



Capturing non-Amplified Tumor Circulating DNA with Ultrasound Hydrodynamics



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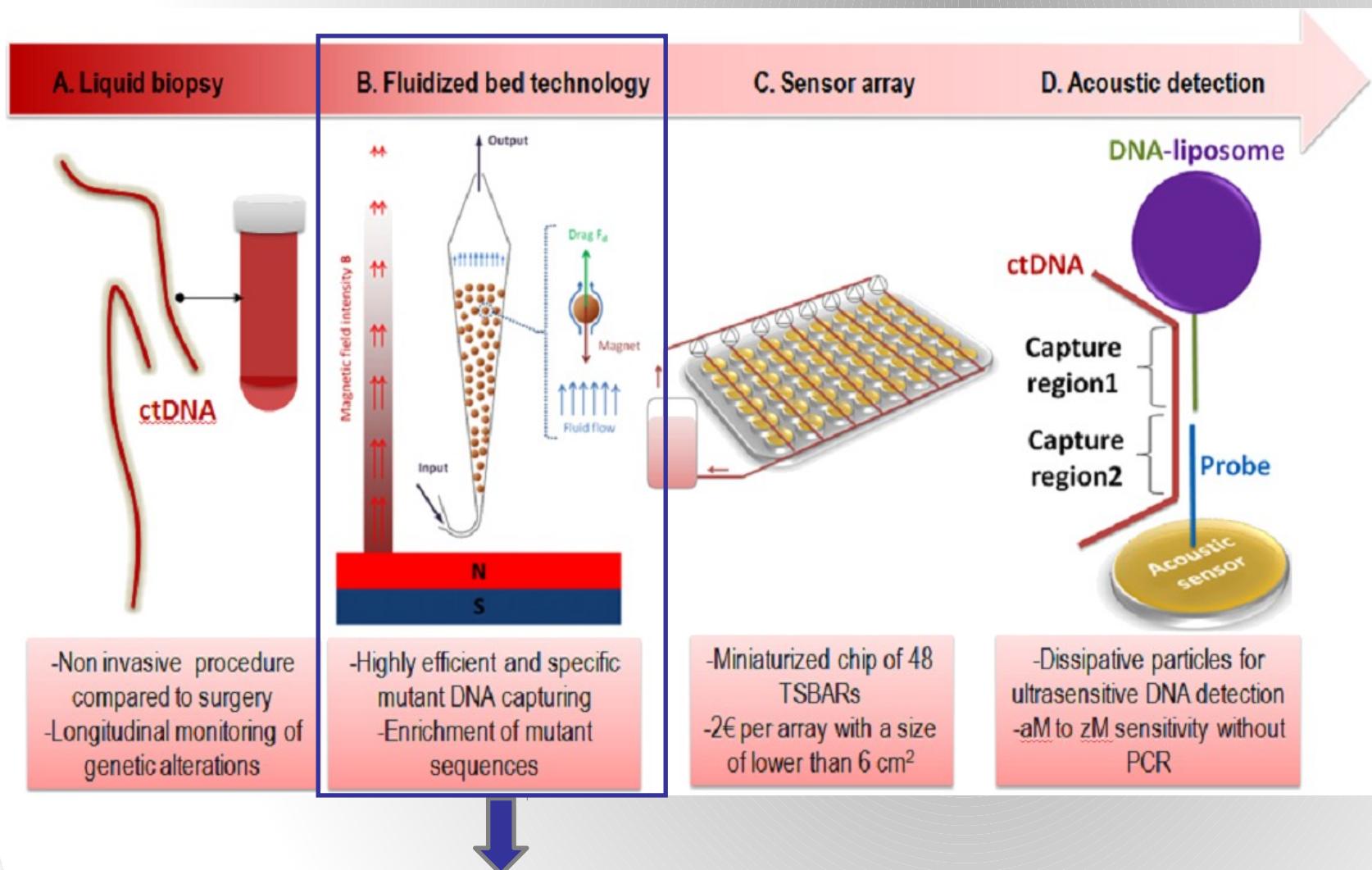
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21ST MAY VALENCIA, SPAIN



CATCH-U-DNA Global Work Plan



WP3: ctDNA enrichment strategies using magnetic beads

WP3-TASKS

ACTIVITIES

Task 3.1

Optimization of ctDNA selective capturing on magnetic beads in serum

- Streptavidin beads functionalization
- Capture based on DNA-DNA interaction
- Improvement of capture efficiency by testing different lengths of oligonucleotide sequences
- Control of hybridization temperature

Task 3.2

Optimization of a ligase-based assay for the selective enrichment of ctDNA targets

- Optimization of the different parameters :
 - 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 T_m

Task 3.3

Optimized protocol for ctDNA isolation and enrichment

- Combination of the results from tasks 3.1 and 3.2 : captured ctDNA on beads and *in situ* LCR or release of ctDNA captured on beads and following LCR

