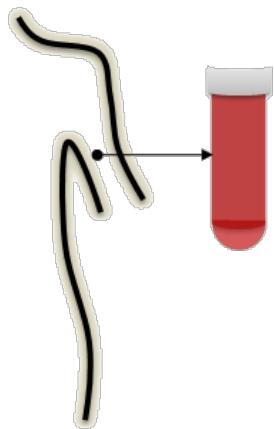


Overview of project work

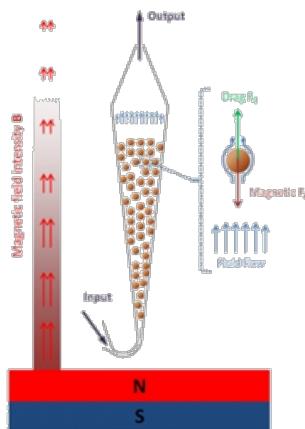
- Develop of ligase chain reaction protocol (WP3)
- Develop of acoustic assay for ctDNA detection (WP4)

1. Liquid biopsy



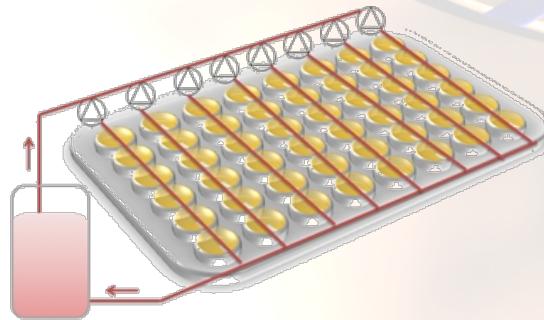
-Non invasive procedure compared to surgery
-Longitudinal monitoring of genetic alterations

2. Fluidized bed technology



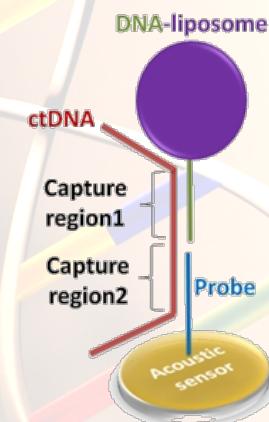
-Highly efficient and specific mutant DNA capturing
-Enrichment of mutant sequences

3. Sensor array



-Miniaturized chip consisting of 48 TSBARs
-2€ per array with a size of lower than 6 cm²

4. Acoustic detection



-Dissipative particles for ultrasensitive detection of DNA
-aM to zM sensitivity without PCR

WP3

WP4

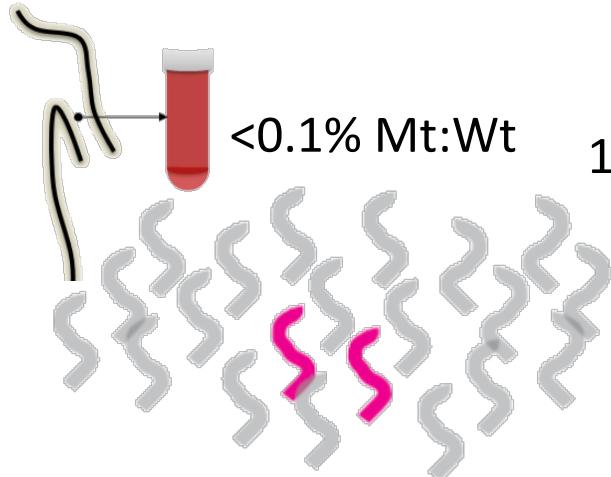
WP3 & WP4: specific objectives (FORTH)

Task 3.2: Optimization of a ligase-based assay for the selective enrichment of ctDNA targets

