

Dual Hydrodynamic and Electrokinetic Actuation in a Capillary Assembly Enables DNA in Line Concentration and Separation

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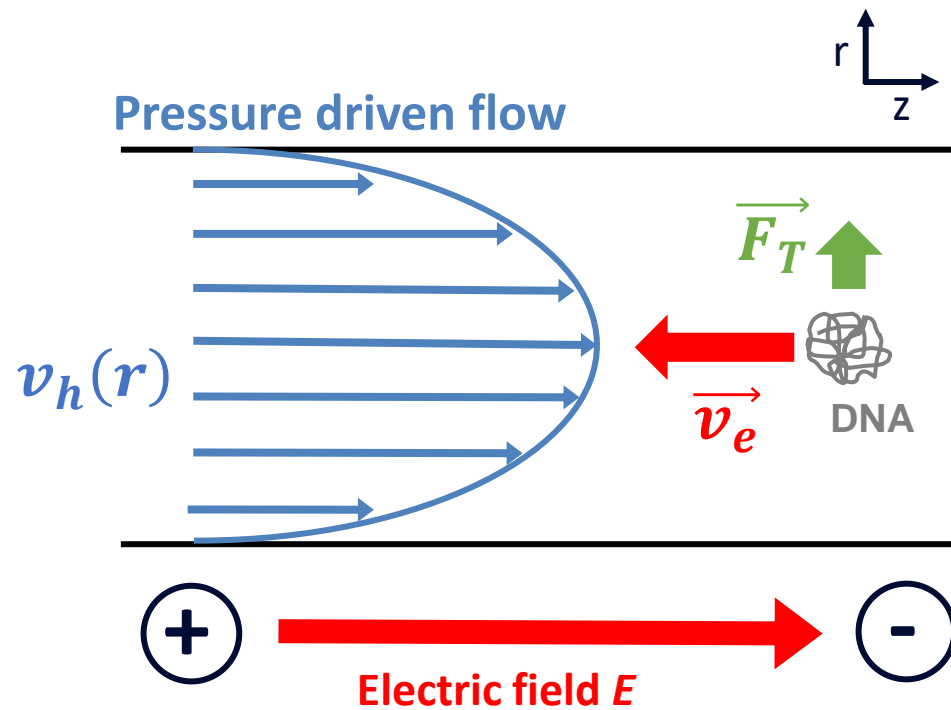
Presentation Plan

- 1. Basics of the dual hydrodynamic and electrokinetic actuation**
- 2. Unrivalled sensitivity**
- 3. In-line purification**
- 4. Large DNA, up to 150 kb**
- 5. DNA fractionation**
- 6. A new Multichannel-CE**

Basics of the dual hydrodynamic and electrokinetic actuation



μLAS Physics



Pressure driven flow

$$v_h(r) = v_{h,max} \left(1 - \frac{r^2}{R^2} \right)$$

{ Fastest at the center
 Zero at wall

Capillary radius

Counter-electrophoresis

$$v_e = \mu E$$

Viscoelastic fluid

$\eta \rightarrow$ Viscosity

$\tau \rightarrow$ Relaxation time

Lift Force

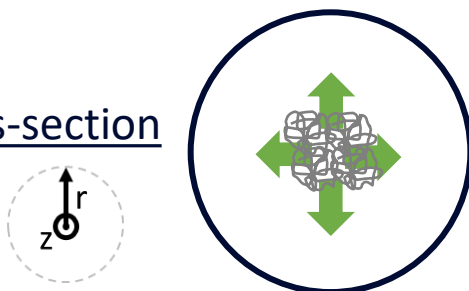
$$F_T \sim \underbrace{(\tau \eta)}_{\text{Fluid properties}} \underbrace{v_{h,max} v_e}_{\text{Actuation parameters}} \frac{r}{R^2} \underbrace{a}_{\text{Molecule size}}$$

