TOWARDS AUTOMATED STR-TYPING: DIFFERENTIAL EXTRACTION AND PCR AMPLIFICATION WITH PLASTIC MICROFLUIDIC DEVICES*

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Conventional short tandem repeat (STR) typing is comprised of several laborious and time-consuming steps: sample solubilization, DNA extraction, DNA quantification, PCR amplification, capillary electrophoresis, and data analysis. We have developed microfluidic lab-on-a-chip solutions to automate and accelerate the process. A plastic cartridge with on-board heaters, pumps and valves was fabricated for the differential extraction of sexual assault samples. The fully-automated assay performed by the cartridge is based on the differential lysis of epithelial and sperm cells, combined with magnetic bead based extraction of DNA. Magnetic bead based DNA capture kits from different companies (Dynal, Agencourt, Invitrogen and Agowa) were compared for DNA capture efficiency and ease of automation. The Invitrogen kit which was found to perform best was used for the fully automated cartridge extraction. The extracted DNA was amplified using the AmpFlSTR® IdentifilerTM multiplex PCR amplification kits (ABI), and was quantified with the QuantifilerTM Human DNA and QuantifilerTM Y Human Male DNA quantification kits (ABI). First experimental results indicate that the developed cartridge can efficiently separate and extract DNA from epithelial and sperm cells that can be used to generate high-quality STR-typing data. In addition to the differential extraction cartridge an injection-molded PCR chip and an in-house designed PCR-cycler was fabricated and tested. The efficiency of the chip-amplifications as tested with the AmpFlSTR® IdentifilerTM multiplex PCR amplification kits (ABI) were found to be comparable to that of commercial bench-top PCR instruments. First examples of an integrated sample-prep module combining differential extraction and PCR will be presented. *This work was supported by the U.S. Department of Justice, Federal Bureau of Investigation under contract J-FBI-03-085. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. Government.