

MICROFLUIDIC CAPILLARY FLOW OF DNA SAMPLE AND SEALANT FOR GENETIC TESTING

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In resource-limited settings such as field testing of environmental biological samples, it is impractical to get access to a diagnostic laboratory having sophisticated instruments, and it is desirable to use disposable genetic testing biochips that do not require liquid handling or pumping instruments for sample distribution among an array of reactors. In addition to the pump-less sample loading method, the challenge to seal an array of reactors without the use of microvalves or mechanical parts still persists. Implementation of microvalve array adds complexity to the chip fabrication and operation processes, and also reduces the space on the microchip. In this paper, we report the development of a high-throughput quantitative PCR chip platform for parallel analyses of multiple gene targets. The PCR mixture distribution among an array of 80 microreactors and subsequent isolation of the reactors were solely realized by a two-step surface tension-based microfluidic scheme, which eliminates the use of pumps, valves and liquid handling instruments. Confinement of the PCR mixture inside the micro reactors was achieved by implementing hybrid flow-restriction passive valves. The microreactors were isolated from each other by the flow of a curable liquid sealant delivered through microchannels by capillary action. We also investigated the effect of detergents that are present in most commercial PCR buffers. Presence of detergents makes the PCR buffer much more wetting on the passive capillary valve surface and this imposes another challenge to the design of the conventional hydrophobic patch valves which has been successfully used for deionized water. We demonstrated a successful capillary valve array with a common air venting channel having a hydrophobic surface for restricting the flow of PCR buffer containing surfactant. The interconnected microreactor array was fabricated on a glass chip substrate with approximate volume of 250 nl microreactor volume for PCR. A different set of PCR primers were preloaded into different microreactor on the PCR array chip for simultaneous amplification of multiple genes. Fluorescent signals from all the microreactors were simultaneously detected at every PCR thermal cycle using EvaGreen fluorescent dye on an in-house real-time PCR instrument. The capability of the scalable PCR array chip was demonstrated by amplifying a fragment of uidA gene for beta-glucuronidase of E.coli genome.

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