Automated Isolation of Genomic DNA from Large Volume Blood Samples

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ABSTRACT

A key source for genomic DNA (gDNA) is blood drawn into a standard 10ml Vacutainer® tube. The Promega ReliaPrep™ Large Volume HT gDNA Isolation System, integrated on Freedom EVO®- HSM workstation, provides a unique and dependable system for isolating genomic DNA from large volumes (1ml–10ml) of blood. The novel chemistry and instrumentation resolve many challenges encountered when processing large-volume samples in a high-throughput format such as: loss of sample pellets during decanting of fluids, transport of full 50ml tubes to various locations on a liquid handling robot, and manual re-suspension of final DNA pellets. Liquid handler resource constraints were removed by creation of a new accessory, the ReliaPrep HSM 32 LV instrument, which provides heating, shaking and magnetization of samples at one deck position. The combination of this device, the Freedom EVO®- HSM workstation and the ReliaPrep Large Volume HT gDNA Isolation System allows automated recovery of pure gDNA from up to 32 ten milliliter blood samples within 4.5 hours.

We present verification studies demonstrating automated system performance. Comparisons between the Freedom EVO®- HSM workstation and a standard precipitation-based method were made for duplicate blood samples from multiple donors. Yield, purity, and integrity of extracted gDNA were assessed using UV absorbance spectroscopy and gel electrophoresis. Genomic DNA yields from normal 10ml whole blood samples were 200 to 400µg (depending on white blood cell count) in an eluted volume of 1ml. Recovered DNA exhibited good purity with A_{260}/A_{280} ratios greater than 1.7 and A_{260}/A_{230} ratios between 1.8 and 2.2. Isolated DNA was suitable for storage and was used in many downstream analysis applications. Results of genomic DNA purification from frozen (hemolysed) blood samples and blood collected using common anticoagulants (EDTA, heparin, citrate) are also compared to demonstrate the efficacy of the new system

System Requirements

Operator intervention:	Minimal - fully automated processing
Throughput:	Process 32 samples 4.5 hours (two runs/working day)
Scalability:	Each tube individually scaled
Chemistry Performance:	
Yield*:	Average yields >3 pg DNA/White Blood cell
Sample Storage:	Fresh or frozen blood fractions
Purity:	A_{260}/A_{280} > 1.7 A_{260}/A_{230} 1.8-2.2
Size	Greater than 25 KB
DNA ready for:	Downstream assays and archival storage

Hardware accessory enables automated processing

The HSM 2.0 provides three functions at one deck position

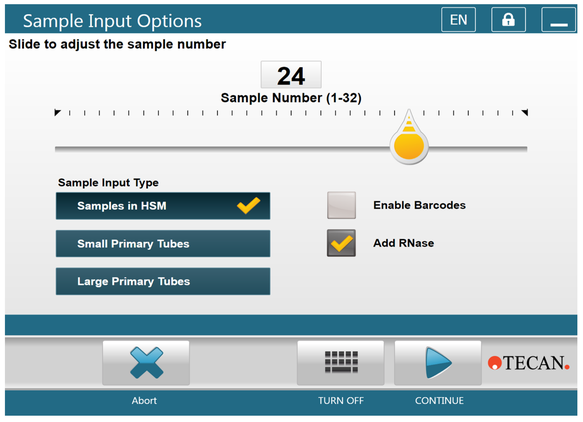
- •Heating
- •Shaking
- •Application of a magnet

This eliminates the need to move samples and reduces the chance for error in automated methods

Tecan Freedom EVO® workstation

The Freedom EVO®- HSM workstation is uniquely qualified for processing large volume samples

- •Delivery of bulk reagents to samples
- •Evacuation of waste by vacuum
- •Pipetting of small volume reagents/sample eluates with 1 ml tips
- •Touch screen interface simplifies instrument set up



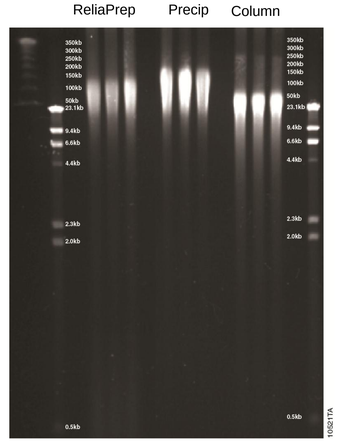
Cross-Contamination testing using Whole Blood

Concentration of male DNA (ng/µl) in ReliaPrep purified DNA. Samples are displayed as they were arrayed on the HSM. Samples from male donors are shaded while samples from female donors are unshaded. Samples with concentrations >250 ng/µl had C_T values less than the highest standard on the standard curve. By this assay there was no detectable sample-sample contamination when processed with an automated method.

