

μ LAS

High sensitivity DNA sorting and separation

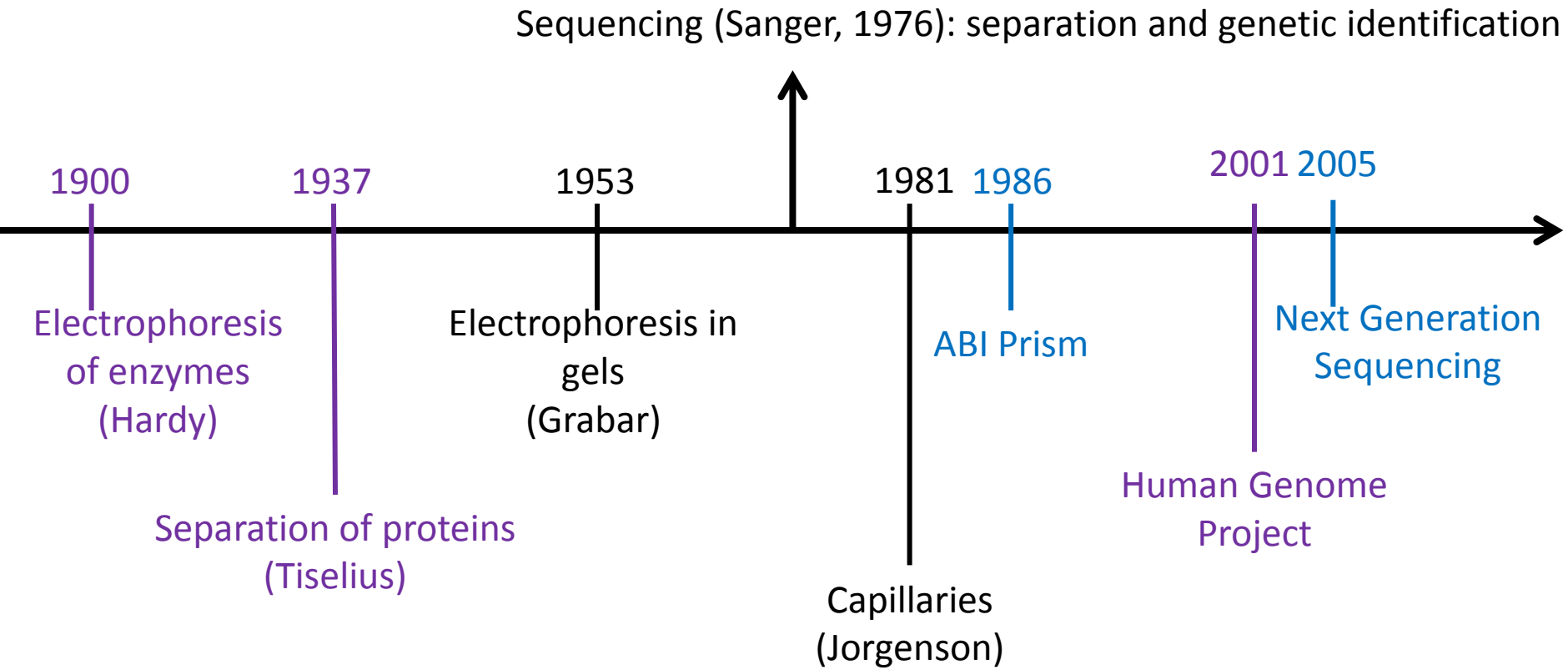
Aurélien Bancaud

abancaud@laas.fr

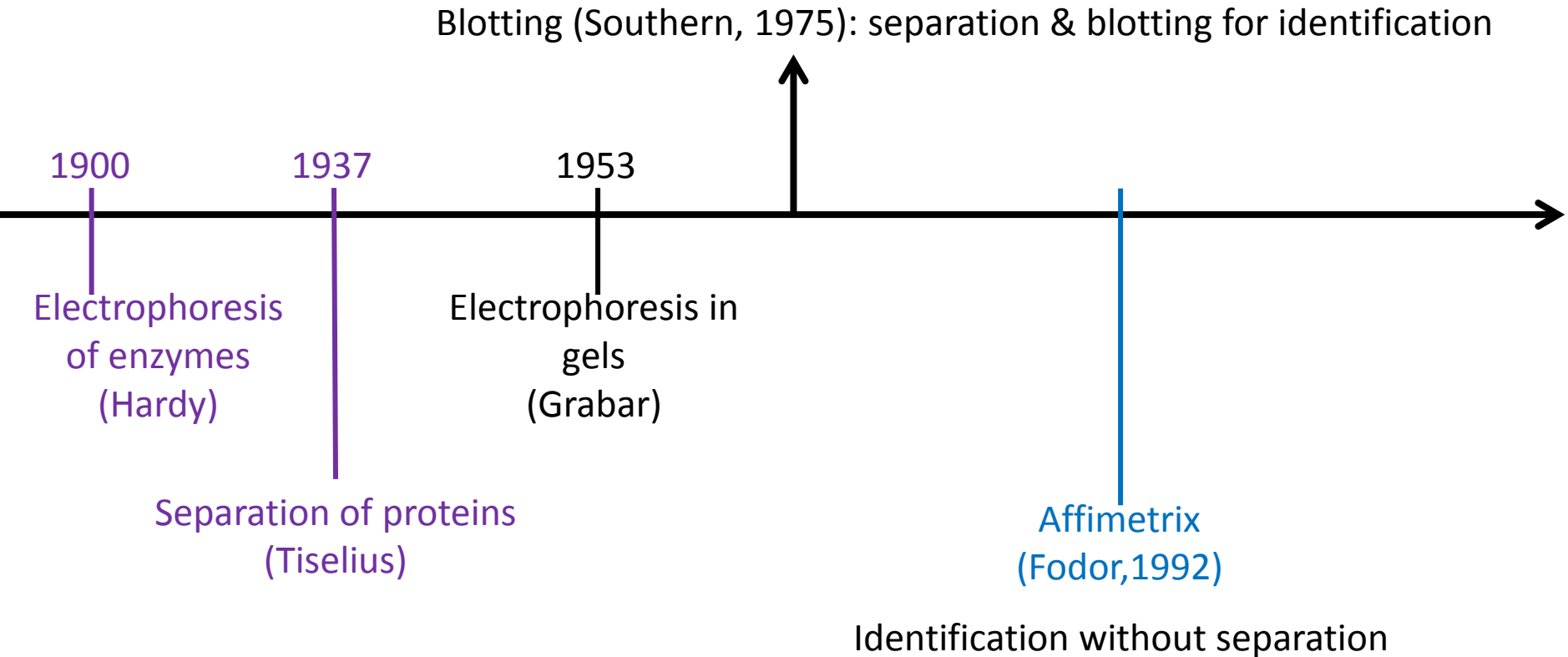
INRA, 24/06/2016

- **Technologies de processing de l'ADN: point de vue de l'ingénieur**
Quelles opportunités pour la microfluidique
- Description du phénomène μ LAS
- DNA concentration and separation by μ LAS
- Industrial transfer to Picometrics Technologies
- Longs ADN et fractionnement en flux continu
- Conclusions

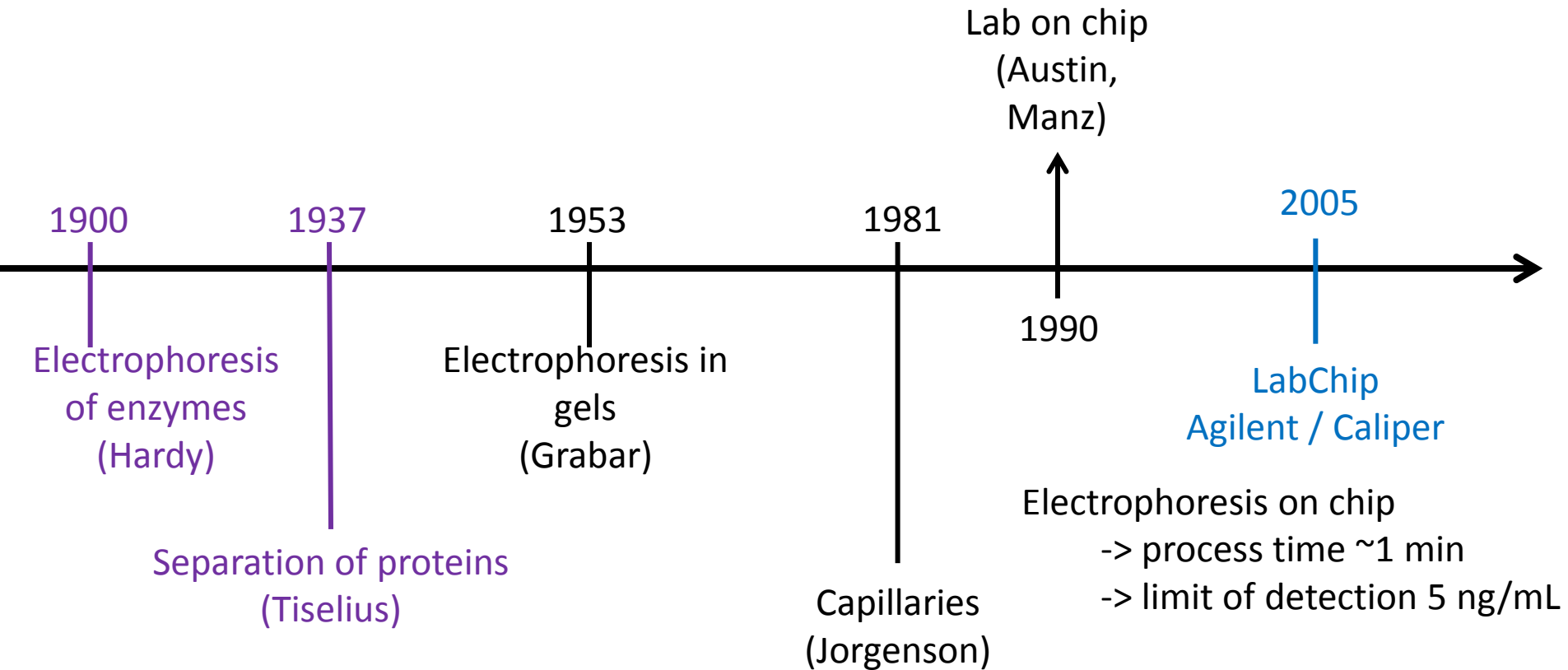
Timeline for DNA processing technologies