Fluid properties

Actuation parameters



Cross-section

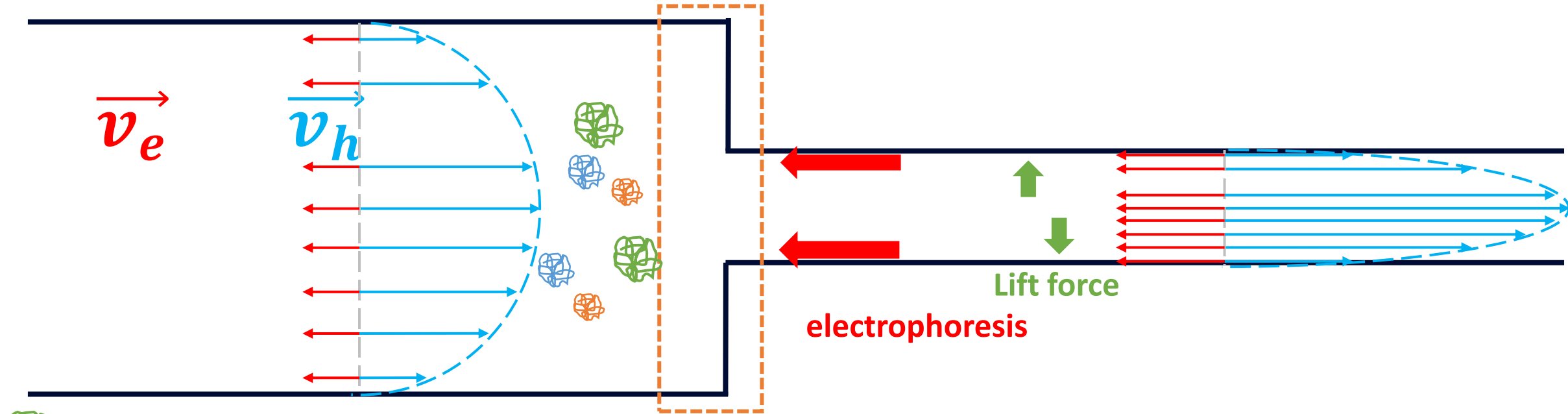


μ LAS concentration phenomenon



large diameter, weak lift force
DNA is carried away by the flow

small diameter, strong lift force
DNA slides backward along the walls



- Large DNA
- Medium DNA
- Small DNA

Stable concentration area

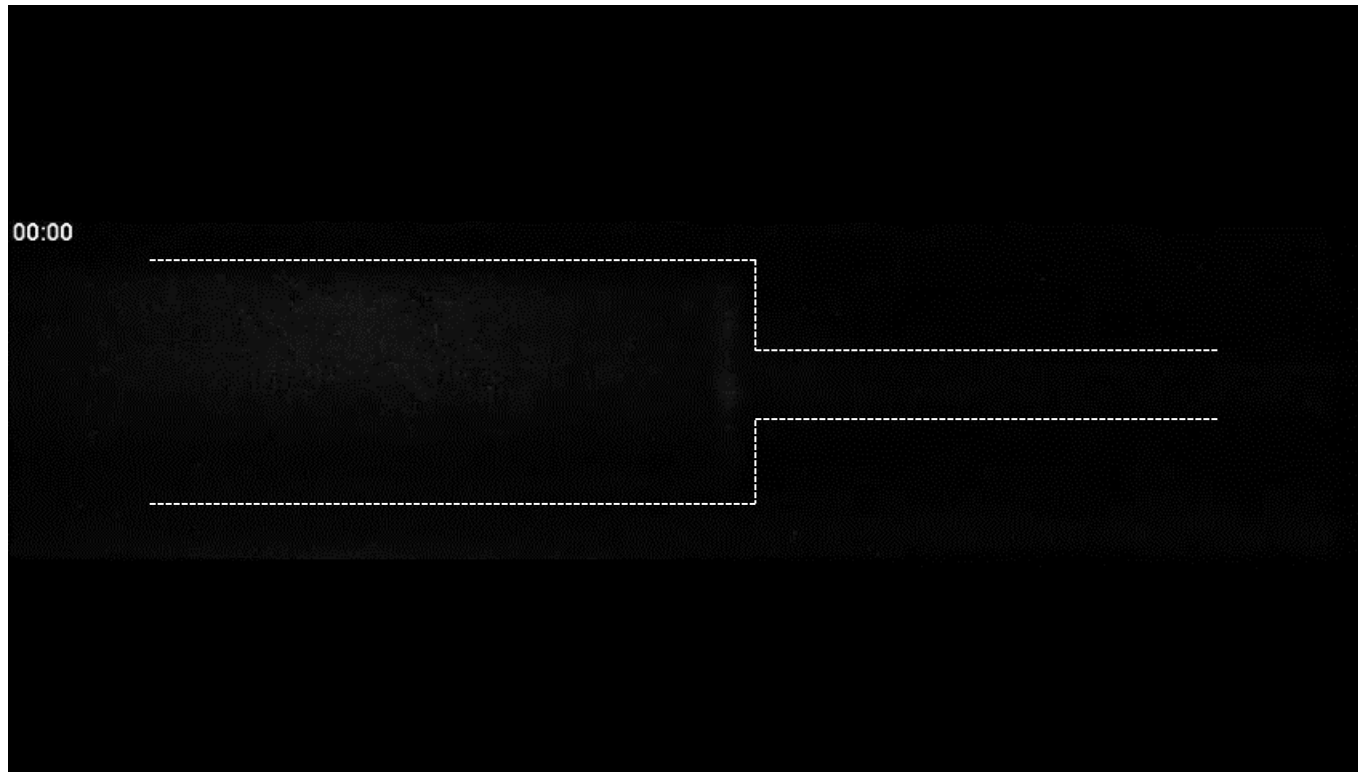
Concentration video



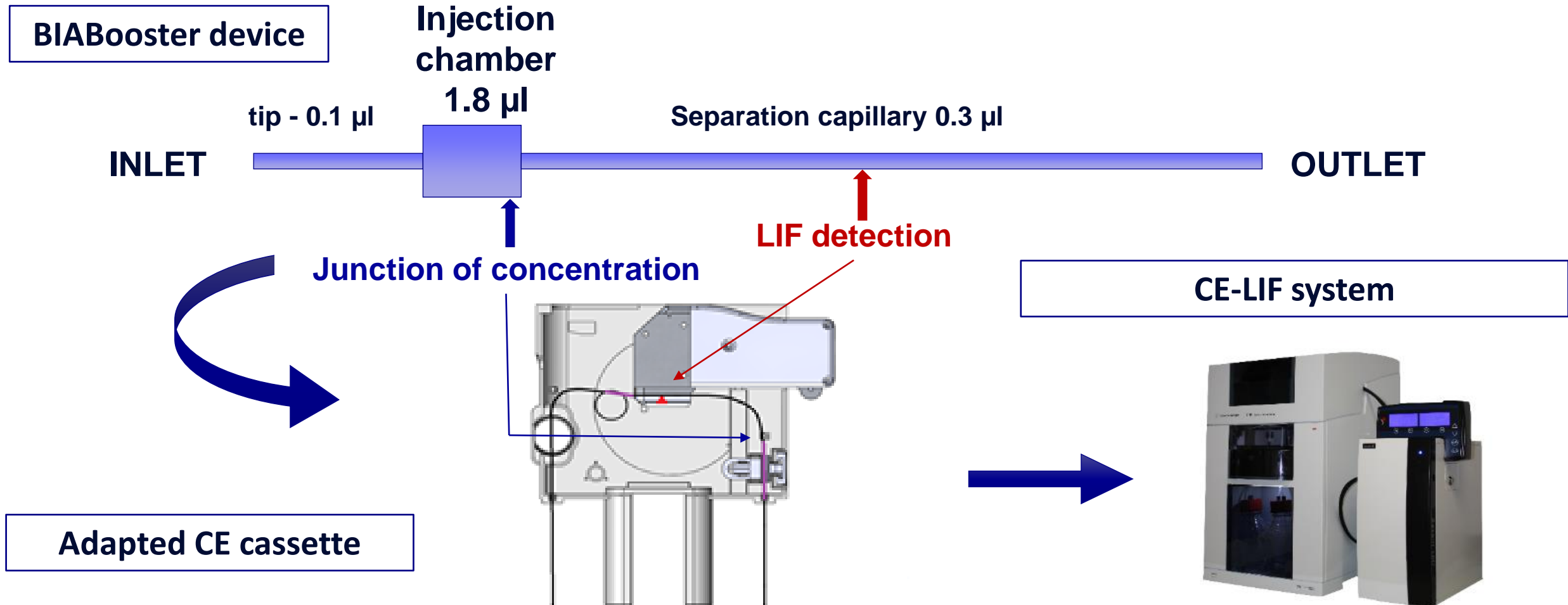
pressure



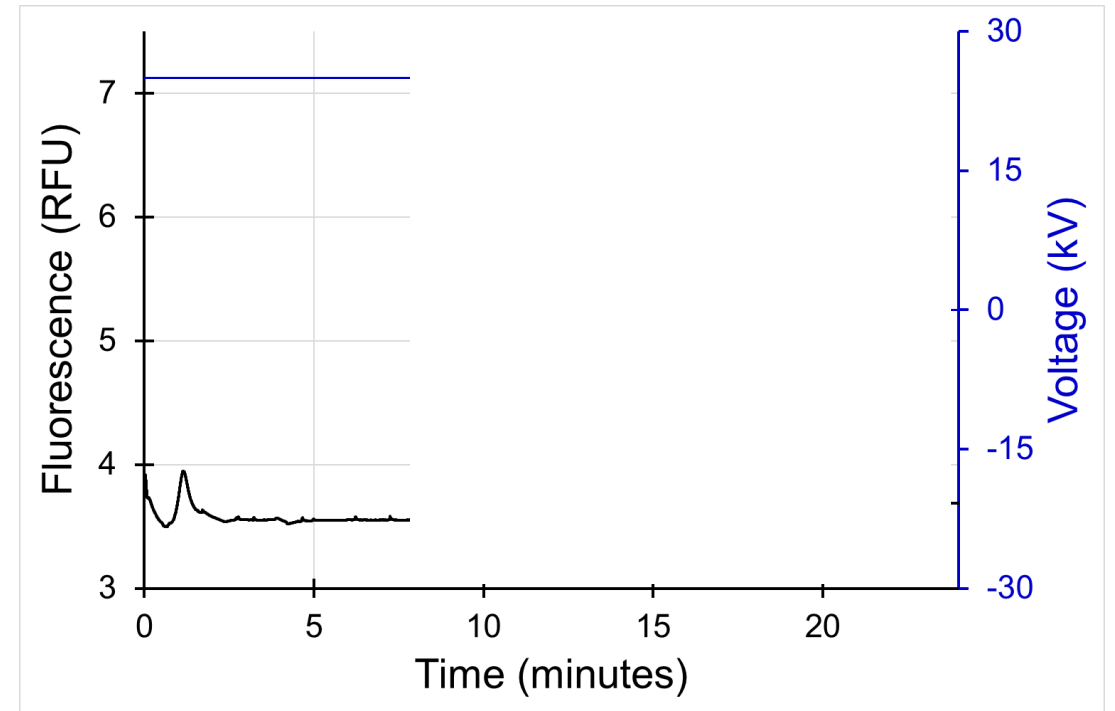
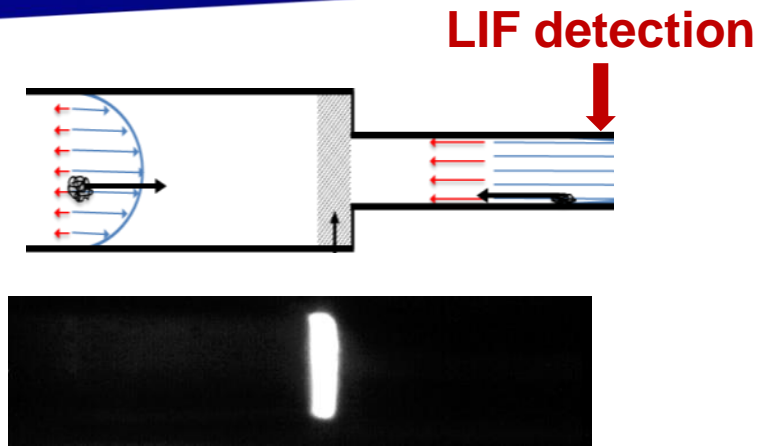
counter-electrophoresis



