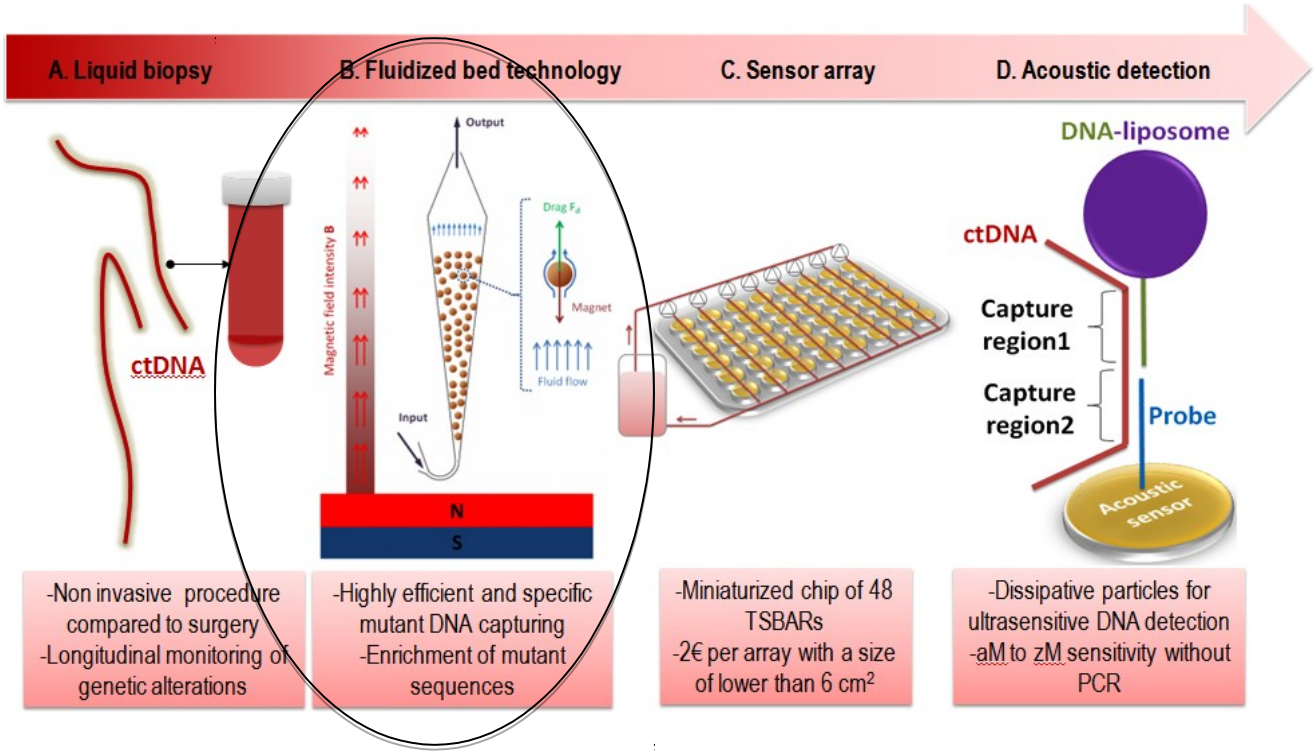
WP3: ctDNA enrichment strategies

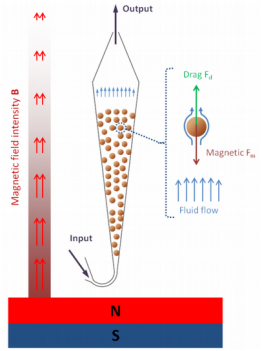


This WP is focused on innovative microfluidic-based strategy for processing of patient samples.

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

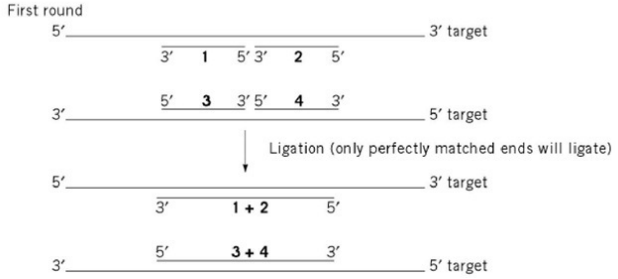
Task T3.1: Optimization of ctDNA selective capturing on magnetic beads in serum (Curie, M1-M24): (D3.1)

