# MICROCHIP INTEGRATED ROLLING CIRCLE AMPLIFICATION FOR SINGLE DNA MOLECULE DETECTION IN MINUTE SAMPLE VOLUMES

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## ABSTRACT

We have recently successfully integrated Rolling Circle Amplification (RCA) into microchannels in microchips using bead immobilized DNA oligos. Using RCA amplification, sensitivity was increased to detection of only 9000 molecules, which is not possible using flow cytometry based methods, and is an important step towards single analyte molecule detection.

**KEYWORDS:** DNA, rolling circle amplification, solid-phase amplification

## INTRODUCTION

Genomic analysis is currently an important technique for biological, medical and food analysis, and microchips have been utilized for DNA analysis based on PCR technology. Even though quantitative PCR provides single-molecule sensitivity in principle, practically, the technique only allows for averaged measurements. Also, the actual number of DNA molecules cannot be counted. Single molecule counting methods, not utilizing PCR, based on padlock probes and RCA have been reported [1, 2]. This technology is capable of single DNA molecule detection through the selective recognition of a padlock probe and amplification of individual target sequences with RCA, where identified target sequences become micron-sized fluorescent spots that can be counted using a fluorescence microscope. Hence, this method is well suited for quantification, counting, of single amplified DNA sequences, without prior PCR-amplification.

## THEORY

Molecular recognition of DNA targets through padlock probe ligation results in the formation of a unique circular DNA. The circular molecule acts as an endless template for a RCA reaction. This produces a long single-stranded concatemer DNA molecule complementary in sequence to the DNA circle, repeated approximately 1,000 times, which spontaneously collapses into a random coil of DNA. Fluorescent molecule–tagged probes are hybridized to the repeated sequence, resulting in a confined cluster of up to 1,000 fluorophores, visible in a fluorescence microscope as a bright dot with a diameter of approximately 1 μm.

## EXPERIMENTAL

Firstly, we demonstrated that microchip based on-bead RCA is possible using the same conditions as in microtube experiments. A Y-channel microchip (see Fig. 1), with a 210 μm wide and 40 μm deep channel containing a dam structure with a 10



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μm opening was fabricated. Agarose beads (34 μm) with the primer oligo (35-mer) was introduced into the channel and used for the RCA experiments. The system set up was shown in Fig.2. Experiment procedure was summarized in Table 1.

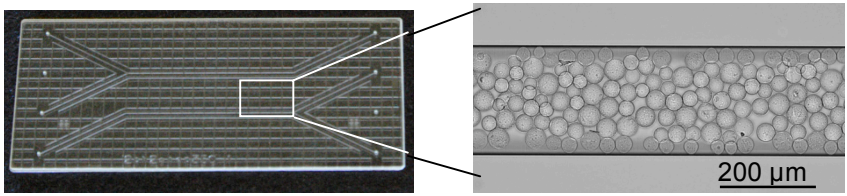


*Figure 1. Rolling circle amplification (RCA) Chip.*
*Microchip : Tempax glass plates, 30 mm × 70 mm.*

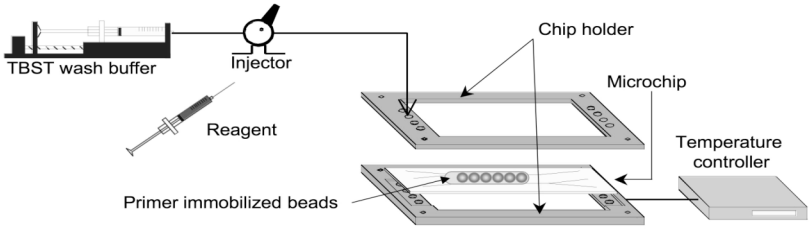


*Figure 2. The system set-up*

*Table 1. Experiment procedure*

| Solution | Temperature | Flow rate | Reaction time | |
|---|---|---|---|---|
| 1.Padlock probe solution<br>400 pM 5 μl | 37℃ | 0.5 μl/min | 60 min | |
| TBST (washing) | | 20 μl/min | 10 min | |
| 2.Ligation mixture<br>20 μl (Ampligase) | 55℃ | Stopped flow | 30 min | |
| TBST (washing) | | 20 μl/min | 10 min | |
| 3.RCA mixture<br>20 μl (phi29 Polymerase, dNTP) | 30℃ | Stopped flow | 60 min | |
| TBST (washing) | | 20 μl/min | 10 min | |
| 4.Fluorescence probe solution<br>2 μM 5 μl (TxRed-DNA) | 37℃ | 0.5 μl/min | 60 min | |

## RESULTS AND DISCUSSION

RCA was successfully performed in the microchip, and individual RCA products were visible with a fluorescence microscope as bright dots, which could be easily

counted (Fig. 3C). The microchip based on-bead RCA reaction allowed detection of as few as 9000 molecules (Fig.4), which could not be detected using a flow cytometer. Of these, about 10% where visible and counted, and enough for quantification, however, for further sample dilution the efficiency must be increased. The optimization z-axis scan and image processing are under investigation. Efforts are also underway to decrease the analysis chamber and injection tube surface to decrease loss of analytes through non-specific binding.

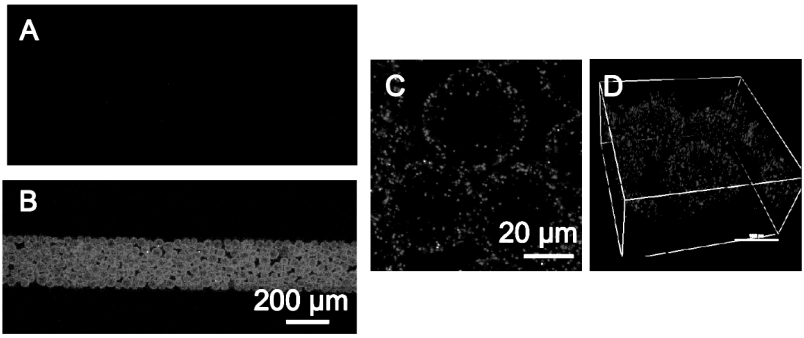


*Figure 3. Detection of a single RCA products on-bead in a microchip. (A) Negative control. (B) Positive control. (C) Enlarged image of (B). (D) 3D image*

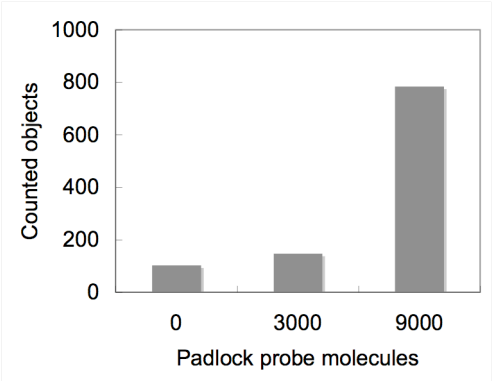


*Figure 4. Observed individual fluorescent signals in the bead filled analysis chamber.*

**ACKNOWLEDGEMENTS**

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