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# Human Plasma Cell-free DNA

## from Healthy Donors for Analytical Validation & Clinical Assay Development

Application Note

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# Introduction

Cancer is a disease arising from genetic alterations that alters cell survival, growth, differentiation and proliferation (1). The gold standard of obtaining a tumor sample via tissue biopsy is invasive and sometimes is not possible due to the location of the tumor. In recent years, liquid biopsy has come into the forefront of precision oncology. The genetic analysis of alterations in circulating cell free tumor DNA (ctDNA) in body fluids such as plasma, urine, or saliva promises new possibilities for cancer diagnosis, treatments, and disease progression monitoring.

Although much research has gone into development of liquid biopsy tests, the medical community has been slow at implementing the technology into clinical practice (2). One major reason is the lack of reference material for validation and benchmarking of liquid biopsy assays. Concentrations of circulating cell free DNA (cfDNA) has been reported to range from 1.8 to 44ng/mL of blood plasma (2) and with most healthy individuals having less than 10ng per mL of plasma (3). Many technologies for assessing genetic mutations require much higher concentrations of cfDNA for accurate assessment (4). Traditionally, researchers have solved this problem by creating artificial cfDNA. The most common methods are shearing of genomic DNA, generation of nucleosome DNA, or enzymatic digestion of human cell line DNA (2). These methods however, have their own limitations. Sonication has been shown to cause oxidation damage to the DNA which can result in sequencing errors and subsequently lead to false positives in variant calling (5). Even though enzymatic digestion does not introduce DNA damage or generation of nucleosome cfDNA from human cell line DNA is more biochemically similar to naturally occurring cfDNA, these methods may not serve well as a baseline or reference for cell free circulating tumor DNA due to cell lines' inherent genetic alterations (4).

# Methods

## Sample collection and cfDNA extraction

Plasma: Up to 10 mL of whole blood was collected in Cell-Free DNA BCT blood collection tubes (Streck) from healthy human donors (Table 1). Plasma was isolated from whole blood using a double centrifuge method according to manufacturer protocol.

Cell free DNA extraction: cfDNA was extracted using the cfPure® Cell Free Nucleic Acid Extraction kit (BioChain Institute Inc.) as per manufacturer's protocol. Each cfDNA pool contains a randomized donor group with mix of ages, sex and ethnic backgrounds. Researchers can also specify and customize the donor pool by contacting the manufacturer.

| Donor | Age | Gender | Race |
|---|---|---|---|
| Donor 1 | xx | M | x |
| Donor 2 | xx | F | y |
| Donor 3 | xx | F | xx |

Table 1: *Baseline characteristics of healthy disease-free donors.*

## Quantification and size evaluation of cfDNA

Concentration of extracted cfDNA was quantified with Qubit High Sensitivity dsDNA Assay (ThermoFisher Scientific) as per manufacturer protocol. Size evaluation, quality and percent cfDNA were performed on both the BioAnalyzer High Sensitivity DNA Kit and 4200 Tapestation Cell-free DNA ScreenTape assay (Agilent Technologies) following manufacturer protocol.

# Results

Extraction using 10mL plasma from a healthy donor yielded about 400ng of cfDNA (BioChain Institute Inc). Size evaluation using the BioAnalyzer with Agilent High Sensitivity DNA assay showed dominant DNA peak at around 170bp, which indicates the abundance of DNA fragments resulting from apoptotic cells. The 170bp peak size corresponds to the length of DNA wrapped around a nucleosome plus a fragment of DNA on the histone that serves as a linker for nucleosomes (6). The two peaks at ~340bp and ~450bp correspond to DNA fragment lengths associated with di-nucleosomes and tri-nucleosomes (Figure 1). Little or no genomic DNA was seen in the sample. Evaluation of cfDNA using the Agilent 4200 Tapestation Cell-free DNA ScreenTape assay also confirmed the presence of these DNA peaks at the corresponding base pair sizes (data available upon request)..

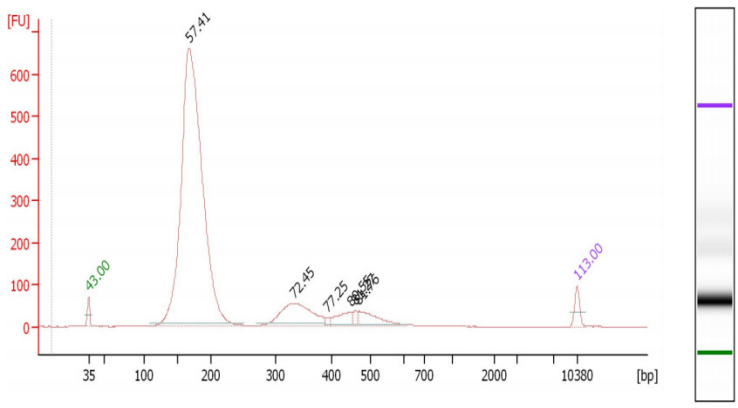


Figure 1: *Representation of a BioAnalyzer trace of cfDNA pool. A dominant DNA peak at ~170bp can be seen, which is the typical size characteristic of cfDNA. The size corresponds to the length of DNA wrapped around a nucleosome plus the histone linker. DNA fragments can also be seen at ~340bp and ~450bp ranges which indicates DNA fragment lengths that correspond to di-nucleosomes and tri-nucleosomes. Minimal genomic DNA contamination was observed.*