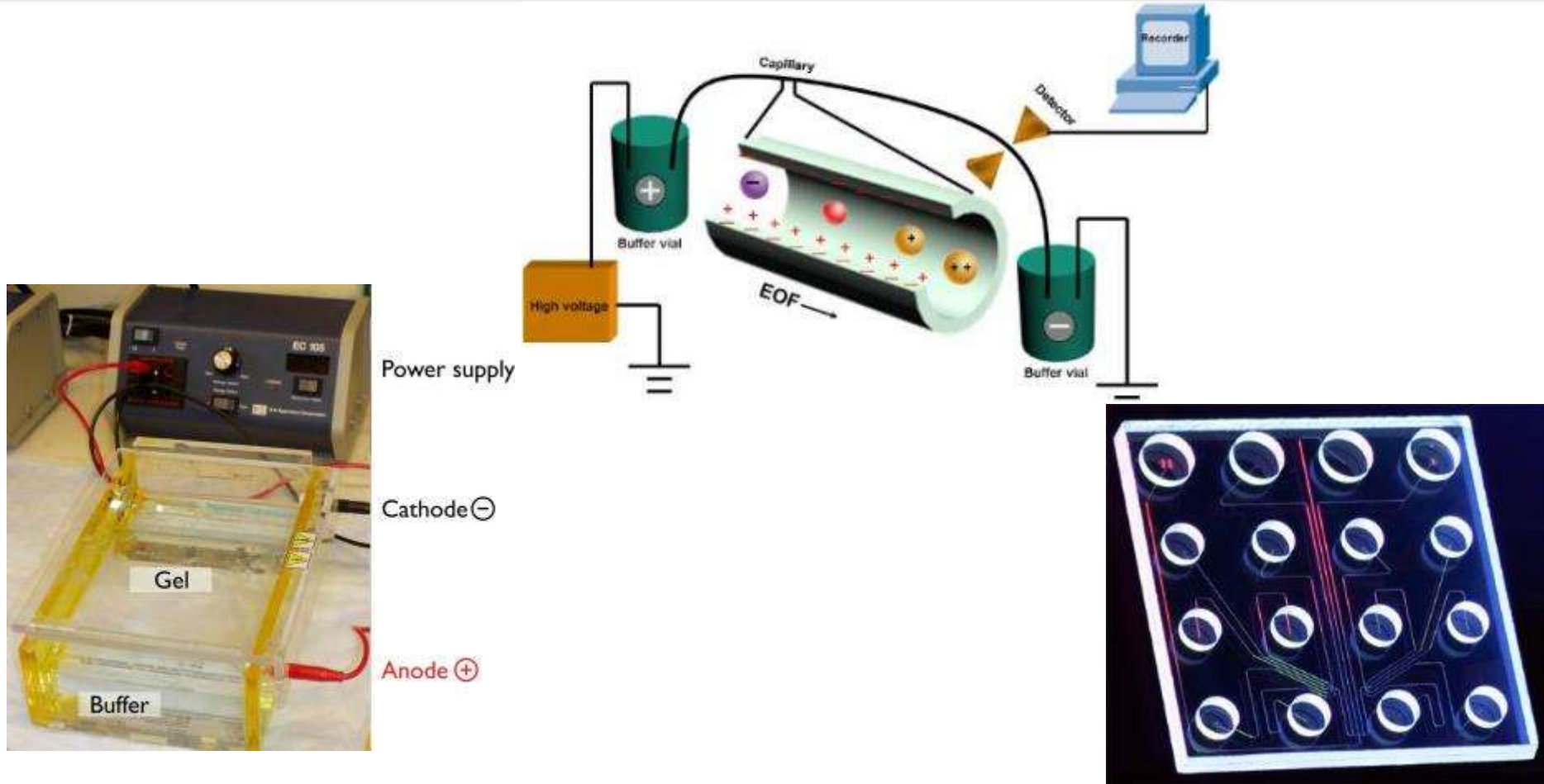
Timeline for DNA processing technologies



Timeline for DNA processing technologies



Evolution of the format for separation

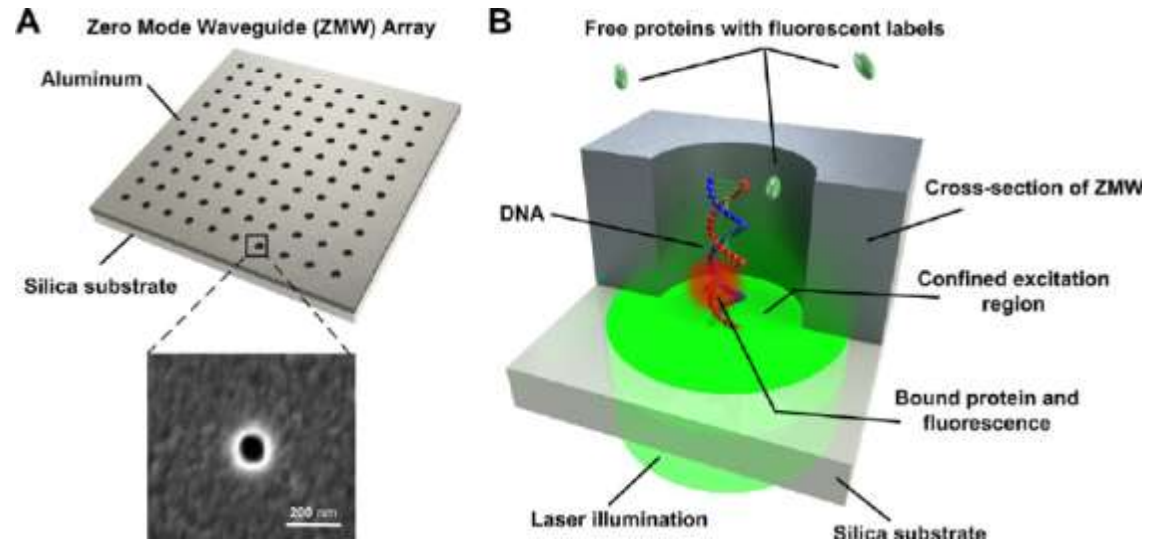


DNA separation: electrophoresis remains the main technology

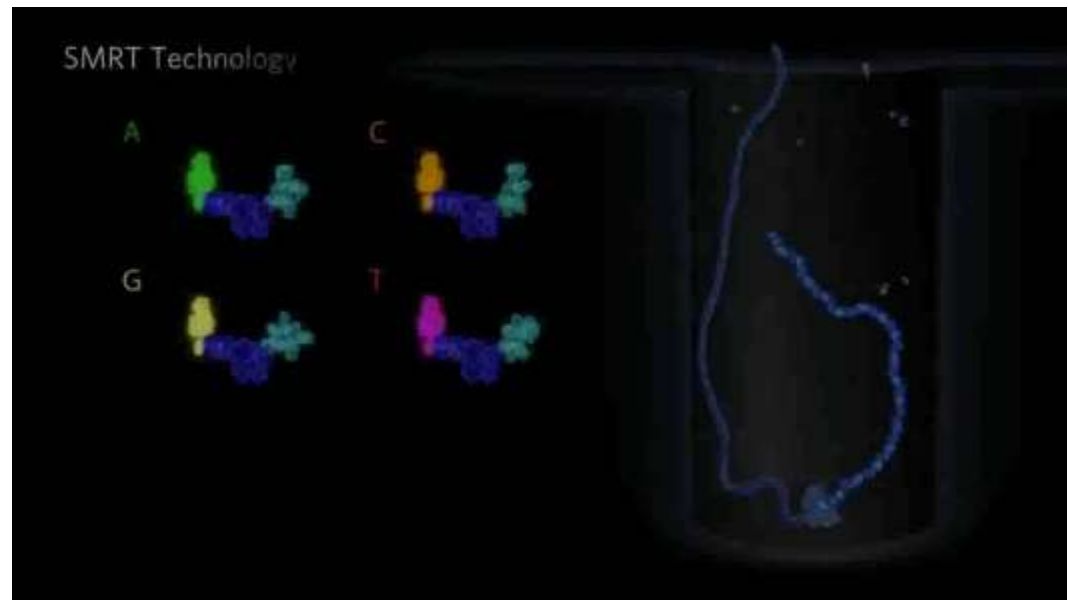
-> are there additional opportunities for microfluidics ?

-> DNA sequencing tells us that some opportunities are still available

Sequencing by Pacific Biosciences (2003)

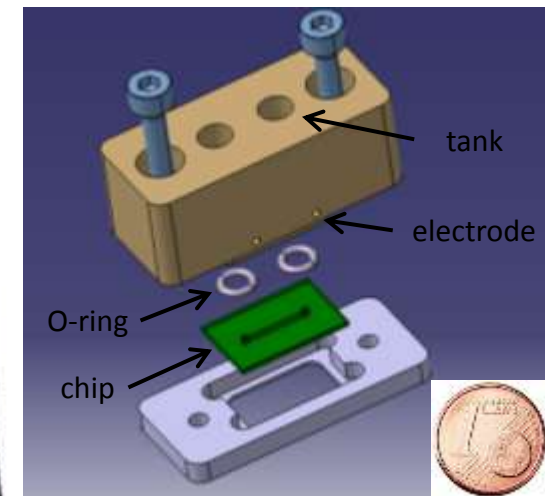
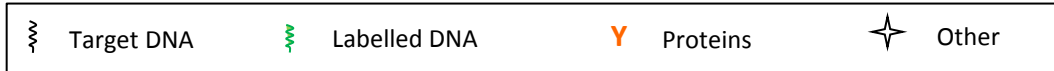
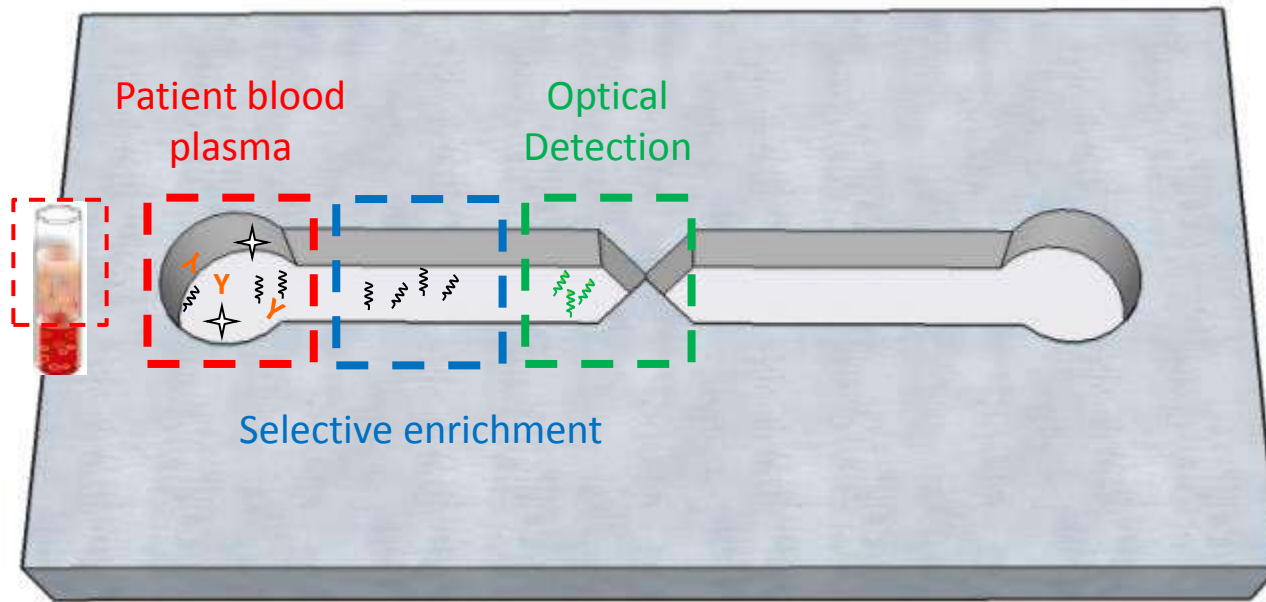


Nanofabrication & single molecule detection



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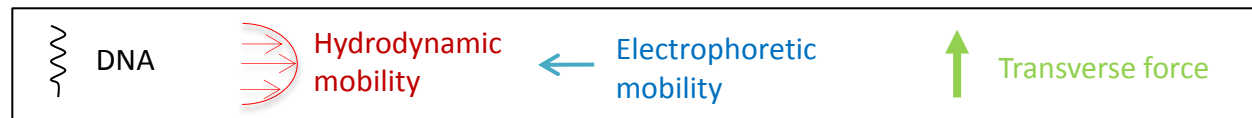
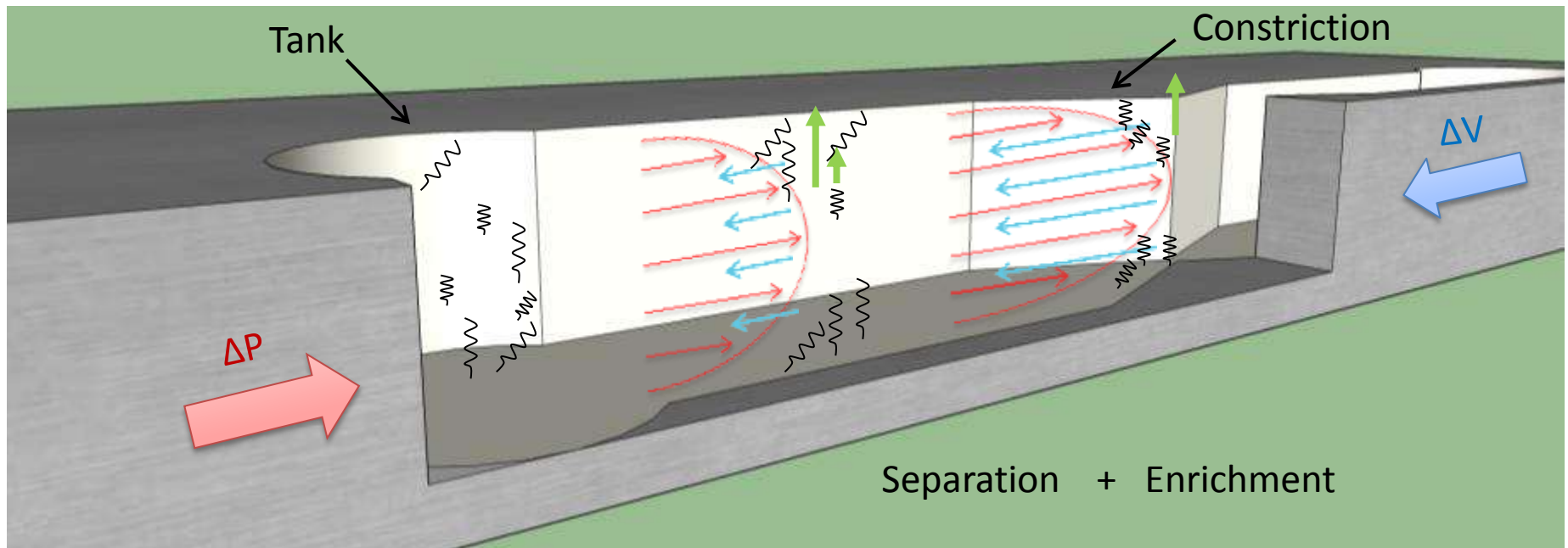
Revisiter technologies de séparation et concentration d'ADN



