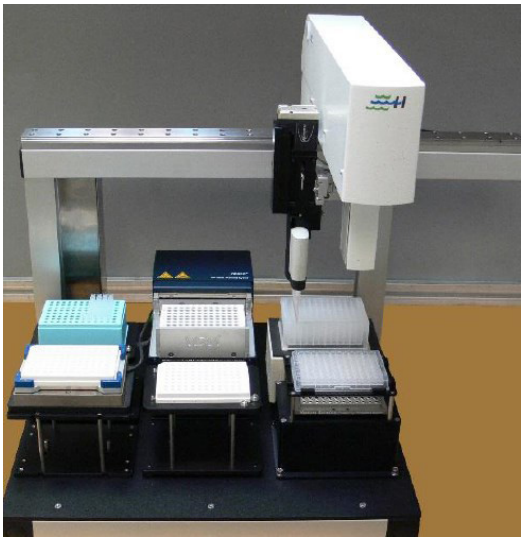
Application Note:

Automated Purification of Cell Free DNA, using the Hudson Robotics SOLO™ Workstation, and BioChain cfPure™ kit.



Cell-Free DNA (cfDNA)

Cell free DNA (cfDNA) is found in body fluids, and is thought to be derived from necrotic or apoptotic cells, which shed their genomic DNA, or release DNA-containing exosomes, into these fluids[1,2,3]. In general, the DNA is degraded, with a characteristic fragment of 170-180bp present in virtually all samples, with other fragments of roughly 300-400bp and 500-600bp being present at lower levels . The sizes of these fragments suggest association with, and protection from nucleases by, histones. In some cases, such as immediately after strenuous exercise, larger DNA species can become prominent - possibly due to genomic DNA being freshly released into the blood, and not yet fully fragmented[3].

Medical uses for cfDNA

In recent years, cfDNA has been identified as a promising tool for early cancer diagnosis and prognosis. Current research suggests that sequence mutations found in cfDNA may also be useful to make better informed cancer treatment decisions[4]. Cancer cells that have become necrotic or apoptotic release cfDNA into the blood stream. Since these cells exhibit mutations or epigenetic changes that distinguish them from the genomic DNA in healthy cells, the DNA they release will frequently contain useful biomarkers. For example, aberrantly methylated Septin 9 sequences are closely associated with colorectal cancer, and are used for cancer screening[5] in blood tests referred to as “liquid biopsies.” Other uses for this DNA species include noninvasive prenatal testing (NIPT). Fetal cfDNA can cross the placenta and be present in the mother’s blood. This fetal cfDNA can be used to detect chromosomal anomalies, such as Down’s syndrome. Therefore, a simple blood draw from a pregnant woman can provide information on the developing fetus that previously required amniocentesis - a far more invasive technique that carries risks, such as infection and miscarriage. Research showing increases in cfDNA levels in response to environmental insults or strenuous exercise suggest that additional applications will arise in the future[3].

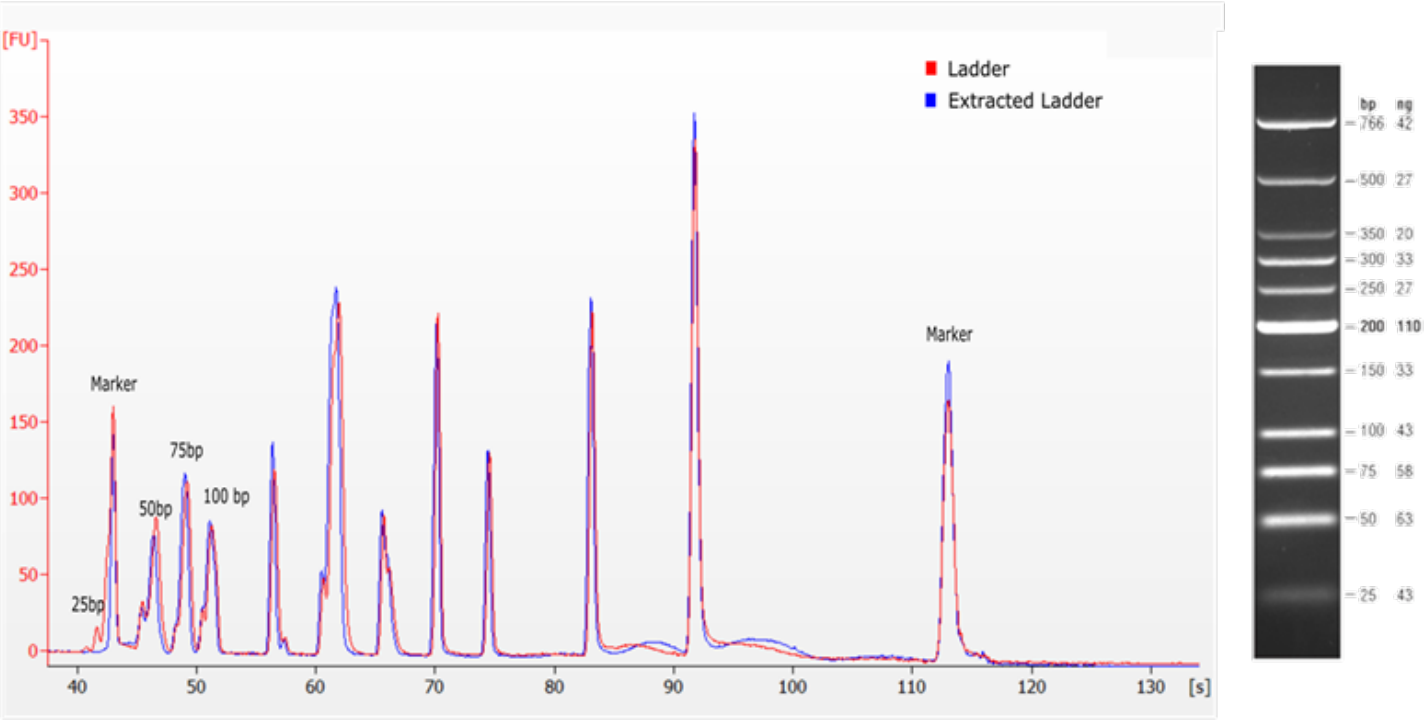
cfDNA extraction and the need for automation