The BIABooster : a practical implementation



The BIABooster : concentration + separation

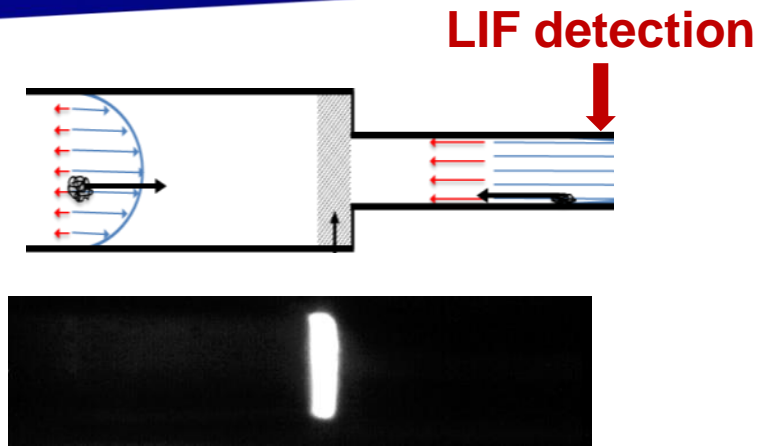


~1 μ L injected \rightarrow high sensitivity

Unrivalled sensitivity

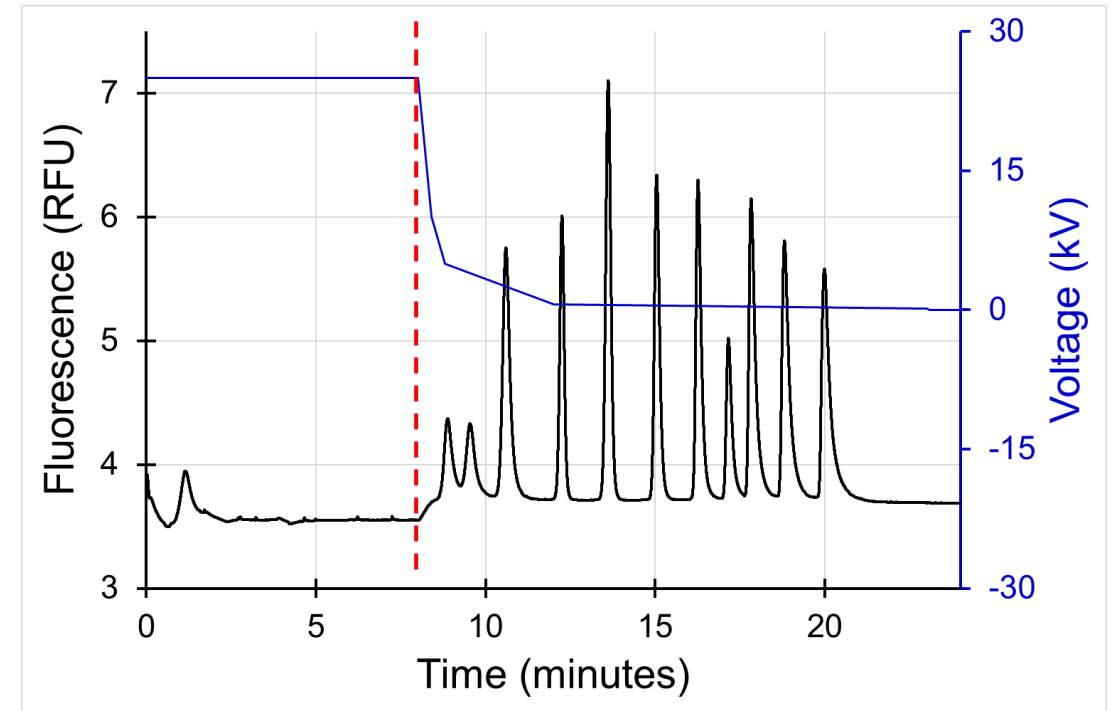


The BIABooster : results 0.1 – 1.5 kb range



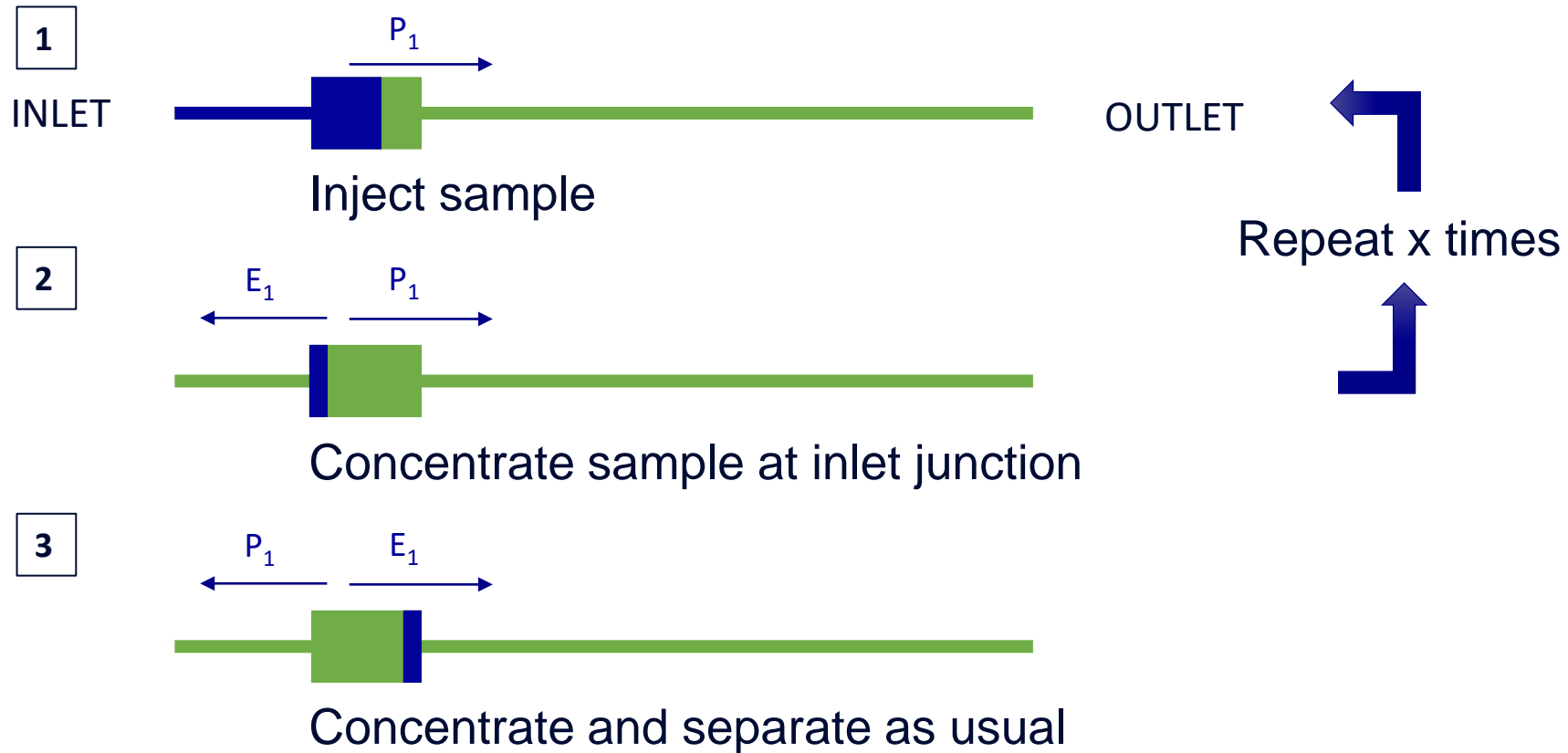
DNA 1K method

- Sizing range : 0.1-1.5 kb
- **LOD :** **10 fg/ μ L at 1 kb**
- Sizing precision : 2% CV
- Sizing accuracy : 3%
- Quantification precision : 15% CV
- Quantification accuracy : 20%
- Dynamic range : 1000

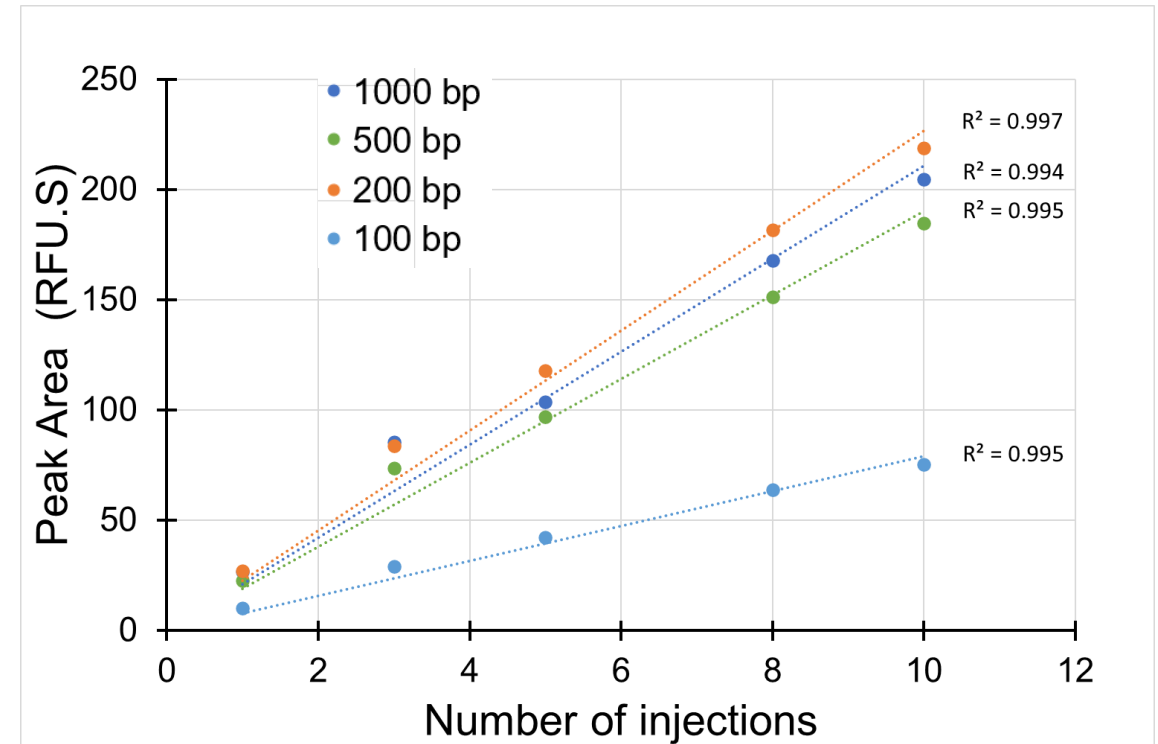
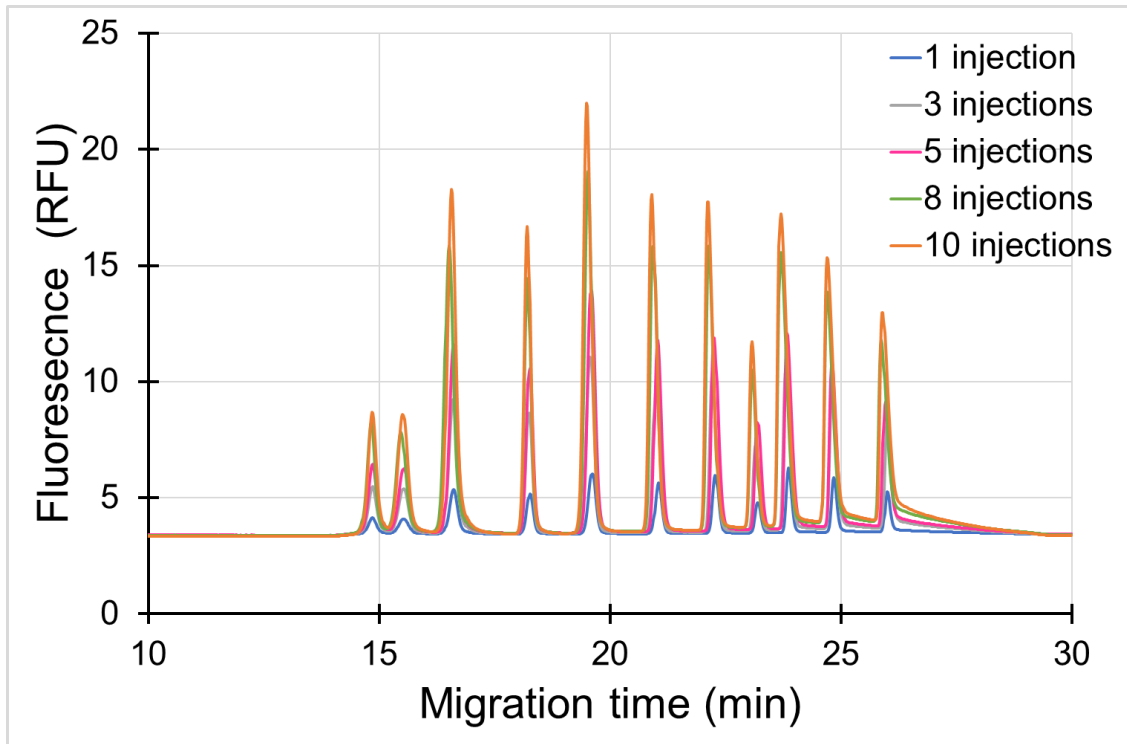


