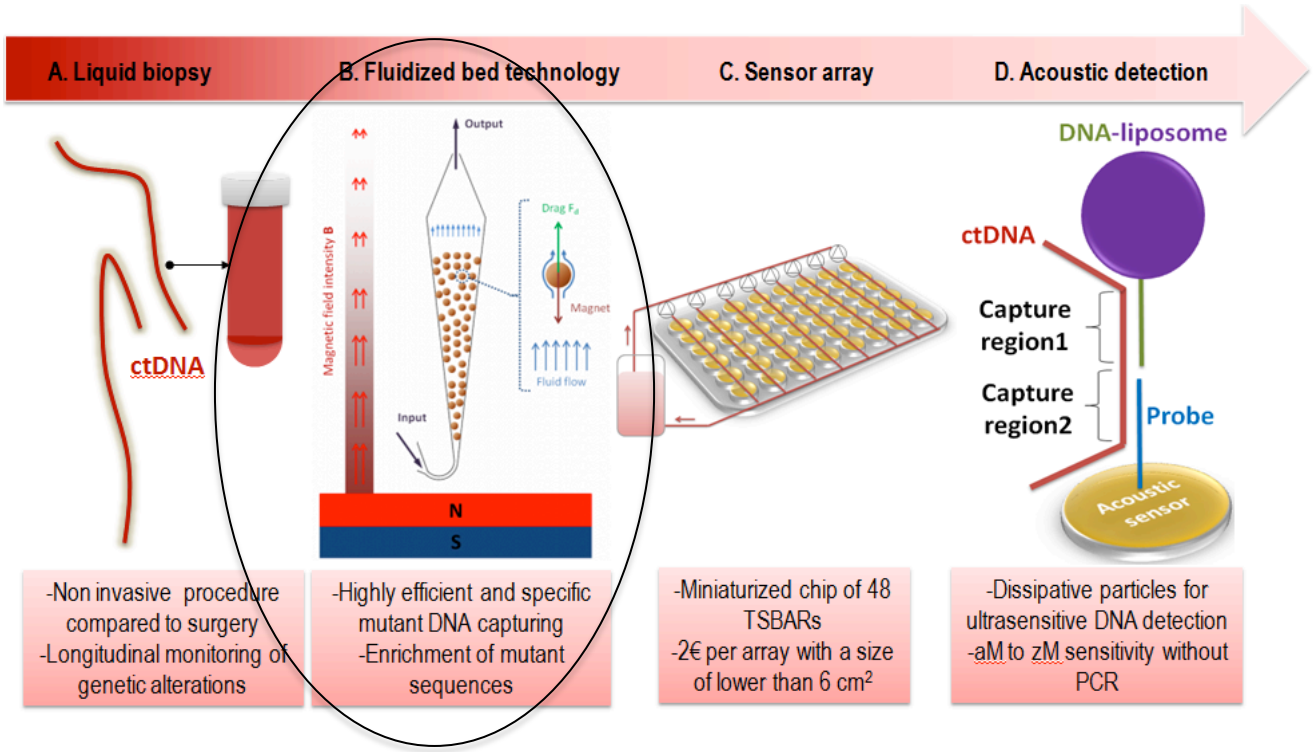
WP3: ctDNA enrichment strategies

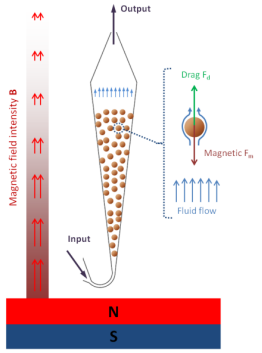


This WP is focused on innovative microfluidic-based strategy for processing of patient samples.