Task 4.1: Design, synthesis and evaluation of DNA oligos

Task 4.2: Optimization of surface hybridization conditions

WP3 & WP4: Development of LCR & detection

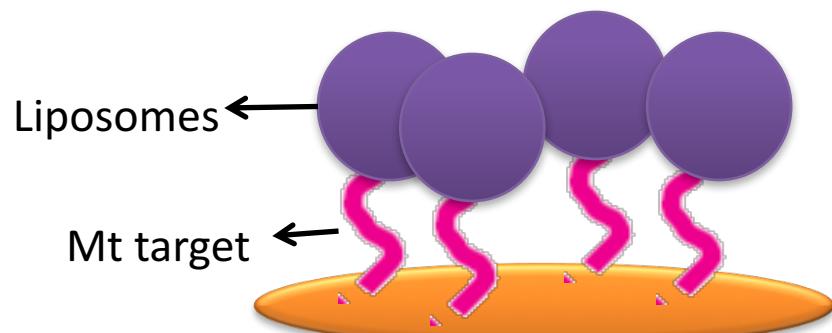


CURIE

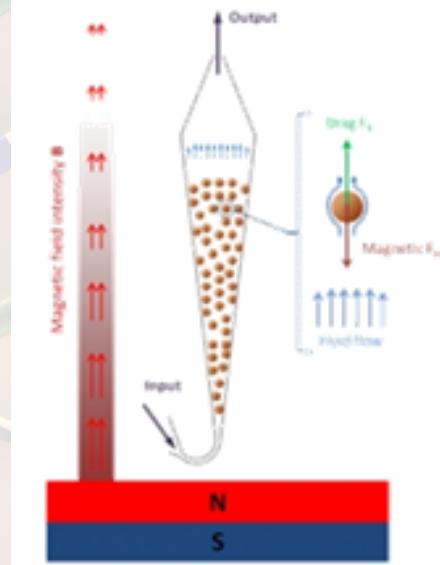
1. Extraction of total cfDNA using kits or fluidized bed technology (Curie)



