A MODULAR MICROFLUIDIC SYSTEM FOR MUTATION DETECTION

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ABSTRACT

A modular microfluidic system was designed to detect point mutations of K-ras gene, which have high diagnostic values for colorectal cancers, in human genome. Genomic DNA, extracted from cell lines of K-ras genotype1, is used to get a number of DNA segments of interest through polymerase chain reaction (PCR), a method to amplify a specific sequence of nucleotides within a double-stranded DNA (dsDNA). A PCR sample, two oligonucleotides, and DNA ligase are needed to perform ligase detection reaction (LDR). A single base change, called a point mutation, can be detected to join two adjacent oligonucleotides using a DNA ligase, when both are annealed to the complementary strand of the target DNA including a point mutation during the LDR. Therefore, this PCR/LDR analysis can discriminate low-abundant mutant DNAs from wild-type DNAs2 (See Figure 1).

Four functional modules were designed to develop a microfluidic system to realize the PCR/LDR analysis (See Figure 2). For a modular approach to the analysis, the amplified target DNA should be mixed with a variety of reagents after PCR to allow LDR. A passive micromixer was added to continue biological processes in the system. Two continuous flow (CF) reactors were designed to perform the PCR and the LDR. As both reactions are required to do thermal cycling between two temperature zones, a module for thermal isolation was added to reduce thermal crosstalk between two CF reactors in vertical direction. To stack up the modules to construct a system, passive alignment structures were used. A combination of hemispherical pins in slot and a plate-plate lap joint was designed by a screw theory. It can help to align a module with others, specially the interconnection parts between the modules.

Thermal management must be considered to design the modular microfluidic system including two microfluidic reactors, which have need of thermal cycling, either to block thermal crosstalk among functional devices or to isolate each temperature zone on microfluidic reactors. Air pockets and commercial temperature insulation material between modules and grooves between each temperature zone were considered to do thermal management. Figure 3-2 shows the results of thermal simulation using ANSYS® v.10.0. The system consists of polycarbonate modules fabricated by hot embossing process. Thermal simulation was done to check the thermal aspects of system in case both reactors were working together. The results were referred to decide the dimensions of system.

Polymer, continuous flow ligase detection reaction (CFLDR) devices with integrated passive micromixers, were designed, fabricated and tested to check the mixers’ performance (See Figure 4). The devices each consisted of: a passive mixer for mixing a PCR sample, a cocktail of primers, and ligase, an enzyme of DNA; an incubator channel (95°C) for preheating the mixture; and a thermal cycling channel for LDR. They were produced by hot embossing polycarbonate (PC) substrates with brass mold inserts manufactured by micro-milling. Both mixers were operated with pretreated PCR samples, primers, and DNA ligase with a buffer. The results from the LDR showed that mixer can deliver a mixture with appropriate concentrations to the cycling channel. G12D point mutation of K-ras gene in human genome was detected at the ratio of 1 to 10 of mutant DNA to wild-type DNA.

Figure 1. A schematic representation of the PCR/LDR analysis for mutation detection1,2
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