DNA CAPTURE - BRAF gene

PREVIOUS RESULTS

- Capture of ssDNA WT (80 bp) : Fluidized Bed (FB) generation 1.0
 - Optimization of the conditions of capture in batch
- Capture of ssDNA WT (80 bp) : FB generation 2.0
 - Strategies to improve the homogeneity of the bed of beads (bimodal + vibration)
 - Capture in complex matrices : Mix of DNA (WT/MUT), FBS and human serum
- Capture of dsDNA MUT (80 bp) : FB generation 2.0
 - Capture in simple matrix (buffer) and in complex matrix (FBS)

 **Work in progress : Capture of DNA 277 bp - Detection by qPCR**

FB generation 1.0 : microfluidic device 50 µm height

FB generation 2.0 : microfluidic device 250 µm height

FBS : Fetal Bovin Serum

WT : Wild Type

MUT : Mutant



Capturing DNA 277 bp BRAF MUT in simple and complex matrix

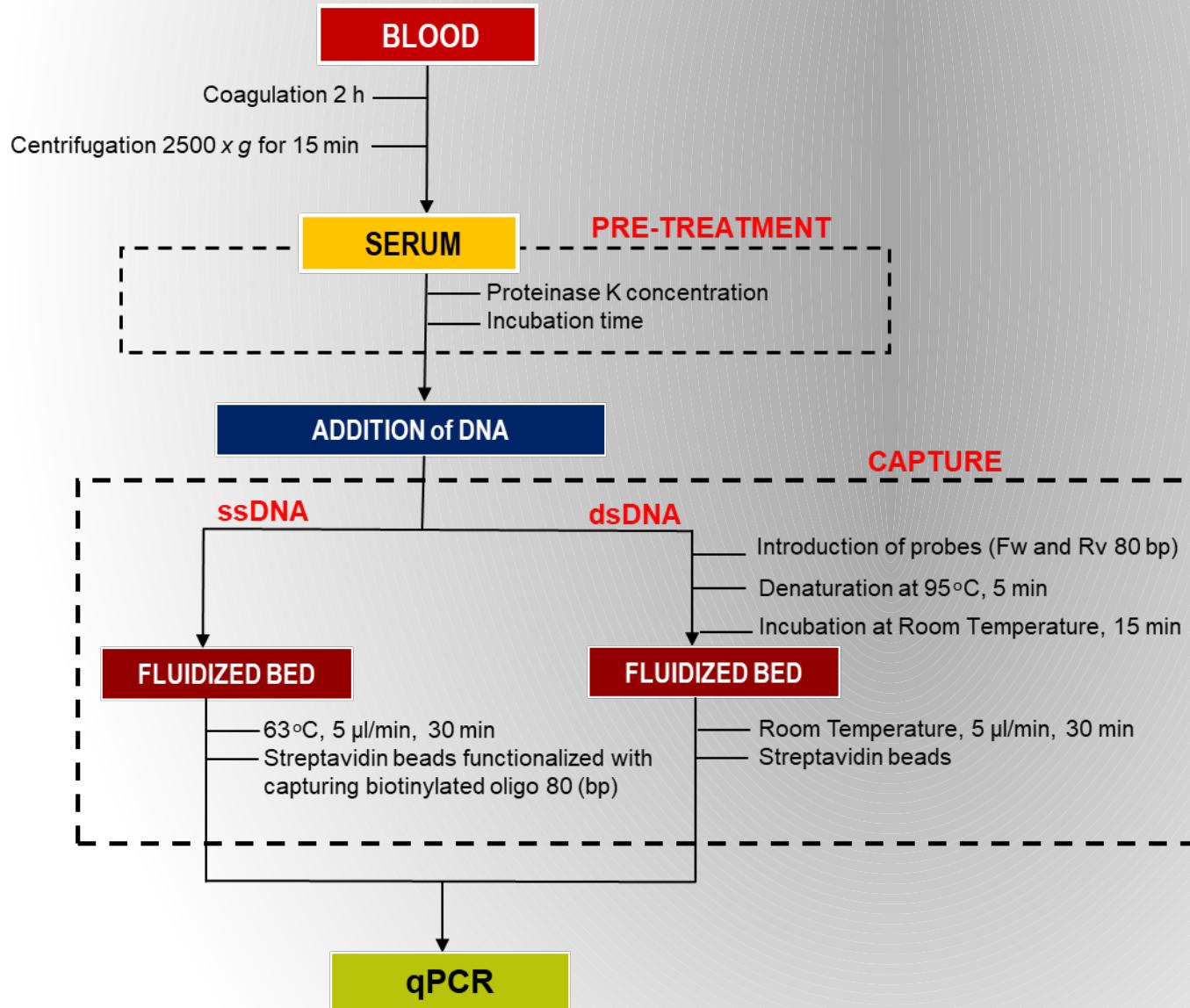


Preliminary RESULTS

SUMMARY

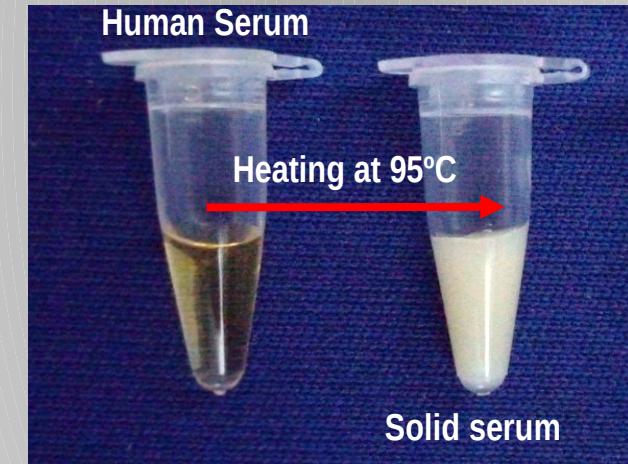
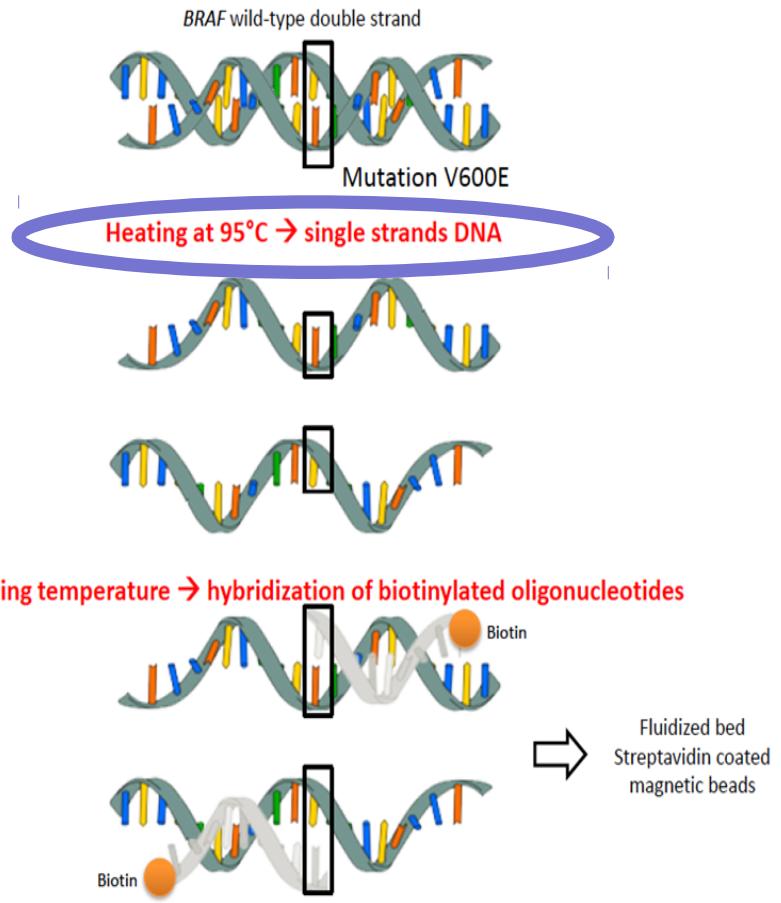
- Methodology for DNA capture
- Detection by qPCR
- Capture of ssDNA 277 bp : FB generation 2.0
 - in buffer and human serum
- Capture of dsDNA 277 bp : FB generation 2.0
 - in human serum

Methodology for capturing DNA 277bp BRAF MUT in human serum



« Houston, we have a problem... John Swigert-Apollo13 »

Strategy developed to capture dsDNA 277 BRAF MUT...

