# Engineering versatility, robustness and speed into a fully automated DNA extraction process

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## Summary

High-throughput laboratories are challenged by the need to efficiently process different sample types and specimen collection devices in a single high capacity workflow. Color Genomics has developed a novel DNA extraction procedure utilizing Omega Bio-tek's Mag-Bind® Blood and Tissue DNA HDQ magnetic bead-based chemistry on the Hamilton Star liquid handling system to extract high quality gDNA in a single, walk-away, automated and high-throughput process. Using Hamilton's HSL programming language, we developed an algorithm to create on-the-fly labware sequences by translating identifier characters on primary sample collection tube barcodes into the appropriate array of tube types. This allows the technician to load different tube types to any location on the instrument deck while still allowing the Hamilton to automatically perform the appropriate, tube-specific, sample handling procedure. In addition, DNA quality assessment was integrated into the extraction procedure to fully automate the pre-analytical process. A Trinean DropSense-96 on a custom-built platform was attached and integrated into the Hamilton HSL environment via an API and custom-built mounting platform. Removing the need to physically and temporally separate extraction processes based on sample type reduced consumable and instrument amortization costs (42 % and 50 %, respectively) and decreased assay step turn-around-time (50 %). Additionally, combining specimen types into a single extraction workflow removes the need to maintain robotic processes and technician training with multiple, sometimes rarely used workflows. Hitherto, high-throughput laboratories required manual processing and/or multiple sample processing workflows for extraction of high-quality DNA from different specimen types or collection devices. By developing a novel robotic process, Color has resolved this issue and increased our ability to provide high-quality and high-throughput genomic products while maintaining low cost, flexibility and rapid turn-around-times.

## 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 providing 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 delay patient care. 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.

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 walkaway protocol. The method has two distinct features that allow parallel processing.

1. The Hamilton Autoloader 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, size and shape parameters are assigned to the collection device via on-the-fly generated consumable specific labware sequences.
2. 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 Microlab 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 walkaway extraction process.

## 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 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.

## 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 transferring 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.
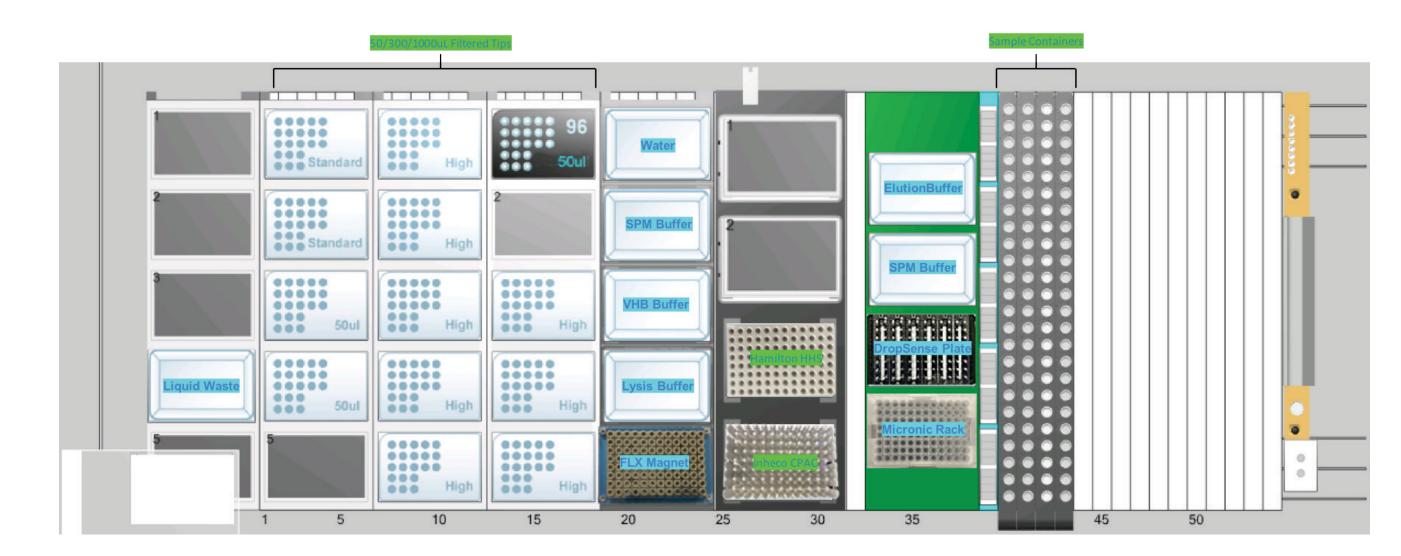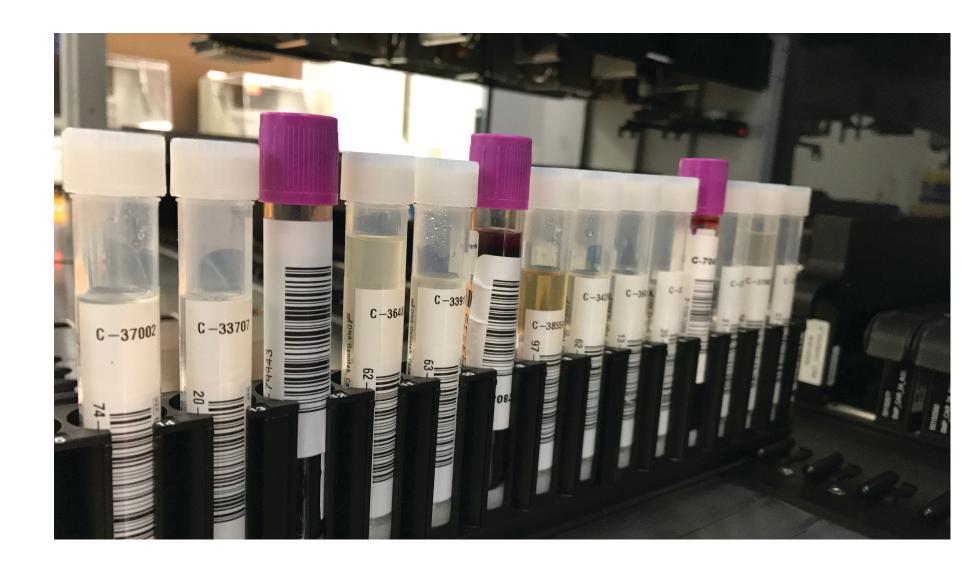