Our approach is to combine microfluidic technology with molecular biology to select and enrich ctDNA materials

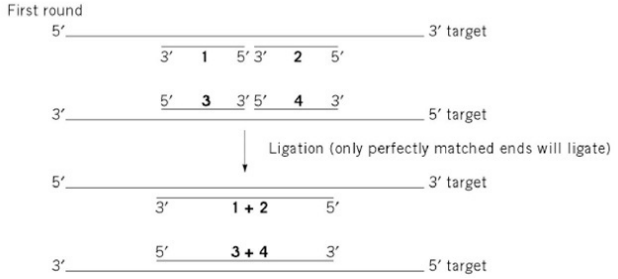
Task T3.1: Optimization of ctDNA selective capturing on magnetic beads in serum (Curie, M1-M24): (D3.1)

- Streptavin-biotin system for capture oligo functionalization
- Capture through DNA-DNA interaction
- Improvement of pretreatment throughput and capture efficiency
- Control of hybridation T°C



Task T3.2 : Optimization of a ligase-based assay for the selective enrichment of ctDNA targets (Curie, FORTH, M1-M30)

- Oligonucleotides sequence composition and length
- Number of cycles and temperatures
- Use of single-base 3' overhangs
  5' phosphorylation of oligos
  Introduction of non-complementary tails
- Use of cycling conditions near the oligos Tm.



Task T3.3 : Optimized protocol for ctDNA isolation and enrichment (Curie, FORTH, M1-M36)

- Combination of tasks 3.2 and 3.3 outcomes
- Two strategy direct LCR or DNA release + LCR

Task T3.1: Optimization of ctDNA selective capturing on magnetic beads in serum (Curie, M1-M24): (D3.1)

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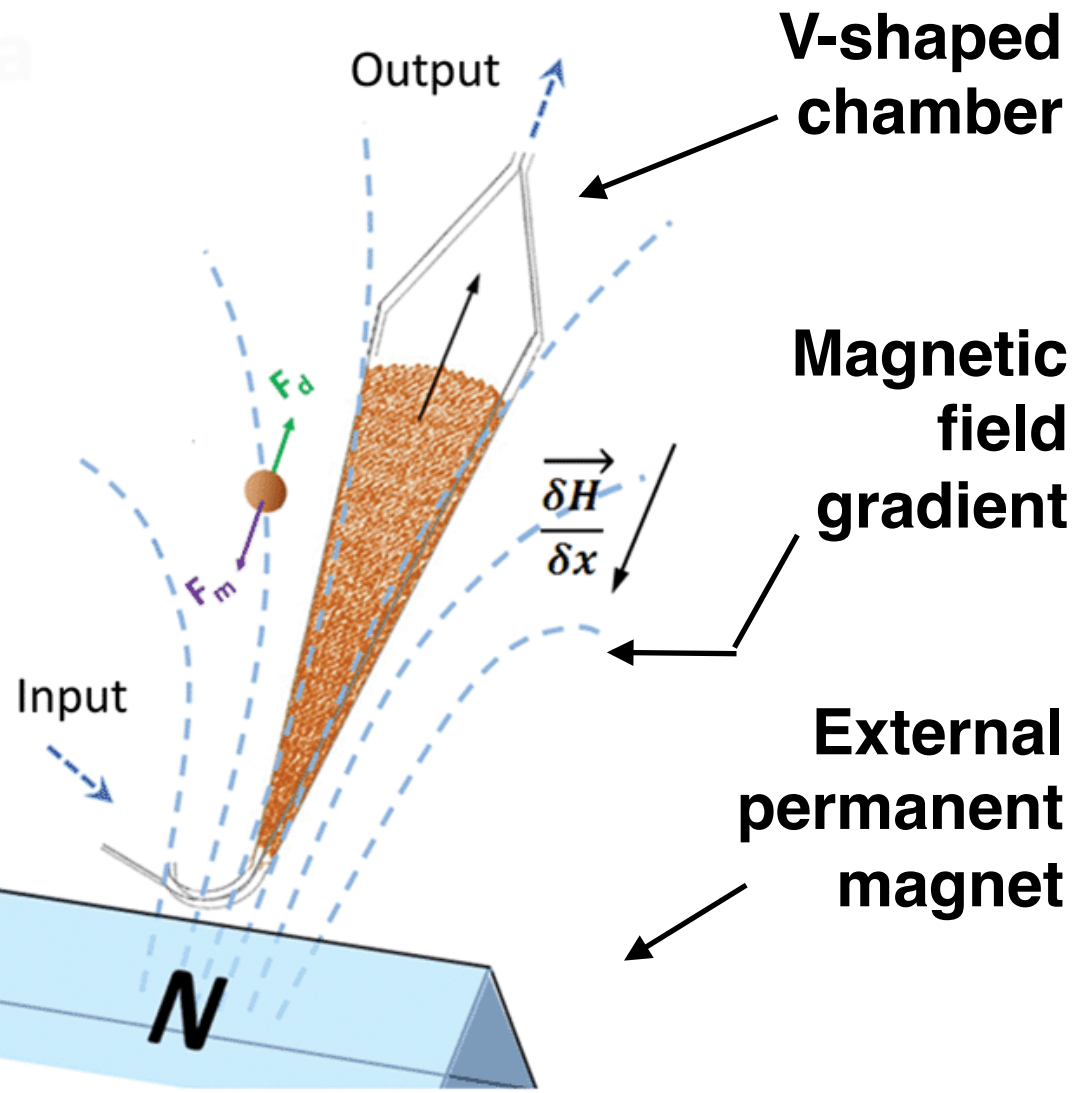
THE CATCH-U TECHNOLOGY : MICROFLUIDIC FLUIDIZED BED