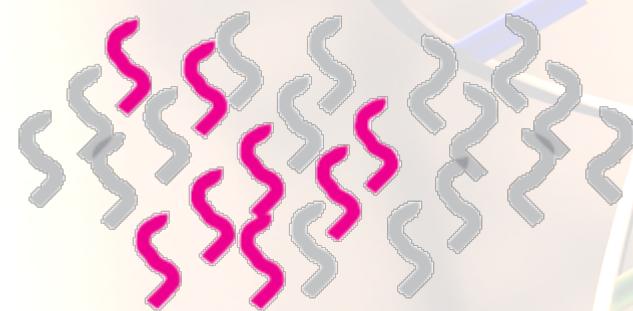
3. Surface immobilization & detection



FORTH

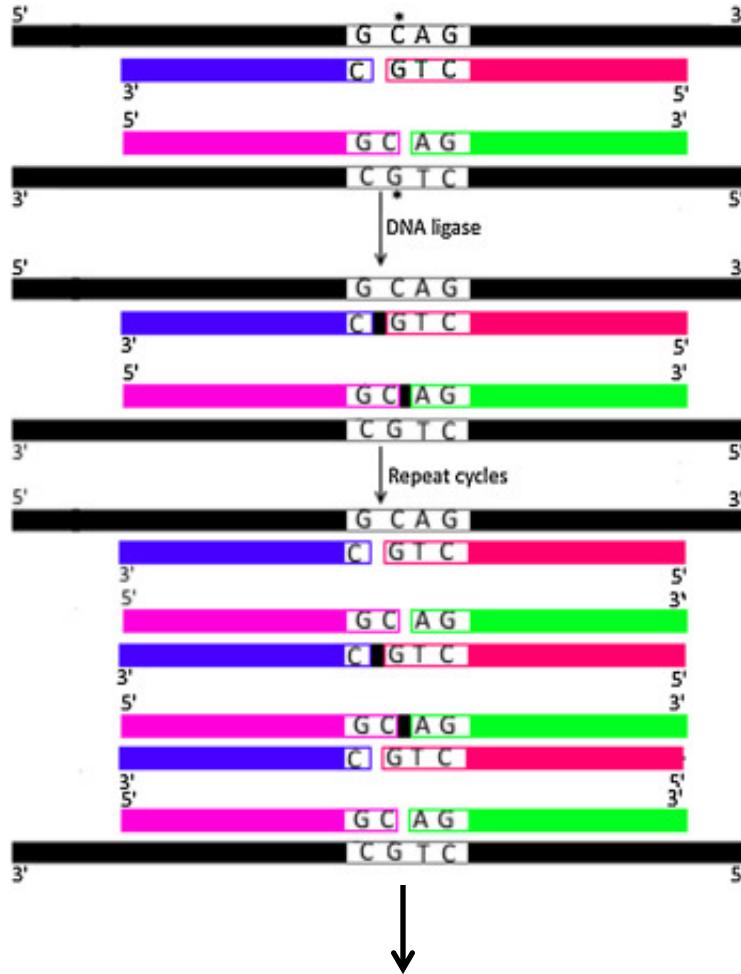


2. Enrich mutant targets with LCR



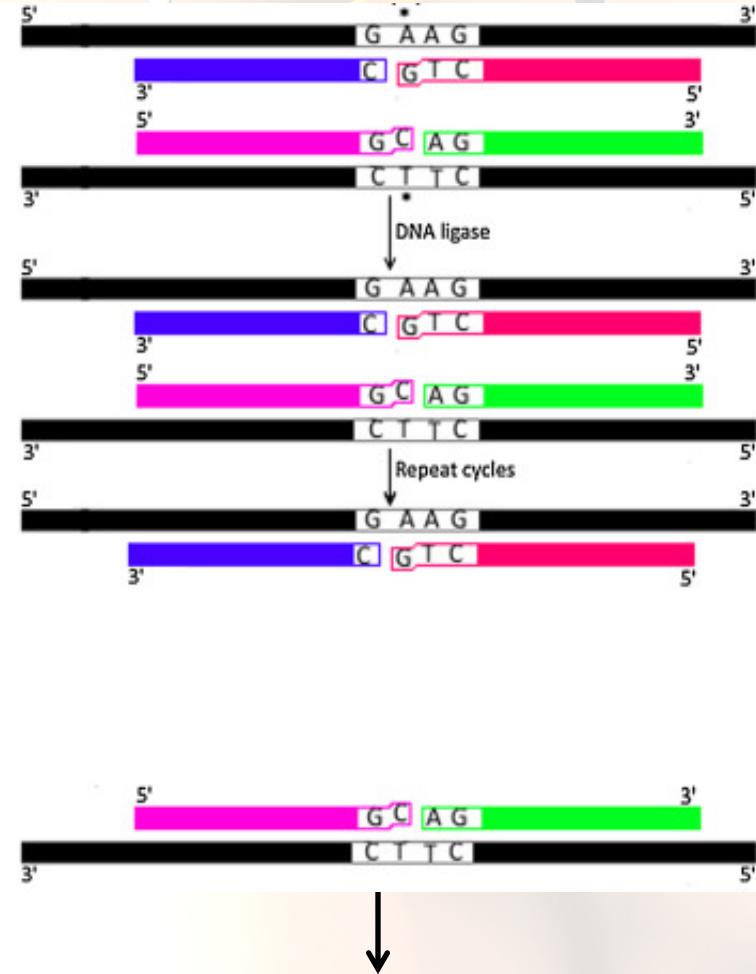