# Summary and Application of cfDNA

Availability of reference controls and standards is imperative in validation and benchmarking of liquid biopsy assays for clinical use. BioChain's disease free human pooled cfDNA control has been shown to contain high concentration of DNA fragments with characteristics of mono-nucleosome and histone linker DNA with minimal genomic DNA contamination. It is available at high concentrations such as 10ng/µL along with a donor pool that is customizable according to the researcher's needs. BioChain also offers cfPure DNA Free Plasma for validation of extraction methods. Using a proprietary method of removing contaminating genomic DNA without affecting plasma components, it has been shown to contain less than 10pg/mL of DNA. The cfPure DNA Free Plasma can be used in conjunction with the Mutation Quantification Control Plasma, which contains spiked in synthetic ctDNA at specific allele frequencies, to allow researchers to evaluate the sensitivity and specificity and use as a process validation and quality control of their assays. The Human Cell-free DNA Control along with other BioChain liquid biopsy products can serve as an unbiased reference and standard for the development and optimization of liquid biopsy assays.

# About the Author

Shukmei Wong has over 15 years of experience in the science industry. She has contributed to research in development of vaccines for HIV and researching the genomic alterations of various human and canine cancers utilizing next generation sequencing technology. In her free time Shukmei enjoys crocheting and spending time with her family along with her furry and feathered army of 1 dog, 2 cats, 3 parakeets, and 6 chickens.

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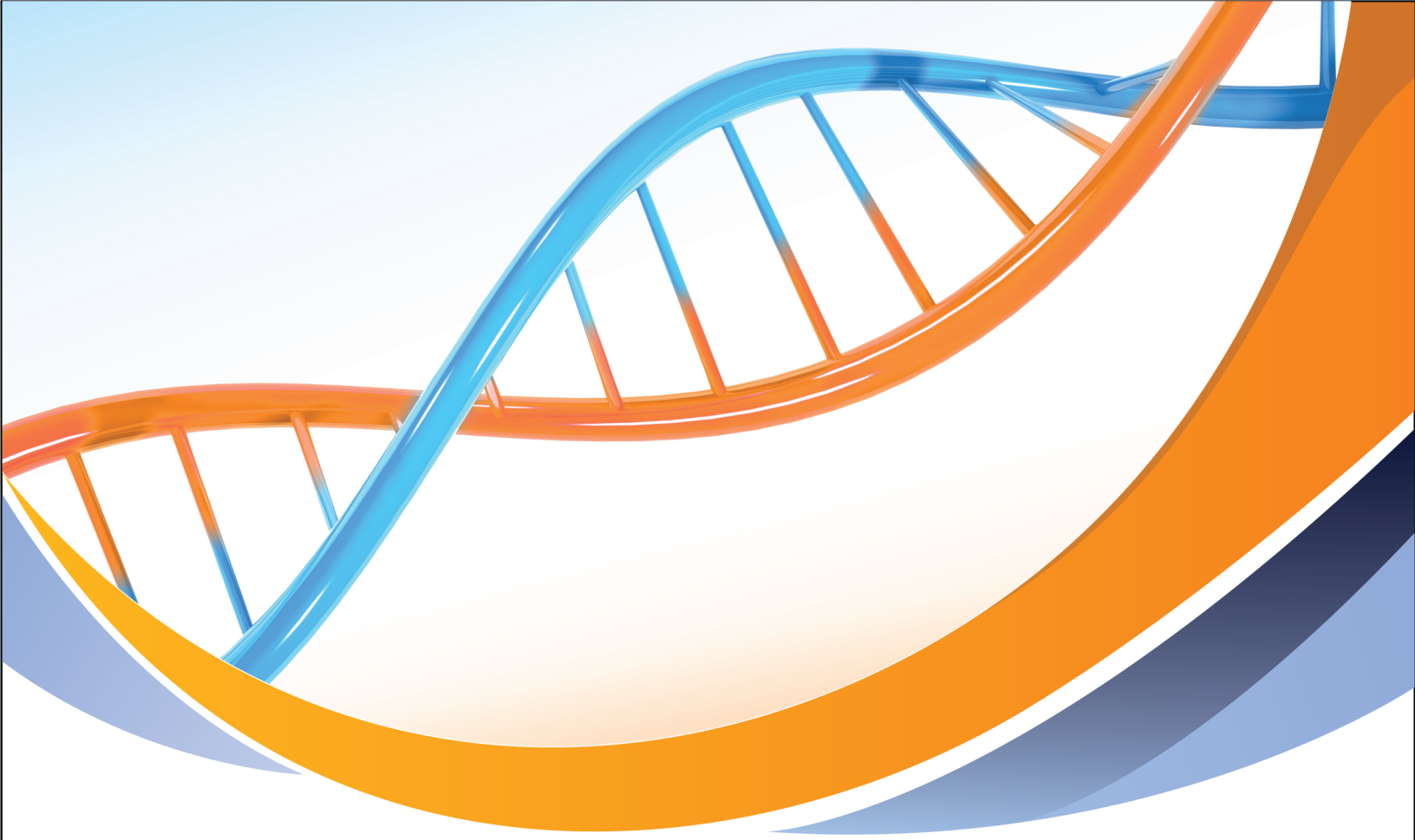

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