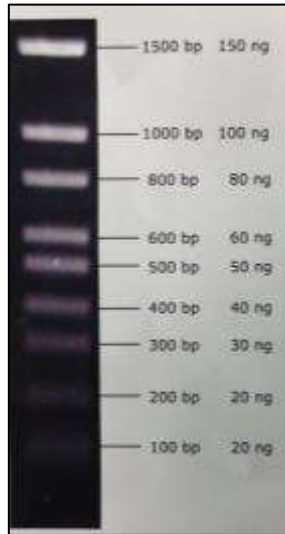
Integrated lab on a chip prototype

Selective enrichment by size

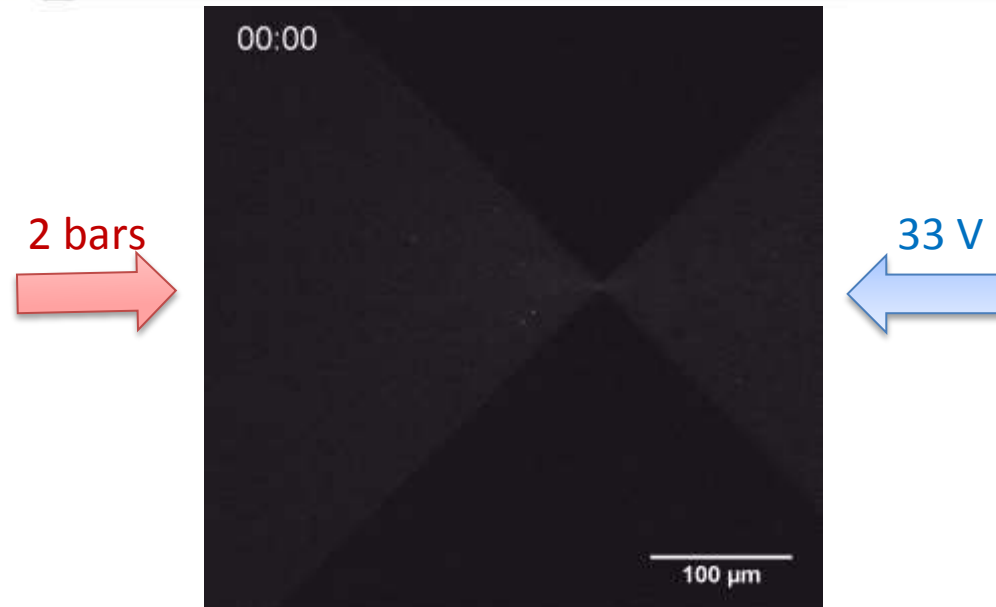
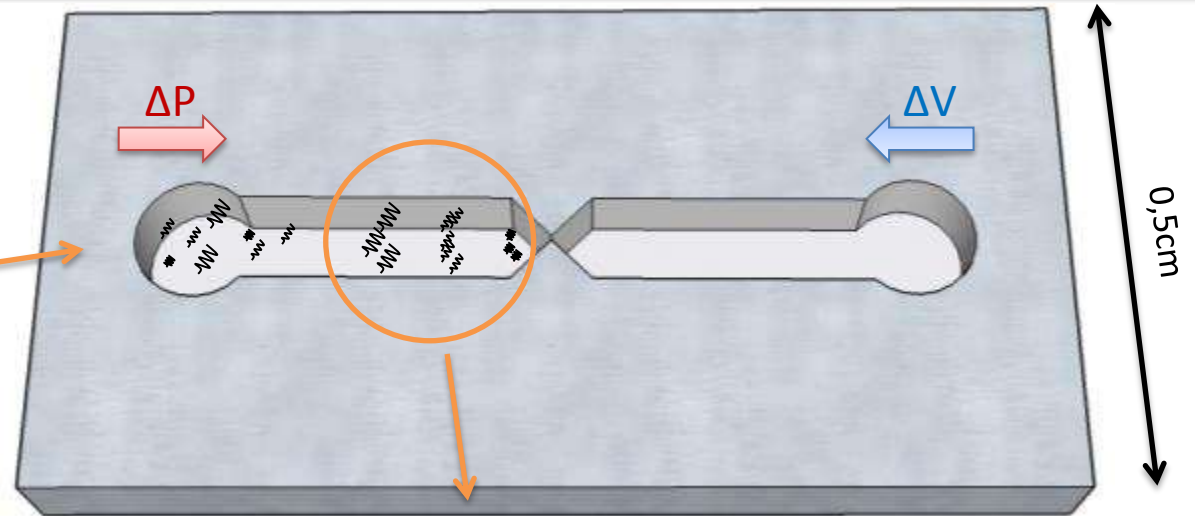
Combined action of an *hydrodynamic* actuation and an *electric field* in a *viscoelastic* fluid.



Mise en œuvre expérimentale

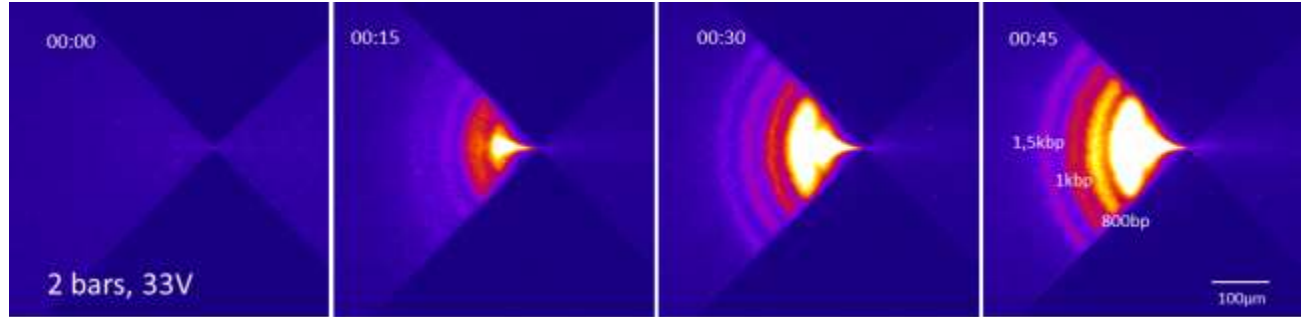


100 bp ladder,
YOYO labelled



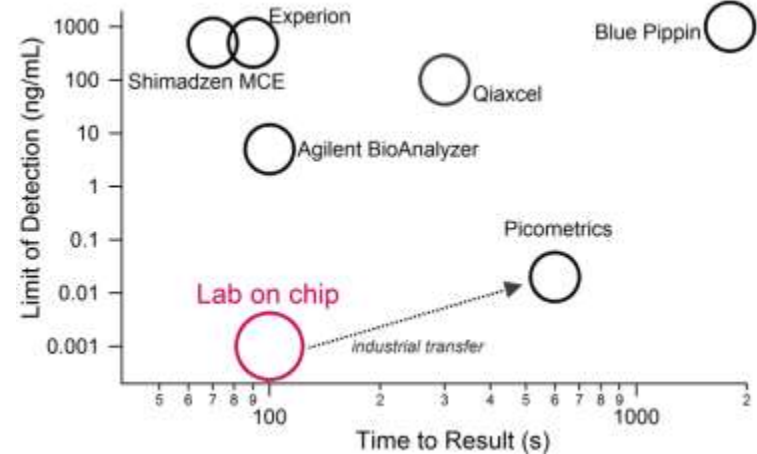
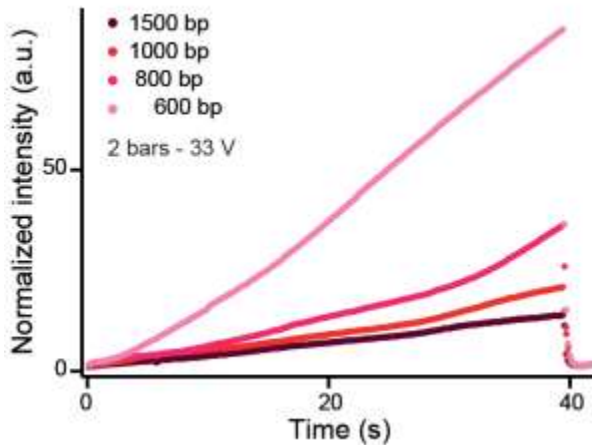
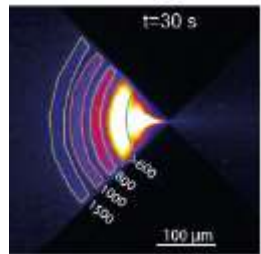
- Time lapse of DNA ladder processing

Ranchon et al, Lab on a chip 2016



- Enrichment factors determination

- Comparison vs. commercial devices sensitivity performances



✓ Enrichment (up to x1000/min)