**Figure 1: Deck layout of high-throughput automated extraction process**
The deck contains all necessary consumables and reagents for a fully walkaway extraction process. The Hamilton is coded with built-in error-handling and the user is notified if any error that requires intervention occurs.

## Method Overview

The linear barcode label attached to input samples is scanned by the Autoloader 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. 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.



**Figure 2: Automating loading of random tube-types**
Different sample types can be loaded by the user in any arrangement. The Hamilton robot is programmed to identify sample types and process them appropriately.

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. Mixing is performed at the bottom of sample wells to prevent formation of bubbles and to ensure settled cells are resuspended during lysis. After lysis is complete, the lysate plates are removed from the heated position and 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 EX magnet. The water wash eliminates the need to dry the Mag-Bind® beads before the final elution step, greatly improving the final DNA yield and ensuring 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 before transfer to an Alpaqua EX magnet to collect the magnetic particles. The eluted DNA is then transferred to storage plates.

When extraction is complete, gDNA quality is assessed by loading an aliquot of 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 Hamilton 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 3 - 6 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:

1. Extracted gDNA concentration is significantly higher (Unpaired t-Test$_{137}$ = 4.905, p < 0.001) for saliva compared to peripheral blood samples (Figure 3).
2. Protein contamination assessed by the A260:A280 ratio was significantly lower for peripheral blood compared to saliva samples (Unpaired t-Test$_{137}$ = 3.859, p < 0.001) (Figure 4).
3. The fraction of reads successfully mapped to the reference sequence GRCh37 was significantly lower for saliva compared to peripheral blood samples (Unpaired t-Test$_{157}$ = 11.01, p < 0.001) (Figure 5). 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 prior to cell lysis significantly improved DNA quality (Figure 6) and mitigates the effect of contaminants such as phenolic compounds (common in chocolate, wine or coffee) in saliva samples affecting down-stream applications.



