# On-chip quantification of miRNA using digital droplet PCR

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## Introduction

Recent advances show that miRNA has a great potential in diagnostics. Several studies demonstrate that the expression profile of miRNAs, which are short (18-22 nucleotides) but powerful regulators of a wide range of biological processes, can aid in distinguishing between healthy and diseased patients. As these regulators are detectable in peripheral blood, they can perfectly serve as blood-based biomarkers. An interesting way to profile miRNAs involves the use of lab-on-chip tools, which allow automated quantification. Therefore, it is of interest to implement miRNA release, reverse transcription (RT) and detection of miRNA into a single microfluidic device or chip. As digital droplet PCR (ddPCR) has shown to be more accurate and has an improved reproducibility compared to regular qPCR, microfluidic chips for ddPCR as well as for thermal lysis and RT were fabricated using in house silicon technology.

## miRNA detection workflow

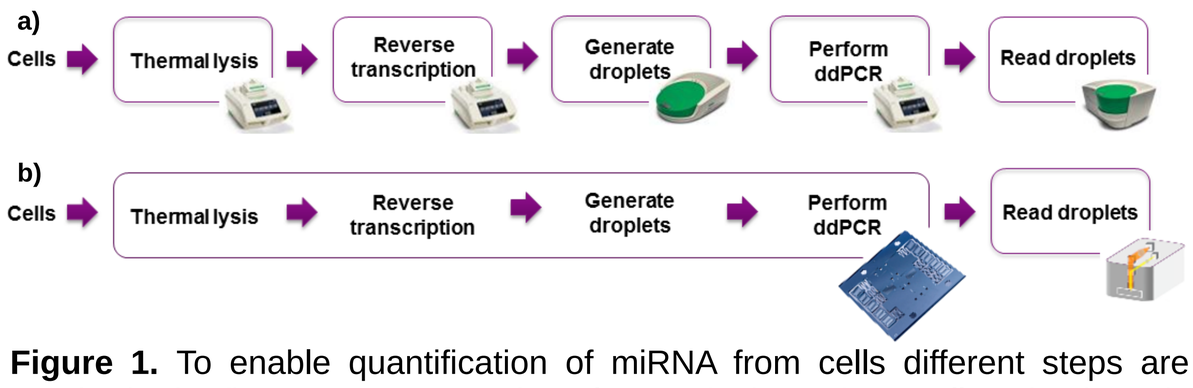


**Figure 1.** To enable quantification of miRNA from cells different steps are required. The first step concerns the release of miRNA from cells using heat (5 min. At 95°C). The next step requires the mixing of the lysed cells with the reverse transcription mix (30' at 16°C, 30' at 42°C and 5' at 85°C). The last step consists of quantifying the miRNA copy number using digital droplet PCR. Current detection systems for miRNA imply the use of several bench-top tools as well as sample handling (a). Using Imec's state-of-the-art silicon technology, it is possible to implement these steps fully on chip and to simplify hands on work (b).