✓ Sensitivity 1pg/mL vs. 10ng/mL for commercial devices

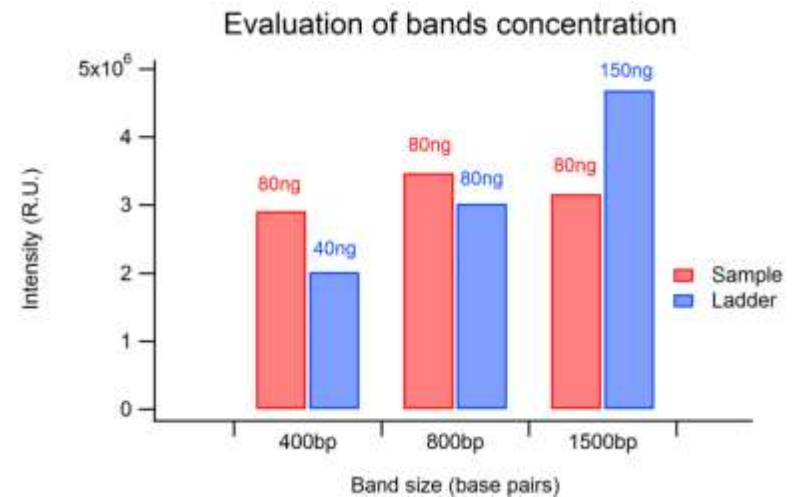
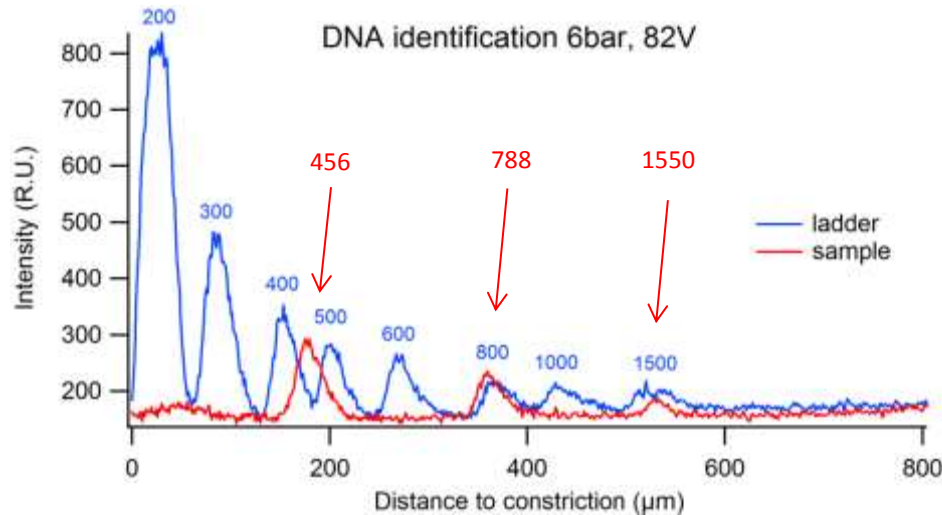
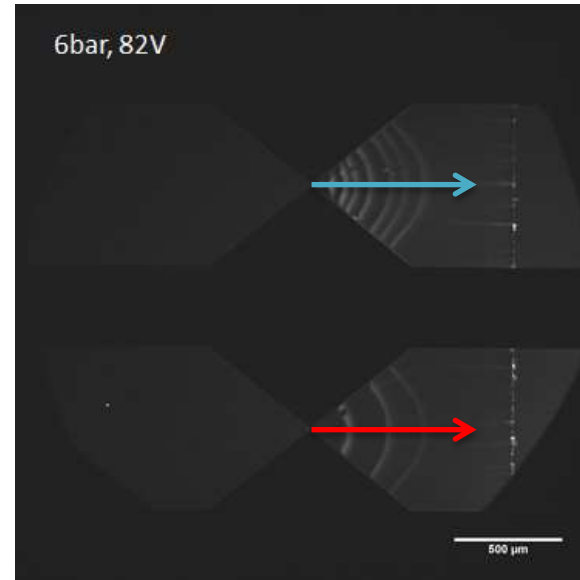
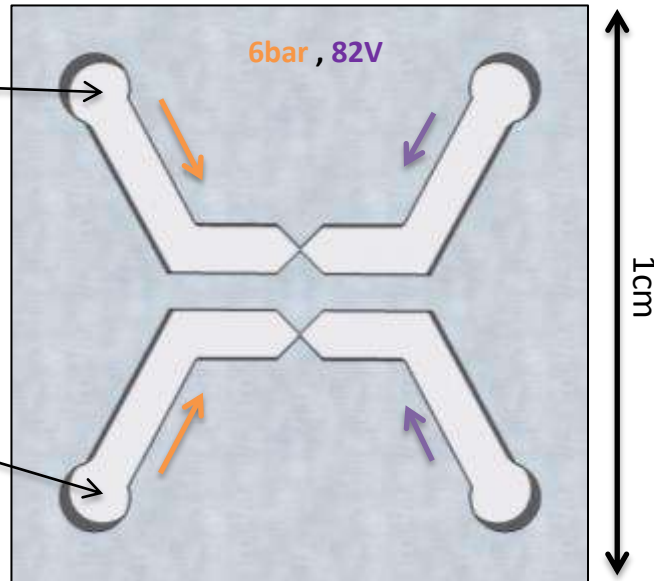
✓ Processing time < 5 min

Performances: Mesure de taille à 3%

Reference:
100bp
ladder



Sample:
• 466bp
• 798bp
• 1512bp



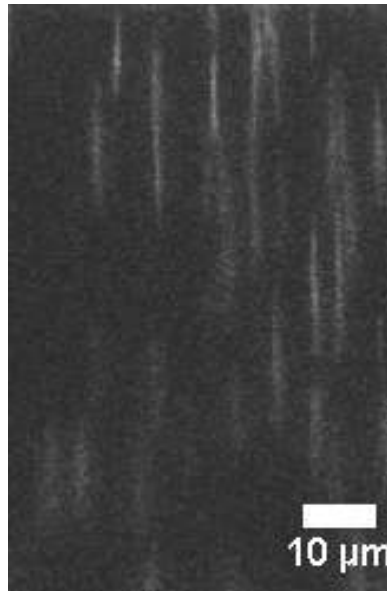
Electric & Hydrodynamic actuation

→ Objective: maintain the molecule in the field of view

λ -ADN (48.5 kpb) in a microchannel of $H = 2 \mu\text{m}$

- High intensity illumination -> DNA breaks occur in real time!
- Addition of Poly-vinylpyrrolidone (PVP)

Hydrodynamic
Force

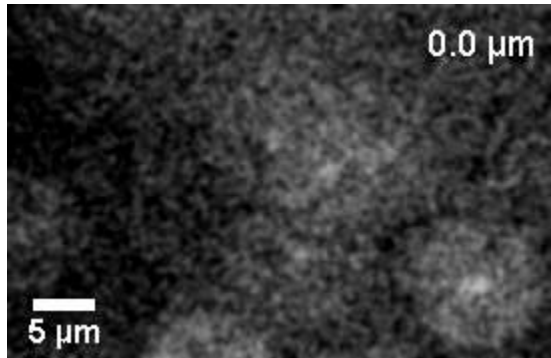


Electrophoretic
Force

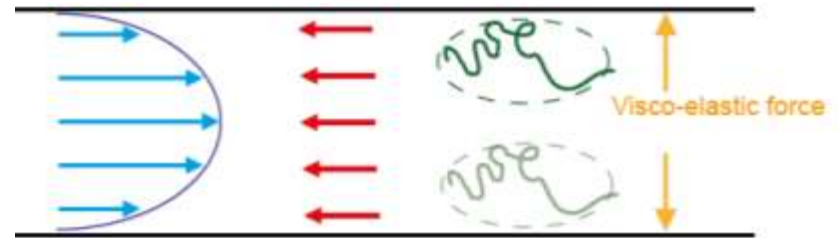
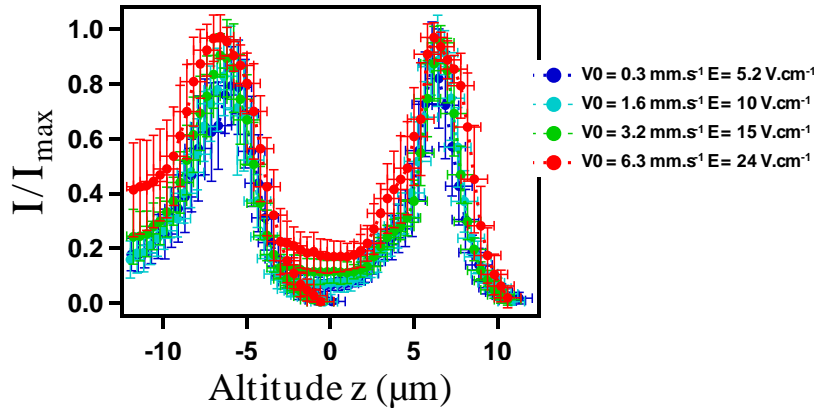
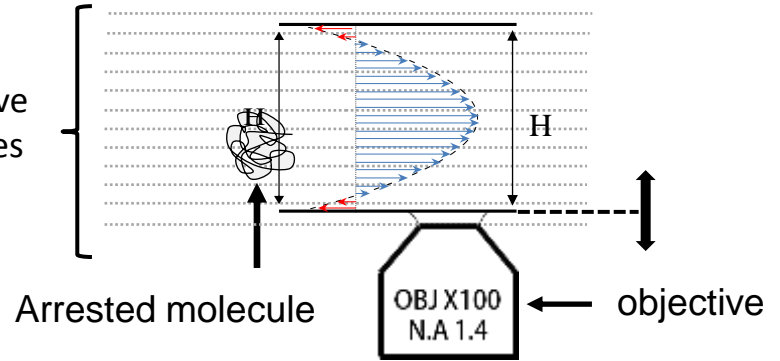


Transverse migration across the channel height

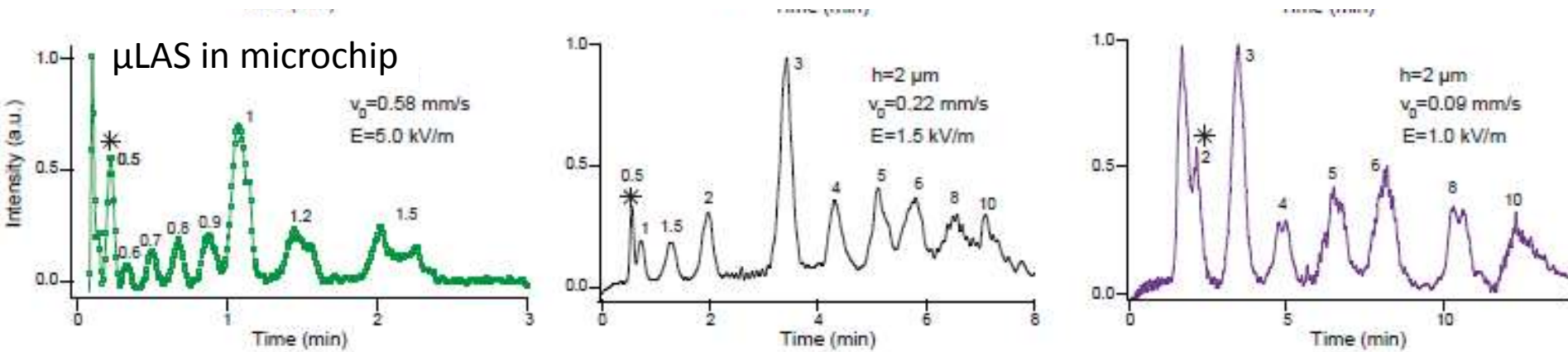
$H = 12 \mu\text{m}$



Consecutive focal planes



Optimal separations



kb-extend separation takes 3 hours according to NEB supplier

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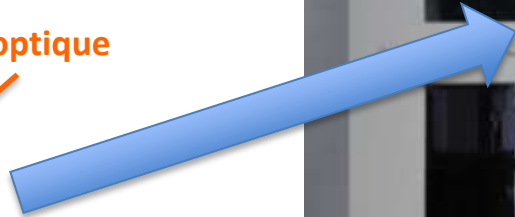
Capillaire en bobine