Extracting cell-free DNA is somewhat different than isolating genomic DNA, due to the small size of many of the key fragments. A good cfDNA extraction kit recovers virtually all of the low molecular weight DNA species from roughly 50bp to 700bp. Recovery of larger fragments is less important in most cases. The most two common formats for cfDNA extraction kits use silica membrane spin columns, or silica-coated magnetic beads as their key component. Magnetic beads confer several advantages. For high-throughput, low sample volume applications, they are much more cost-effective. With a column-based kit, one spin column will be used for each cfDNA extraction, regardless of the volume of a sample. With a magnetic bead-based kit, the amount of beads used is proportional to the volume of sample extracted. In many cases, this results in a 10-fold reduction in cost - as the spin column-based extraction methods are typically designed to process up to 5 mLs of plasma, while some applications require the amount of cfDNA found in 0.5mLs of plasma. The scalable nature of bead-based systems also confers advantages when processing larger (5-10mL) sample volumes. Additionally, magnetic bead-based DNA extraction kits can easily be adapted to fully automated laboratory instruments, which will be critical in high throughput situations - both for cost-effectiveness and for reproducibility. Since the liquid biopsy market is projected to exceed $3Billion over the next five years, with millions of samples being processed, high throughput will be the norm and not the exception.

Hudson Robotics provides automated liquid handlers that are capable of fully automating laboratory processes - including those required for magnetic bead-based nucleic acid extractions. Hudson Robotics specializes in consulting with their clientele to deliver automation platforms tailored to the needs of their clients, using components such as the SOLO liquid handling system, the 96-well Mag Nest, the Shaker Nest multiwell vortexer, and the PlateCrane microplate stacker. The modular nature of Hudson Robotics systems makes them scalable. As throughput increases in a laboratory, additional modules can be incorporated to handle the increased workload without requiring significant changes to overall workflow.

Experiment

Confirmation of BioChain's cfPure™ chemistry



To confirm that cfPure™ efficiently recovers low molecular weight DNA, 50ng of a Low Molecular Weight DNA ladder (New England Biolabs, Cat#N3233S) was added to 1mL of a simulated plasma sample. This DNA was then purified using BioChain's cfPure™ cell free DNA extraction kit, eluting the DNA in a final volume of 50ul. At 100% recovery, the expected concentration of purified NEB ladder would be 1ng/ul. This purified DNA was then analyzed by electrophoresis, using a BioAnalyser DNA 1000 chip (Agilent). The resulting electropherogram was compared with one produced at the same time, using the same DNA ladder, diluted to 1ng/ul.

