RESEARCH ABSTRACT

SWINE HEALTH

Title: PCR-on-a-Chip for the Identification and Control of Porcine Reproductive and Respiratory Syndrome Virus – NPB #04-198

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Abstract

We have been working on the development of a micro-fluidic DNA assay with the goal to quickly identify the introduction of PRRSv in pig herds in order to eliminate the transmission and spread of the virus. The project involved developing and integrating many structural components of the micro-fluidic DNA assay. Our work has led to the development of successfully operating micro PCR chambers, micropumps and capillary electrophoresis systems. A thermal cycler with flexible PCR cycle control was designed and implemented using Labview software. We have also completed fabricating and testing of gel electrophoresis micro-channels. Our results have shown that we can achieve ramp-up and -down heating times that are almost $1/10^{th}$ of any conventional system. Amplification has been tested using two different dilution ratios with extremely low (picogram level) template DNA concentration on this platform. We have developed a chamber design which demonstrates negligible non specific binding of the template DNA. We have been able to successfully demonstrate multiple usage of this chip without cross contamination from the previous run by introducing a wash step in between with elution buffer and RNase free water. This observation and the fluorescence studies provide us a basis of demonstrating the reusability of this platform for multiple PCR runs. The capillary electrophoresis system was fabricated using a 225 micron PDMS glass capillary filled with ethidium bromide doped agarose sample. Two thin platinum wires were used as electrodes and 300 V DC was supplied in the agarose capillary. A cross capillary was used to load a 100-1000bp DNA ladder and this was successfully electrophoresed inside the capillary in about 50 secs. Additionally, we amplified a 527-bp viral DNA of Infectious Bovine Rhinetracheotis (IBR) on a microchip scale because of its high gel fluorescence response of this stain (reported earlier). We have also amplified 750 bp PRRS isolate. In addition, we developed a novel gel material using doped Platinum nanoparticles in which a substantially enhanced mobility of the IBR and PRRS stains during electrophoresis was achieved.