Multiple injections, concept

Sensitivity can be further enhanced by increasing the sample volume injected in the device.



Multiple injections is quantitative



LOD for 10 injections : 1 fg/ μ L at 1 kb

In-line purification for analysing unpurified samples

Application to cfDNA and other biological fluids



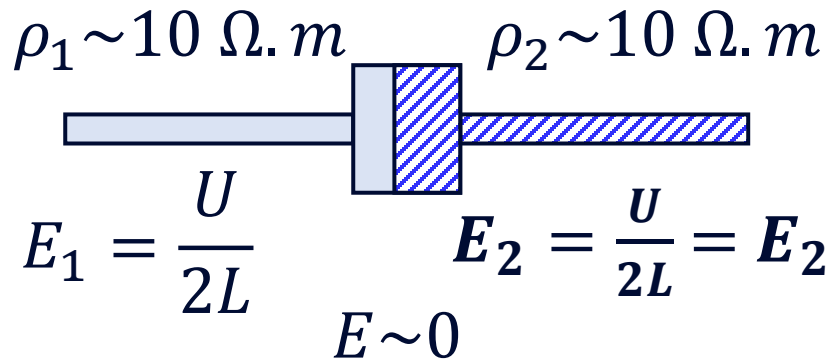
In-line purification principles

DNA is a large molecule, even at 100 bp (~63 kDa), and highly negatively charged.

Smaller molecules, or less charged, or positively charged, will be washed away by the buffer during concentration.