- 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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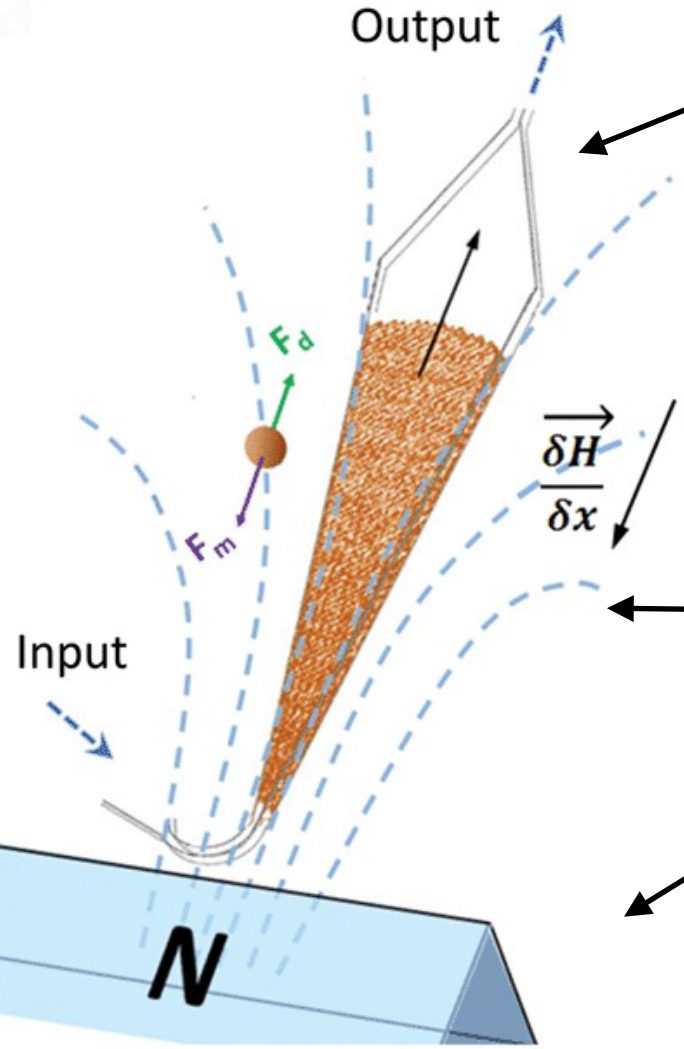
Catch U DNA – Review Meeting 28th June Heraklion