WP3: Ligase Chain Reaction

Target



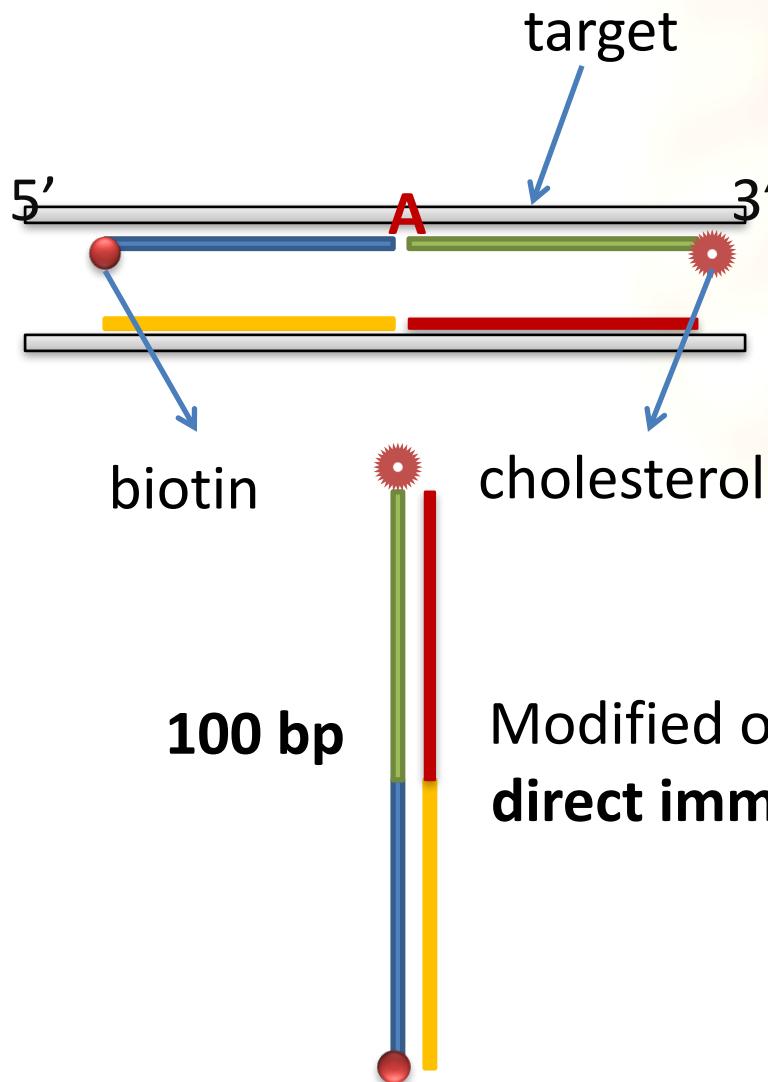
Exponentially amplified product

Mismatch



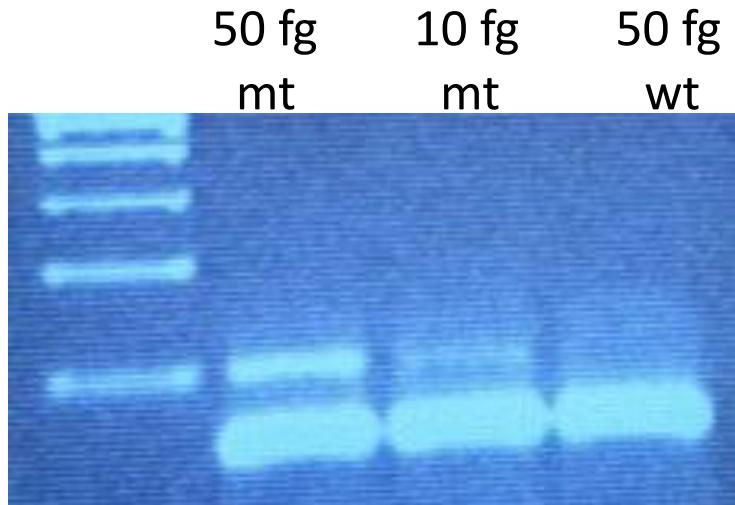
No amplified product

LCR in CATCH-U-DNA



