

Abstract

The design and implementation of miniaturised systems for analysis of nucleic acids from various biological samples has undergone extensive development. Several advances have been made particularly with the integration of nucleic acid amplification and detection, where amplification is most often polymerase chain reaction (PCR). Sample preparation remains a major obstacle for achieving a quantitative analysis employing full miniaturised integration. Miniaturised devices for nucleic acid sample preparation, amplification and detection have to be further developed in order to achieve a fully integrated system, which ultimately can perform single cells genomic analysis with sample-in-answer-out ability.

In this thesis, three miniaturised systems have been presented, which can be used for purification and preconcentration of DNA, pre-amplification and long-term storage of DNA, and amplification with real-time detection of DNA, respectively. The first miniaturised system applies isotachopheresis for pretreatment of DNA, where the DNA sample can be purified and concentrated using a discontinuous electrolyte system. Both qualitative and quantitative information can be acquired simultaneously. The second miniaturised system employs simple isothermal multiple displacement amplification, (MDA) for whole genome amplification (WGA) of human genomic DNA. The miniaturised WGA process showed a high efficiency of 95.8%, and the fidelity of the amplified products is extremely high as suggested by single-nucleotide polymorphisms analysis. For the last system, we developed a bidirectional shunting PCR microdevice equipped with real-time fluorescence detection, which allows higher flexibility and fast thermocycling by combining both advantages of stationary PCR and continuous-flow PCR. Real-time monitoring of RNase P PCR amplification from lower concentration human genomic DNA down to ~24 copy numbers or 12 cells was achieved.

The three systems described in this thesis can be readily adapted to current reported miniaturised platforms. Such a fully integrated device capable of quantitative nucleic acid analysis remains an enigma, and with further development will represent significant importance for the development of point-of-care device.