Capillaire installé dans la cassette de l'instrument



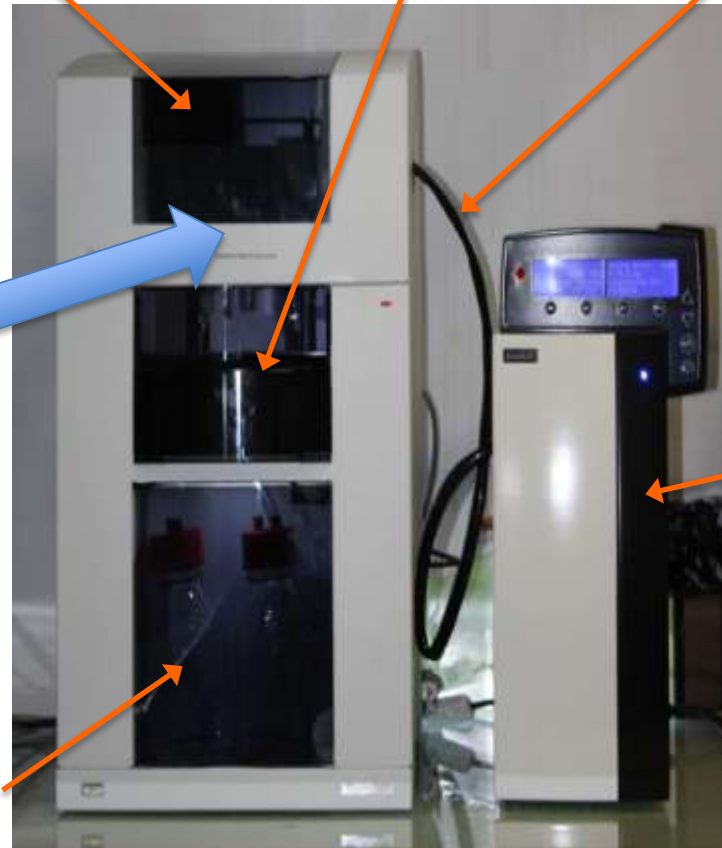
Tête optique



Chambre thermostatée

Carrousel 50 positions

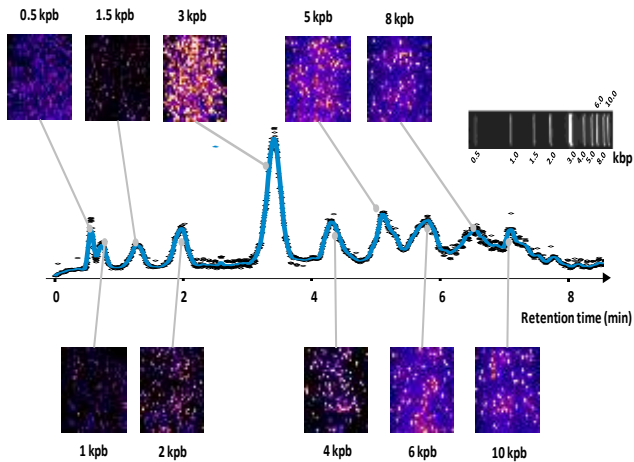
Fibre optique



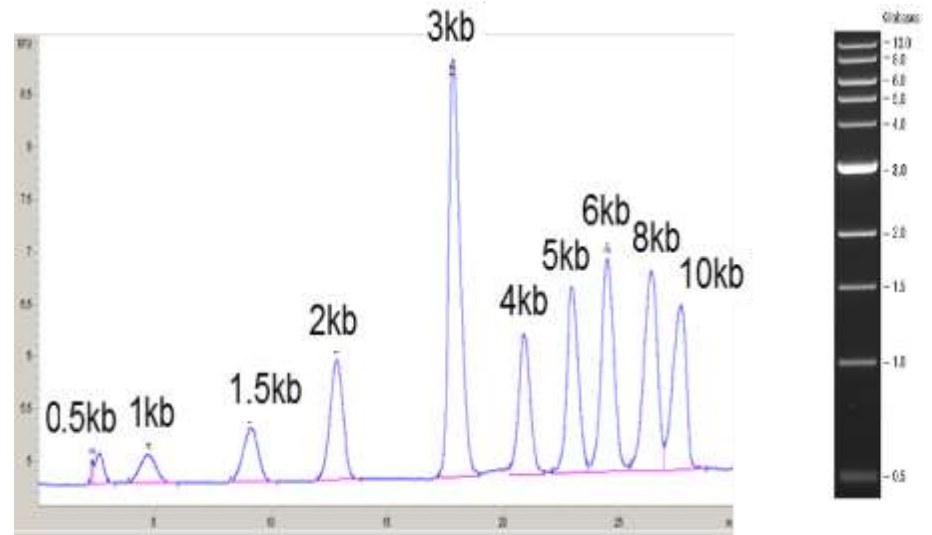
Détecteur de fluorescence

Remplissage et vidage du tampon d'analyse

Microchip format



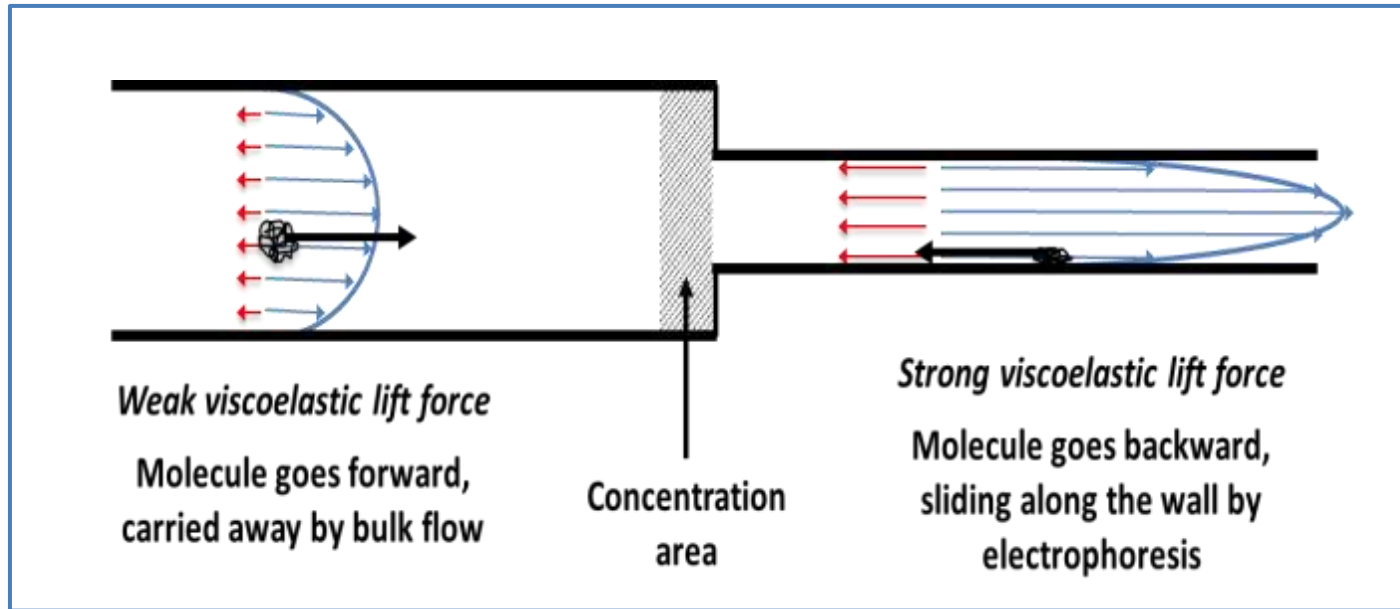
Capillary Electrophoresis format



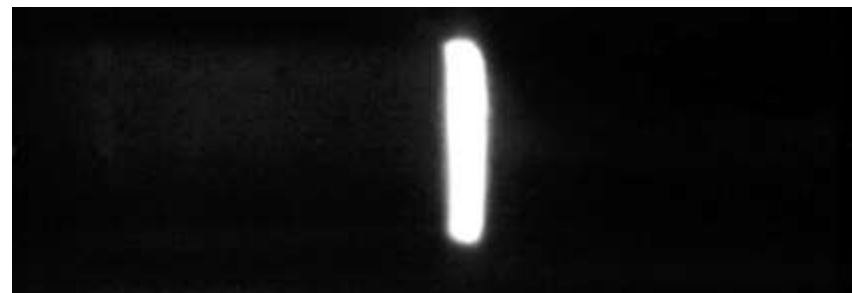
CNRS-LAAS results – courtesy of A. Bancaud

Picometrics results

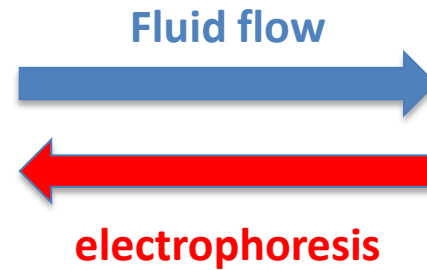
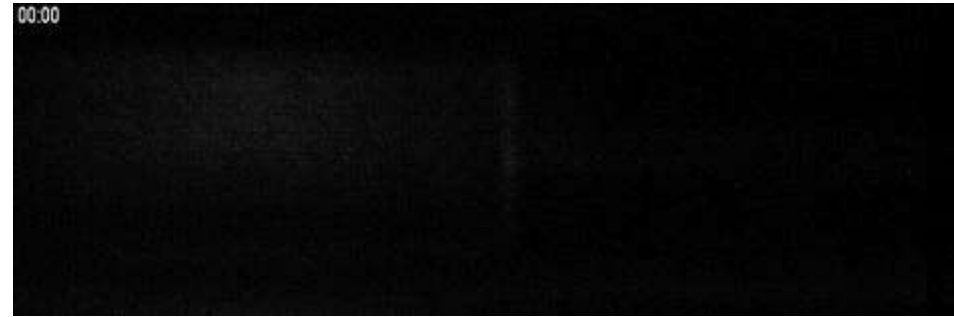
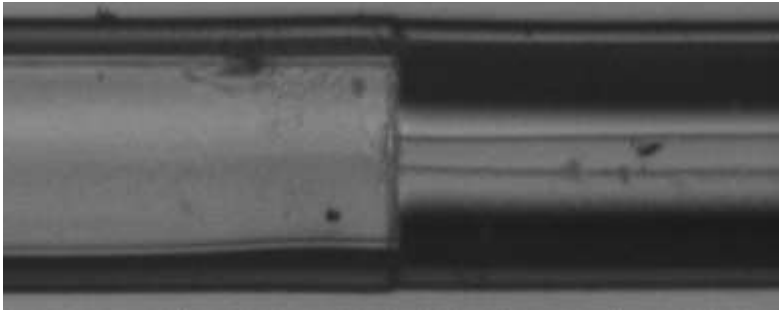
μ LAS concentration – capillary format



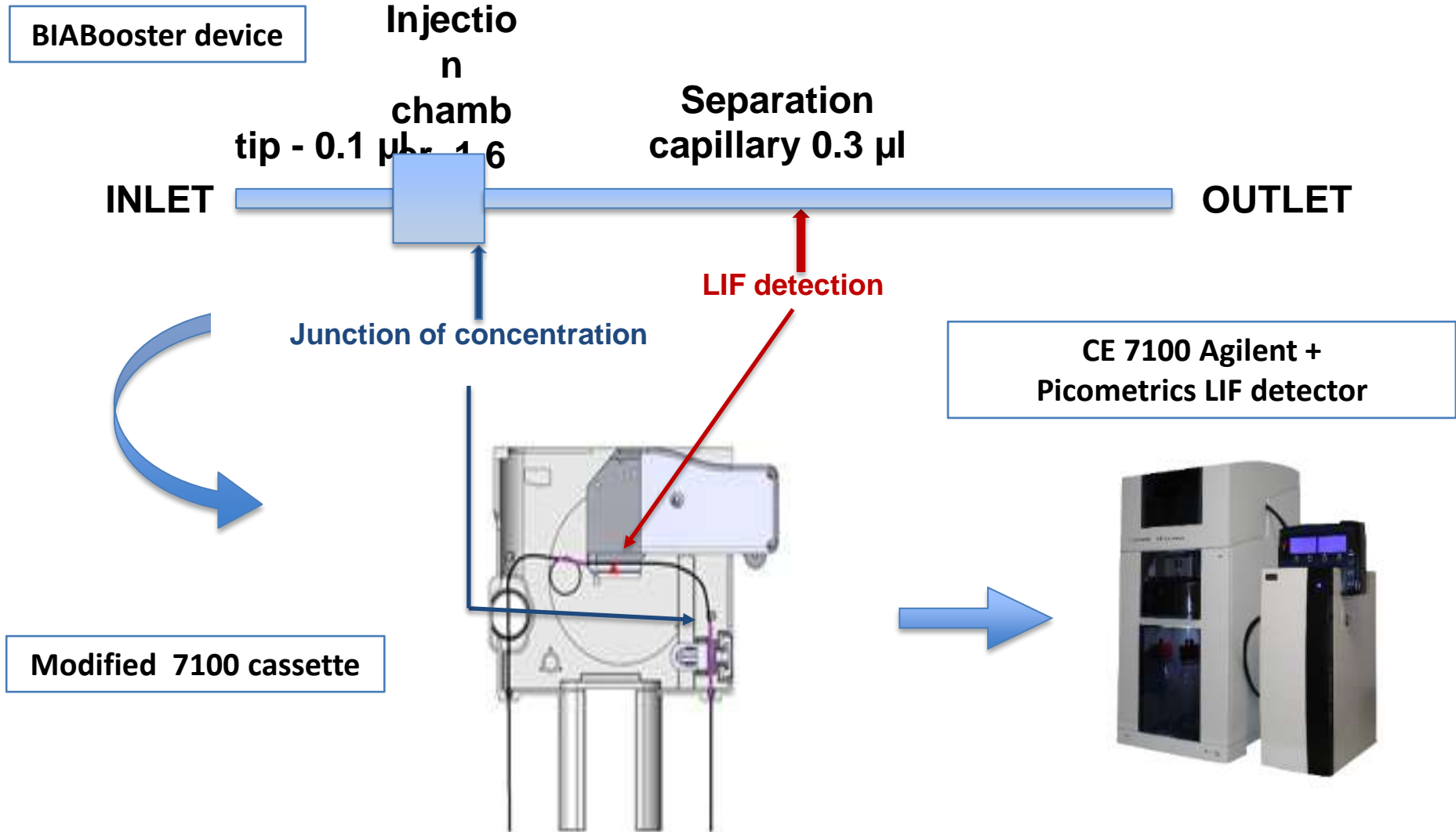
Capillary junction – 100/20 μ m



DNA concentration at capillary junction



At $t=0$, there is DNA both in the large and the small capillary
Video speed : 2X