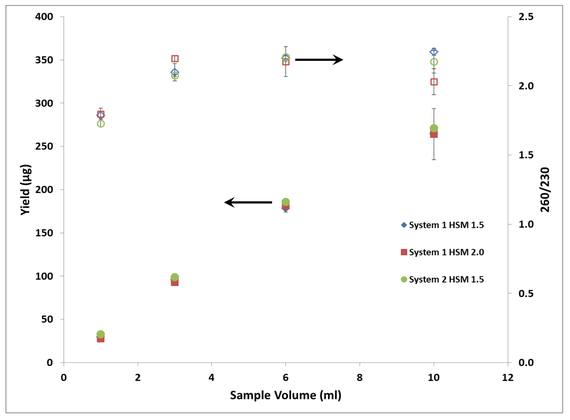
	1	2	3	4
A	152	No C_T	175	No C_T
B	No C_T	>250	No C_T	>250
C	>250	No C_T	>250	No C_T
D	No C_T	>250	No C_T	244
E	119	No C_T	235	No C_T
F	No C_T	>250	No C_T	208
G	165	No C_T	191	No C_T
H	No C_T	185	No C_T	>250

DNA Integrity

DNA was isolated from whole blood via three methods, separated by CHEF gel electrophoresis and visualized by ethidium bromide staining. DNA isolated using the ReliaPrep™ Large Volume HT gDNA Isolation System provided DNA with a size range of 20-125kb precipitation based purification isolated DNA with a size range of 20-200kb while column based methods demonstrated gDNA with a size of 20-75kb



Linear DNA yield is independent of HSM model and individual systems

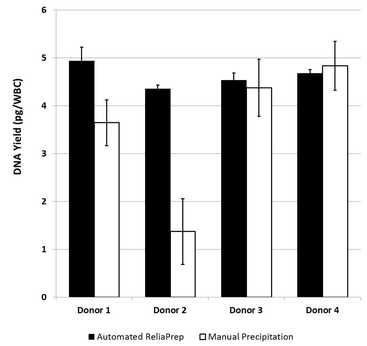


Samples from a single donor were processed on two different Tecan Freedom EVO® - HSM systems. One system is fitted with both an HSM 1.5 and an HSM 2.0. The second system is a Standard Solution configured robot equipped with an HSM 1.5.

Data shown was measured using a Nanodrop spectrophotometer. Each point is the mean of n=4 values with error bars of 1 standard deviation.

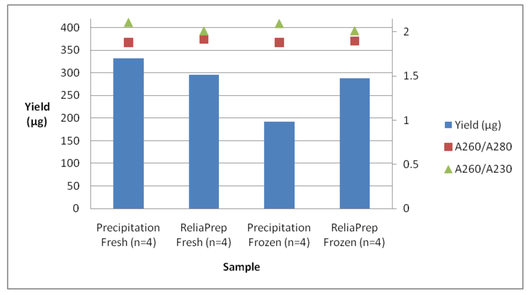
Solid symbols (◆) represent yield and outlined symbols (◇) represent purity.

gDNA recovery from multiple individuals: comparing to precipitation



Normalized DNA yields across multiple donors for both automated and manual methods. DNA yields are normalized based on the white blood cell counts for each donor. Each bar is the mean of quadruplicate samples with error bars of one standard deviation.

Extraction of gDNA from Fresh and Frozen blood samples



Freshly drawn blood samples were split into two groups one group of 10ml samples were processed directly and a second set were subjected to two freeze thaw cycles. Samples were either processed using the ReliaPrep Large Volume HT gDNA Isolation system or by a precipiation-based method. After elution of gDNA or re-suspension of DNA pellets, yield and purity were assessed by UV Absorbance spectroscopy.

Summary

The ReliaPrep™ Large Volume HT gDNA Isolation System provides an effective means for isolation of gDNA from 32, 1 to 10ml whole blood samples. Liquid level sensing and EVOware instrument operating software allow scaling of chemistry to sample input volume for each individual sample reducing reagent waste and expense. Samples are processed in one 50ml conical tube per purification. The system is robust, providing purification with no operator intervention once samples and reagents are placed onto the deck of the instrument. The chemistry is equally effective at recovering DNA from fresh blood as blood that has been lysed through freezing.

For more information email: genomics@promega.com