CH-U TECHNOLOGY : MICROFLUIDIC FLUIDIZED



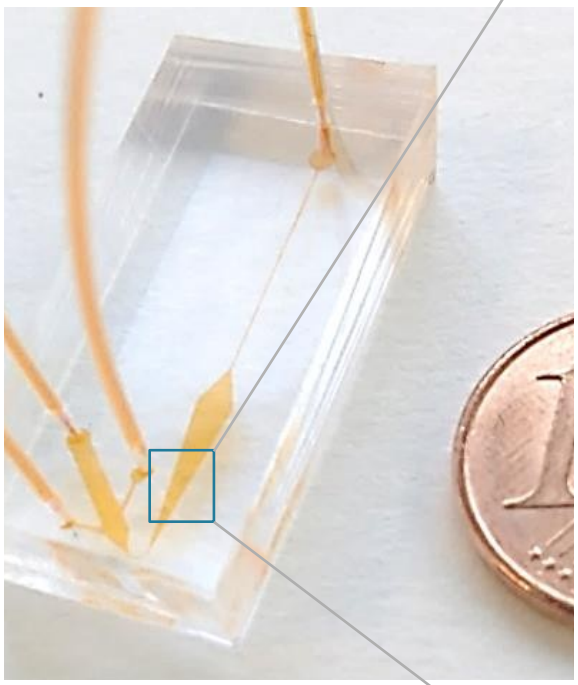
V-shaped chamber

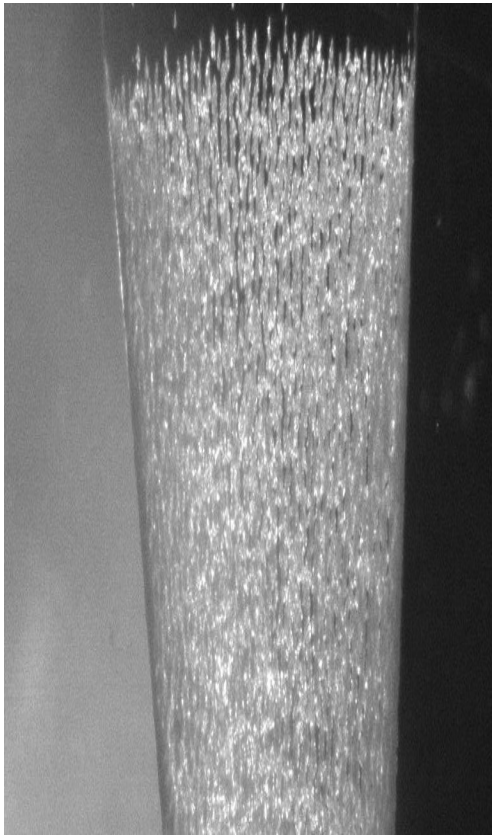
Magnetic field gradient

External permanent magnet

- **Simplicity of fabrication and operation**

THE 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

✝ 2nd generation : Achieved
Bimodal beads composition +vibration

2/ ctDNA capture : two strategies

✓Non specific capture - Achieved
(K. Perez-Toralla Sensors and actuators 2019)

✓Specific hybridization : Ongoing

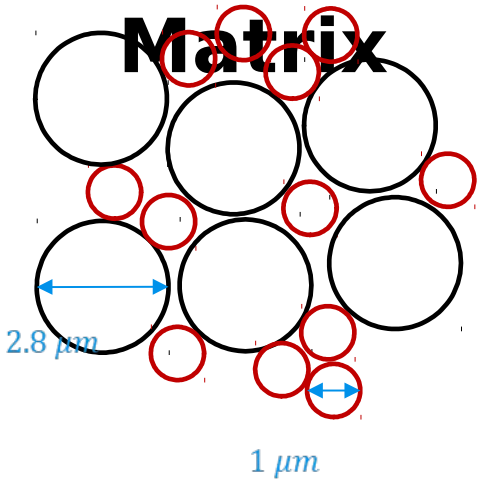
Second generation of fluidized bed

Similar design except : hx5 : 250 µm
Flow rate increase up to 15 µL/min

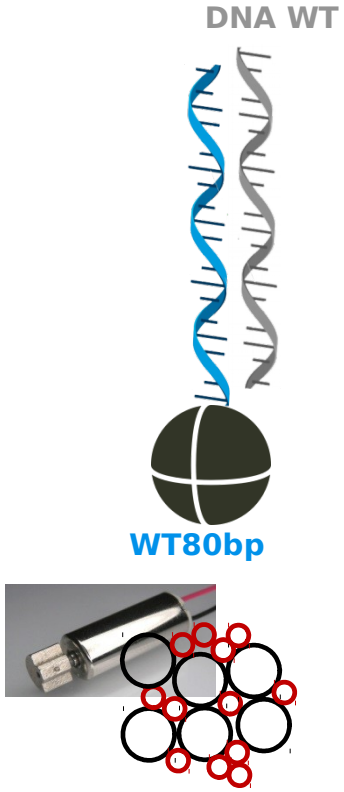
To avoid non uniformity in the bed of beads