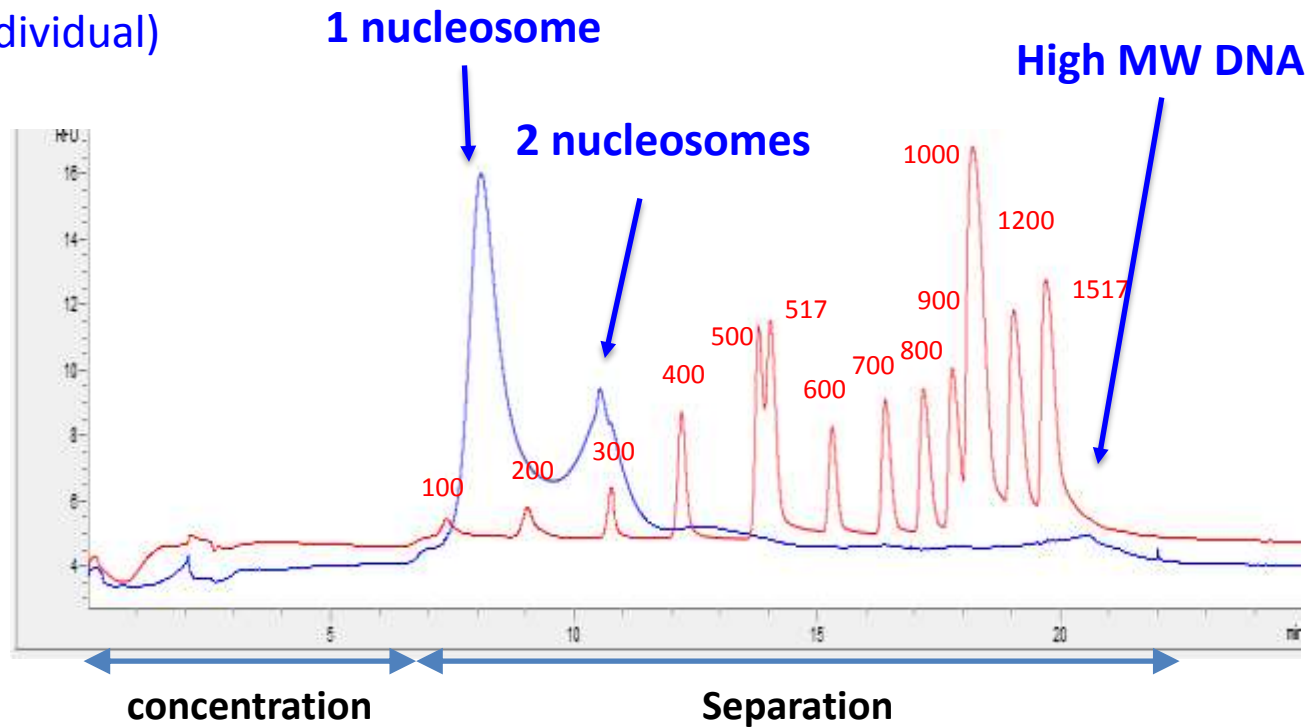


Example of application : Cell-free circulating DNA

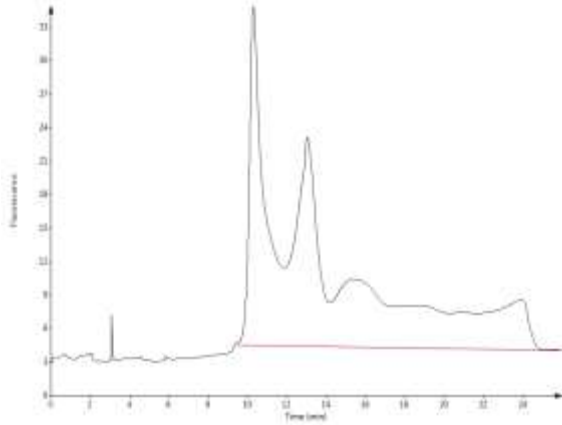
Getting a DNA profile of plasmatic free DNA is impossible using existing electrophoresis systems.

But it is an easy thing with the BIABooster :

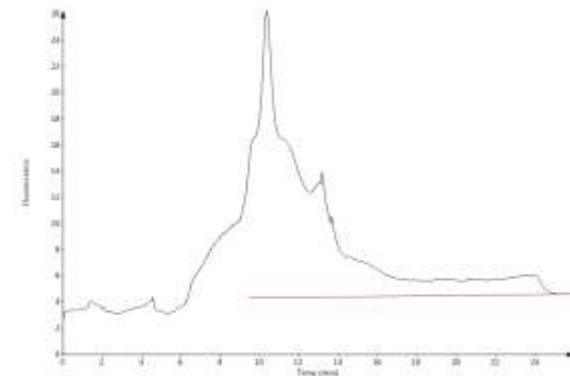
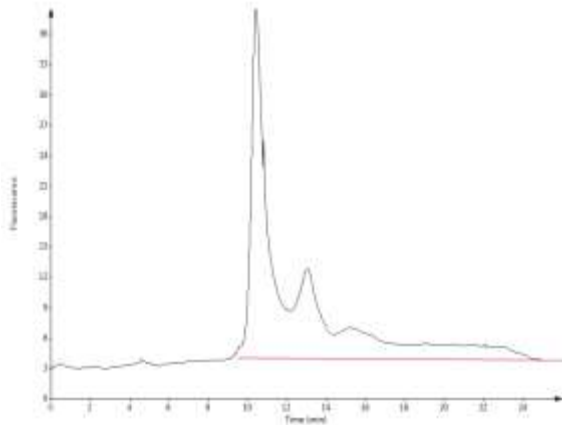
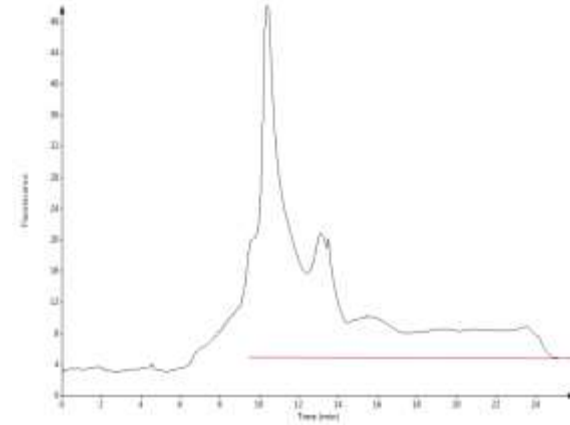
100 bp ladder
cfDNA (healthy individual)