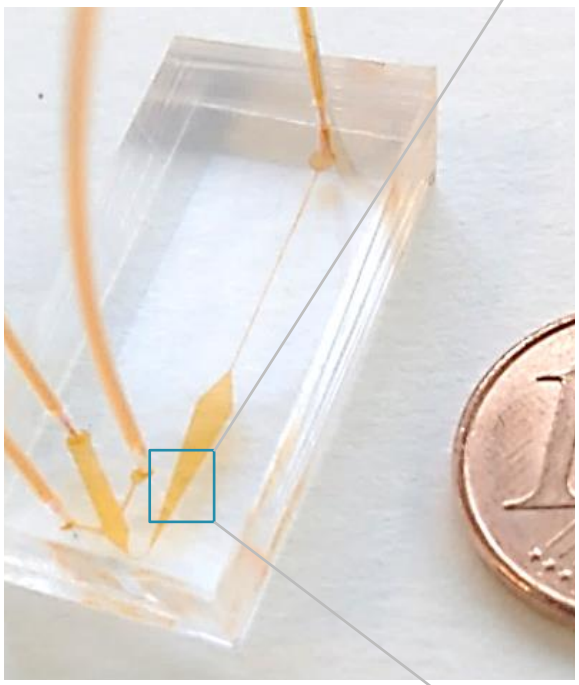
V-shaped chamber

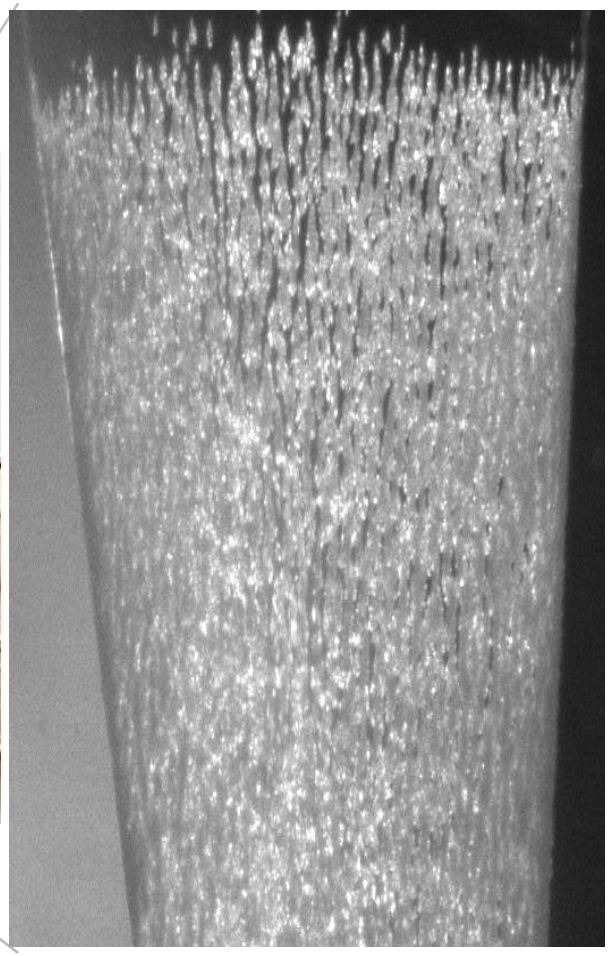
Magnetic field gradient

External permanent magnet

- Simplicity of fabrication and operation

MICROFLUIDIC FLUIDIZED BED





- Simplicity of fabrication and operation
- Well controlled particle recirculation and bead density

For Catch-U the beads will be functionalized to extract DNA from patients samples

Task T3.1: ctDNA CAPTURE WITH THE FLUIDIZED BED TECHNOLOGY

Main objectives

1/ Improvement of the fluidized bed technology

✓Ongoing – 2nd generation

2/ ctDNA capture : two strategies

✓non specific capture : proof of concept achieved
✓specific hybridization : ongoing

Fluidized bed 2.0

Main objective: increase the analytical throughput

First generation
H : 50 µm

Generation 2.0
H: 250µm

1 µL/min 1 µL/min 5 µL/min

Channeling effect
=
Decrease of the ratio of contact between the liquid and the solid phase

Necessity **to improve the homogeneity** of the bed of beads

✔ Bimodal magnetic support

✔ Vibration system

Objective 1 : Ongoing work

To improve the fluidized bed throughput:

- Development of the Fluidized bed 2.0
  - ➔ 5 to 20 x Flow rates
  - ➔ Compatible with larger sample volumes

- Homogenization strategies currently investigated : bimodal beads distribution, vibration, chaotic mixing and sequential onf/off state

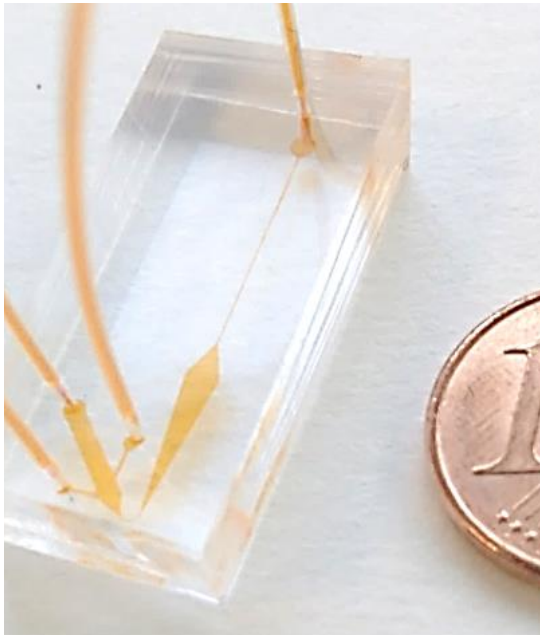
- Improvement achieved with bimodal sizes of beads and vibration
  - ➔ Capture efficiency - streptavidin/biotin model > 90%