External vibration

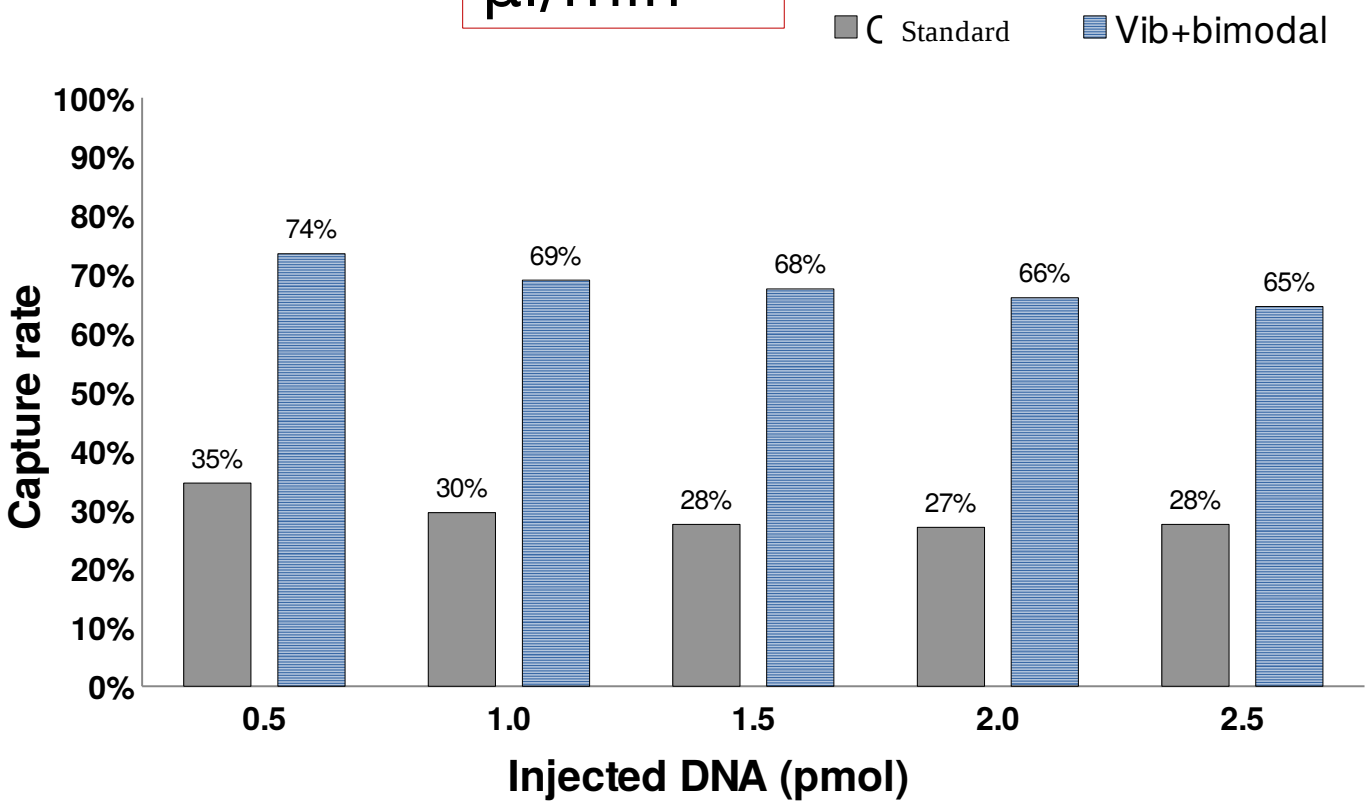
Bimodal Matrix



Model DNA capture with the second generation of fluidized bed



15 µl/min

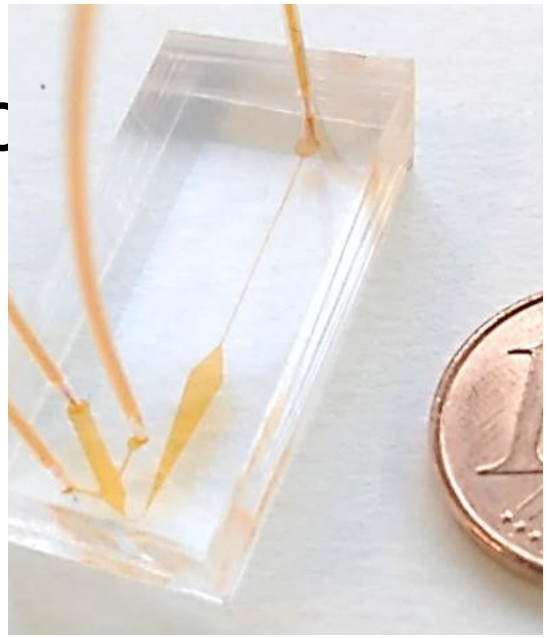


Objective 2 : ctDNA CAPTURE WITH THE FLUIDIZED BED TECHNOLOGY

- ctDNA capture : two strategies