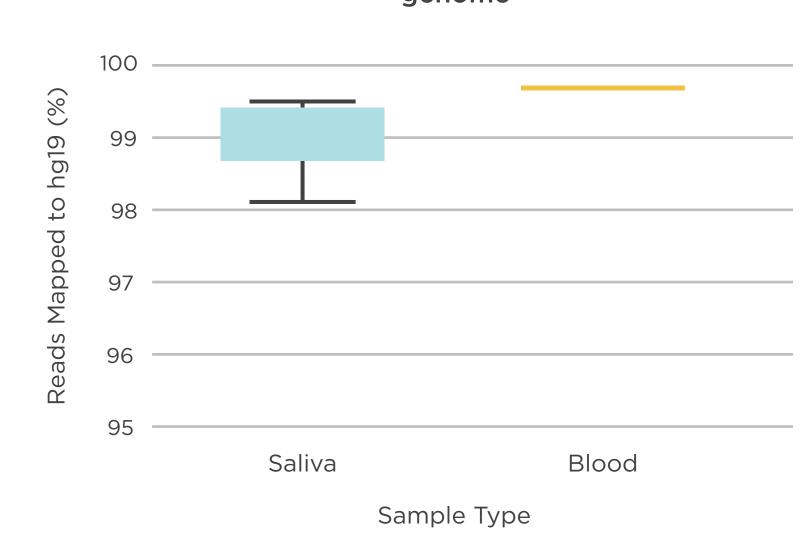**Figure 3:** The concentration of DNA extracted from two specimen types using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ chemistry and automated in parallel using the Microlab STAR platform. The method extracted DNA from 300 μL of starting material (saliva, n = 500 or peripheral blood, n = 95) and eluted purified nucleic acids in 60 μL of 10 mM Tris-HCl before quantification using the Trinean DropSense96 instrument. Whiskers show the 10th and 90th percentiles.
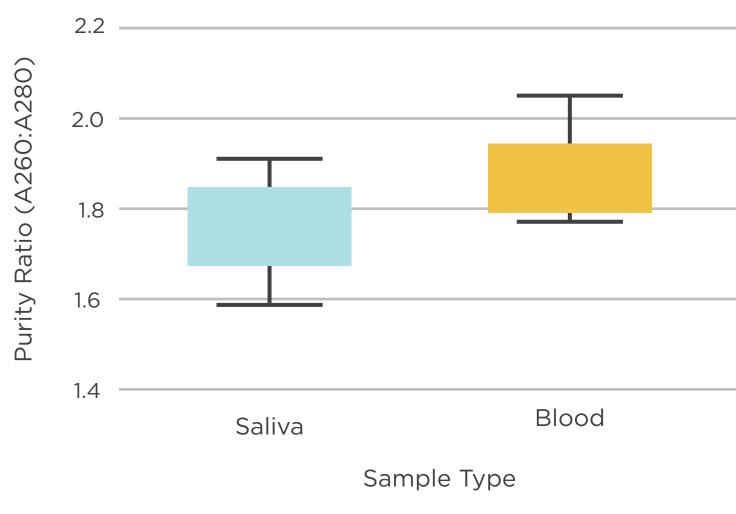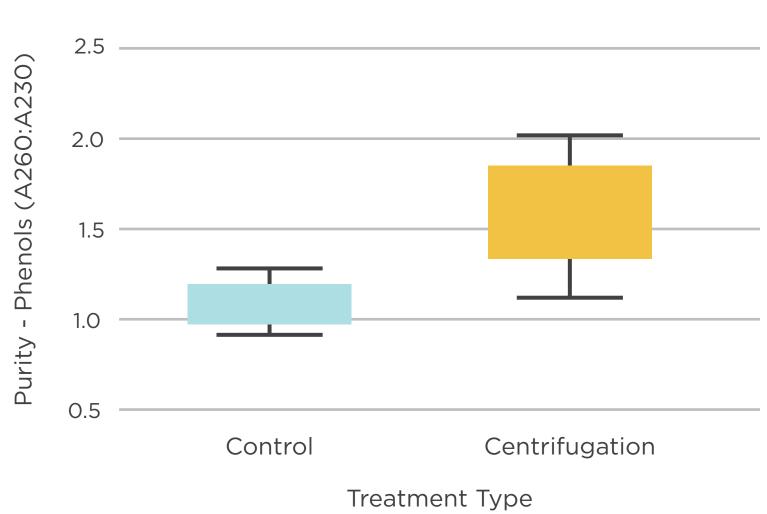


**Figure 4:** The purity of DNA extracted from two specimen types using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ chemistry and automated in parallel using the Microlab STAR platform. The method extracted DNA from 300 μL of starting material (saliva, n = 500 or peripheral blood, n = 95) and eluted 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.



**Figure 5:** 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 using 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.



**Figure 6:** The results of a study to determine the impact of a brief low force centrifugation of Oragene Dx saliva samples prior to extraction on level of phenolic contamination. Centrifugation has a significant positive impact on purity (Paired-Samples t-Test$_{22}$ = 8.353, p < 0.001).