BIABooster CE-LEDIF SYSTEM

BIABooster Platform:

- Agilent Technologies 7100 Capillary Electrophoresis System
- Picometrics Zetalif™ LED Induced Fluorescence Detector (LEDIF)
- Capillary Device

This note describes how the µLAS technology is used with the Agilent Technologies 7100 Capillary Electrophoresis system coupled to Picometrics™ LED Induced Fluorescence Detector.

The BIABooster system provides high sensitivity and extended range of DNA size analysis.
It is also possible to perform all modes of capillary electrophoresis that are available with the Agilent Technologies CE system.

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Instrumental Set-Up

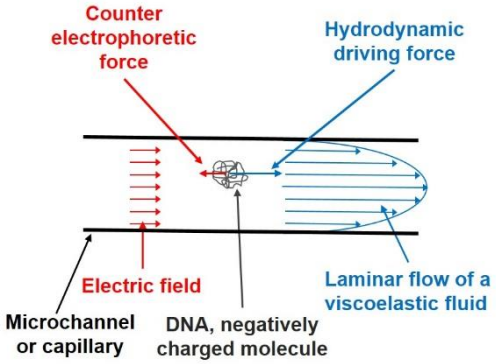
The BIABooster solution consists of the following items:

- Agilent Technologies 7100 Capillary Electrophoresis System including all functional hardware for performing CE separation
- Picometrics Zetalif™ LED Detector including a Detector, Optical Cell, LIF Cassette, LED light source with the corresponding emission filter block, LIF Driver for Agilent Technologies Software.
- Proprietary capillary device for µLAS technology
- BIABooster Analytics software to quantify, qualify and size DNA.

About µLAS Technology

µLAS technology simultaneously uses electric field and pressure in a viscoelastic fluid to analyse DNA. The capillary device is specially designed to take full benefit of the technology.

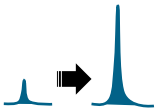
Basic principle : DNA is subjected to a pressure-driven viscoelastic flow in combination with a counter-electrophoresis. In these conditions, DNA undergoes a viscoelastic force oriented toward the channel walls, the amplitude of which depends on its size. Because of the parabolic velocity profile of the flow, DNA molecules are transported by the fluid at a rate which depends on their size, like in gel electrophoresis.



µLAS basically covers three functions:

CONCENTRATE

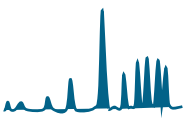
On-line concentration



To concentrate DNA "in line": µLAS enables the design of "DNA traps" within a flow, by joining two capillaries of different diameters. In these traps, molecules are confined precisely at capillary junction, allowing to stack them and/or remove them from the flow. DNA can therefore be concentrated before being separated, for unmatched analytical sensitivity.

SEPARATE

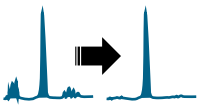
Size separation



To separate and quantify DNA fragments of different sizes, which have equal electrophoretic mobilities. As such, it is a new separation technique, complementary to gel electrophoresis since molecules are separated in solution.

PURIFY

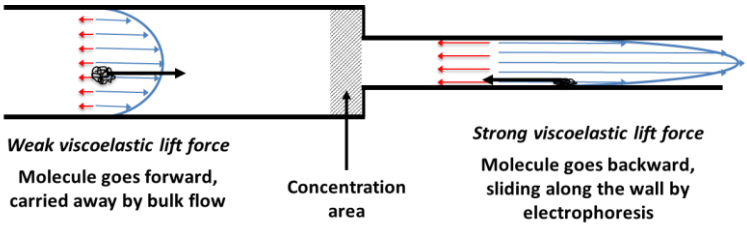
On-line purification

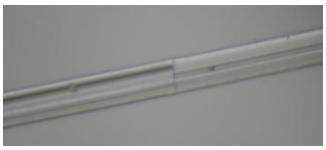


To isolate and purify DNA fragments of interest: this functionality is obtained by combining the two functions of concentration and separation, and by adding a collecting function available with the 7100 CE. DNAs of the desired size can be selected, excluding other DNAs present in the sample.

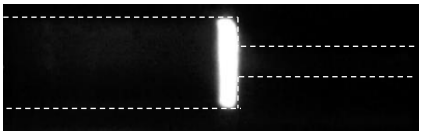
Proprietary Capillary Device

A modified cassette accommodates the integration of a LIF detection system and any type of µLAS capillary device.