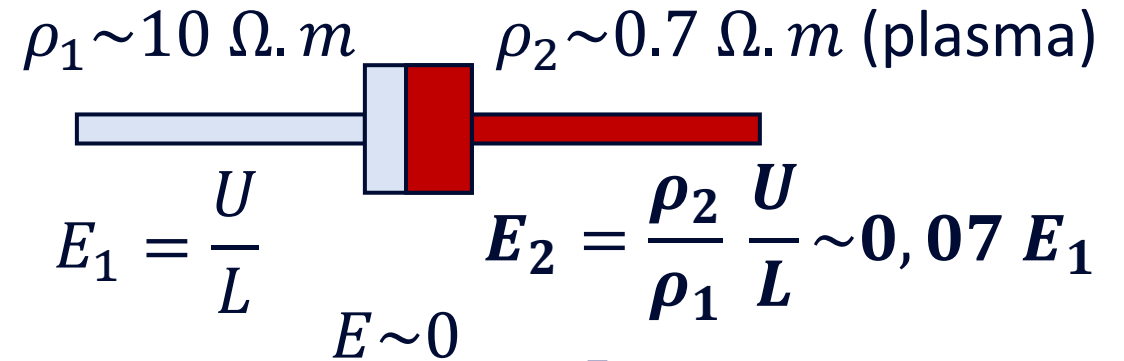
Problematics with salts

DNA in buffer



Small DNA leaks out during concentration,
... but more slowly than salts

DNA in salty solution



E_2 is too small

Solution for salts

1. injection of salty sample; pressure alone



2. Start concentration; pressure + voltage



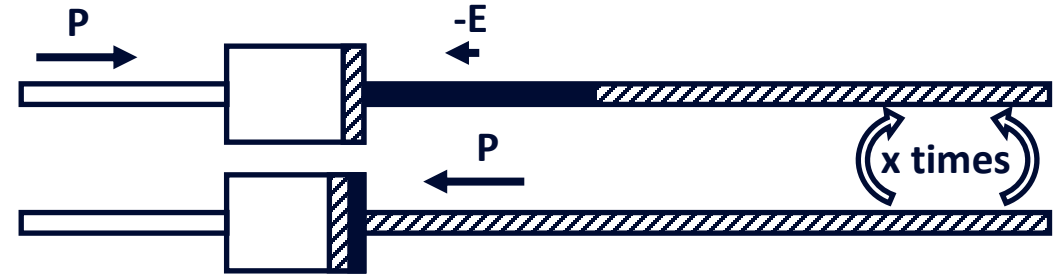
DNA leaks, more slowly than salts

3. Short back pressure



DNA goes back in concentration chamber

4. Iterate concentration & back pressure

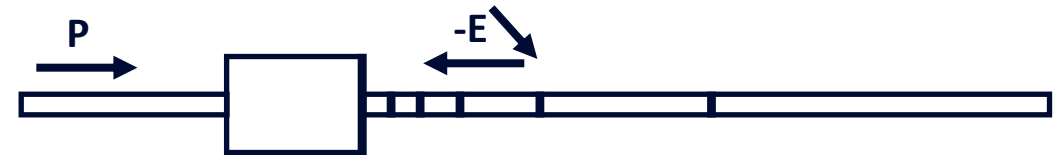


5. Finalize concentration



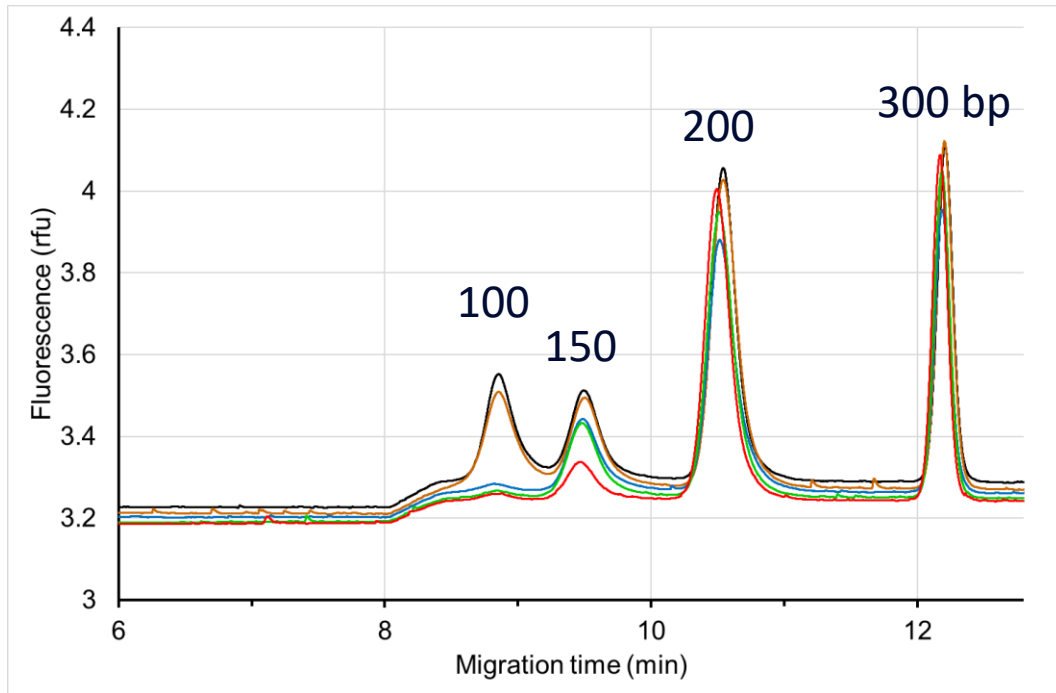
DNA no longer leaks

6. Separate according to size

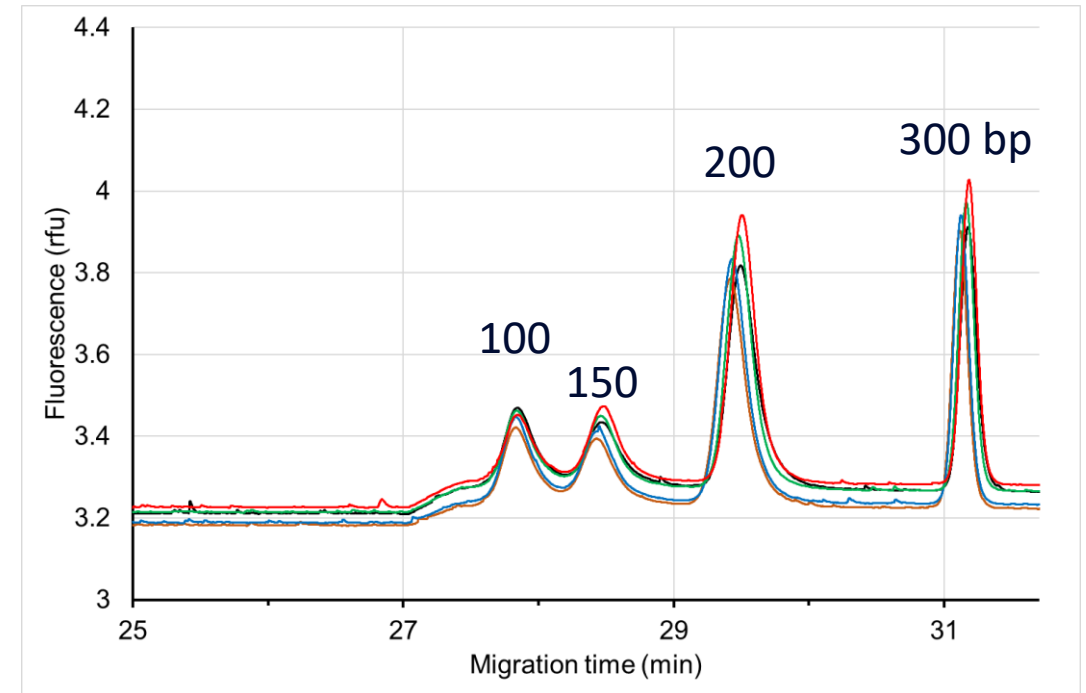


Backflow is effective to extract DNA from salts

No backflow



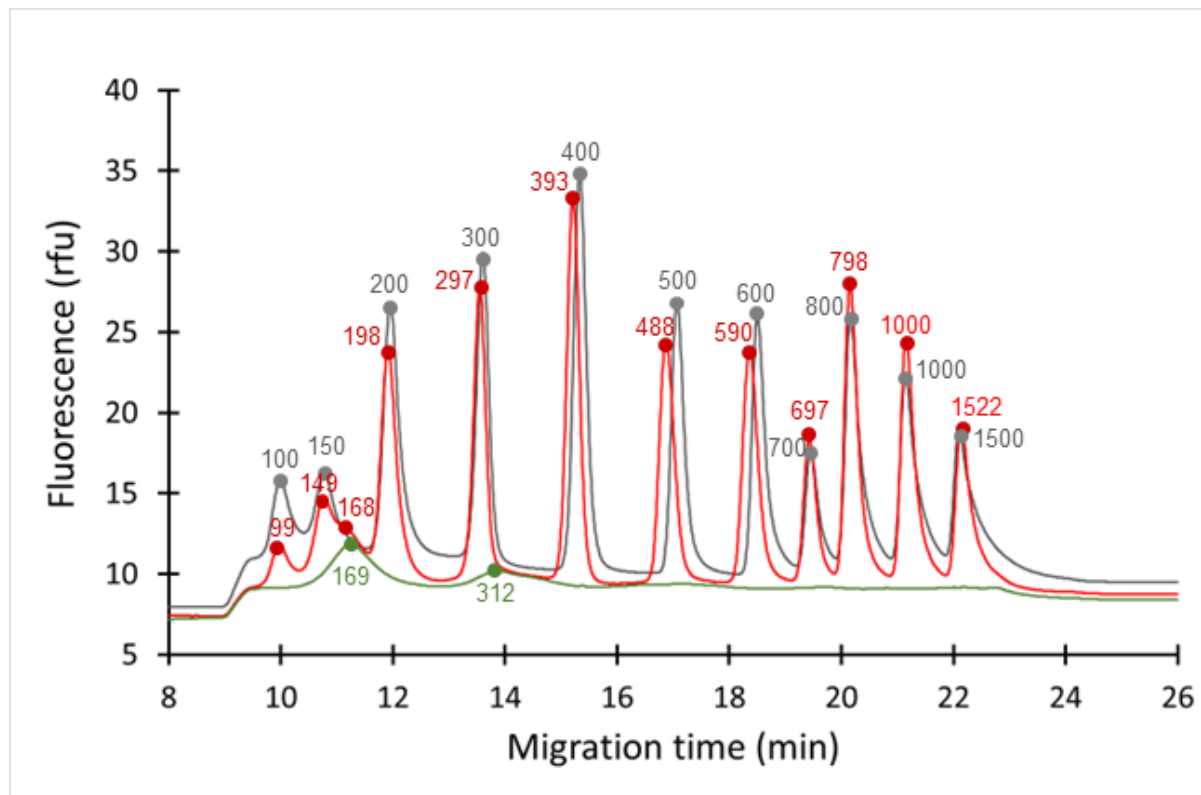
Seven backflows



—0mM NaCl —20mM NaCl —50mM NaCl —100mM NaCl —130mM NaCl

Manage lipids and proteins

Perform a standard proteinase K digestion with detergent before BIABooster analysis