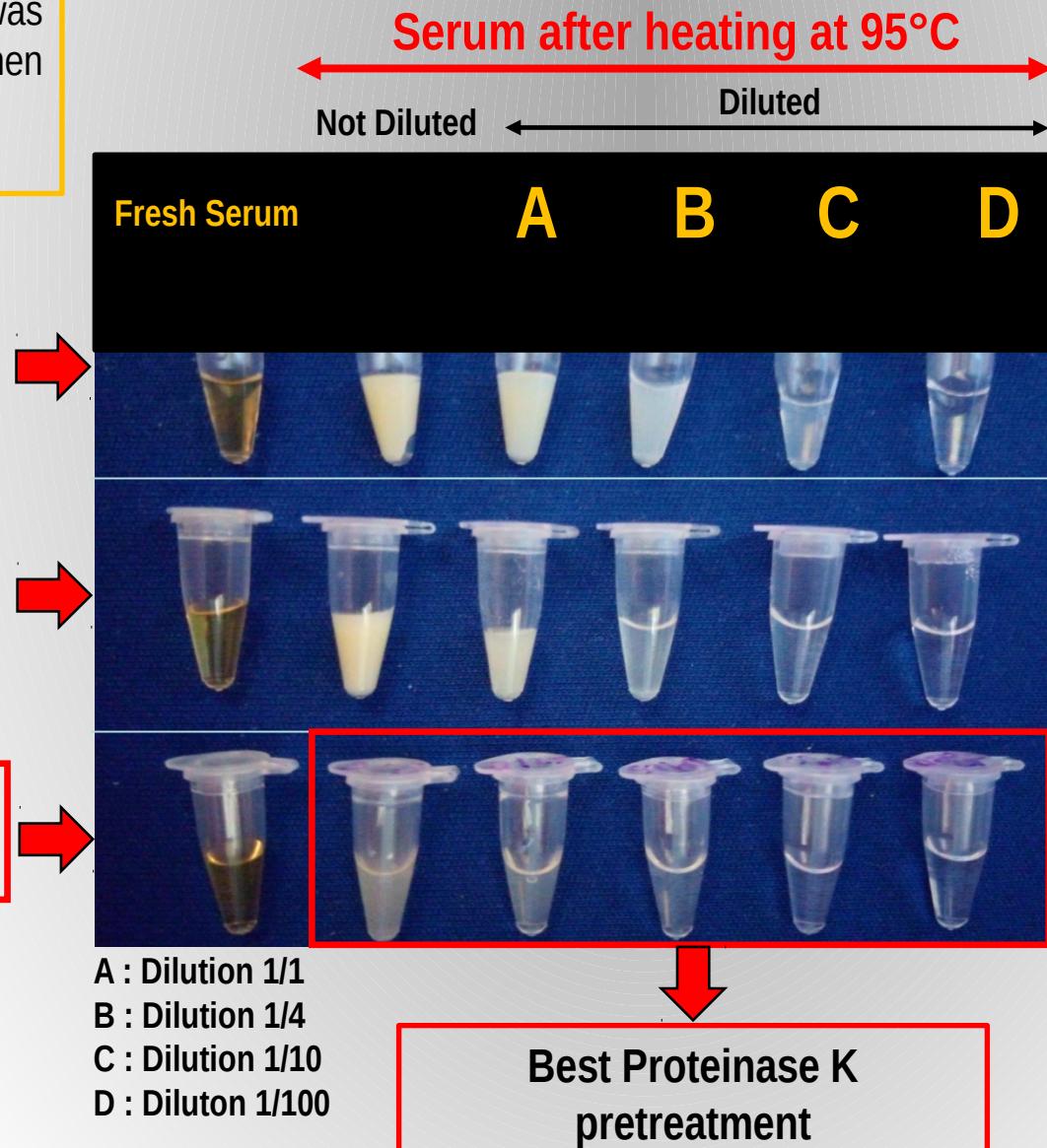


Solution
Pretreatment with Proteinase K

PROTEINASE K PRETREATMENTS

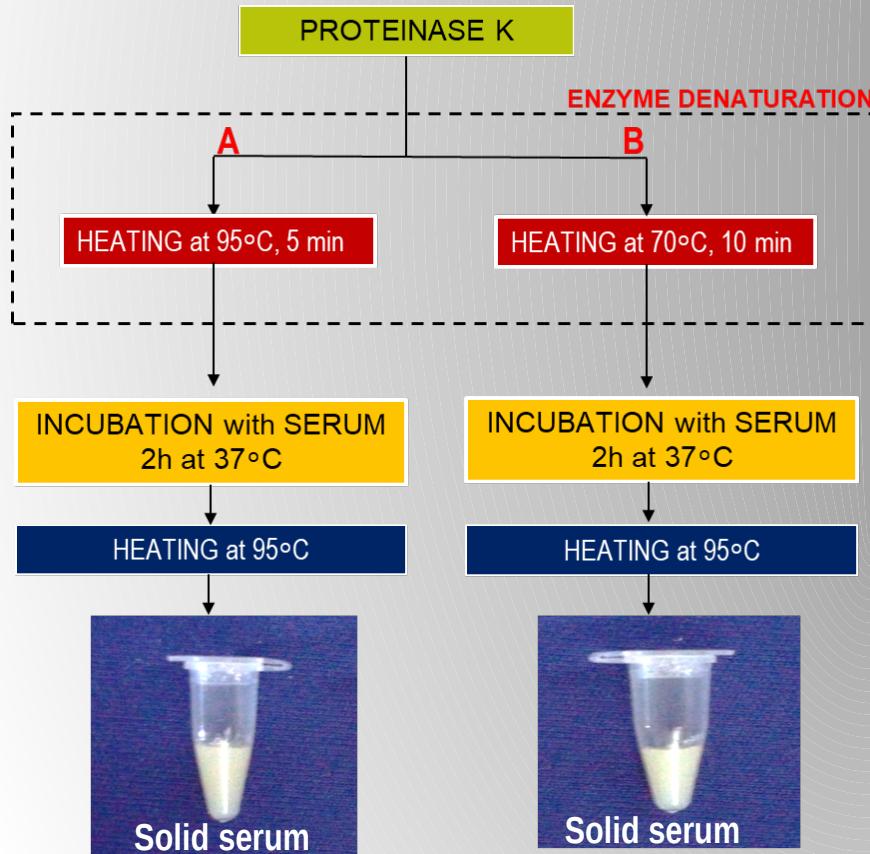
Serum (not diluted and diluted in PBS) was incubated with Proteinase K (PK) and then heated at 95°C for 5 min.
Control pretreatment : without PK