Objective 2 : ctDNA CAPTURE WITH THE FLUIDIZED BED TECHNOLOGY

- ctDNA capture : two strategies

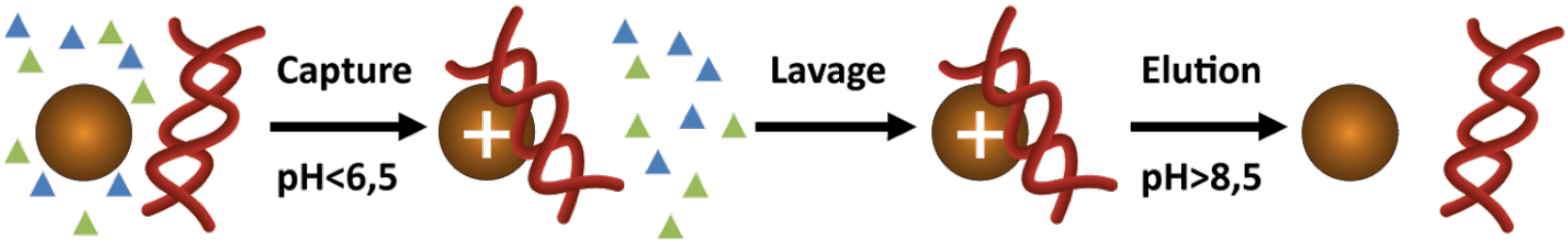
✓non specific capture : proof of concept achieved

