specimens.

Fragment Size After Capture and Sequencing

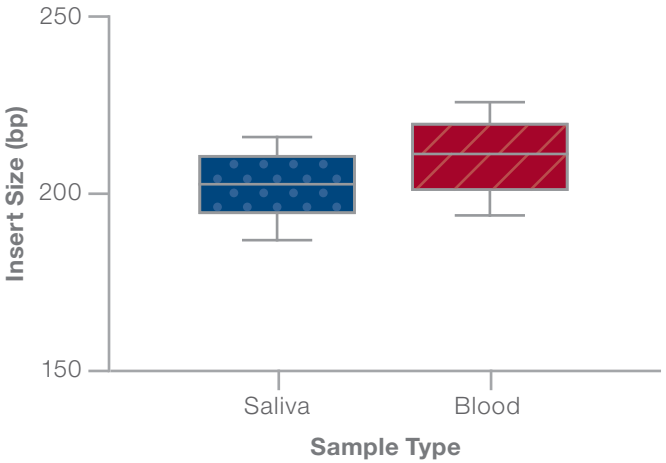


Figure 4: The insert size of library fragments mapped to the human reference genome after library preparation (Using Kapa Biosystems' Hyper Plus Library Prep Kit) and capture (Agilent's SureSelect XT Kit) using input gDNA from two specimen types. Specimens (saliva, n = 500, peripheral blood, n = 95) were extracted with Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ chemistry automated in parallel on the Microlab STAR platform. Generated NGS data was aligned with BWA to the GRCh37 (hg19). Whiskers show the 10th and 90th percentiles.

Phenolic Contamination Before and After Centrifugation

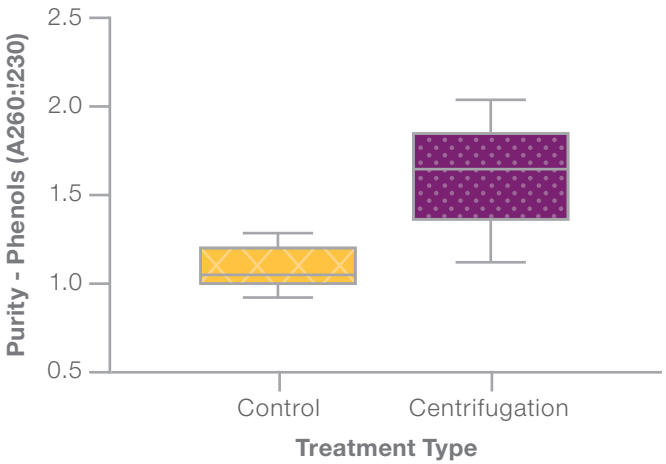


Figure 5: The results of a study to determine the impact of a brief low force centrifugation of Oragene Dx saliva samples prior extraction on level of phenolic contamination. Centrifugation has a significant positive impact on purity (Paired-Samples t-Test22 = 8.353, p < 0.001).

Conclusion

By applying collection device specific labware sequences to input samples based on their scanned barcode, we have developed a method to apply individual and collection tube specific liquid-handling in a high-throughput manner without compromising the quality of extracted gDNA. Moreover, by removing the need for users to manually load samples to specific locations on the deck, the composition of sample types on any single extraction batch is flexible while still keeping the procedure scalable and robust. We demonstrate that using this technique, combined with minor optimization to the OMEGA extraction protocol has allowed Color Genomics to extract gDNA, at a quality that is optimal for NGS, at scale, and in a highly cost-efficient manner.

Lit. No. L50162 — 02/2017

HAMILTON®

Web: www.hamiltoncompany.com/robotics
Email: marketingrequest@hamiltoncompany.com

United States
Tel: +1-775-858-3000
United Kingdom & Ireland
Tel: +44 (0)121-717-0199
Brazil
Tel: +55 (11) 126-50562
China
Tel: +86-21-6164-6567
France
Tel: +33 (01) 69751616
Italy
Tel: +39-39-689-33-93
Denmark, Norway, Sweden, Finland
Tel: +45-70-26-4499
Germany, Switzerland, Austria, Benelux
Tel: +49 (089) 552649-0

To find a subsidiary or distributor in your area, please visit www.hamiltoncompany.com/contacts.