Modified oligo with biotin & cholesterol;
direct immobilization on sensor surface

LCR optimization



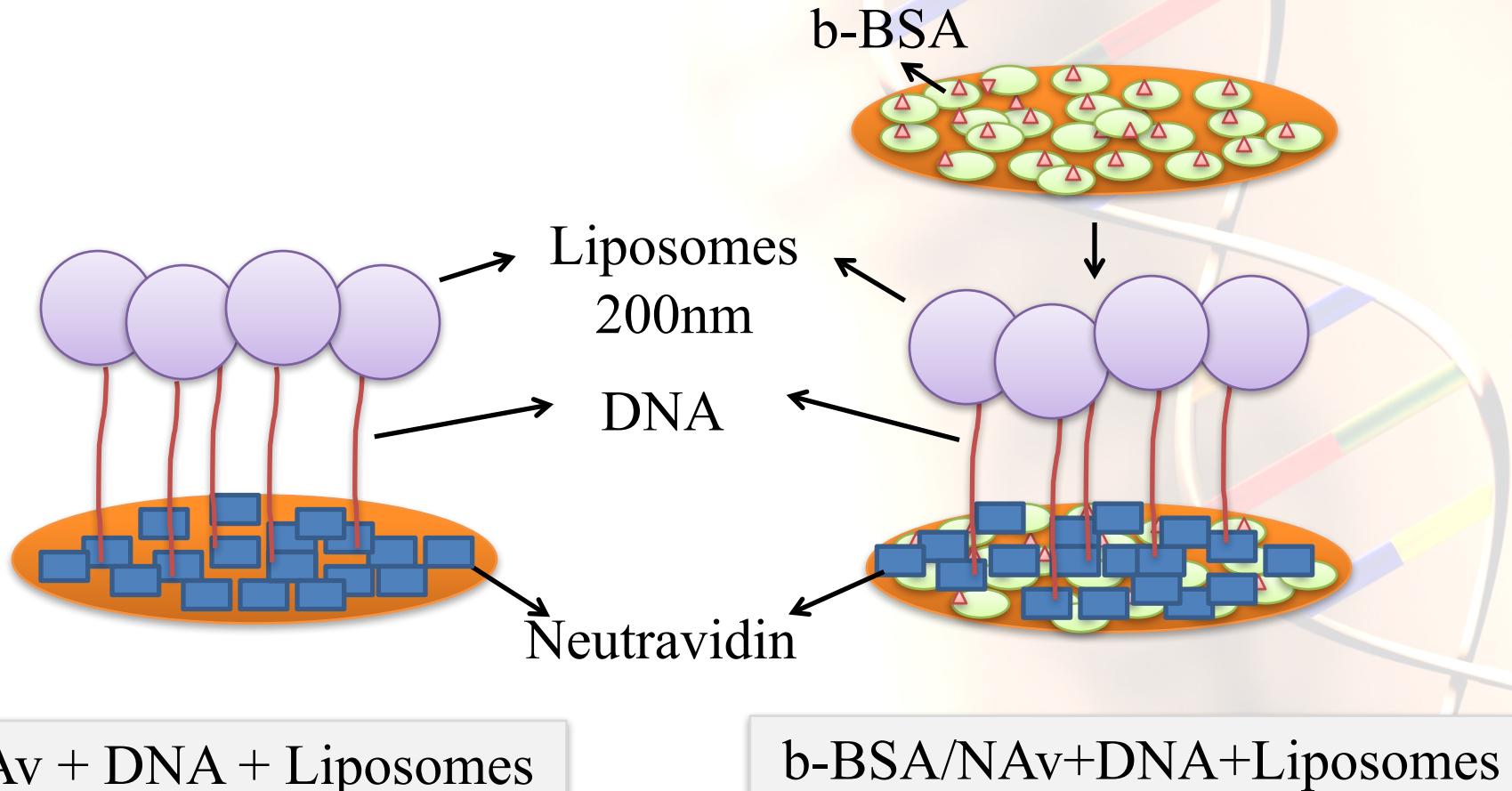
- 149 cycles of exponential LCR
- 10 fg (~33.000 molecules) of mt target