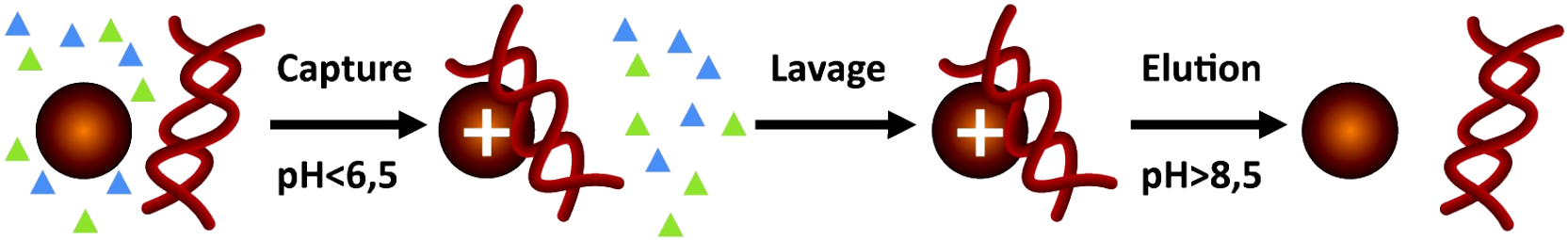
✓ non specific capture : achieved

✓ specific hybridization : ongo

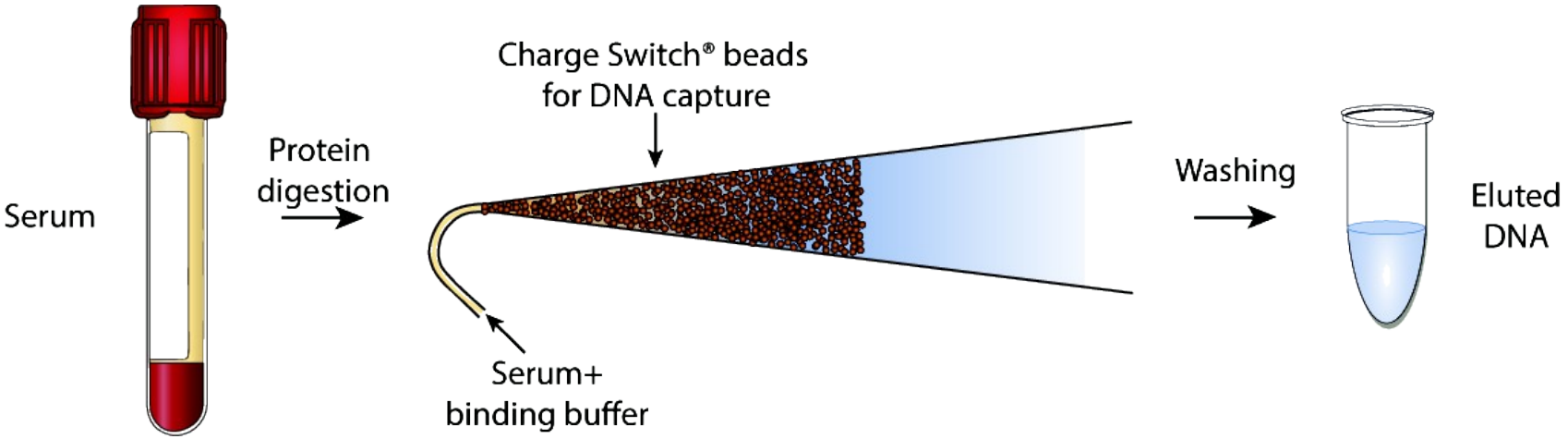


NON SPECIFIC DNA EXTRACTION

- ChargeSwitch® Technology



DNA capture based on electrostatic interactions

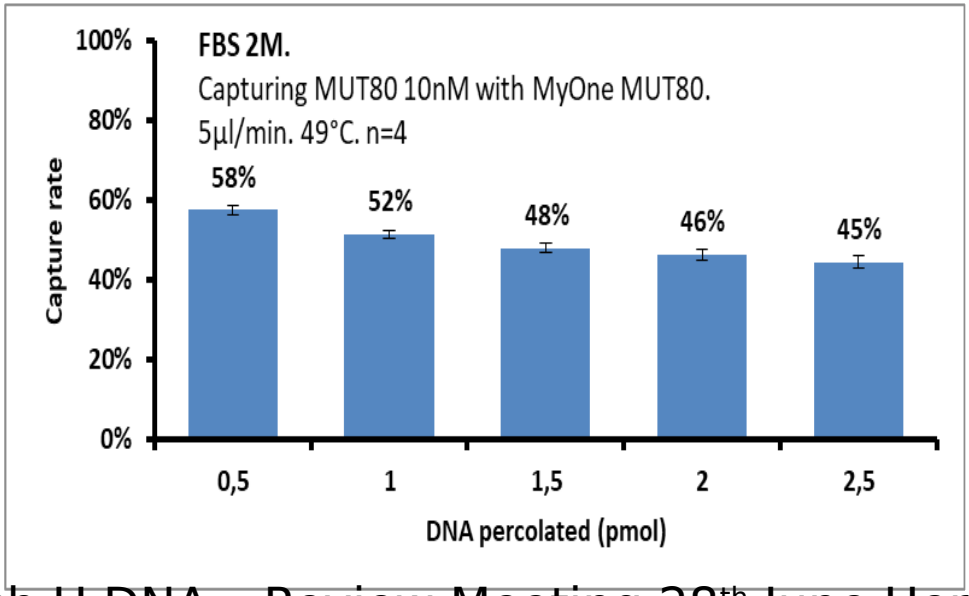


K. Perez-Toralla Sensors Actuators B