Healthy individuals

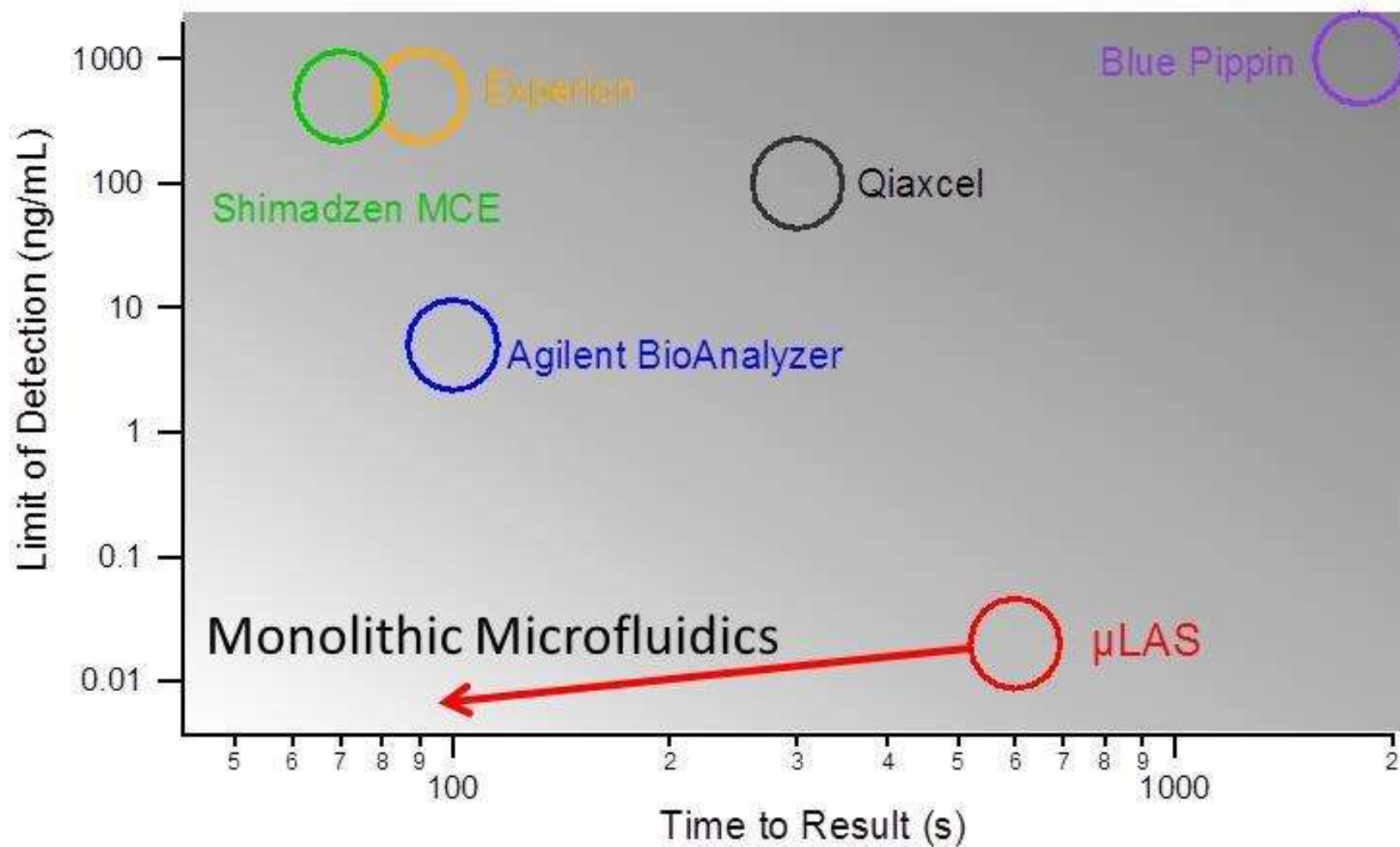


Metastatic patients



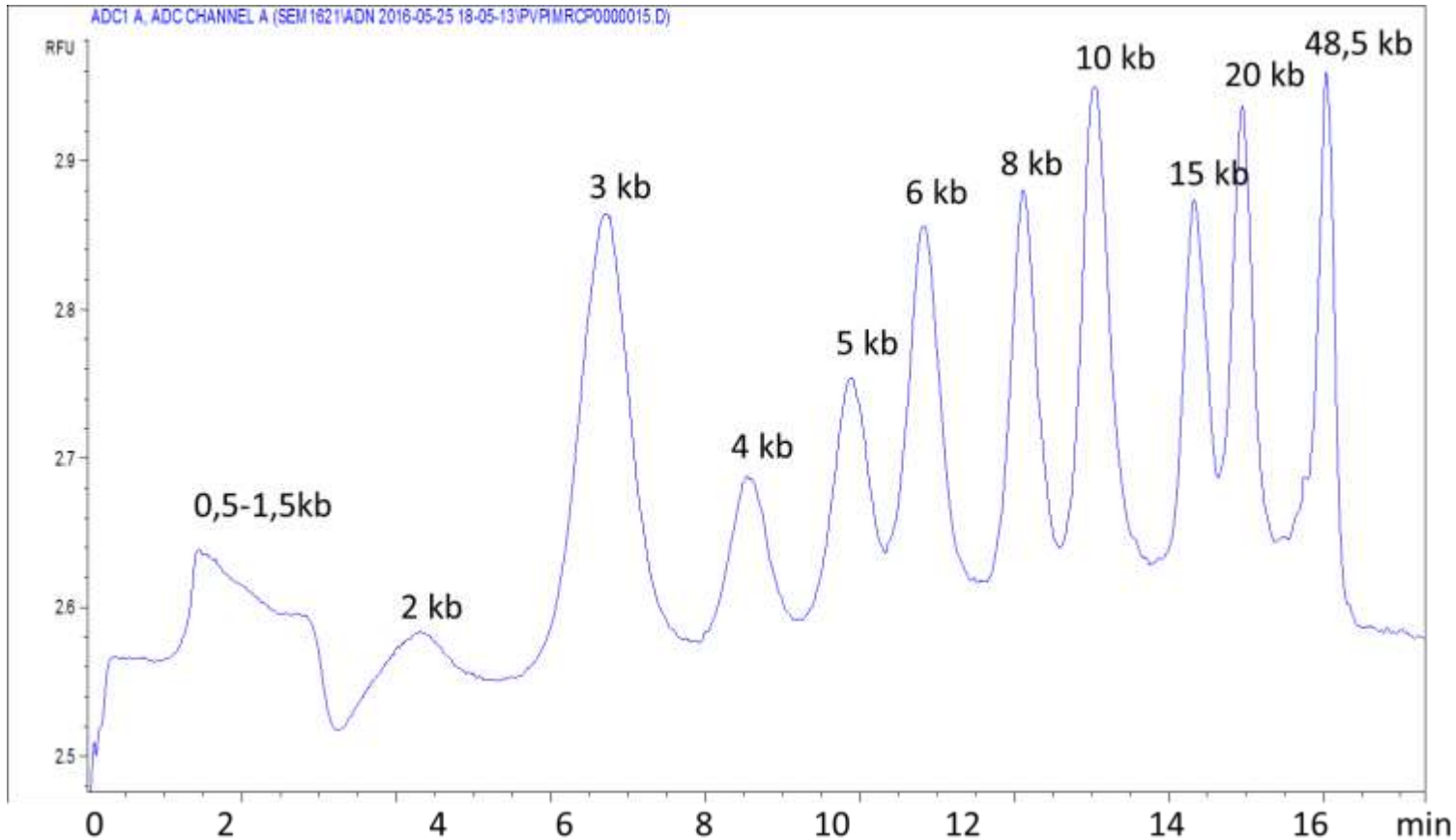
*With the courtesy of G. Favre and A. Pradines, Toulouse
Oncopole*

Benchmark



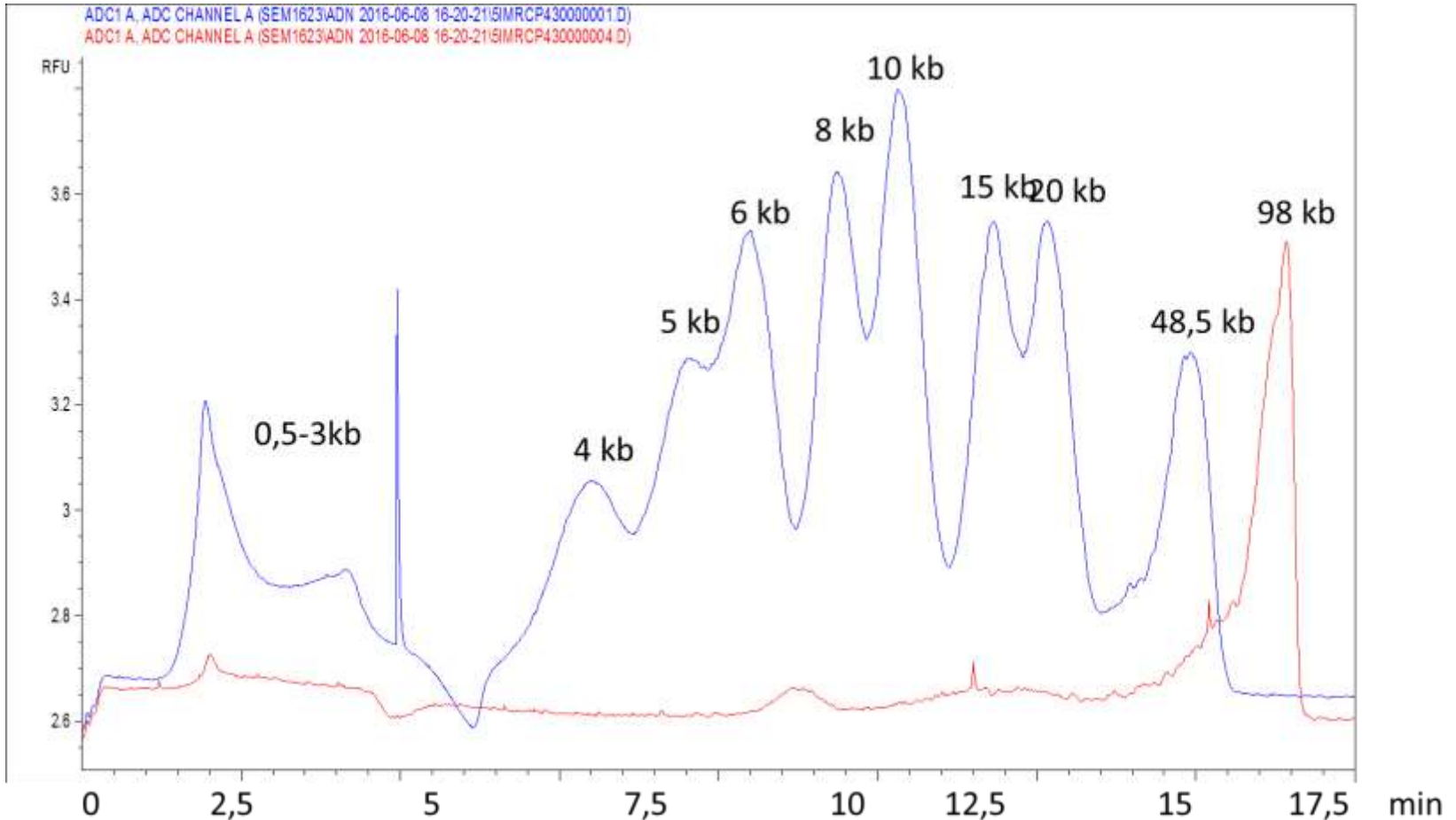
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**PVP 30kDa; 2 bar (1,5 mm/s)- 25 V/cm; kb ladder extend;
capillaire ID=25µm**



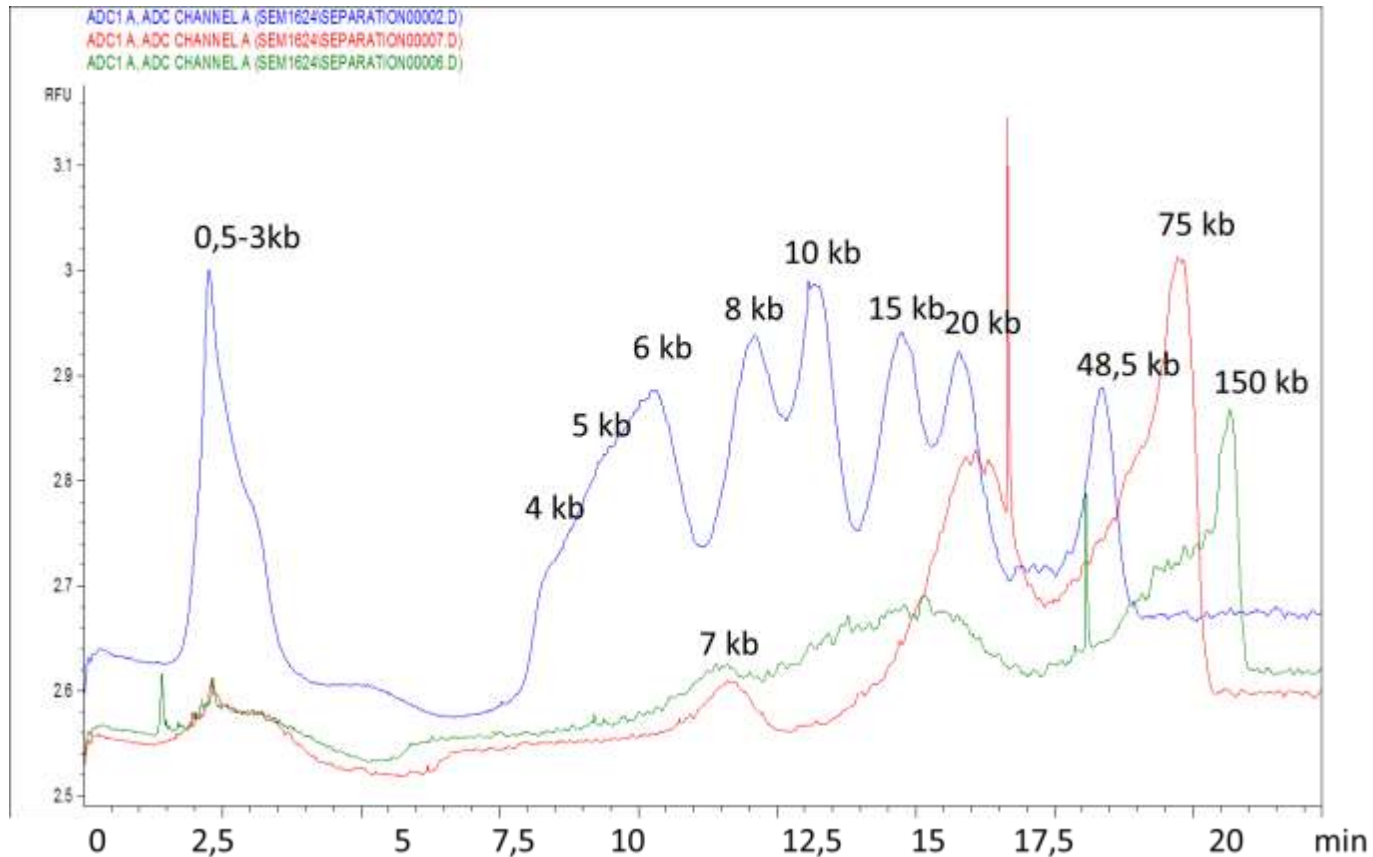
Vers 100 kb en 18 minutes

kb ladder extend (bleu) et 98 kb (rouge); ID=25 μ m
Projet avec CNRGV (BAC purifié)