- Incubation 2h at 37°C without PROTEINASE K
- Incubation 2h at 37°C with PROTEINASE K (0.4 mg/mL)
- Incubation 2h at 37°C with PROTEINASE K (2.75 mg/mL)



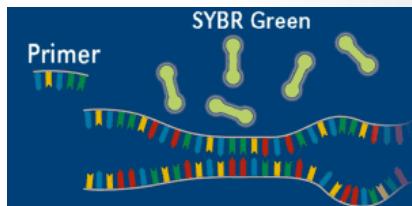
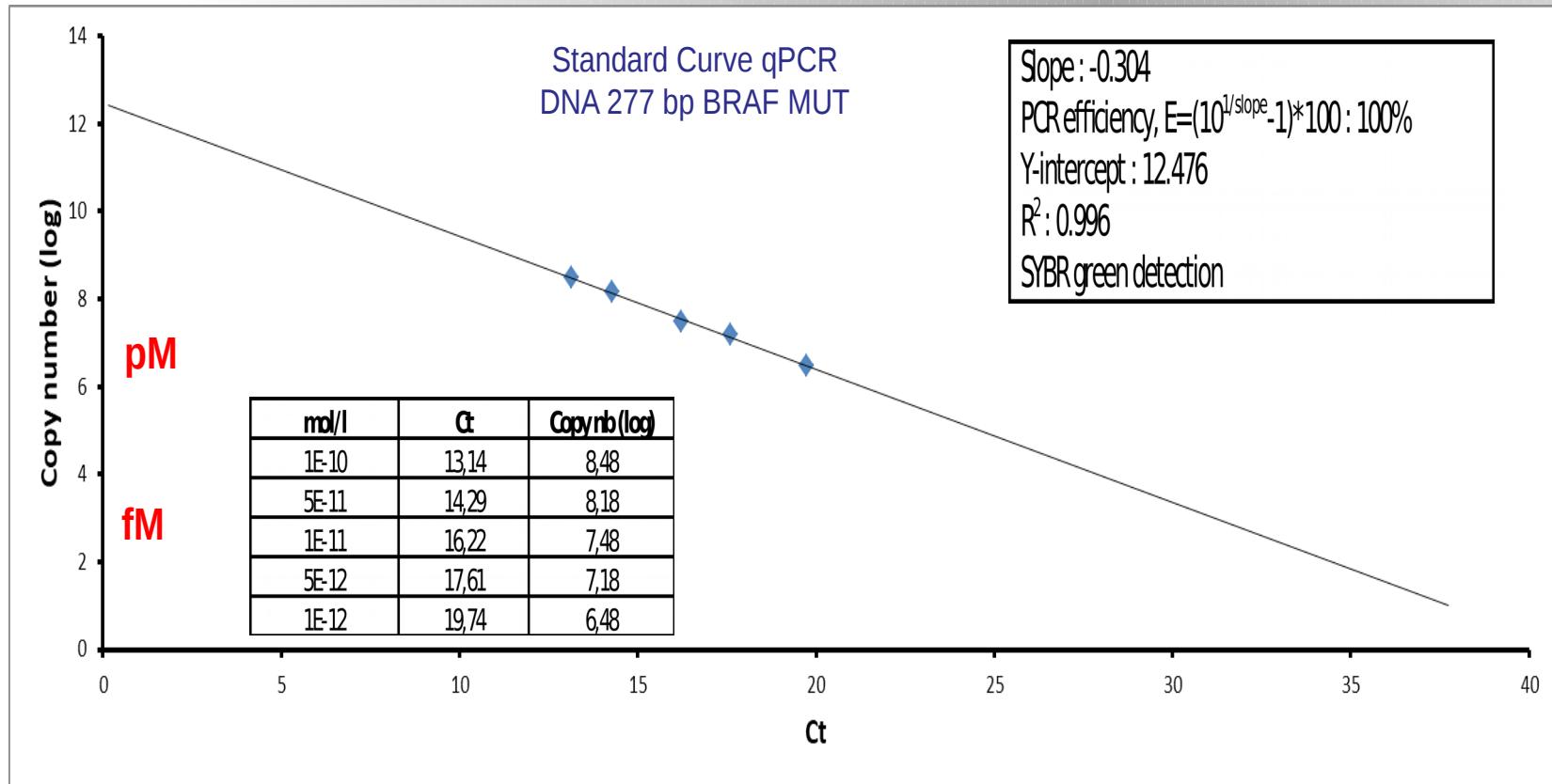
...but PROTEINASE K, Could it affect the *streptavidin* beads ?