SPECIFIC DNA EXTRACTION WITH THE

First period : optimization and demonstration of single strand DNA capture

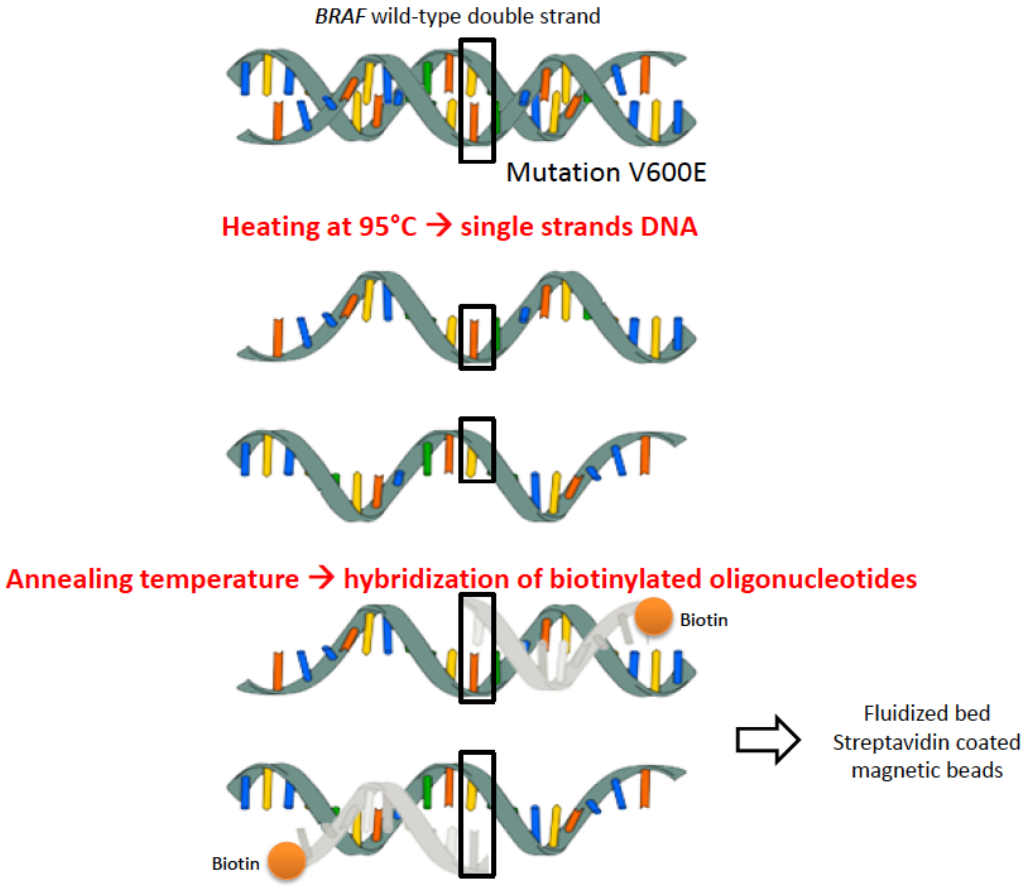
- Buffer salinity optimization
- Optimal temperature
- Optimization of the size of the capture oligo
- Study of the capture efficiency and sp