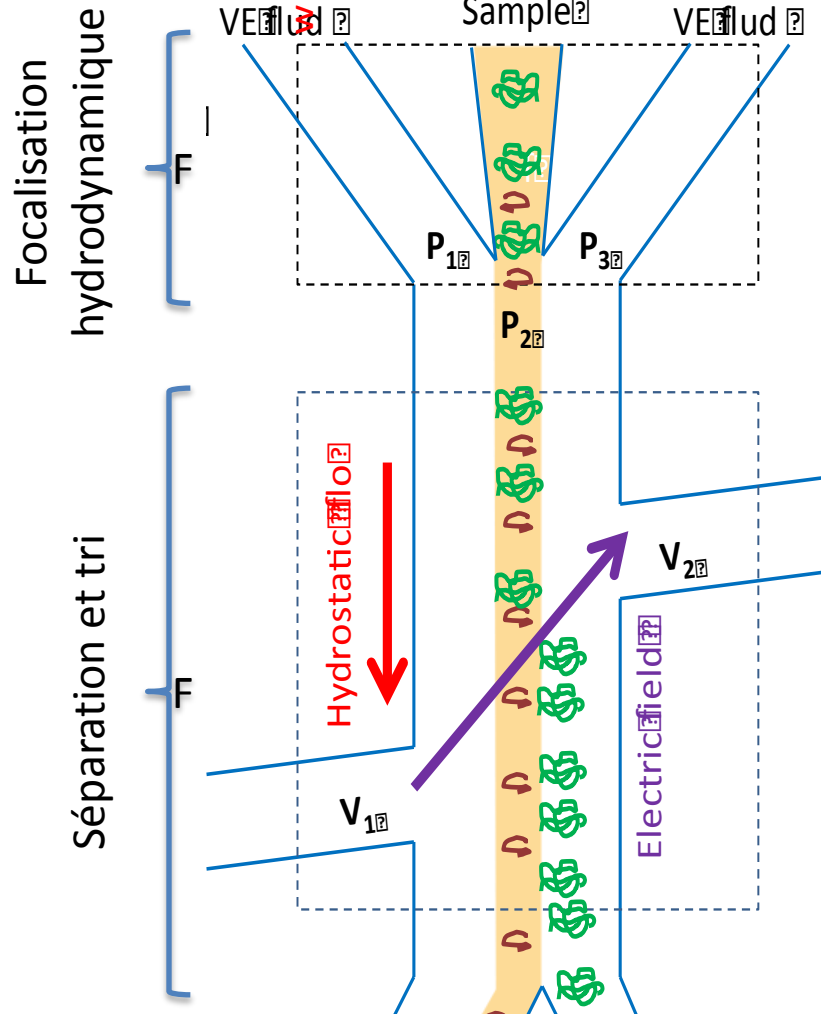


Vers 200 kb en 20 minutes

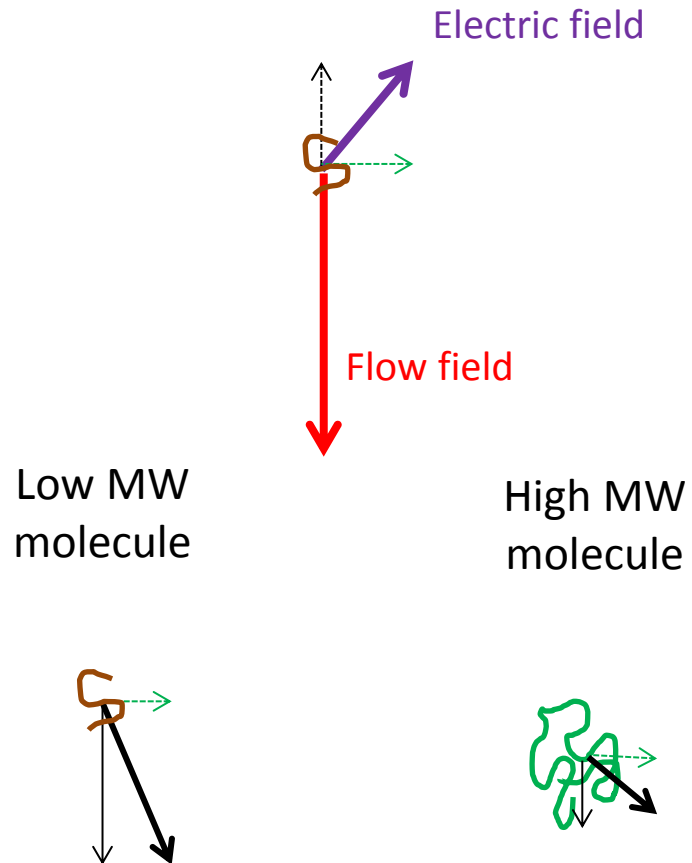
**kb ladder extend (bleu), 75 kb (rouge) et 150 kb (vert); ID=25 μ m
Projet avec CNRGV (BAC purifié)**



Tri d'ADN sous flux continu



Anisotropic transport



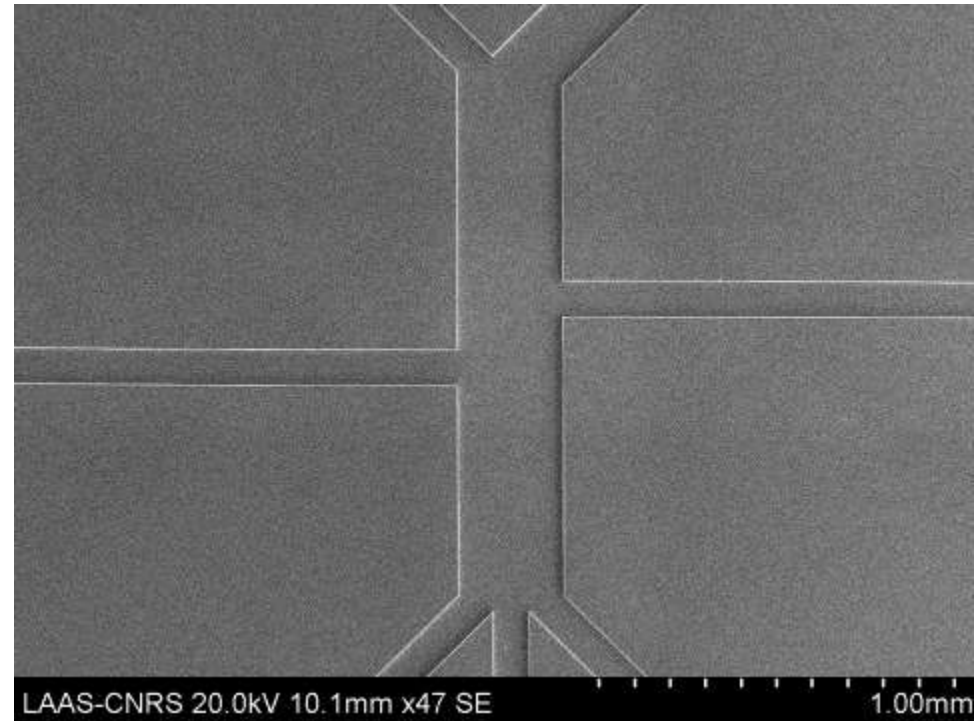
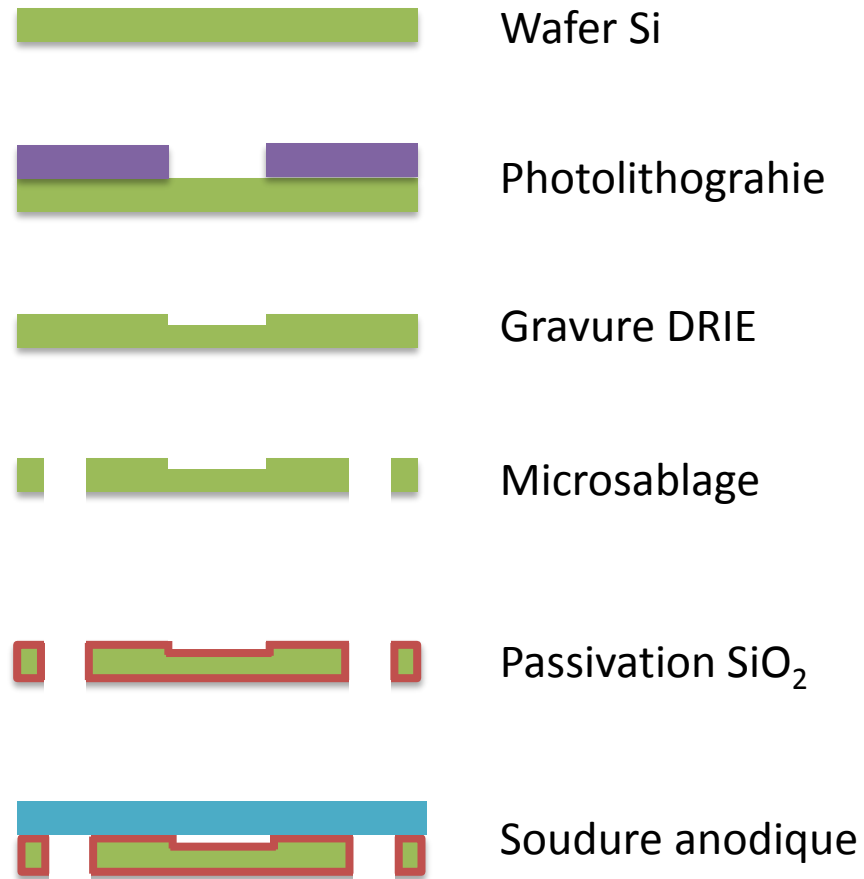
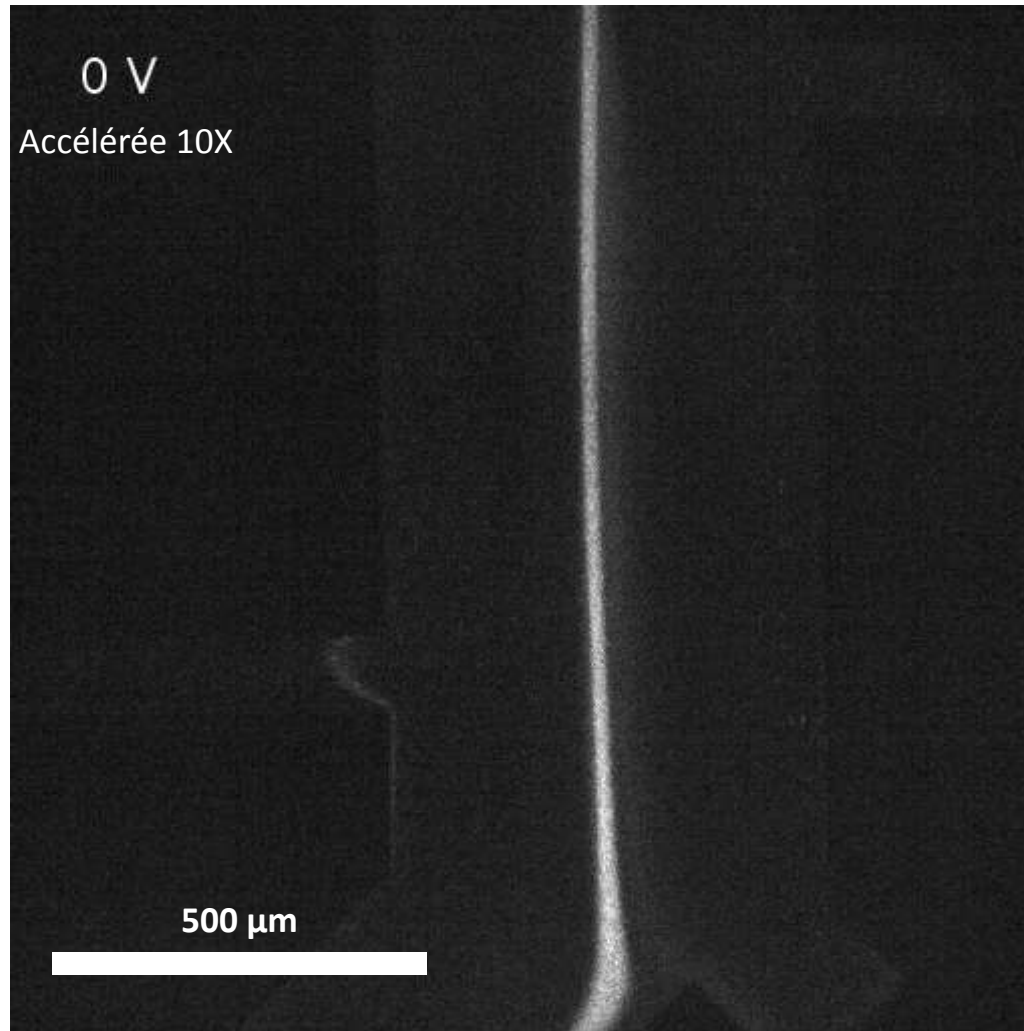


Image MEB puce l=300μm, h=20μm



- S. Méance, B. Chami,
L. Boyer, C. Blatché,
A. Bancaud
MicroTAS 2016 SOUMIS

- **μLAS: empilement des fonctions en ligne, séparation, enrichissement, tri et détection**
- **Transfert démontré pour l'ADN de faible MW**
- **Pistes de développement pour l'ADN de haut MW**
- **Fonction d'identification en cours d'évaluation brevet**

- **Perspectives**
 - **Consolider le tri d'ADN -> en lien avec les séquençages**
 - **Travail sur le plasma direct sans purification**
 - **Manipulation optimisée d'échantillons en petite quantité**

Remerciements

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Vincent Picot