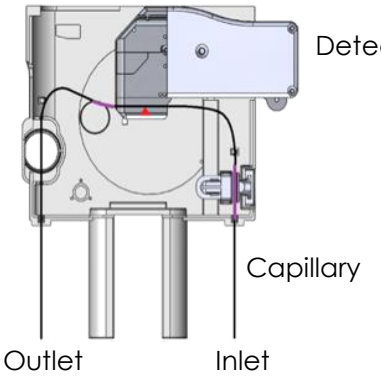


Capillary junction



DNA stacking at the capillaries junction

On-line DNA concentration at a µLAS capillary junction before separation



Inside View of the LIF Cassette

Specifications for cfDNA Analysis

cfDNA is a promising biomarker for non-invasive monitoring of cancer disease. Getting a DNA profile of plasmatic free DNA is difficult with existing electrophoresis systems. But it is an easy thing with the BIABooster system using the **DNA 1K** and **DNA 10K Kits** for Quality Control of circulating DNA previously purified from plasma.

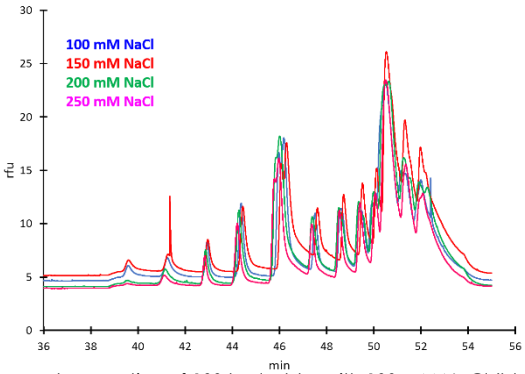
Analytical specifications	Kit DNA 1K	Kit DNA 10K
Sizing range	**0.1-1.5 kb**	**1-10 kb**
Limit of detection (S/N = 3)	10 pg/ml at 1 kb 100 pg/ml at 100bp	10 pg/ml at 10 kb 30 pg/ml at 1 kb
Sizing accuracy*	+/- 3%	+/- 3% (from 1kb to 6kb) +/- 5% (from 6kb to 10kb)
Sizing reproducibility	3% CV	3% CV
Quantitative range*	5-1000 ng/ml for cfDNA	0.5-400 ng/ml for DNA smear
Quantitative precision	20% CV	20% CV
Quantitative accuracy	20%	20%
Minimum sample volume	10 µl (1 µl injected)	10 µl (1 µl injected)
Maximum salt concentration	15 mM	15 mM

** Determined using a commercial ladder as a sample, different from the standard used for reference.*
Excitation wavelength : 488nm Laser or LED option available

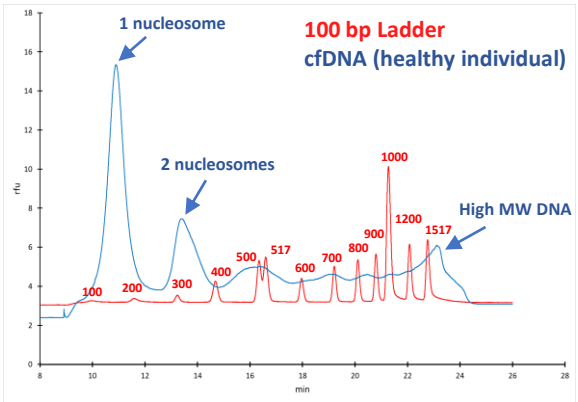
Some BIABooster Applications

cfDNA Analysis in Plasma

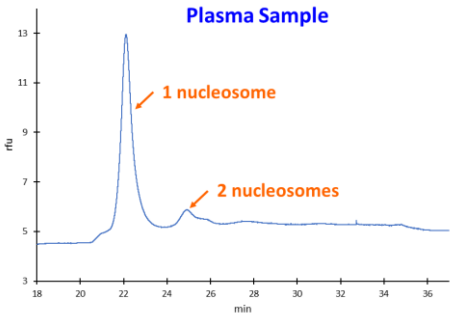
- Unrivalled Sensitivity
- Unrivalled Robustness
- Purification + Concentration + Separation in 30 minutes



Concentration and separation of 100 bp ladder with 100 mM NaCl (blue), 150 mM NaCl (red), 200mM NaCl (green), 250 mM NaCl (pink)



Analysis of purified cfDNA (blue) and 100 bp ladder (red)



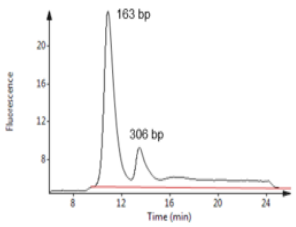
Analysis of endogenous DNA of plasma (with Proteinase K + SDS treatment)

The BIABooster system is able to provide a profile of circulating DNA directly from **plasma**.