SPECIFIC DNA EXTRACTION WITH THE FLUIDIZED BED

Second period : optimization and demonstration of double strand DNA capture

1. Heating to separate double stranded DNA
2. Introduction of biotinylated oligos
3. Capture on streptavidin beads



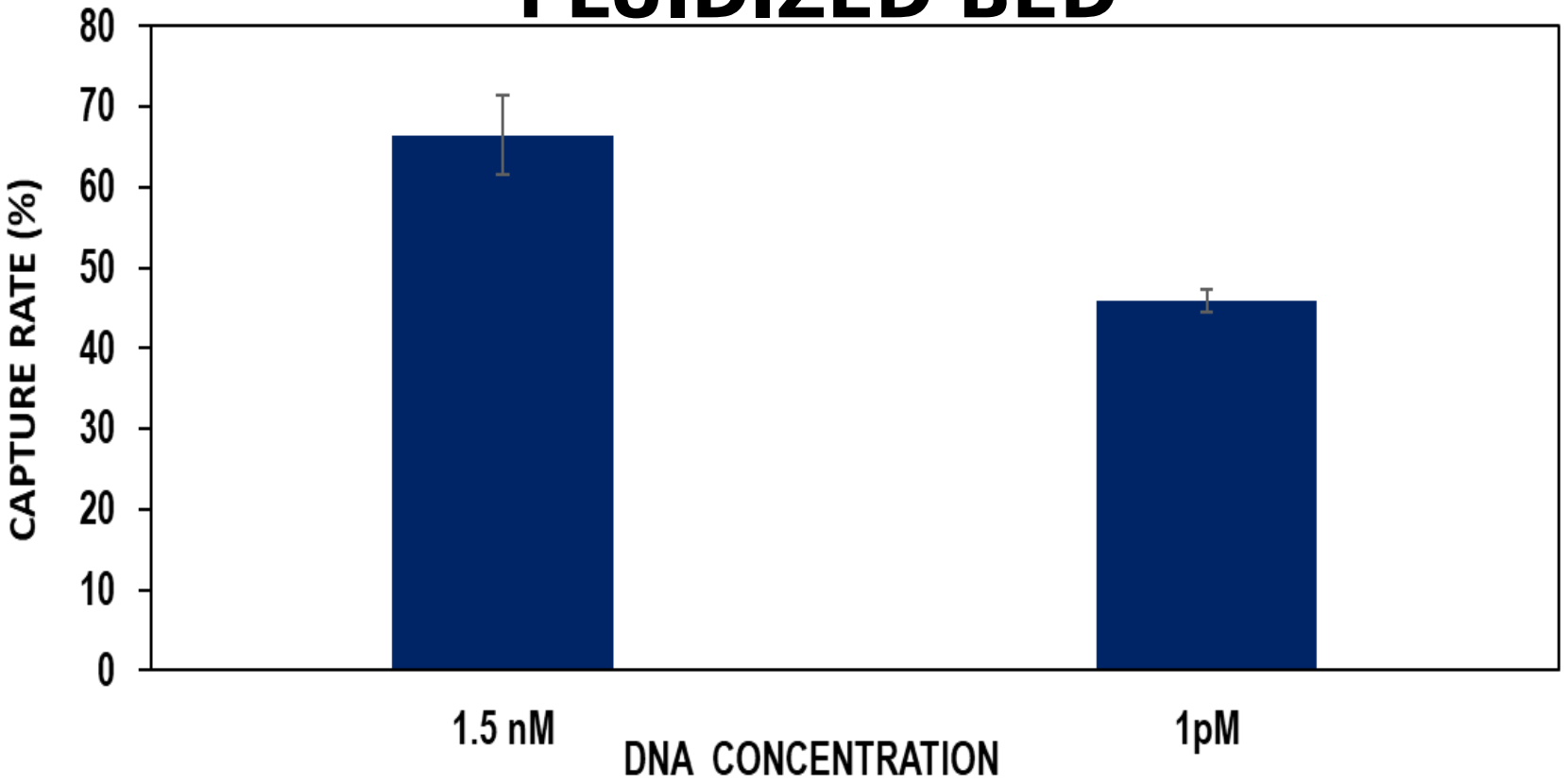
SPECIFIC DNA EXTRACTION WITH THE FLUIDIZED BED

- Optimization of the sample pretreatement : Proteinase K
- Incubation 2h at 37°C with PROTEINASE K (2.75 mg/mL)

- Inactivation of the enzyme to avoid ligands digestion
- Heating at 95°C is a ‘Win-Win’ condition : DNA and PK denaturation

- Evaluation of DNA capture in buffer and in serum

SPECIFIC DNA EXTRACTION WITH THE FLUIDIZED BED



Capture Rate (%) of dsDNA 277 BRAF MUT in human serum by using FB generation 2.0.
Error bars represent mean ± SD of duplicate.

