# Detection of non-Amplified circulating tumor DNA (ctDNA) with Ultrasounds

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## ABSTRACT

Cancer is the 2nd leading cause of death worldwide. The best way to achieve effective and efficient management of cancer is the early diagnosis and the frequent monitoring of patient response to treatment. Herein, we aimed to develop a non-PCR based approach for the detection of circulating tumor DNA (ctDNA) point mutations presented in colorectal (CRC) and lung cancers, in the context of liquid biopsy. Liquid biopsy is a non-invasive technique to track the disease through the analysis of circulating biomarkers, like nucleic acids, presented in the peripheral blood (plasma or serum) of cancer patients. This technique has been called the “Holy Grail” of future cancer diagnosis. The project is part of the CATCH-U-DNA research project awarded within the Horizon2020 FET-OPEN call.

The approach involves the use of a ligase-based method for the amplification of the ctDNA targets combined with acoustic biosensors for the detection. In more detail, the first step is the selective enrichment of specific ctDNA targets from serum samples carrying the BRAF-V600E point mutation, which has a strongly positive correlation with CRC and lung cancers. For the enrichment is used the fluidized bed technology developed by Curie Institute. Following this step, the mt targets are exponentially amplified with high specificity and sensitivity with the Ligase Chain Reaction (LCR); this method is known to obviating any enzymatic polymerization and bias amplification. The LCR products are subsequently selectively immobilized on the surface of the acoustic sensor via the NAv-biotin interaction. The final step of the assay involves the specific capturing of POPC liposomes by the mutant target. This step causes high changes in the signal of the acoustic wave (frequency, dissipation) achieving ultra-sensitive DNA acoustic detection. With the currently established LCR protocol, we have managed to detect down to the 3 x $10^3$ molecules of mt targets using a 35 min LCR protocol.

The last part of the approach involves the use of a novel acoustic micro-sensor array and a platform developed by Advanced Wave Sensors, partners in the CATCH-U-DNA project. This array operates at 150 MHz and allows the multiple performance of 24 measurements.