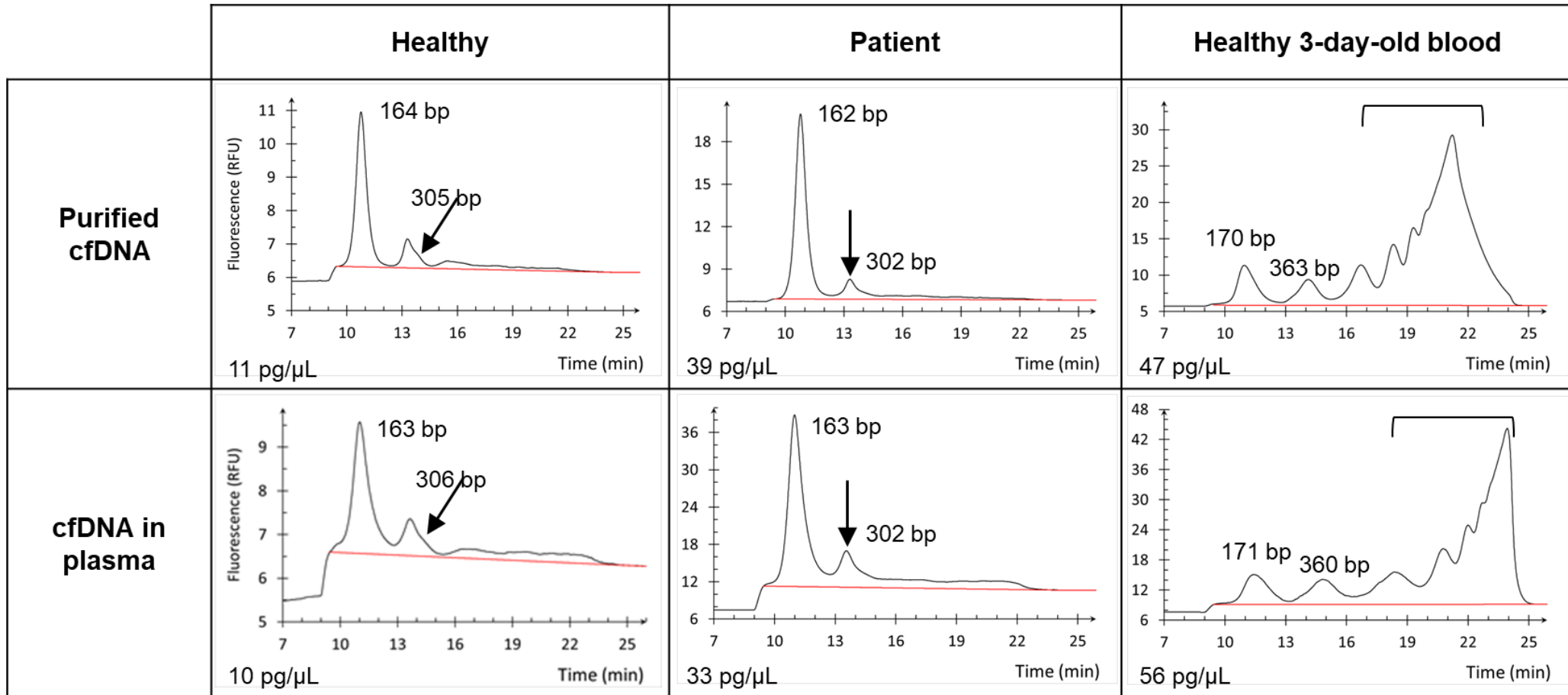
DNA ladder

Human plasma sample

9 μ L plasma + 1 μ L 10 \times DNA ladder

DNA migration is not affected by plasma matrix

Application to cfDNA in human plasma



More than 3000 clinical plasma samples have been analysed in this way

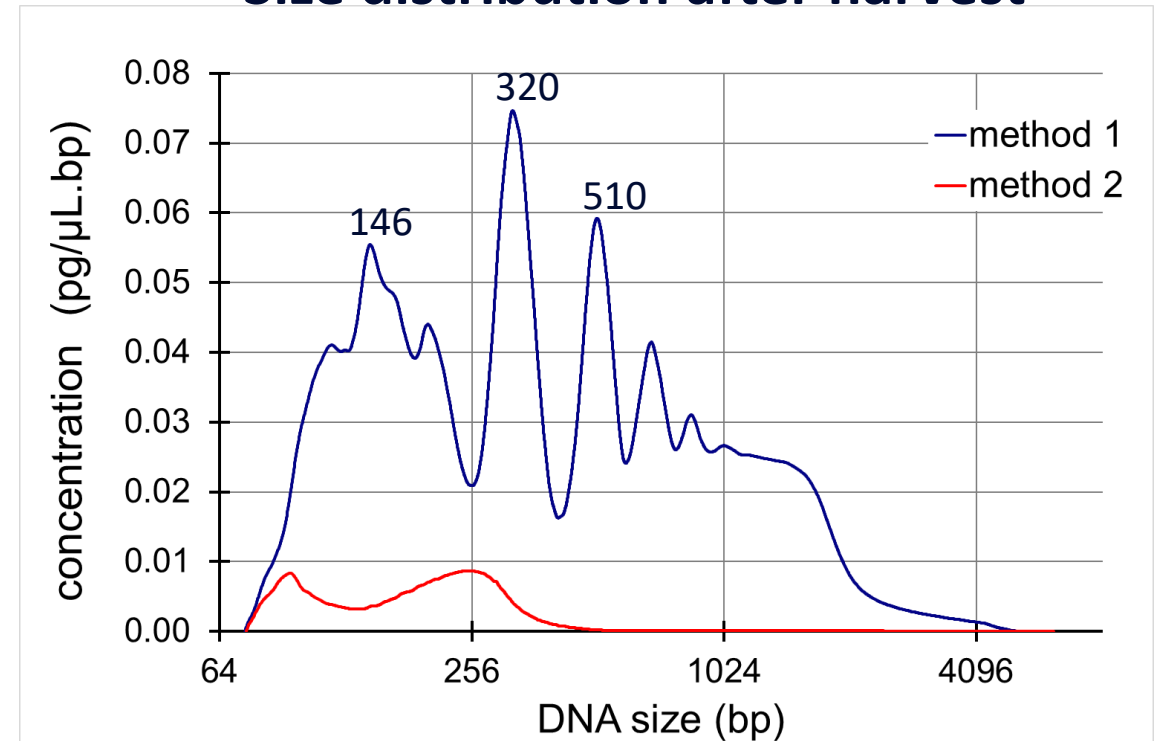
Monitoring Residual DNA

DNA concentration

Harvest method 1 : 58 pg/ μ L

Harvest method 2 : 2 pg/ μ L

Size distribution after harvest



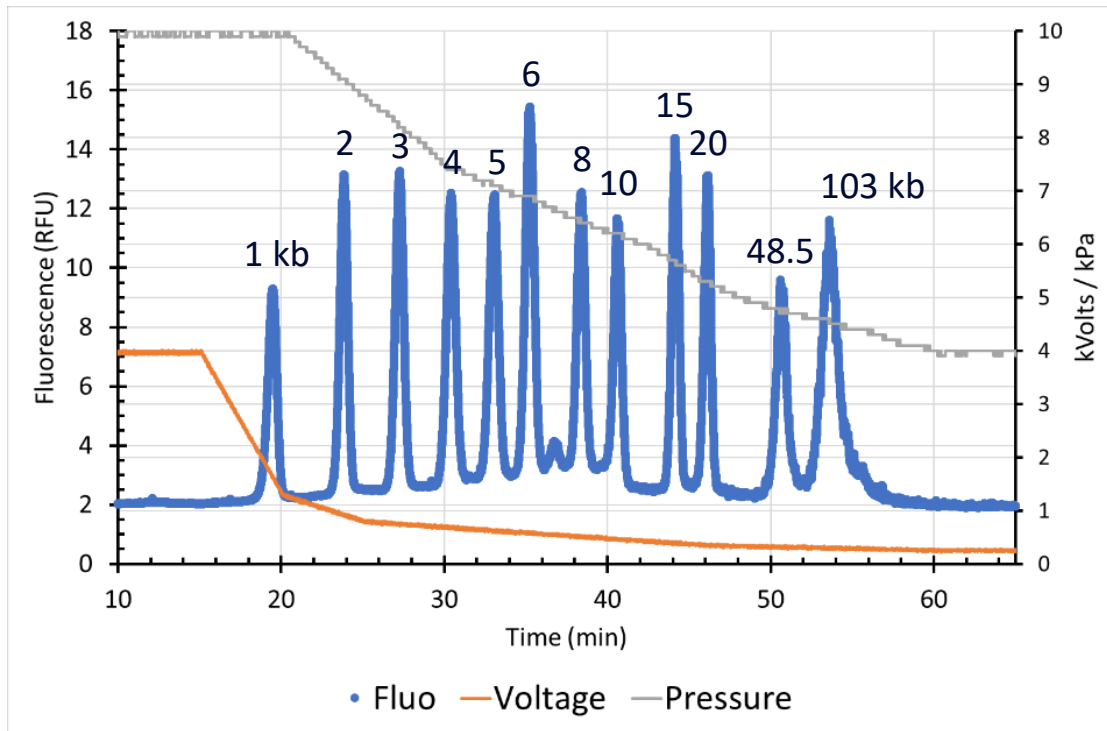
The method enables cellular DNA characterisation along bioproduct purification

Large DNA, up to 150 kb

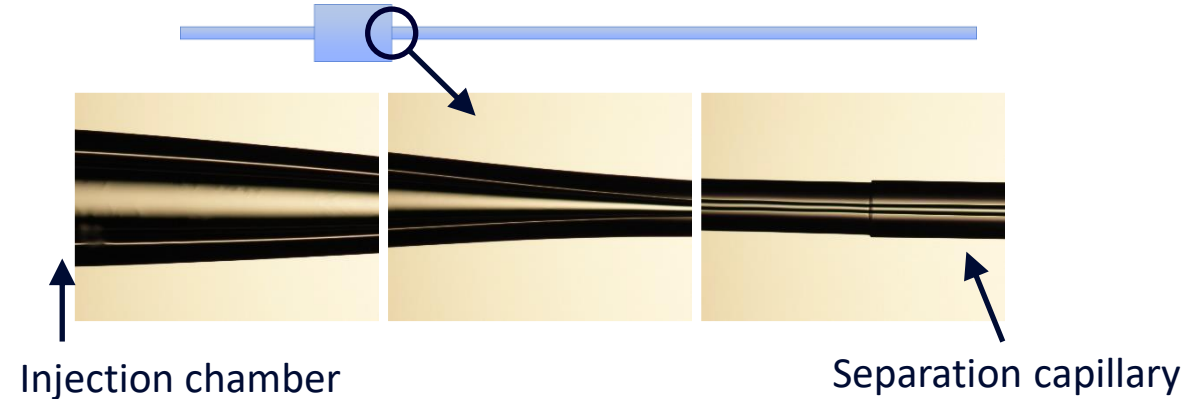


Additional innovations for large DNA

Pressure gradual decrease
superposed to voltage decrease



Concentration area without dead volume

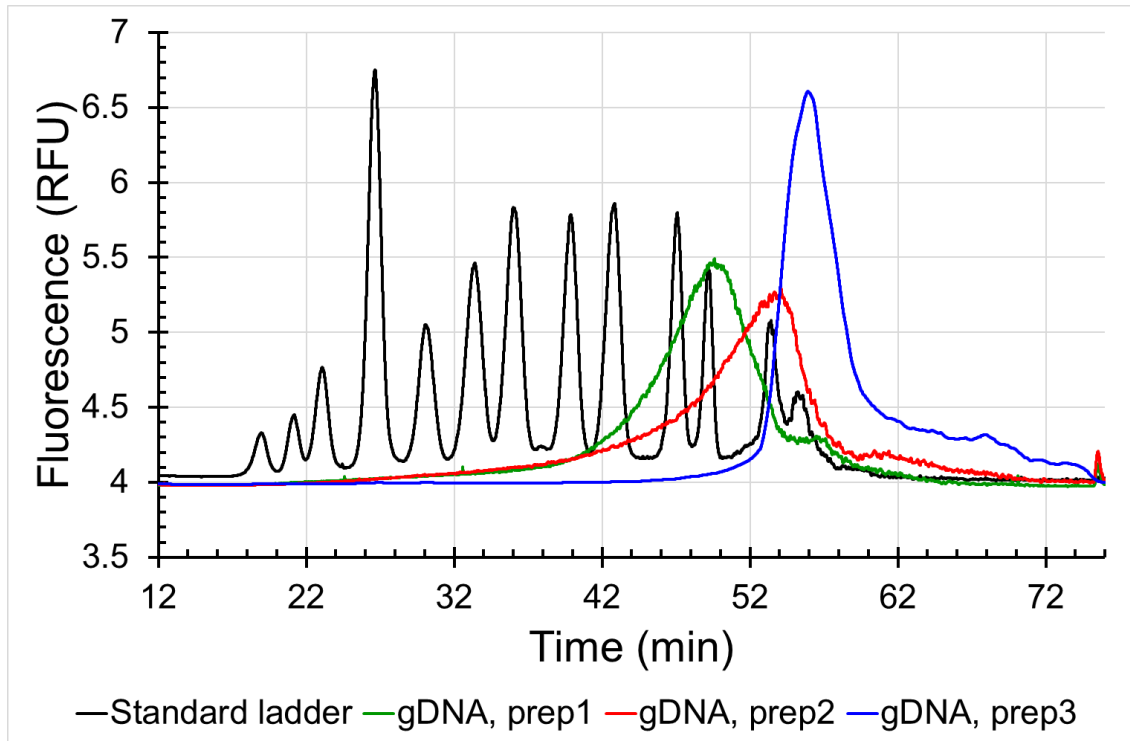


New manufacturing technology for capillary device
The diameter changes progressively along 3 mm
No dead volume in the concentration area