✓specific hybridization : ongoing

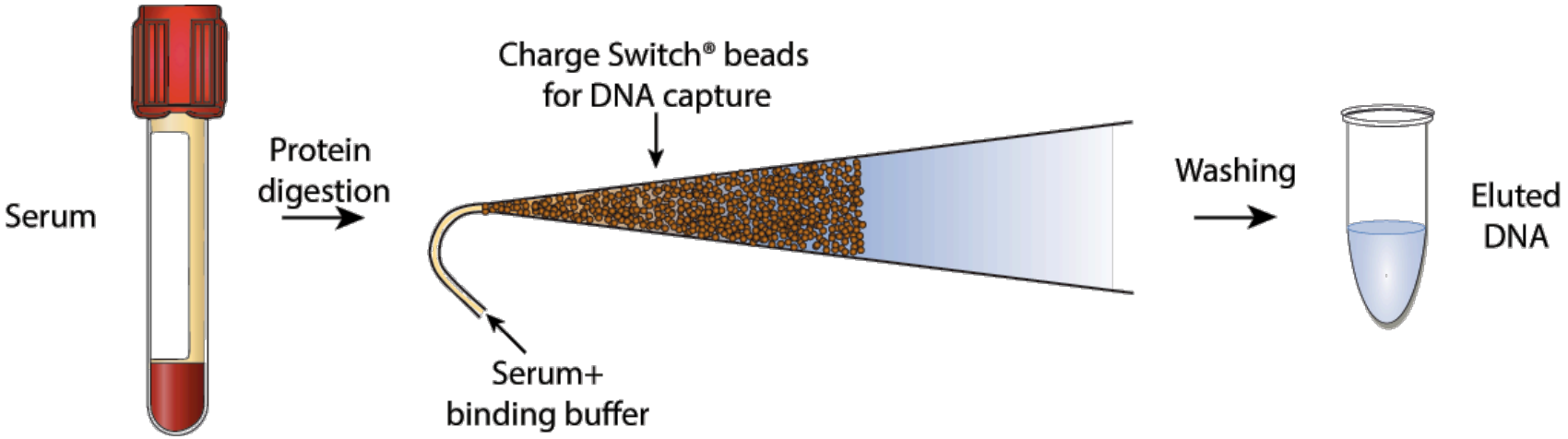


NON SPECIFIC DNA EXTRACTION

- **ChargeSwitch® Technology (Invitrogen)**

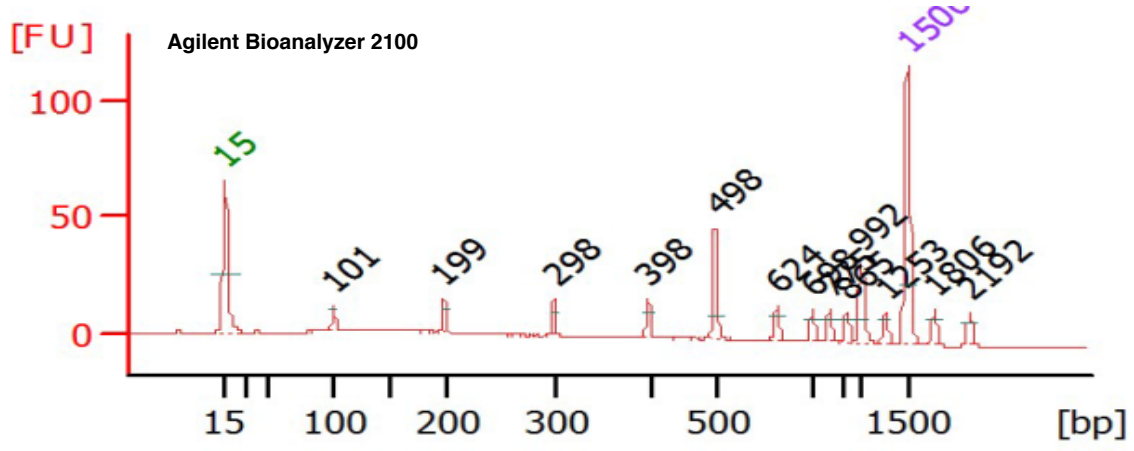


DNA capture based on electrostatic interactions

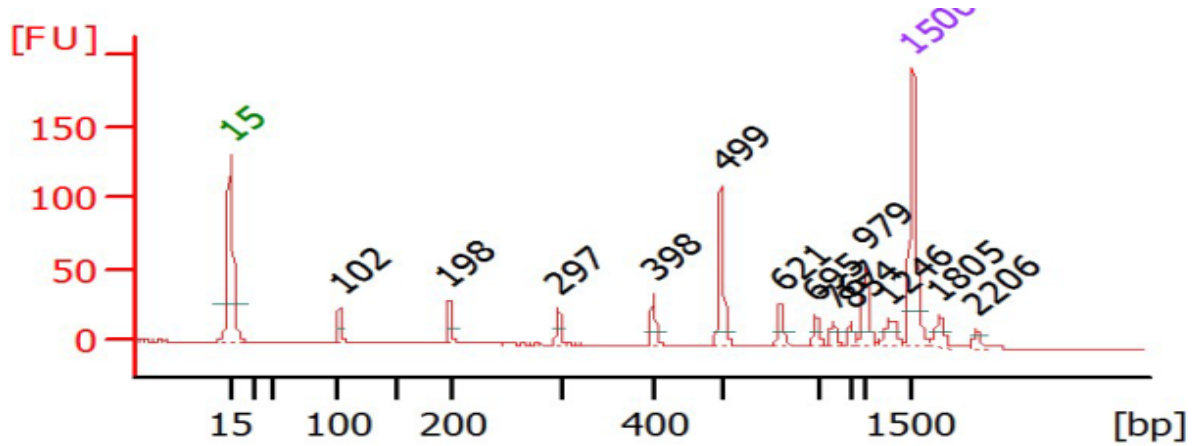


NON SPECIFIC EXTRACTION OF FRAGMENTED DNA (100-1000 bp)