Capture was performed with streptavidin beads at Room Temperature, 5 µL/min. Standard matrix + vibration. Probes (0,4 µM). Human serum was pretreated with Proteinase K (2.75 mg/mL) 2h at 37ºC following by a denaturation at 95ºC (5 min) and an incubation at room temperature (15 min) before performing the capture on FB.

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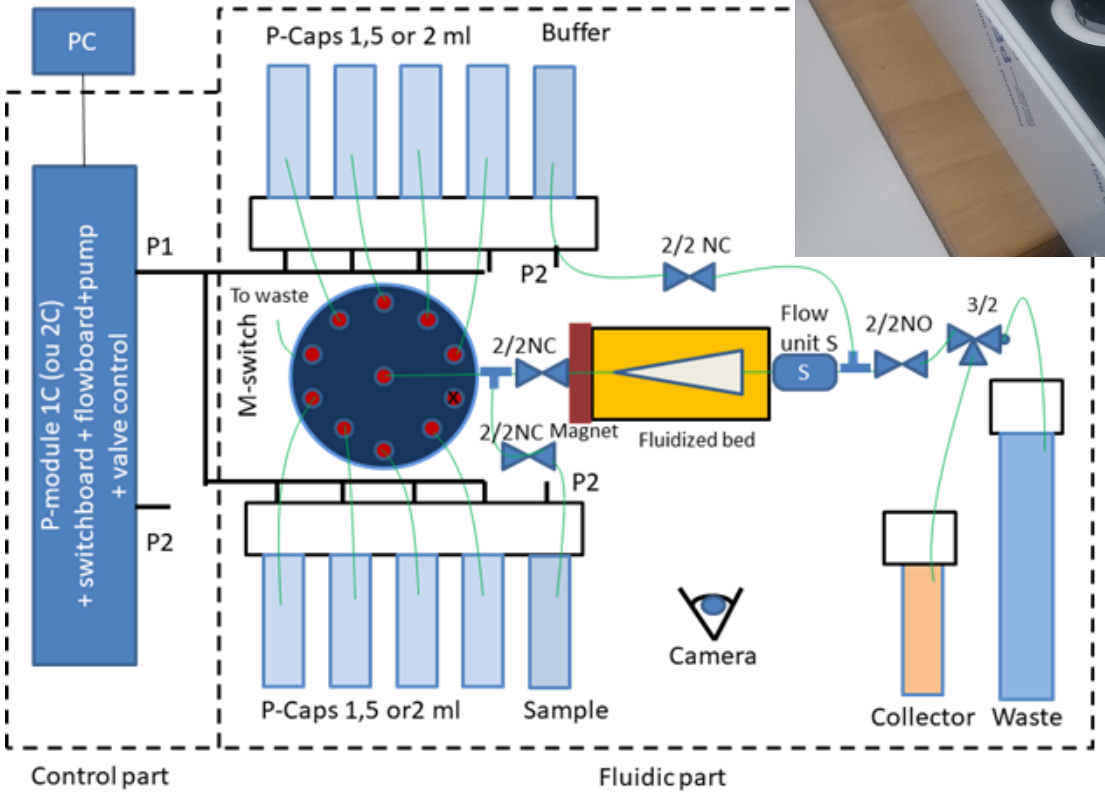
Fluidized Bed platform



First Experiments to combine fluidized + LCR here in Heraklion

Conclusion and Perspectives

Technology

- Development of fluidized 2.0 able to accommodate large volume

- Demonstration of the use of this second generation for DNA capture

DNA capture (D3.1 Delivered)

- Demonstration of non specific DNA capture on chip with patients samples

- New approaches for DNA specific capture from serum samples

DNA capture combined with LCR