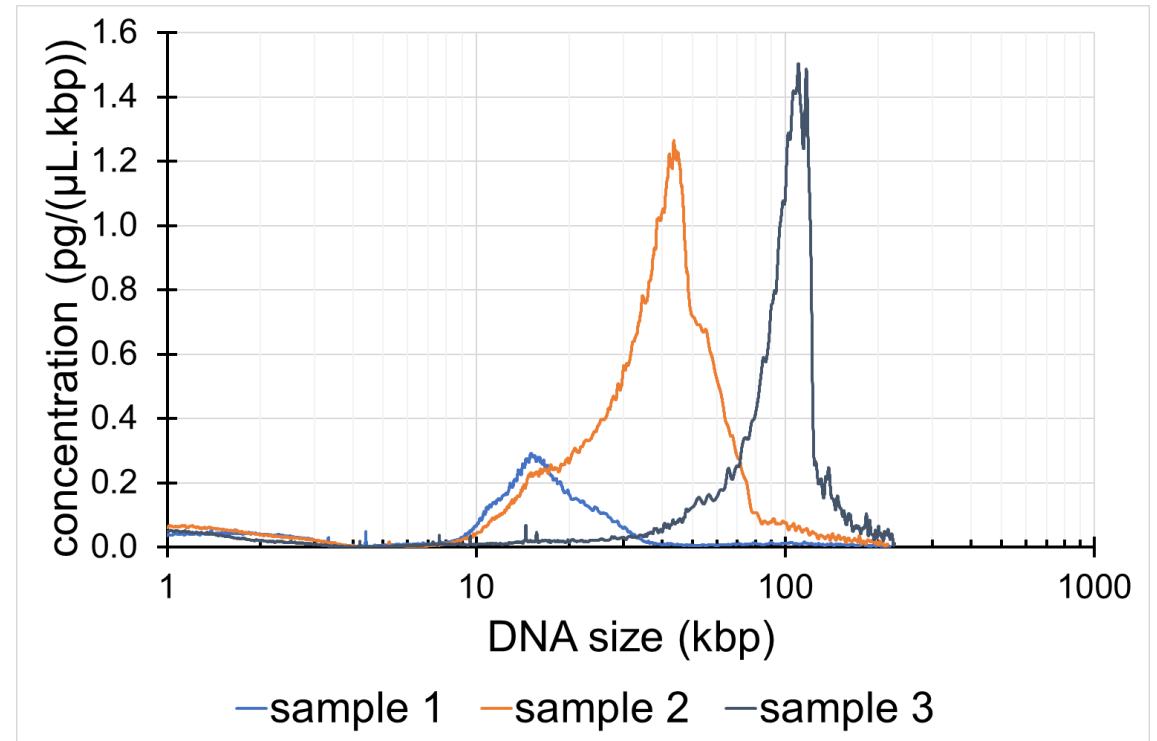
- Sizing range : 1-150 kb
- 0,8 μL injected
- Limit of detection: 20 fg/ μL at 5 kb – 50 fg/ μL at 100 kb

