Topic No.6.07 Nucleic Acids/Genomics Abstract Reference No: 0052
Oral or Poster

NOX: INNOVATING SICKLE CELL DISEASE GENETIC DIAGNOSIS WITH MICROFLUIDIC DISPOSABLE CHIPS

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Sickle Cell Disease (SCD) refers to a genetic blood disorder that affects the haemoglobin protein found within red blood cells (RBCs). This condition is caused by a point mutation in the gene that encodes the β-globin chain (HBB), leading to a structural variant of the normal adult haemoglobin tetramer, Haemoglobin A (HbA), into Haemoglobin S (HbS) or Haemoglobin C (HbC), leading the RBC to change their shape [1]. While high-income countries have achieved significant progress in managing SCD, low-income countries face challenges in providing accessible and effective interventions, where more than 80% of individuals born with SCD remain undiagnosed, and less than half of them survive beyond the age of 5 [2]. This paper introduces a novel PCR-based genetic diagnostic testing technology for SCD, designed for implementation on a disposable microfluidic chip.

NOX technology is based on the kinetic hairpin oligonucleotide blockers model proposed by Jia et al. 2014 [3]. By adjusting the stem length and temperature conditions of hairpin oligonucleotide blockers, we tailored the binding process to target sequences effectively, while by optimizing the blocker binding temperature within specific steps of the PCR process, we enhanced its preference for hybridizing with the target template (Fig. 1). Because with this technology we "block" our targets, avoiding the primers to bind and amplify them, a positive result is read as the absence of fluorescence, therefore, we called this novel approach **NOX**.

Our preliminary results, depicted in Figure 2, demonstrate that the incorporation of the hairpin blocker effectively retards the amplification process of 1,000 to 10,000 copies of the target sequence. These results indicate that the hairpin blocker effectively inhibits the amplification of their matched target up to 100 copies per reaction.

To increase the potential of this technology, we designed a three-layer disposable microfluidic chip (Figure 3A-B). The 3x3 cm chip, consisting of a single inlet, eight reaction chambers, and outlets, was designed in a snowflake shape to aid the loading of the DNA samples by centrifugal force. The fabrication materials were chosen following the work of Bae et al (2018)[4] for a disposable multi-chamber film-based PCR chip. The top and bottom layers are PET film, while the middle layer holding the chambers and channels is PVC double-adhesive film. Inlets, outlets, reaction chambers, and channels are obtained by cutting with a plotting cutter, and fabrication is done by sequentially stacking the films. A 3D view of the chip can be seen in Figure 3B.

The implementation of this microfluidic chip by the hand of our new technology promises to bring affordable genetic testing, contributing to better management of SCD in underserved regions.

Acknowledgements
This work is supported by the Macau Science and Technology Development Fund (FDCT) [FDCT 0168/2023/RIA3]; University of Macau [MYRG-GRG2023-00034-IME]

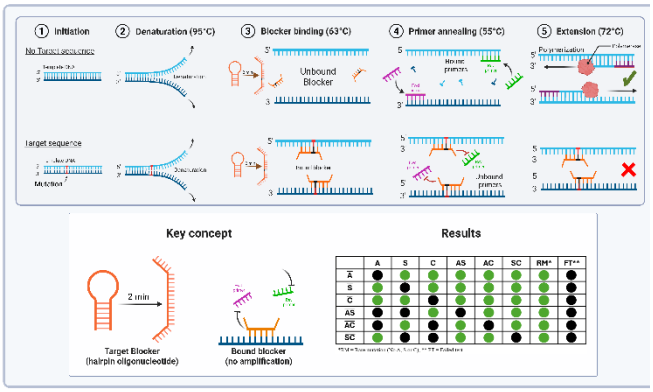

Figure 1: Schematic description of selective amplification by using the hairpin blockers

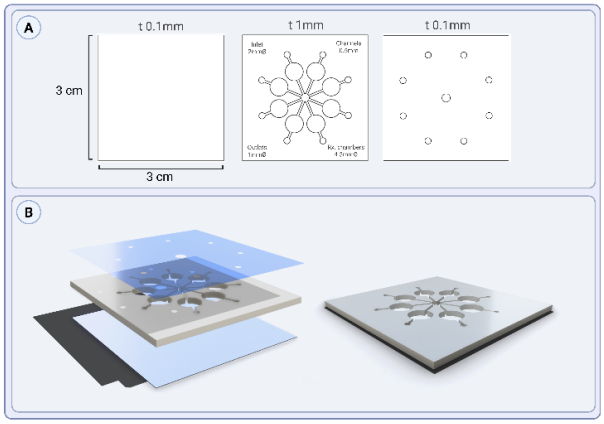

Figure 3: A. AutoCAD design of the three layers of the disposable chip with measurements. B. 3D view of stacking layers and final result after assembling.

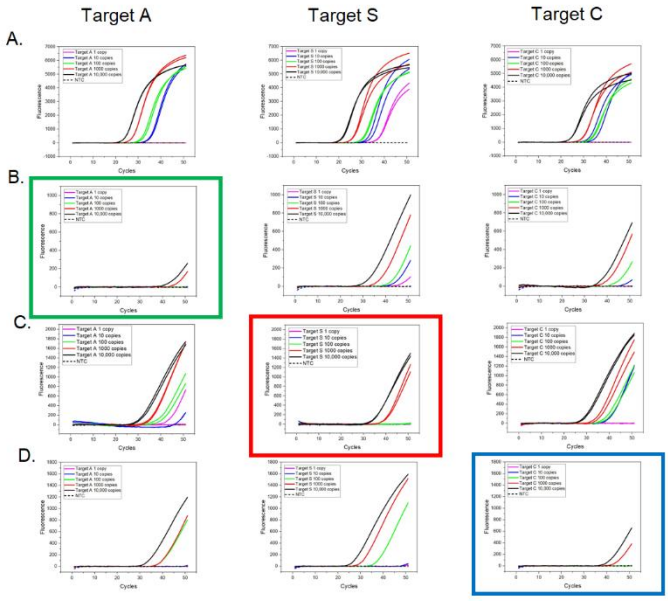

Figure 2: PCR fluorescence reading during NOX-PCR amplification on synthetic DNA. A. Control. B. Blocker A. C. Blocker S. D. Blocker C. Green box, blocked target A, Red box, blocked target S, Blue box, blocked target C.

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