Optimization of 0.5 pg after **99** cycles of exponential LCR:

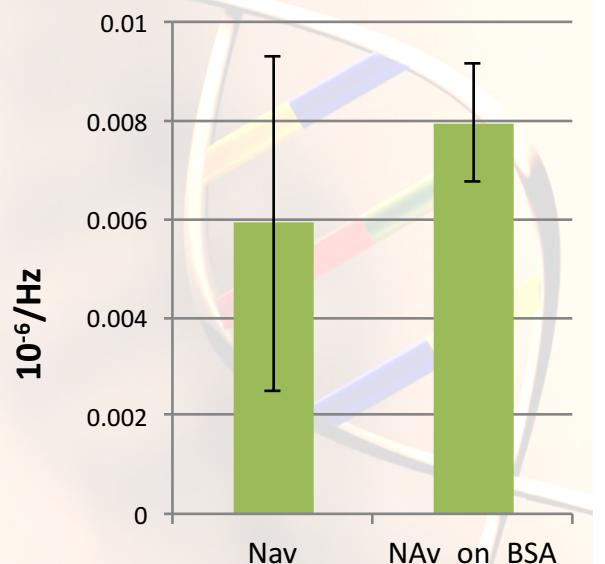
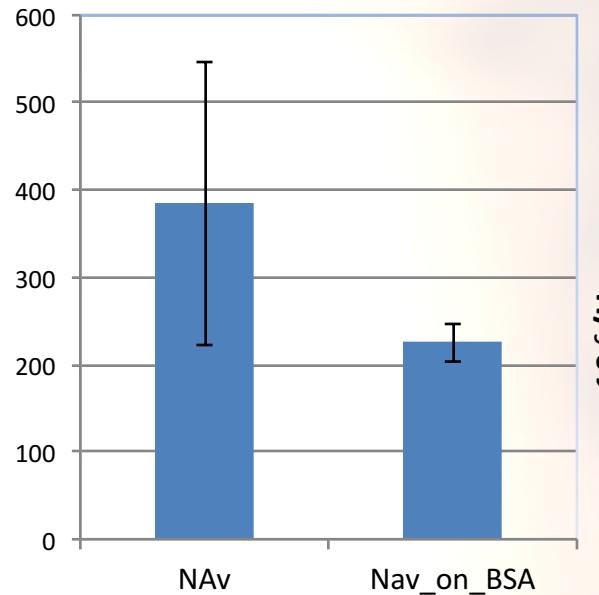
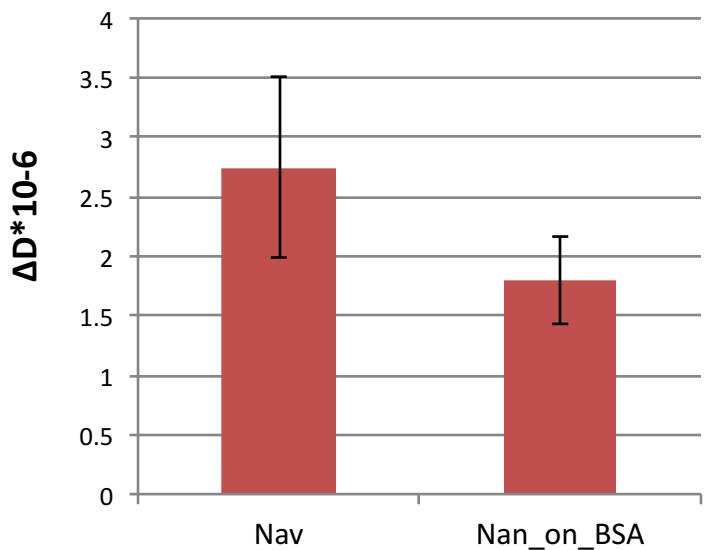
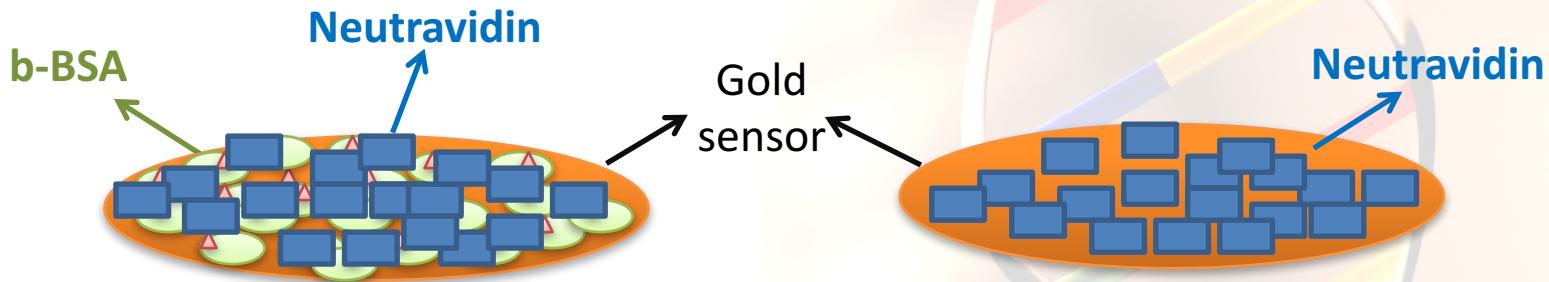
- annealing temperature
 - number of cycles
 - amount of each oligo