Test to verify the residual activity of PK

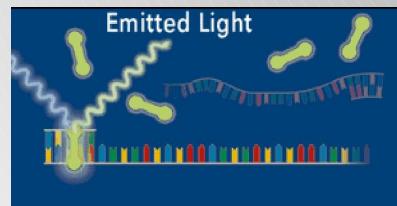


- Both conditions (A and B) seem to inactivate the enzyme
- Heating at 95°C is a 'Win-Win' condition : DNA and PK denaturation

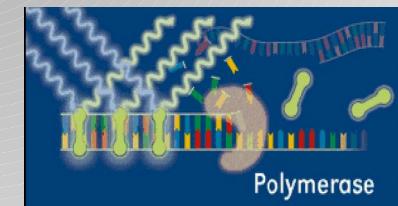
qPCR DETECTION



SYBR Green does not fluoresce until binding to ds DNA



Upon binding primers, the dye starts to fluoresce



As more dsDNA are formed more Syber Green molecules bind and fluorescence increase



Capture of ss DNA 277bp

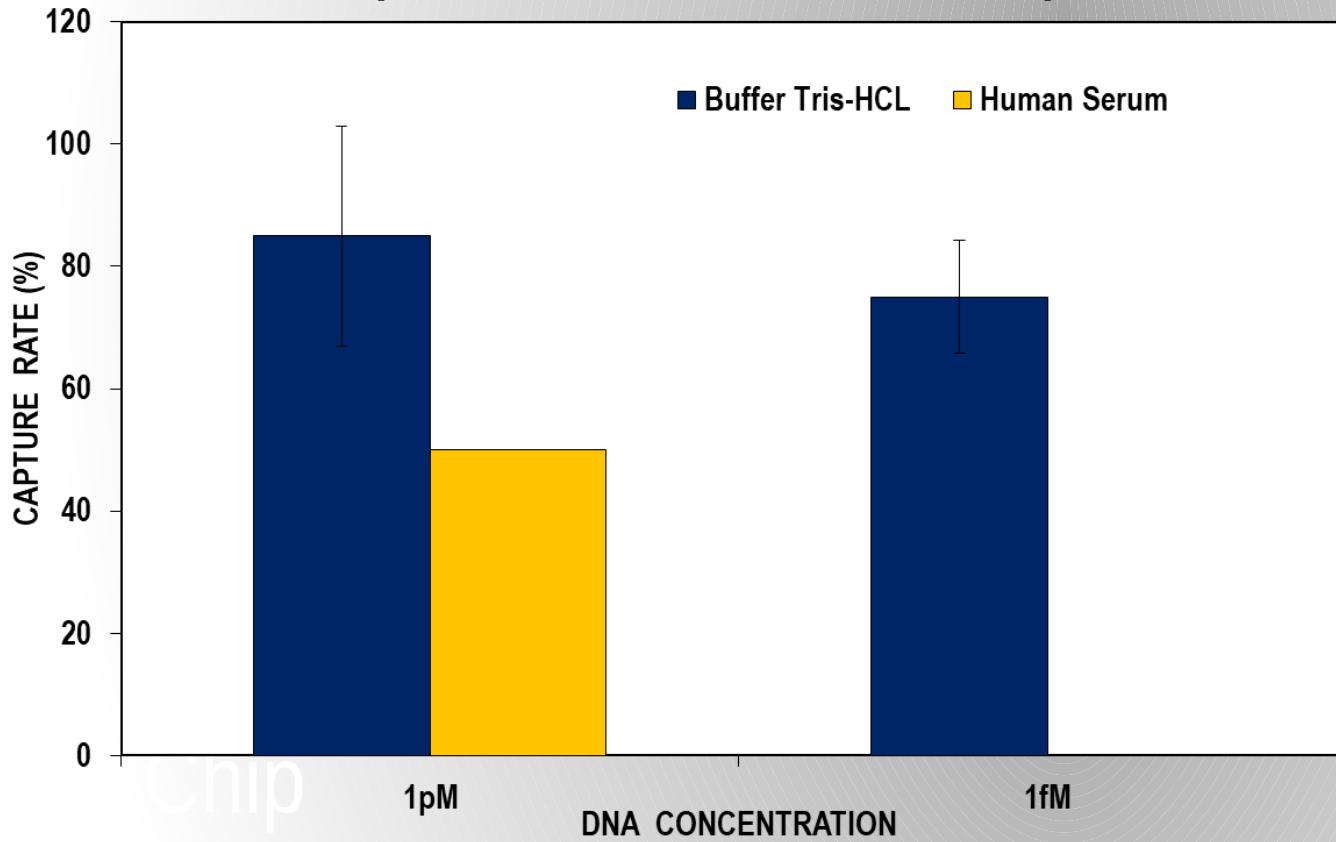


Fig 1. Capture Rate (%) of ssDNA 277 BRAF MUT in simple and complex matrix by using FB generation 2.0.
Error bars represent mean \pm SD of triplicate . One value for human serum.

Capture was performed with streptavidin beads functionalized with biotinylated oligo BRAF MUT 80 (bp). Temperature 63°C, 5 μ L/min, Bimodal + vibration. Human serum was pretreated with Proteinase K (2.75 mg/mL) 2h at 37°C before performing the capture on FB. Buffer Tris-HCL : Tris-HCL (10 mm) EDTA (5 mM) NaCl(1M). Concentration of DNA (1fM) in human serum was not determined.

