

Forensics



Automation of the INVITROGEN ChargeSwitch® Forensic DNA Purification kit for Genotyping from forensic samples

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To manage the analysis of the increasing number of samples, the DNA isolation process was automated. The ChargeSwitch® Forensic DNA Purification Kit (Invitrogen) was chosen, because it allows the purification of genomic DNA from a wide range of forensic samples. The kit was adapted for a MICROLAB® STARlet with 8 channels. The method is validated for the DNA-rich samples bloodstain and cigarette butts/paper. The samples are processed on the system in sets of 24 or 48 samples. After the extraction, samples are successfully quantified by real-time PCR and then genotyped by STR analysis. The automation of extraction from other sample types, like sexual assaults, will be done in the near future.

Equipment and Material

Equipment

- Microlab® Starlet, 8 Channels, Autoload
- CO-RE Gripper for plate and lid handling
- Multiflex Heating Module, for incubation (at 55°C)
- Carriers for plates and tubes
- Variomag Teleshake (Thermo Electron Oberschleißheim GmbH)
- Magnet Agencourt SRI Plate 96R



Figure 1: The method is automated on the MICROLAB STARlet.

- PTC-200-Chromo4 Real TimeThermocycler (BioRad)
- Thermal Cycler GeneAmp 9700 (Applied Biosystems)
- Genetic Analyzer 3130XL (Applied Biosystems)

Reagents

- ChargeSwitch® Forensic DNA Purification Kit (Invitrogen)
- AmpF/STR Identifier PCR Amplification (Applied Biosystems)
- Quantifiler Human DNA Quantification (Applied Biosystems)

Protocol

Application Software

All methods were programmed on the HAMILTON Vector standard software including specific liquid handling features. For integration into the LIMS system, the standard work list handling commands of the Vector software are used.

Application

The method is designed to process 24 or 48 samples, including positive and negative controls. A file with the barcode list of all sample-lysates, created by the LIMS, is provided to the STARlet at the beginning of every method. All plate transports are done automatically with the CORE gripper. The full protocol takes 50 minutes for 24 samples and 1h25min for 48 samples.

For binding, the sample lysates (500µl) are transferred to the deep well plate, positioned on the shaker. After the addition of 100µl CST purification buffer and 20µl CST beads to the samples, the plate is shaken for 60s at 1400rpm and then incubated for 5min at room temperature.

For washing, the plate is transferred with the CORE gripper to the Ring Magnet adapter. After an incubation of 2min 30s, the beads are attracted to the magnets and the supernatant is aspirated for disposal. The plate is then transferred back to the shaker, where 500µl of CST wash buffer is added to every well. After shaking of the plate to



resuspend the beads, the plate is transferred back to the magnet. After a 90s incubation, the supernatant is aspirated and discarded. The beads are washed a second time with 450µl wash buffer.

For elution, the beads are resuspended in 50µl CST elution buffer on the shaker for 60s at 1400rpm. For higher elution efficiency, the plate is transferred to a heating module for 5min at 55°C. After incubation, the plate is transferred to the round magnet for 60s. The supernatant is then aspirated and transferred to micro tubes labeled with barcodes for storage.

Results

DNA quantification

The quantity of DNA is measured by real time PCR with the Quantifiler Human DNA Quantification Kit (Figure 2). A volume of 2µl of DNA is used for 23µl of Master Mix. The standards range is diluted by the STARlet and dispensed twice onto the PCR plate. Following PCR conditions are used on the PTC-200 Chromo4 Thermocycler: Hot Start at 95°C for 10 min and 40 Cycles with 95°C for 15s and 60°C for 60s.

The results of the real time PCR quantification are accepted, if the Ct-value of the internal positive control measurement is within the limits of 28 to 32.

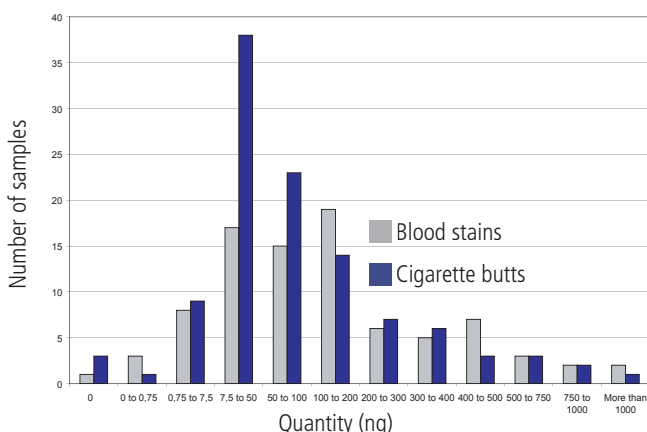


Figure 2: DNA yield from different typical forensic samples. The minimum amount of DNA required for STR analysis is 3ng.

In order to test for cross contamination, blank samples next to positive samples are positioned in a check board pattern on the purification plate. No cross contamination was detected when analyzing the samples by quantitative real time PCR.

STR Genotyping

For genotyping, 3ng of purified DNA is amplified with the Amp/STR Identifier PCR kit. This kit allows the simultaneous amplification of 16 STR loci including Amelogenin, which is used in sex deter-

mination. After fragment separation and detection on the Genetic Analyser 3130XL, the data is analyzed with the GeneMapper ID Software (Figure 3). The percentage of successful STR profiles from different forensic samples purified on the STARlet robot is always higher than 90%.

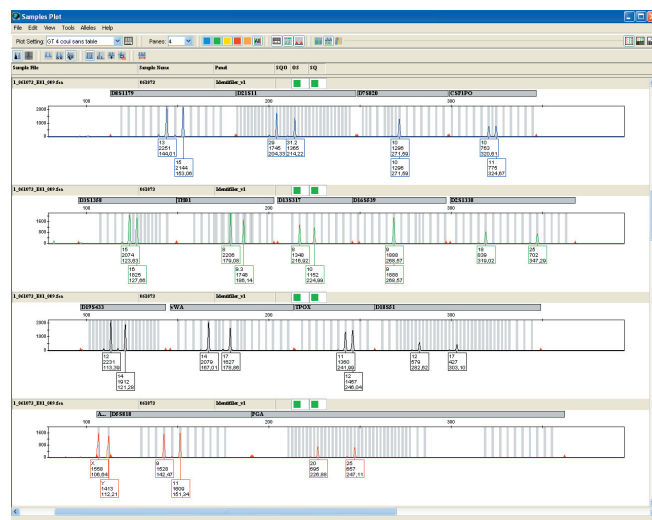


Figure 3: Example of STR profile obtained from a blood spot using the described protocol.

Discussion

In order to achieve higher DNA yields with a good robustness of the protocols, several changes have been made to the original manual ChargeSwitch® protocol. The optimized method is so far validated for rich samples (bloodstains and cigarette butts/paper). The validation of other samples types, like sexual assault samples, will be done at a later time point.

The autoload system for barcode reading of the STARlet allows to increase the security of the sample identification and traceability of the whole process.

Features and Benefits

- Complete sample traceability and loading security with the Auto-load barcode reading system of the STARlet
- Easy integration with the LIMS by work list handling
- Fully automated hands-free processing with built in robotic plate handler (CORE-Grip)
- Good reproducibility of the process due to optimization of the manual protocol
- Contamination free process

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