## Implementation on chip

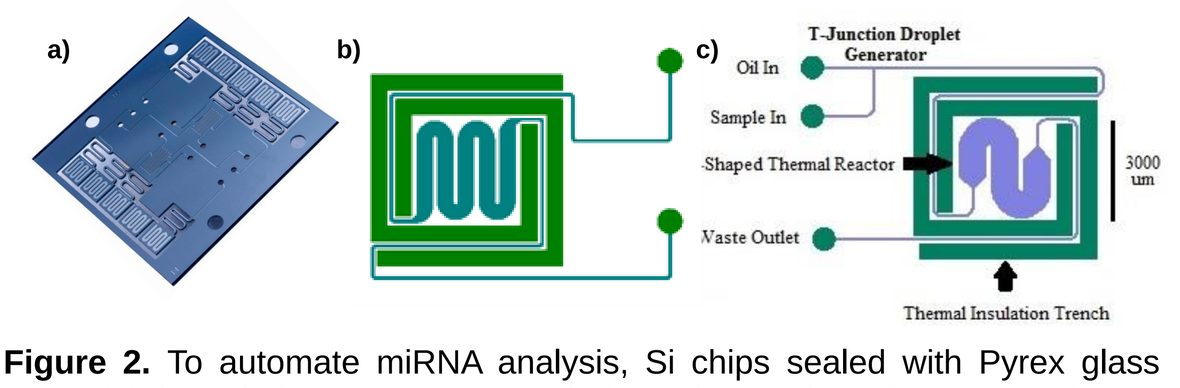


**Figure 2.** To automate miRNA analysis, Si chips sealed with Pyrex glass were fabricated (a). Every step of the miRNA detection protocol was performed on different chips, the solutions were pre-mixed before loading. Lysis of cells for miRNA release and RT were performed in S-shaped cavities (b). Whereas for the ddPCR chips, a T-shaped droplet generator and a thermally insulated PCR reactor were designed (c). A thermoelectric Peltier element was used to accurately control the temperature of the reactors up to 100°C.

## Results

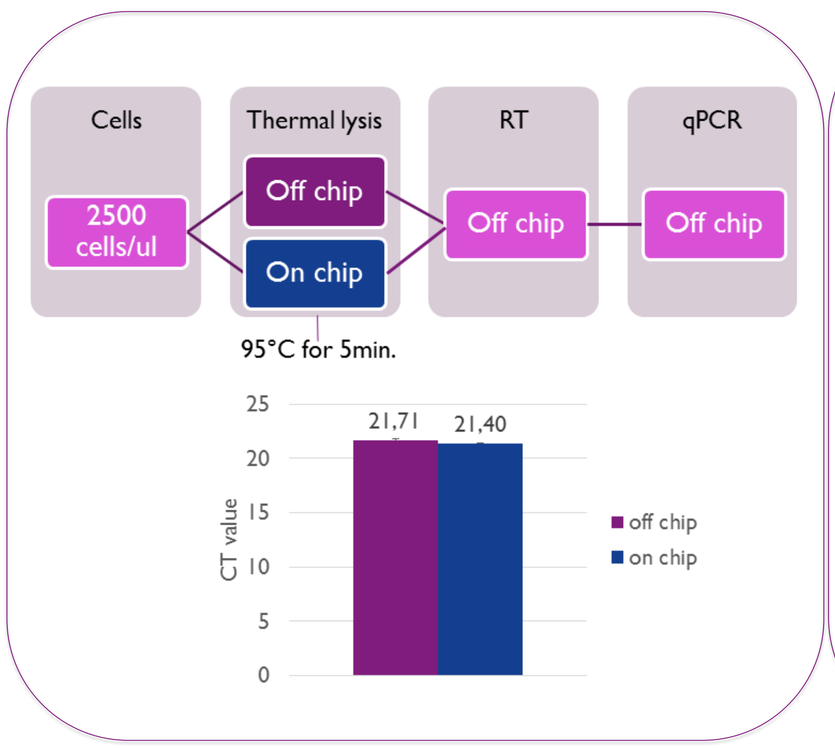


**Figure 3.** Thermal lysis was performed on chip and compared to off chip (on bench-top tools) lysis. Both thermal lysed samples were subjected to RT and qPCR off chip for comparison purposes. The difference in CT is smaller than 0,3.