*For every point mutation find **optimized conditions**
(set of oligo probes, T, No of cycles etc.)*

WP4: Development of acoustic assay; surface immobilization

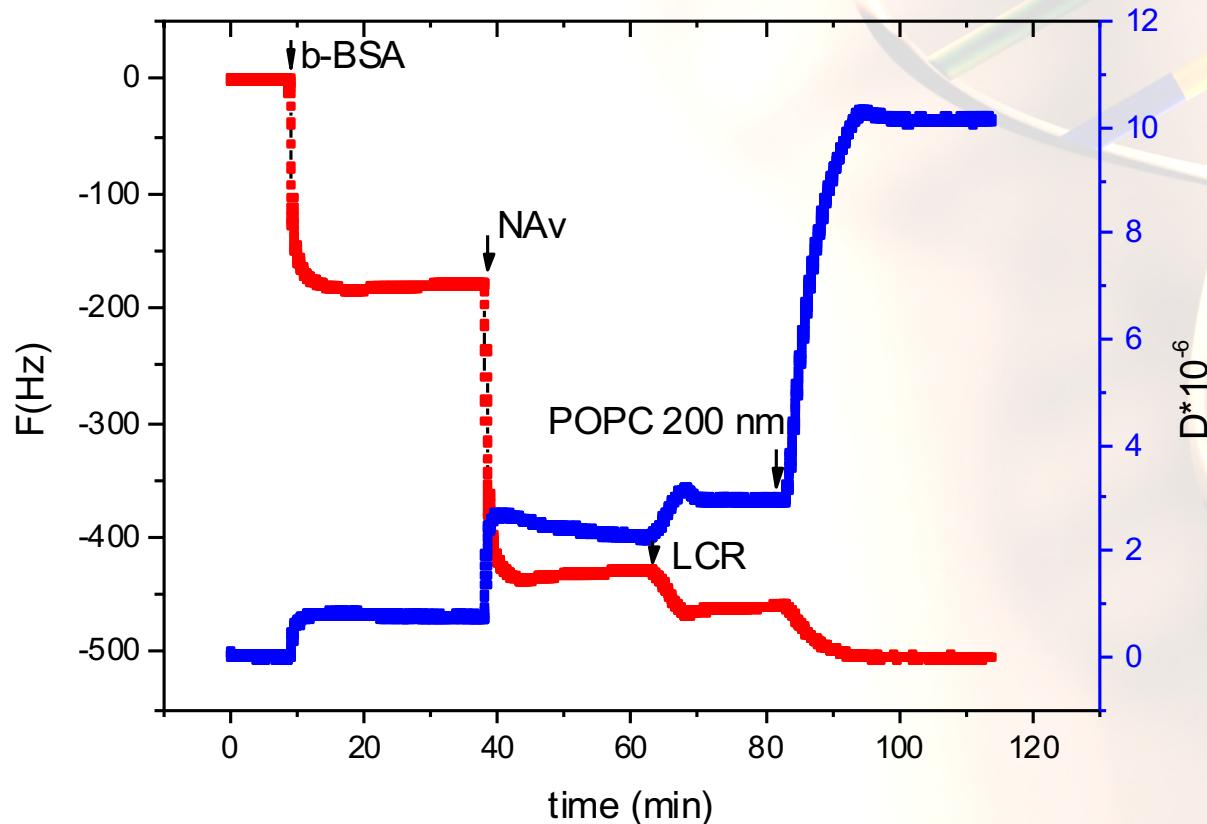


NAv vs b-BSA/NAv

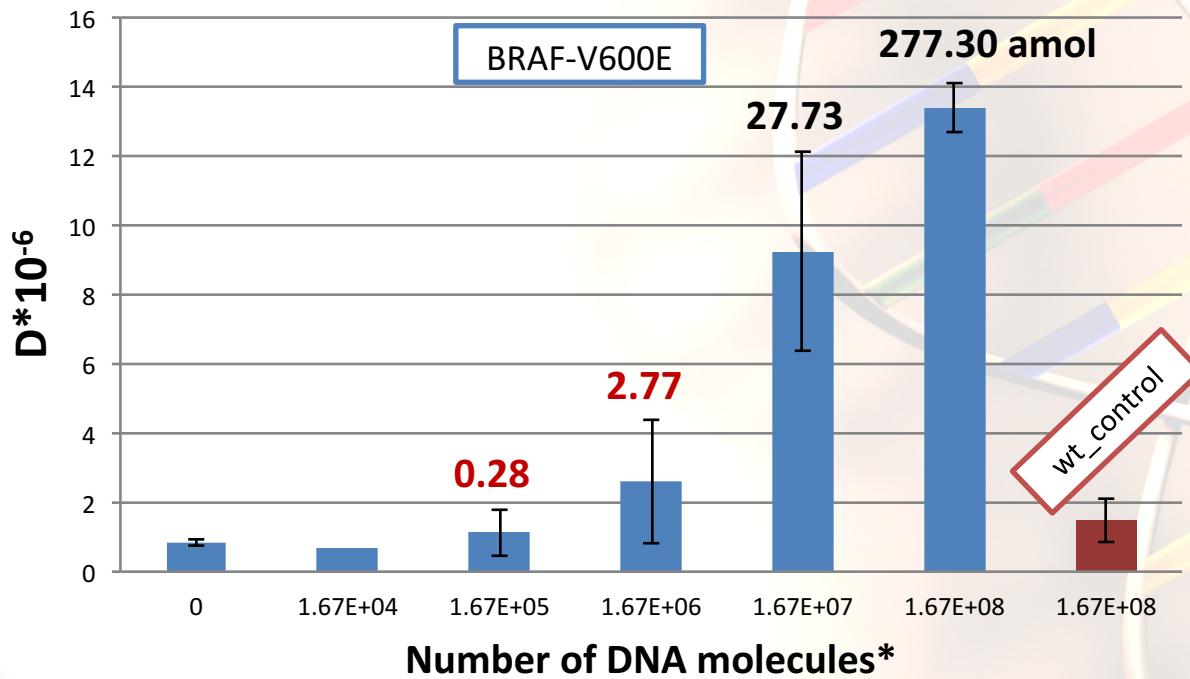
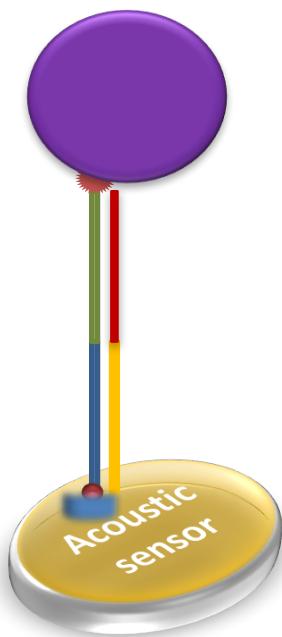


Acoustic detection of BRAF-V600E

- **b-BSA / Neutravidin**
- **LCR:** 99 cycles (50 min total duration) of **100bp** DNA
 - **200 nm** POPC liposomes



Detection limit of LCR-produced BRAF-V600E



Control 0: w/o template in the LCR mix

Control wt: 1.67E+0.8 molecules of wt

* In the presence of wt (50:50)

