Automated Circulating DNA Purification from Large Volumes of Plasma

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Abstract 1516

Abstract

The circulating DNA found in human plasma is enriched for genomic DNA from minority tissues such as tumors, fetuses, and transplanted tissue. This sub fraction of blood has become an area of interest for development and disease as the nucleic acid recovered may represent a source of biomarkers for study. One milliliter of plasma typically yields between 1 ng and 50 ng of DNA. The current commercial DNA purification methods limit researchers' ability to scale their samples in both volume and number. Automated solutions are not available to process large numbers of samples with volumes greater than 1 ml, and manual methods can process up to 5 ml of plasma.

Here we present a fully automated solution to extract DNA from 1-64 samples up to 8 ml in volume. The system provides an intuitive user interface to select run options and enter barcodes for reagents and samples. Any combination of sample volumes may be processed together in a single run. Samples are eluted in 50 µl of nuclease-free water in either plates or tubes. We have found that the performance of our system meets or exceeds common commercial alternatives over their common sample input volume.

Promega Heater Shaker Magnet (HSM) Processes Large Volume Samples

The HSM 2.0 provides three functions at one deck position:
- Heating
- Shaking
- Magnetization

Single-position processing of large volume samples reduces risks of lost samples.
- Enclosed tube cavities
- PC control
- Spill resistant
- Tecan integration

Expanded Tecan ReliaPrep™ System Processes Large Volume Plasma Samples

User interface provides intuitive access to all script features.
No prior automation experience is needed

DNA Yield Scales Linearly with Input Volume

Sample DNA yields across the sample input volume range (1 – 8 ml). Samples were prepared from pools of single-source human plasma and were processed across several days. Reagents were automatically metered for individual samples based on detected initial volume. All samples were eluted in 50 µl of nuclease-free water. DNA was analyzed by TaqMan® qPCR using primers/probe for the single-copy locus TERT. Each column is the mean of 4 replicates with error bars of 1 standard deviation.

Conclusions

The circulating DNA purification method provides excellent performance across 1 – 8 ml input sample volumes
- Up to 64 samples
- Elute in as little as 50 µl

The robotic system is a expansion of the proven Tecan Freedom EVO HSM Workstation:
- User interface provides method flexibility in an intuitive environment.
- No prior automation experience is required.
- Reagents are automatically metered depending on sample volume.
- Retains potential to process other whole blood fractions

This system is currently available as a custom product. Contact genomic@promega.com or your local Promega branch for additional information.

Performance Meets or Exceeds Current Standard Protocols

Sample DNA yields across several individuals at 4 ml. Samples were prepared from pools of single-source human plasma and were processed across several days. Reagents were automatically metered for individual samples based on detected initial volume. All samples were eluted in 50 µl of nuclease-free water. DNA was analyzed by TaqMan® qPCR using primers/probe for the single-copy locus TERT. Each column is the mean of 4 replicates with error bars of 1 standard deviation.

Process All Whole Blood Fractions on One System

Automated DNA yields for whole blood and blood fractions. DNA was purified from both whole blood and blood fractions collected from a single individual. Samples were quantitated by Nanodrop spectrophotometry. Data are averages of 4 replicates +/- 1 standard deviation.

Process Begins in HSM and Transitions to 96-Well Plates

Schematic description of the circulating nucleic acids purification protocol. Lysis, binding, and initial washes are performed in the HSM. The liquid handler transfers the samples to 96-well plates for final washes and elution. Reagent volumes are individually calculated for each sample allowing parallel processing across the full range of sample input volume (1 – 8 ml). Sample information including input barcodes, sample volume, reagent volumes, and destination barcodes are provided in detailed reports.

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Whole blood represents the most stringent condition for cross-contamination. Ten milliliter whole blood samples were processed using the large volume blood gDNA automated method. Samples were arrayed in a male-female sample checkerboard.

The concentration of male DNA (ng/µl) in ReliaPrep purified gDNA is shown in the table. Samples from male donors are shaded while samples from female donors are unshaded. Samples with concentrations >250 ng/µl had Ct values less than the highest standard on the standard curve. By this assay there was no detectable sample sample contamination.

Contact genomic@promega.com or your local Promega branch for additional information.

**Table:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA Yield (ng/µl)</th>
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<tbody>
<tr>
<td>Sample 1</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&gt;250</td>
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<td>Sample 3</td>
<td>&gt;250</td>
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<td>Sample 4</td>
<td>&gt;250</td>
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<td>&gt;250</td>
</tr>
<tr>
<td>Sample 10</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

No C

**Note:** All values within the shaded region are considered to be contaminated.

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