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Extraction of DNA from difficult samples with an automatable portable system for early detection of intestinal cancer

Autor: Wojciech Hahnel
Institut / Klinik: Institut für Molekular- und Zellbiologie der Hochschule Mannheim
Doktorvater: Prof. Dr. M. Hafner

Extraction of DNA from so-called difficult samples like feces and soil is problematic, because of the presence of compounds which are mutagenic and destructive against DNA and inhibitors that influence further processing of DNA. As fecal DNA contains DNA from various sources, like gut flora or intestinal mucosa, it has diagnostic relevance. Analysis of fecal DNA might therefore allow conclusions on the presence of (intestinal) diseases like tumors and infections at early stages in a quick and non-invasive manner. DNA extracted from soil, on the other hand, allows conclusions on the composition of the microflora and the purification and analysis of DNA from specific bacteria. Unfortunately, all methods for extraction of DNA from difficult samples currently available are neither quick nor automated nor easy to use. Thus, the development of an easy-to-use, automatable and portable system for extraction of DNA from difficult samples would be beneficial.

At first, a suitable system was searched as base for further modifications. This system was then scaled down to microchip-size and several modifications to the original protocol were made to adapt the extraction system to difficult samples. A number of microchip designs were developed, built and tested with the new extraction method and the protocol was adapted to it. As examples for difficult samples soil and, in later experiments, human feces were used for further evaluations.

In order to make extraction possible inside a chip without the use of a centrifuge, sedimentation was introduced to replace a centrifugation step in the extraction protocol. Finally, complete DNA extraction was performed inside a chip, from sample addition to final elution. As an advantage, the majority of parts are reusable, except for a small fraction of tubes. The chip itself might be reused as well.

Bacteria DNA was successfully extracted from soil and from stool samples with the microsystem, even from spiked samples with very low bacteria count. However, DNA yield from stool was lower than from soil samples. Extraction and detection of human DNA from stool was not successful. For this, further modifications to the extraction protocol and / or the system itself are needed.

The microsystem developed within this work is easy to use and the established protocol is quick to perform. It is significantly faster than current column-based methods and requires a minimal number of manual steps. The system offers options for automation, so that it might be possible to increase speed and simplicity even further. Additionally, a PCR step could be integrated in the chip as well as specific markers, so that the extraction system may be expanded to a detection system, e.g. for diseases (stool) or specific bacteria (soil).