Automation of cfPure™ on the Hudson Robotics SOLO workstation

For this proof of concept test, the following Hudson Robotics instrumentation and lab supplies were used:

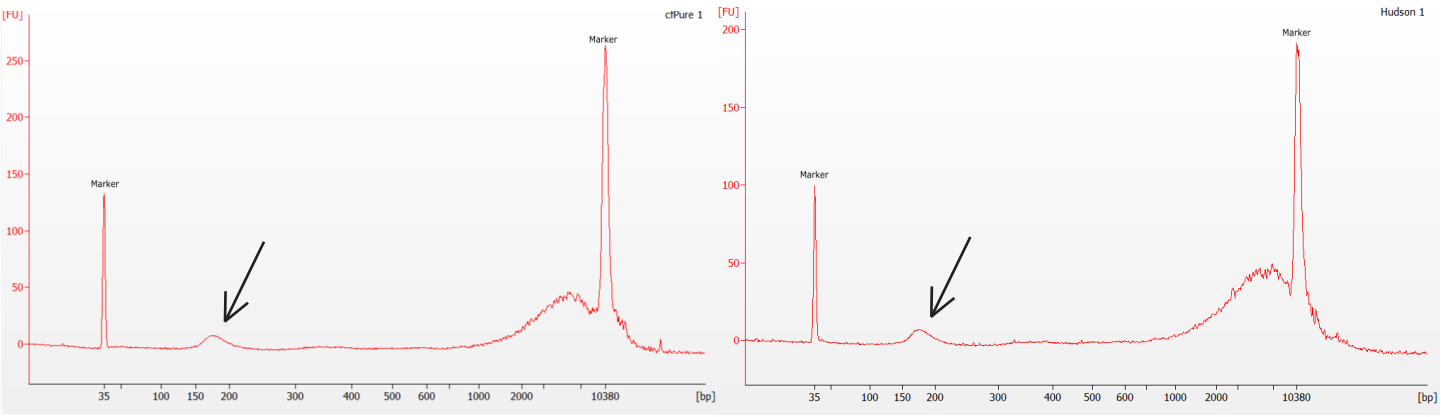
- SOLO MXT Automated Pipette unit
- 8-channel, 200uL Pipette
- 6 position deck
- 1x Hudson Robotics 96-well Mag-nest
- Hudson SOLOSoft v8.47 on Windows 10 Laptop

This setup allowed for extraction of eight 0.5mL cfDNA samples simultaneously, and was used as proof of concept - Hudson Robotics instruments are modular systems, sample volume and throughput can be tailored to a user's needs. A single plasma sample was divided into 11 500ul aliquots. Four of these were subjected to extraction of cfDNA manually using the cfPure™ kit. The remaining 7 aliquots were extracted in a fully automated Hudson Robotics system.

Results

Sample Name	Protocol	Total DNA yield (Qubit) (ng)	CV of Total DNA yield (Qubit)	Yield of Alu Sequence DNA amplified by PCR (ng)	CV of cfDNA yield (qPCR)
1	cfPure™	22.2	23.73%	6.92	40.74%
2	Hudson Robotics	19.09	12.59%	4.81	26.05%

Yields were tested by fluorescence using a Qubit fluorometer (Thermofisher Scientific), or by qPCR amplification of Alu repeat sequence DNA.



A) cfPure™ B) Hudson Robotics

Recovery of low molecular weight cfDNA was confirmed by electrophoresis, using a BioAnalyser DNA 1000 chip, of a representative 0.5 mL plasma sample extracted by cfPure™ chemistry on the Hudson Robotics system (A), or manually, using cfPure™ chemistry. A characteristic 170-180 bp cfDNA peak (marked by an arrow) is visible on both electropherograms.

Discussion

- The Hudson Robotics SOLO MXT Automated Pipette unit, combined with the Hudson Robotics 96-well Mag-nest, provided all of the functions needed for a fully automated cfDNA purification system. No manual steps are required.
- cfDNA extraction using the MXT pipette unit and Mag-nest demonstrated good reproducibility, with low sample to sample variability.
- Hudson Robotics system used is a modular system. Low throughput systems can be handled with a single MXT pipette unit, while multiple units can be used for higher throughput systems.
- This is a system that can be implemented at low cost, and then scaled as needed.
- BioChain's cfPure™ cell-free DNA extraction kit efficiently recovers the critical low molecular weight DNA fragments characteristic of cell-free DNA.
- DNA isolated by cfPure™ is suitable for more challenging applications such as PCR, Next Generation Sequencing, and methylation studies, is also amenable to automation walk-away robotic systems.

References

1. Snyder, Matthew W., et al. “Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin.” Cell 164.1 (2016): 57-68.
2. Salvi, Samanta, et al. “Cell-free DNA as a diagnostic marker for cancer: current insights.” OncoTargets and therapy 9 (2016): 6549.
3. Breitbach, Sarah, et al. “Direct quantification of cell-free, circulating DNA from unpurified plasma.” PloS one 9.3 (2014): e87838.
4. Shu, Yongqian, et al. “Circulating Tumor DNA Mutation Profiling by Targeted Next Generation Sequencing Provides Guidance for Personalized Treatments in Multiple Cancer Types.” Scientific Reports 7 (2017).
5. Tetzner, Reimo, et al. “Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer.” Clinical chemistry 55.7 (2009): 1337-1346.

Notes

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