Genomic DNA samples

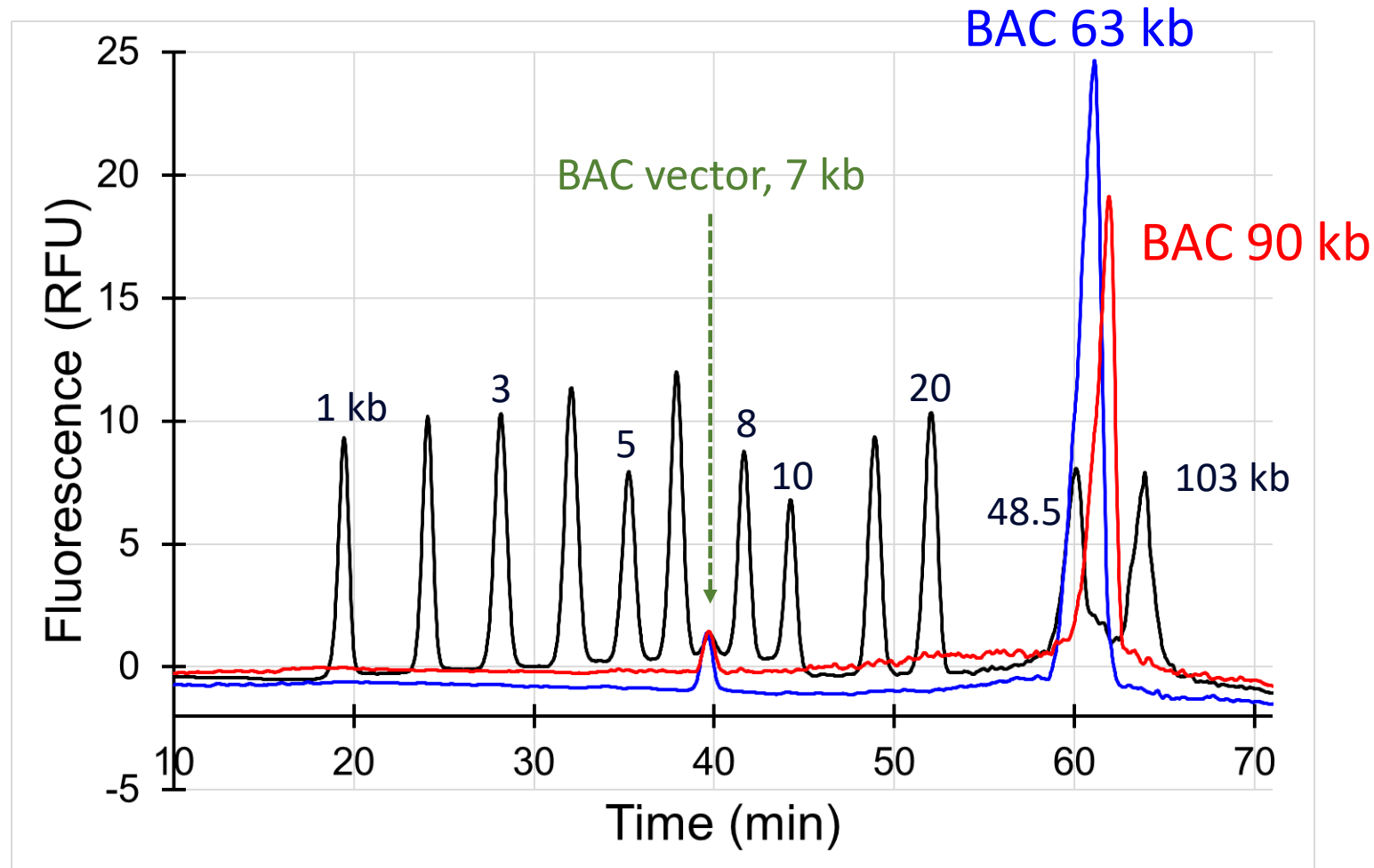
Comparison between extraction methods



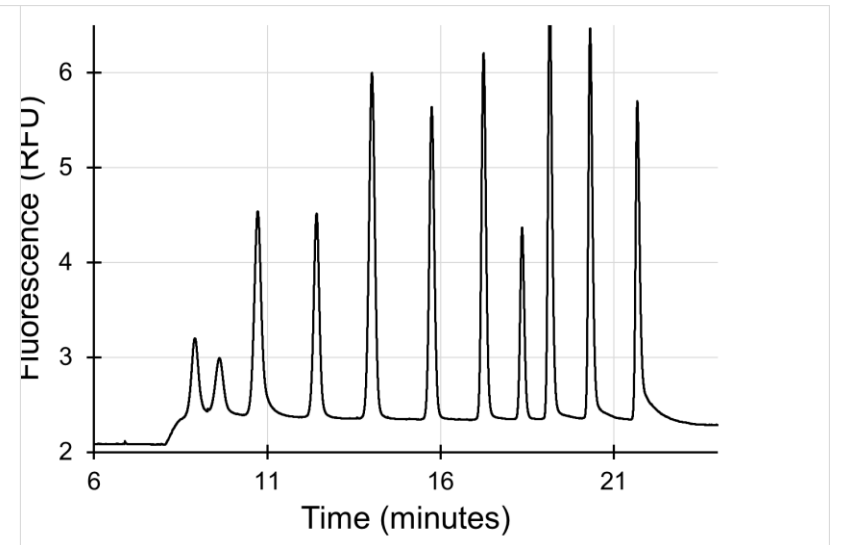
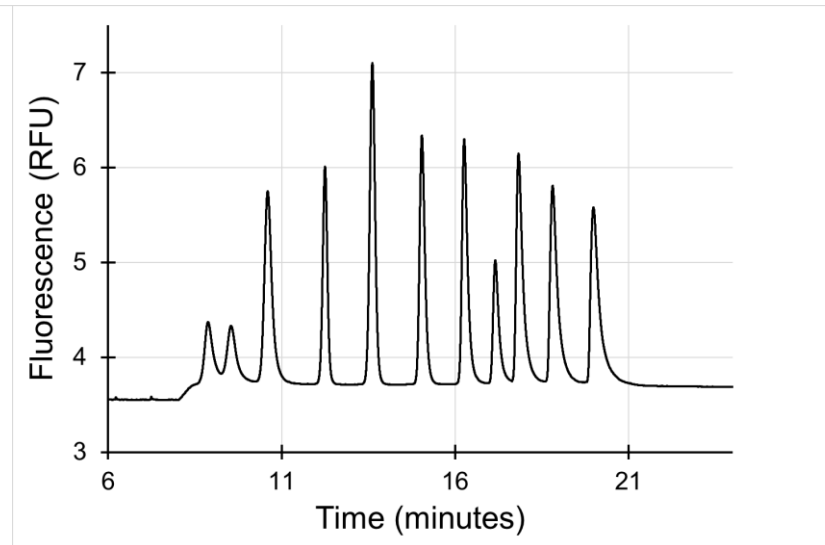
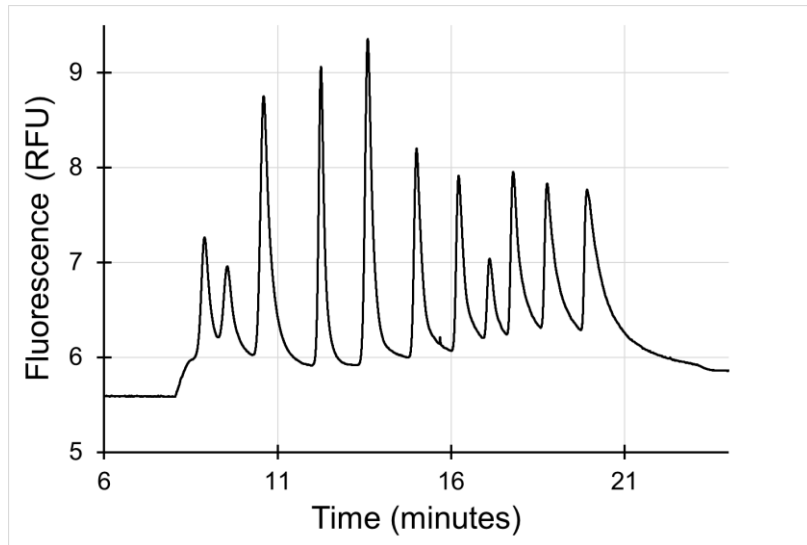
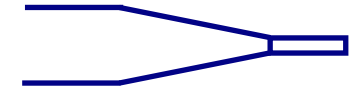
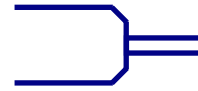
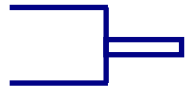
Comparison between sample sources



Bacterial Artificial Chromosomes characterisation



Shape of the concentration junction and resolution



Smooth transition improves resolution also for smaller DNA

DNA fractionation

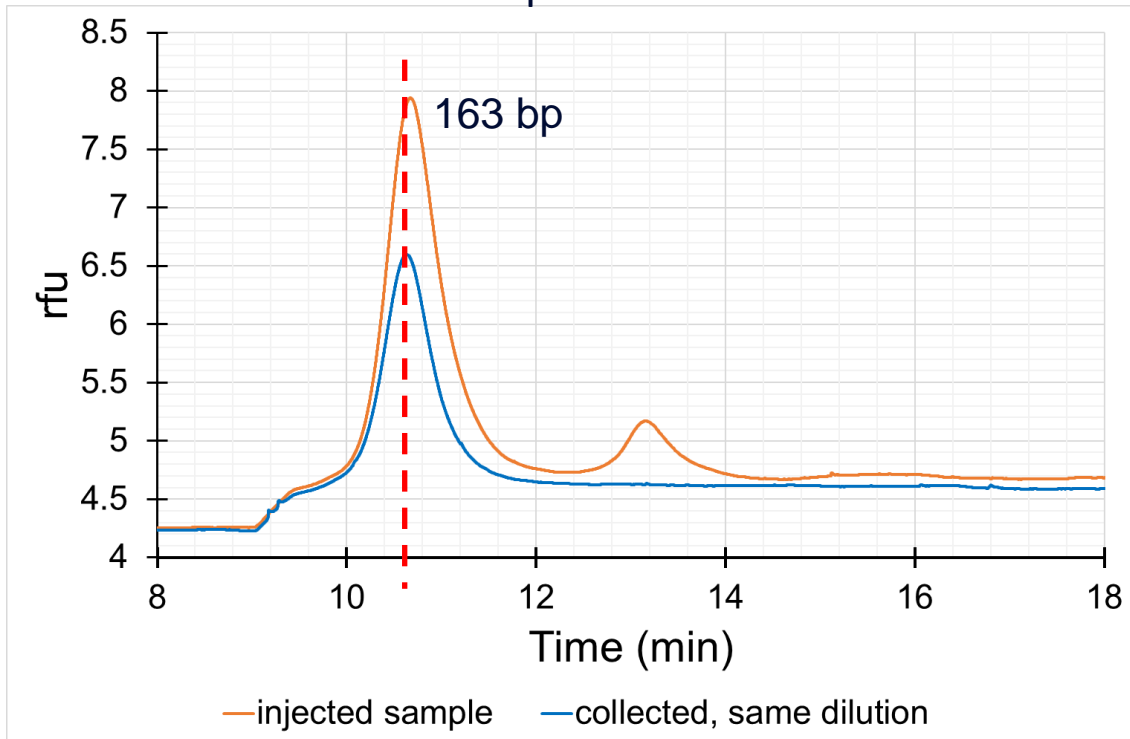
Application to cfDNA for NIPT and genomic DNA for long read sequencing



DNA fractionation at 1-10 μ L scale

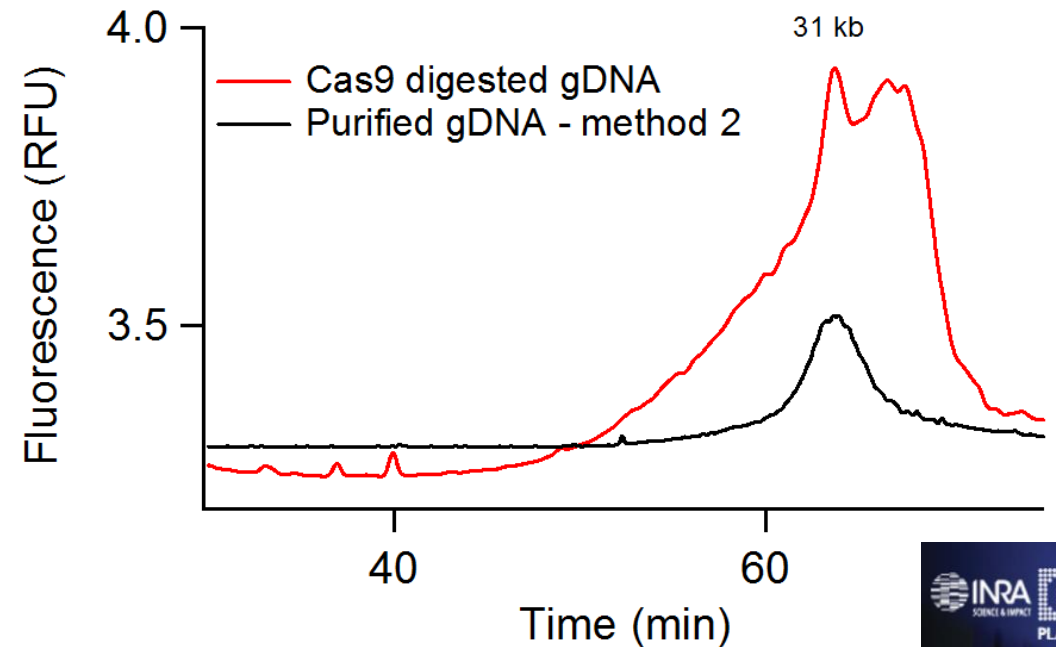
cfDNA for NIPT

Collect ≤ 160 bp



fragment isolation for long read sequencing

Extract fragment from 500 Mb Medicago genome



Yield $\geq 90\%$ - < 10 ng at junction - Fraction volume : 10-15 μ L - sequencable

A new Multi Channel CE-LIF

A new versatile four channels CE-LIF has been developed to take fully advantage of the BIABooster technology

- Continuous pressure range, 20 mbar - 12 bars.
- Current measurement in each channel
- Up to 8 buffers on board
- Refrigerated samples



Acknowledgment



Methods

A. Boutonnet

M. Mano

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Capillary device

J. Fabre

MA El Khaldi



Physics

A. Bancaud

P. Bruand

Capillary device

D. Labat

G. Bouwmans



A. Bellec (BAC samples)



A. Castinel (gDNA samples)

C. Donnadieu



G. Favre (cfDNA samples)

A. Pradines



**Thank you
for your attention**