Capture of dsDNA 277 bp in human serum

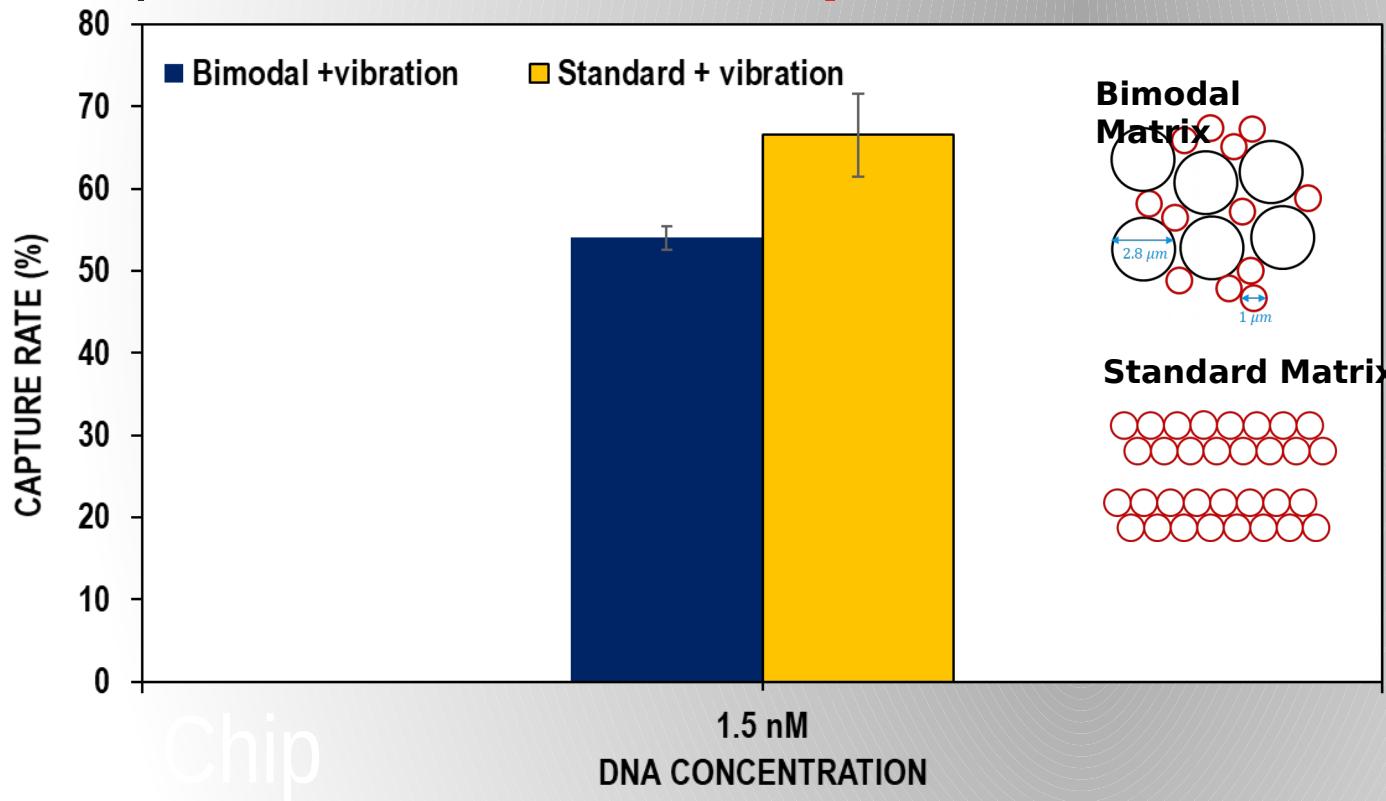
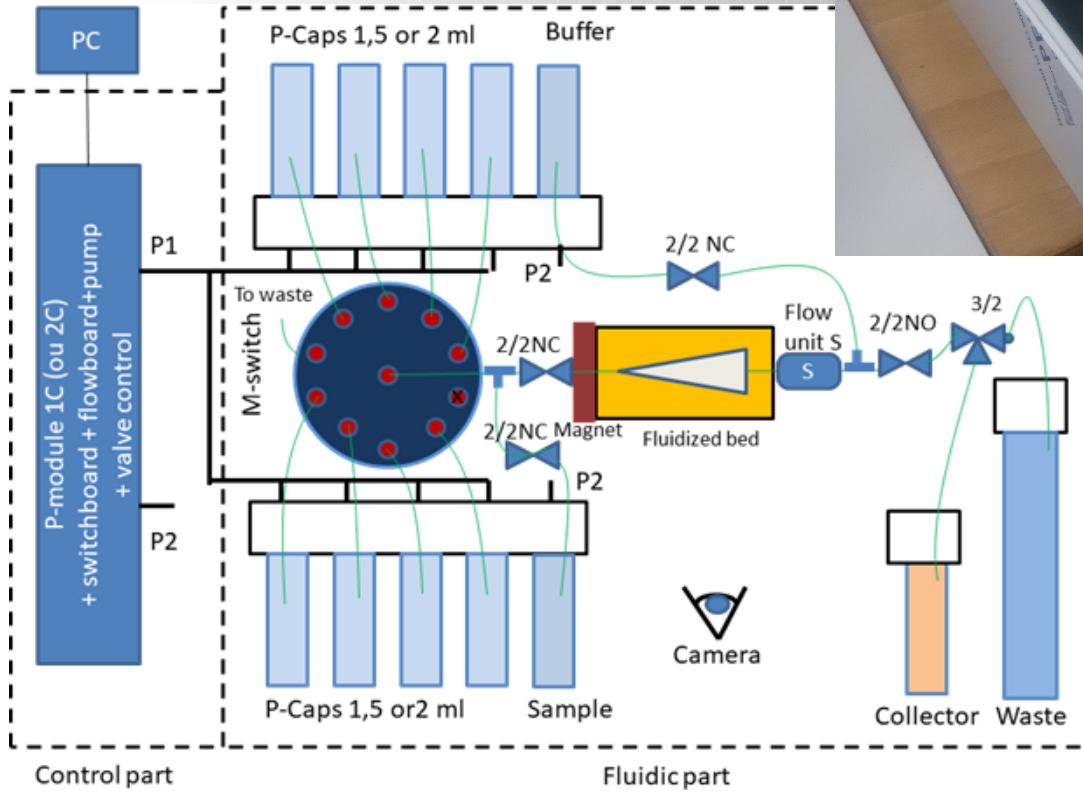