LoD: 3 amol (1.67M molecules) for different liposome preparations

BUT **0.3 amole (167,000 molecules)** for same preparation

Main achievements

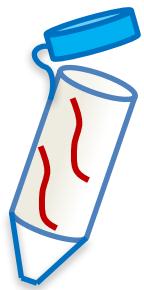
- Optimized LCR reaction in terms of specificity and sensitivity for BREAF-V600E mutation
- Optimized acoustic surface for LCR-product binding
- Achieved detection of 3 amoles or 1.5 million copies in the initial sample through combined LCR and liposomes enhancement
- Within same preparation achieved LoD of 0.3 amole and 150000 copies

Future experiments

- Develop LCR protocol for more mts
- Combine LCR with fluidized bed starting from 1 ml serum
- Demonstrate acoustic detection using the new array & instrument
 - Decrease further LoD

LCR	Non-specific binding	Highly dissipative particles
Increase No of cycles	Reduce non-specific signal	Particles of higher DC Longer DNA (>100 bp)

“LCR”



N_o

167,000

10

$$N \approx N_o * 1.04^{99}$$

167,000

10

240

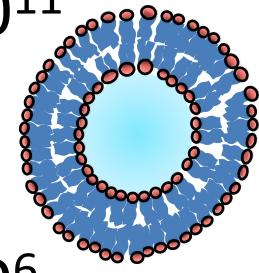
“QCM”

N

6×10^{11}

6×10^6

6×10^4

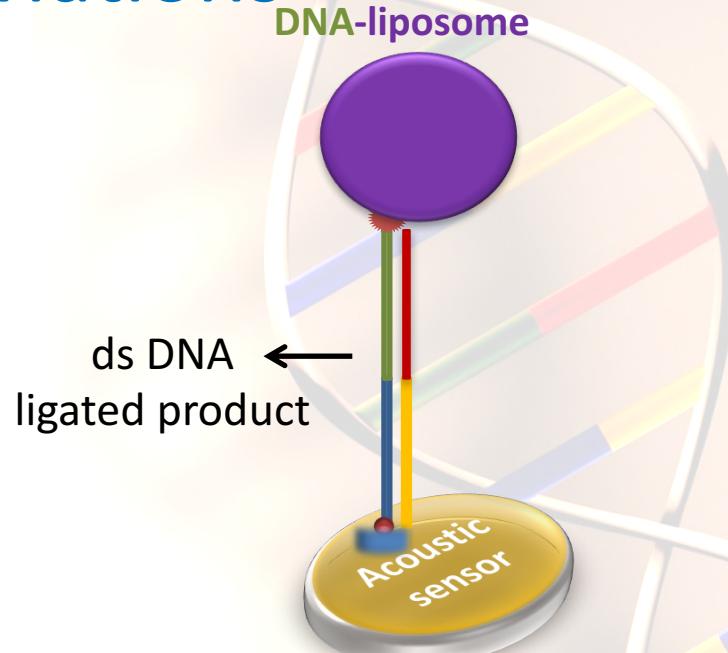
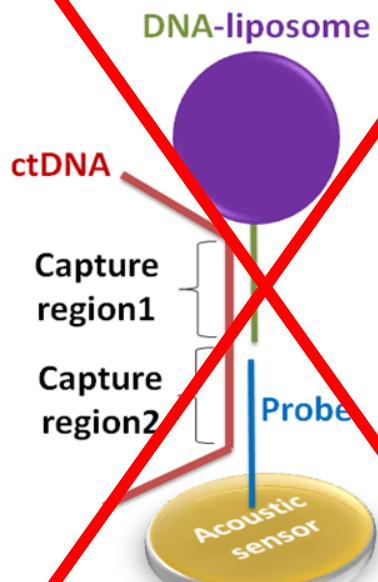


so: if Biophysics $\times 100$
& Biology $\times 2.5$



achieve the target
(one mutant)

Deviations



- Instead of 48 mts, target 12 mts + controls on a 24 array

	A	B	C	D	
1	█	█	█	█	samples
2	█	█	█	█	WT controls
3	█	█	█	█	NT controls
4	█	█	█	█	samples
5	█	█	█	█	WT controls
6	█	█	█	█	NT controls

Panel 1

	A	B	C	D	
1	█	█	█	█	samples
2	█	█	█	█	WT controls
3	█	█	█	█	samples
4	█	█	█	█	WT controls
5	█	█	█	█	samples
6	█	█	█	█	WT controls

Panels 1,2,3

Acknowledgements WP3 & WP4 (FORTH)

- Ms Nikoletta Naoumi (PhD student)
 - Dr Dimitra Milioni
- Ms Fotini Papagavril (MSc student)
- Ms Gesthimani Theodosi (MSc student)

Thank you