Before extraction



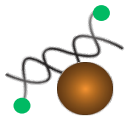
After extraction on chip



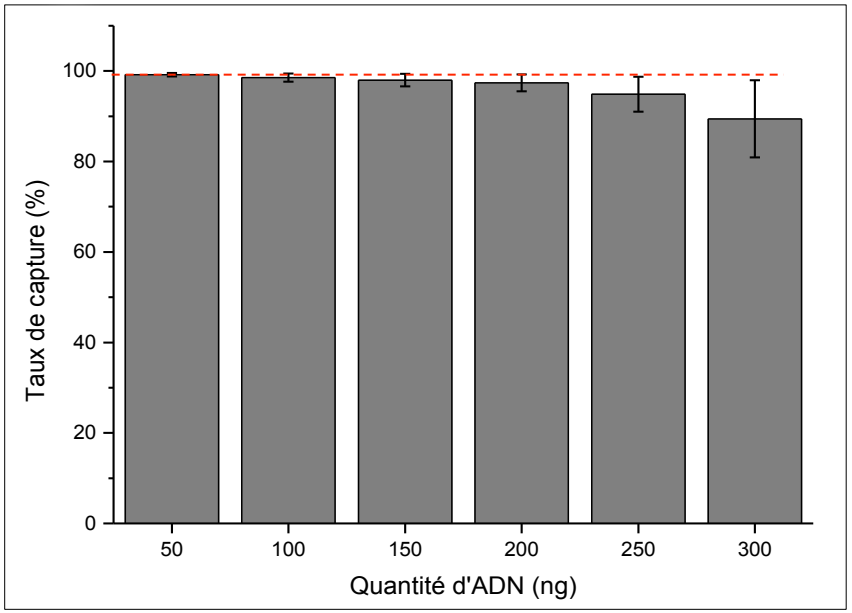
All DNA fragments extracted with same efficiency
Potential use for ctDNA

