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AUTOMATION OF A DNA COMPUTING READOUT METHOD BASED ON REAL-TIME PCR IMPLEMENTED ON A LIGHTCYCLER SYSTEM

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Abstract. *A DNA computer readout approach based on real-time polymerase chain reaction (PCR) for the computation of Hamiltonian Path Problem (HPP) is the main interest in this research. Based on this methodology, real-time amplification of DNA template with TaqMan probes is performed with some modifications to realize the readout. The readout approach consists of two phases: real-time amplification in vitro followed by information processing in silico to assess the results of real-time amplification. The in silico information processing enables the extraction of the Hamiltonian path but the TaqMan "YES" and "NO" reactions produced by real-time PCR need to be identified manually beforehand. Manual identification or classification limits the capability of automated readout. Hence, in this study, the readout approach is further improved by incorporating Fuzzy C-means clustering algorithm in the in silico information processing phase. As a result, automatic classification of the TaqMan "YES" and "NO" reactions is possible as demonstrated by the experimental results. Finally, an automated readout method can be realized as supported by the advantages of real-time PCR and Fuzzy C-means clustering algorithm.*

Keywords: DNA computing, LightCycler system, Readout method, Real-time PCR, TaqMan probe

1. **Introduction.** Real-time Polymerase Chain Reaction (PCR) is based on the revolutionary method of PCR [1], a technique that allows researchers to amplify specific pieces of DNA more than a billion-fold. The simplest method is based on the ability of SYBR Green dye to bind non-specifically to any double-stranded DNA in a reaction mixture, including primer-dimers and other nonspecific products that may be generated during amplification [2]. A more specific strategy depends on different reporter molecules such as TaqMan or hydrolysis probes [3], molecular beacons [4], and hybridization probes [5].

In DNA computing, Ibrahim *et al.* [6] have firstly implemented a TaqMan-based real-time PCR for reading out DNA molecules that encode a Hamiltonian path. The readout method consists of *in vitro* computation and *in silico* information processing. During *in vitro* phase, several TaqMan-based real-time polymerase chain reactions are implemented to investigate the order of the Hamiltonian path. The output of the real-time PCR can be distinguished as either a "YES" or "NO" reaction. The "YES" or "NO" reactions