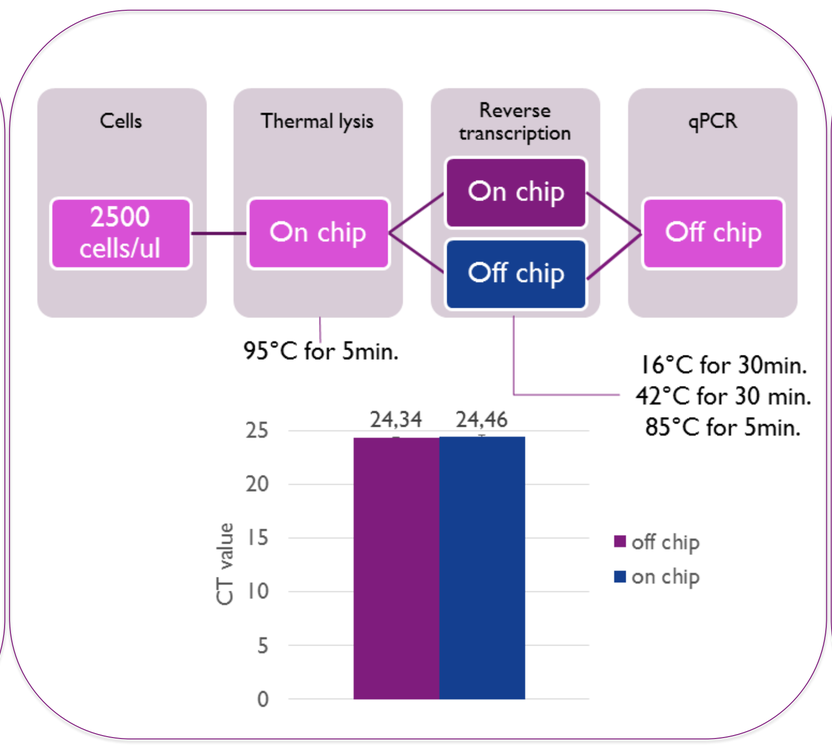


**Figure 4.** Also the RT was performed both on as well as off chip with on-chip lysed cells. Also here, quantification was done off chip using qPCR. Again the difference in CT is smaller than 0,3.

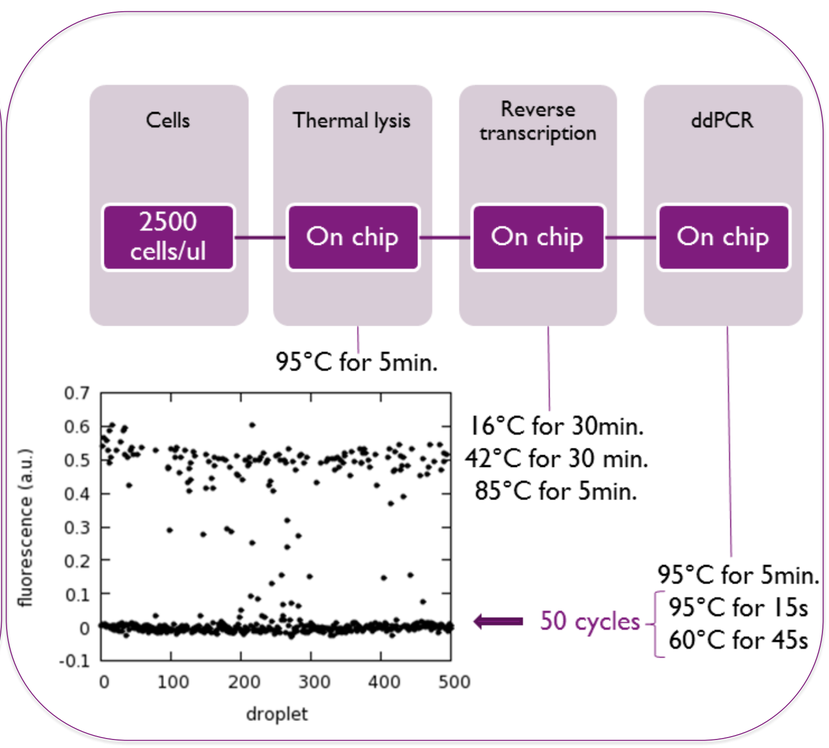


**Figure 5.** For the detection of miRNA on chip, cells were both thermally lysed and reverse transcribed on chip and sequentially used for ddPCR on chip. Results show that the concentration determined by ddPCR (780 copies/μl) was in close proximity with the value determined by qPCR (900 copies/μl).

## Conclusion

Preliminary results show that it is possible to process the different steps of miRNA detection on chip. Initial characterization studies indicate comparable quantification with bench-top tools. The next approach, would be to design a chip which allows all three steps to be performed sequentially. Future experiments also include the improvement of limit of detection as well as the implementation of multiplexing of several miRNAs.