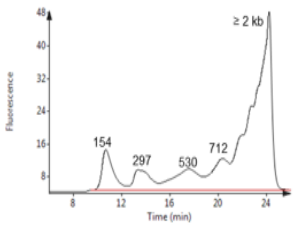
cfDNA Sample Quality Control

Qualify your samples before PCR and sequencing analysis:

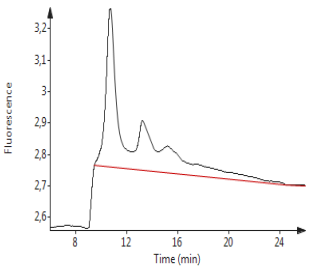
- Concentration
- Integrity
- Genomic DNA contamination



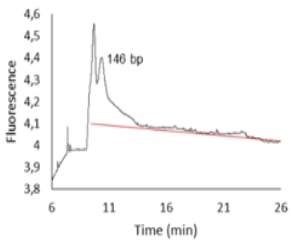
cfDNA at 210 ng/ml
Typical profile



cfDNA at 270 ng/ml
HMW contamination



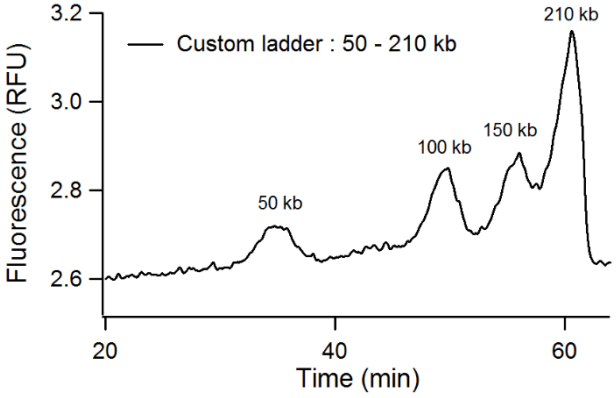
cfDNA at 4 ng/ml
Low concentration of cfDNA with good integrity

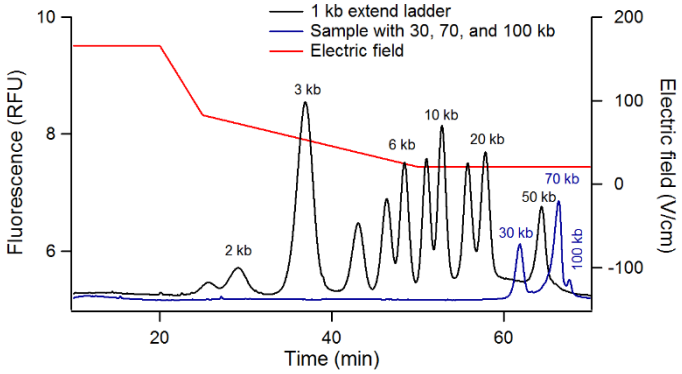


cfDNA at 7 ng/ml
Low concentration of degraded cfDNA

High Molecular Weight DNA Separation

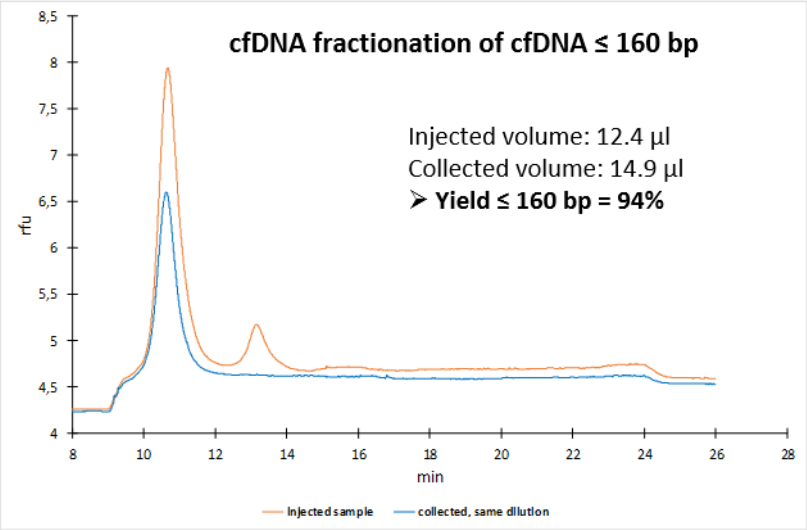
Next generation long read sequencing has increased interest in large DNA fragment analysis. BIABooster platform enables to quantify and qualify DNA fragments up to 150 kb.





cfDNA Fractionation

µLAS technology can be used to select a DNA size range of interest. This has been used for cell free circulating DNA in which tumoral cfDNA has been reported to have a smaller size compared to constitutional cfDNA. The isolation of tumoral cfDNA is expected to provide a better sensitivity for mutation detection.



References:

CfDNA Biomarker research applications, see **article in *Anal. Chem.*, 2018, *90* (6), pp 3766–3774**

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