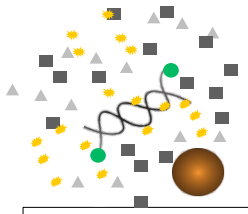
ON-CHIP EXTRACTION EFFICIENCY

200 bp dsDNA

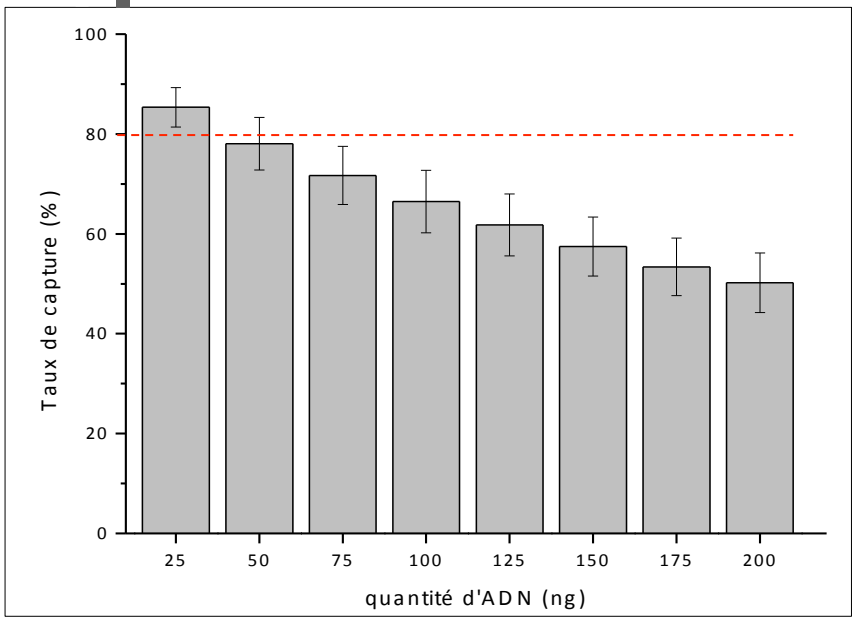


DNA in PBS



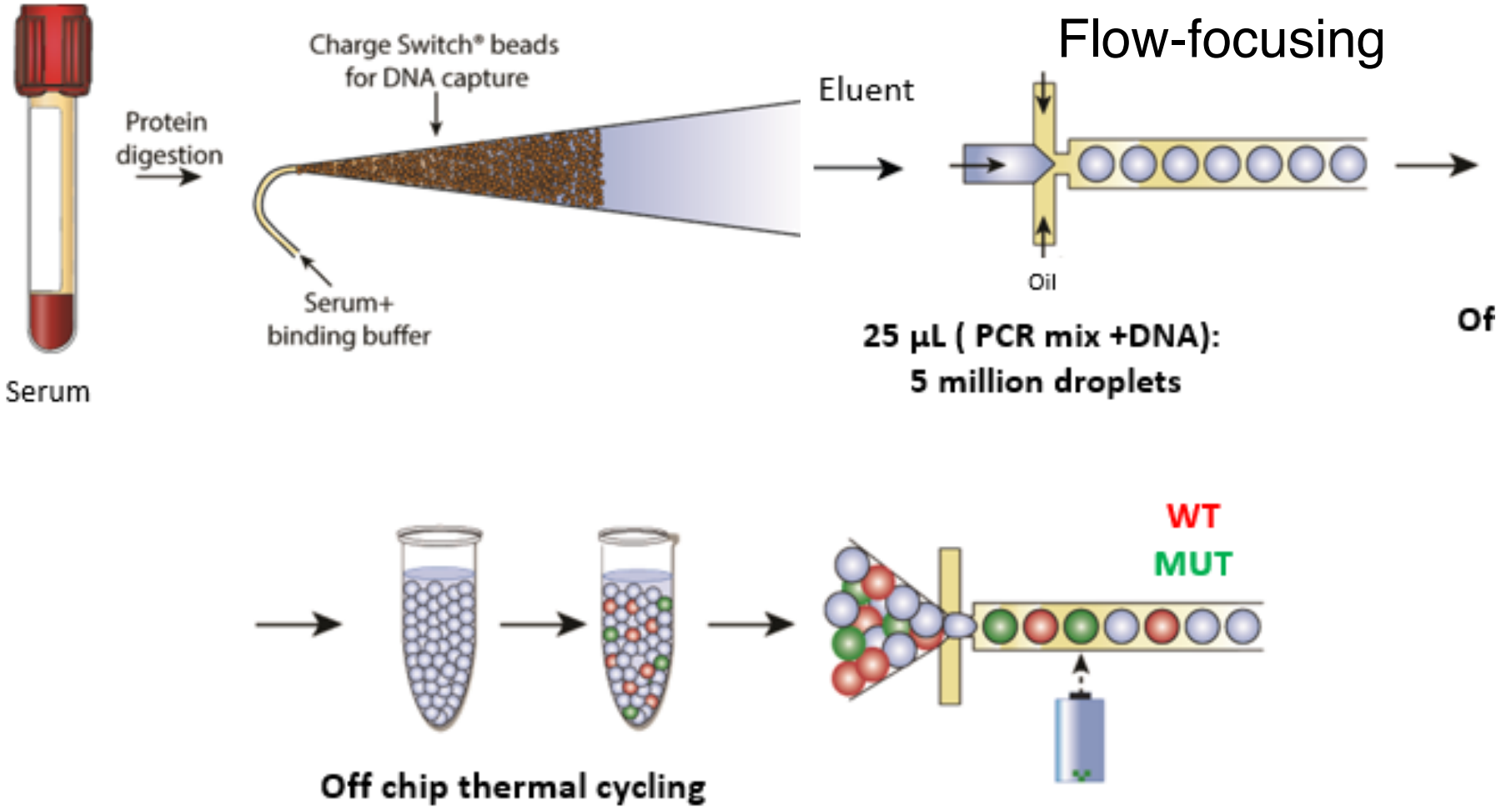


DNA in human serum



High capture efficiency of the system despite an important screening effect of serum proteins

DOWNSTREAM ANALYSIS BY DIGITAL PCR



Conclusion and Perspectives

Technology

- Development of fluidized 2.0 able to accommodate large volume
- Development of different strategies to improve the bed homogeneity

DNA capture

- Demonstration of non specific DNA capture on chip with artificial samples and patients samples
- Preliminary results on specific DNA capture

DNA capture combined with LCR

- Preliminary results on DNA release
- Strategy for LCR on chip – Exchange of PhD in July with FORTH