Fig. 2. Capture Rate (%) of dsDNA 277 BRAF MUT in human serum by using FB generation 2.0.
Error bars represent mean \pm SD of duplicate.

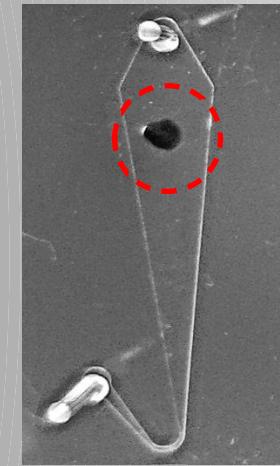
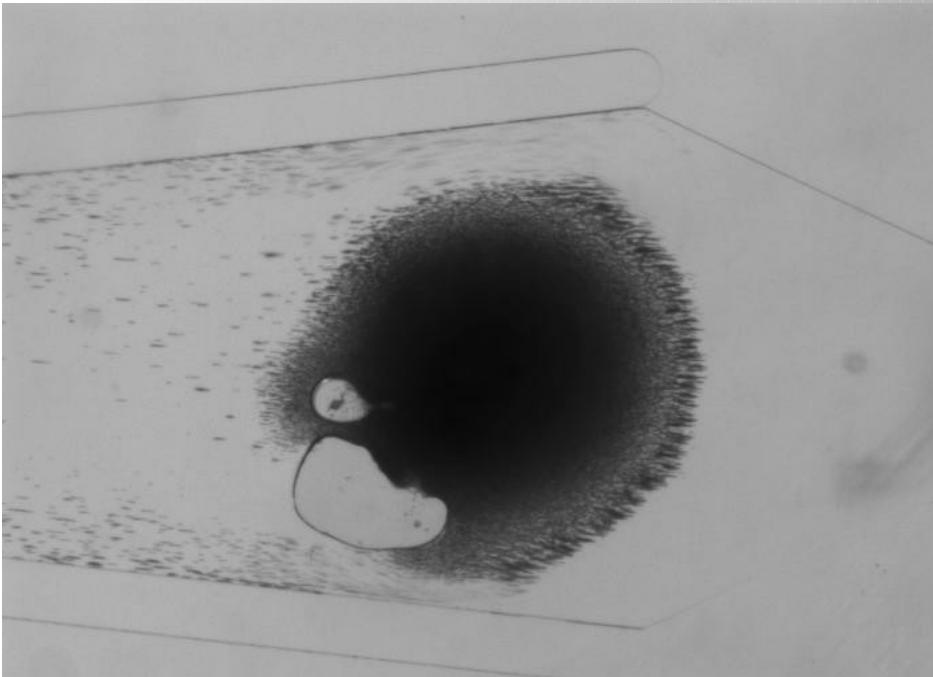
Capture was performed with streptavidin beads at Room Temperature, 5 μ L/min. Bimodal and standard matrices + vibration. Probes (0,4 μ M). Human serum was pretreated with Proteinase K (2.75 mg/mL) 2h at 37°C following by a denaturation at 95°C (5 min) and an incubation at room temperature (15 min) before performing the capture on FB.

Installation of Fluidized Bed platform

Quick view of the system



Pellets of beads



FB chip with pellet of beads

Conclusions and Perspectives

High capture efficiency (>85%) of ssDNA 277bp BRAF MUT in simple matrix at concentrations lower than 1pM

PK-pretreatment have a large impact on the protein digestion and thus on DNA extraction

Successful **dsDNA 277bp BRAF MUT** capture in human serum using FB generation 2.0. Capture rate 55-70%.

COMING UP ...

To perform dsDNA 277 capture in human serum at DNA concentrations lower than 1nM and to find the best FB capture conditions

To optimize the PK pretreatment conditions in order to reduce the incubation time

To carried out a simultaneous capture of dsDNA 277 on beads and LCR (with our partner in Greece)





Thank you for your attention !

